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Dispersal patterns and connectivity among Mediterranean reef fish populations: the case of the saddled sea bream, O*blada melanura* [Linnaeus, 1758] in the Western Mediterranean Sea

Patrones de dispersión y conectividad entre poblaciones de peces de arrecifes Mediterráneos: el caso de la doblada, *Oblada melanura* [Linnaeus, 1758] en el Mediterráneo occidental

Antonio Calò

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Universidad de Murcia

Facultad de Biología

Departmento de Ecología e Hidrología

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Resumen

RESUMEN

Los ecosistemas marinos costeros están sujetos a una multitud de impactos antropogénicos. La pesca intensiva está considerada como una de las amenazas más graves y ha conducido a una sobreexplotación de las poblaciones de peces en todo el mundo. Las áreas marinas protegidas (AMPs) son sectores de la costa o del mar donde las actividades humanas, en particular la pesca, están reguladas, pero no necesariamente prohibidas. Las AMPs constituyen una de las estrategias más importantes para proteger la integridad y la restauración de la salud de los ecosistemas marinos frente a múltiples factores de estrés. Las AMPs son consideradas una poderosa herramienta para la gestión de los recursos marinos y la conservación de la biodiversidad, capaz de producir una amplia gama de beneficios dentro de sus límites. A pesar de la necesidad a nivel mundial de aumentar la superficie de las áreas protegidas, en la actualidad el 2,2% de los mares se incluye dentro de zonas protegidas y solamente el 1% está protegido formalmente ante todas las formas de pesca. Las AMPs bien gestionadas se demuestran muy eficaces para evitar la sobrepesca en áreas específicas y conservar la biodiversidad. Un efecto bien documentado de las AMPs es el aumento de la densidad y del tamaño de los peces dentro de los límites de la reserva, especialmente en las especies objetivo de la pesca. Un aumento de la talla corporal de las hembras y de sus abundancia, debido al "efecto reserva", determinaría una mayor producción de propágulos (huevos y larvas) dentro del AMP, potencialmente capaz de producir beneficios también en las zonas exteriores a través de la exportación o desbordamiento ('*spillover*') de biomasa. Este proceso es especialmente importante para las especies que tienen una fase larvaria pelágica, durante la cual los huevos y larvas pueden ser transportados por las corrientes marinas, pudiendo llegar a áreas no protegidas muy alejadas de las zonas protegidas. Por lo tanto, el estudio de los efectos de las AMPs y los procesos biológicos y ecológicos que los determinan resulta fundamental para evaluar el alcance de los objetivos de protección y aumentar la eficacia de las AMPs.

La dispersión es el proceso mediante el cual los seres vivos expanden activamente o pasivamente el espacio o el rango geográfico en el que viven,

y es una de las características de la historia de vida de las especies que afectan a las dinámicas de las poblaciones espacialmente estructuradas. En la mayoría de las especies de peces costeros la magnitud de la dispersión se debe principalmente a la fase de propágulo. La dispersión determina el nivel de "conectividad" entre las poblaciones, es decir, la dirección y la magnitud de las conexiones demográficas entre las poblaciones a través del intercambio de individuos en cualquier etapa de la vida: huevos, larvas, juveniles y adultos. El grado en el que se conectan las poblaciones de peces tiene implicaciones importantes para sus persistencias, su manera de responder a las amenazas naturales y antropogénicas, y el modo en el que han de ser manejadas: poblaciones altamente conectadas son más capaces de resistir a las perturbaciones locales que degradan o eliminan sus hábitat, y a lo largo de tiempo son menos propensas a la extinción local. En este contexto, el conocimiento de los patrones de dispersión y conectividad es crucial para optimizar las estrategias de conservación, por ejemplo mediante la mejora de las directrices para el establecimiento de AMPs más eficaces.

Aunque la eficacia de un AMP depende de sus objetivos, un fin común y esperado es el restablecimiento de los recursos pesqueros dentro de los límites del AMP, hasta alcanzar niveles que permitan beneficios en áreas exteriores a través de la exportación de los individuos. Por lo tanto, la recogida de información sobre la dispersión y la conectividad resulta fundamental para establecer la escala espacial a la cual la exportación de propágulos de un AMP puede sostener las pesquerías exteriores u otras AMPs que pudieran formar parte de una red. Según la definición de la UICN, una red de AMPs es 'una colección de zonas marinas protegidas que están en funcionamiento de manera cooperativa y sinérgicamente, a varias escalas espaciales, y con un rango de niveles de protección, con el fin de cumplir con los objetivos ecológicos de manera más eficaz y amplia que los sitios individuales por sí solos'. El correcto diseño de redes de AMPs se basa en una serie de criterios espaciales, tales como el tamaño adecuado de cada AMP, su número y la distancia entre ellas. Este último concepto se refiere a la posición relativa entre las zonas protegidas dentro de la red y depende de la escala espacial de la dispersión y la conectividad entre las poblaciones locales de especies p salud de los ecosistemas marinos frente a múltiples factores de estrés. Las AMPs son consideradas una poderosa herramienta para la gestión de los recursos marinos y la conservación de la biodiversidad, capaz de producir una amplia gama de beneficios dentro de sus límites. En este contexto, con el fin de establecer redes de AMPs más eficientes se necesita mejorar nuestro conocimiento sobre las características biológicas y ecológicas de las especies, especialmente durante la fase pelágica, su capacidad de dispersión y los patrones de conectividad, teniendo en cuenta diferentes escalas espaciales y temporales.

El sector sur-oriental de la costa española (Región de Murcia y el sur de la provincia de Alicante) acoge actualmente dos de las AMPs más eficaces del mar Mediterráneo: la reserva marina de Tabarca (establecida en 1986) y la reserva marina de Cabo de Palos e Islas Hormigas (establecida en 1995). Considerando la necesidad (creada merced a la adhesión española a diversos compromisos internacionales) de ampliar la superficie de los ecosistemas marinos que se encuentran bajo protección frente a la pesca y otras actividades antrópicas, existe la intención, tanto a nivel nacional como a escala regional, de establecer nuevas AMPs a lo largo del mencionado tramo de costa, que pueda generar una eficiente red de estos espacios protegidos. Por esta razón, resulta urgente contar con información acerca de los patrones de dispersión y de conectividad entre las poblaciones locales (protegidas o no protegidas), con el fin de establecer la disposición geográfica adecuada de las futuras AMPs dentro de la red, y maximizar así sus beneficios.

El objetivo de la presente tesis fue la evaluación de los patrones de dispersión y de conectividad en una especie de pez costero utilizando un enfoque multidisciplinar a múltiples escalas espaciales: 1) a escala Mediterráneo noroccidental, investigamos los patrones de conectividad genética; 2) a lo largo de la costa del sureste ibérico evaluamos los patrones de conectividad ecológica. En concreto, nos centramos en los patrones de conectividad como resultado de la dispersión de propágulos, ya que esta fase de la vida es considerada la principal responsable de los movimientos de especies de peces costeros. La doblada (*Oblada melanura* [Linnaeus, 1758]) fue seleccionada como una especie modelo por su amplia distribución en los ecosistemas costeros mediterráneos. También es una especie con un valor relativamente alto para la pesca artesanal y recreativa.

En el Capítulo II se revisó la literatura disponible sobre las diferentes metodologías aplicadas en el Mediterráneo para evaluar la dispersión y la conectividad entre las poblaciones de peces. En el capítulo III se analizaron los patrones de conectividad genética a escala regional, considerando AMPs y zonas no protegidas del Mediterráneo noroccidental. Luego, a escala espacial más fina, centrándonos en la costa del sureste ibérico, se investigaron los patrones de dispersión y de conectividad ecológica. En el capítulo IV se determinó la variabilidad espacial de los rasgos biológicos de las primeras fases del ciclo de vida de la doblada, que influyen de manera importante en la dispersión de propágulos a lo largo de la costa. En el capítulo V se evaluó la magnitud de la dispersión de propágulos y el número de posibles orígenes natales que contribuyen a la provisión de larvas a lo largo de la costa. En el capítulo VI se partió de las informaciones obtenidas en los capítulos IV y V para desarrollar un modelo de dispersión de propágulos y determinar la presunta posición geográfica de las orígenes natales. Por último, en la sección de discusión (cápitulo VII) se combinaron todas las informaciones obtenidas a partir de los enfoques complementarios utilizados, y se proporcionaron sugerencias para el futuro diseño de AMPs en el Mediterráneo español, con especial incidencia en el sureste ibérico.

Los resultados de los análisis genéticos indican que las AMPs investigadas en el Mediterráneo occidental están bien conectadas con las zonas cercanas no protegidas. Este es un resultado importante por lo que supone el mantenimiento de las poblaciones de peces, dado que el enlace genético es la única prueba de que efectivamente ha ocurrido un intercambio entre poblaciones de adultos capaces de reproducirse. Por otra parte, se han detectado señales de estructuración genética a escala regional que sugirieren que la presencia de barreras oceanográficas puede afectar los flujos genéticos, y ser potencialmente capaz de generar rupturas genéticas a lo largo del devenir evolutivo. Por esta razón, es importante hacer un seguimiento de la evolución de la dinámica genética entre poblaciones, incluyendo otras especies con diferentes características biológicas y ecológicas.

Las reservas marinas de Cabo de Palos - Islas Hormigas y Tabarca son dos de las AMPs más eficaces desde el punto de vista ecológico y pesquero en el Mediterráneo occidental. En ambas AMPs se ha registrado un aumento de biomasa de peces, especialmente debido al aumento del tamaño y número de depredadores apicales (por ejemplo los meros). Las evidencias de los beneficios de protección y la distancia relativamente corta (menos de 60 km) entre las dos reservas permiten plantear la hipótesis de que las AMPs pueden actuar recíprocamente como fuente/sumidero de propágulos, proporcionando también un suministro importante de propágulos para las áreas no protegidas situadas entre ellas. Los resultados de este estudio sugieren, sin embargo, que ambas reservas marinas - Cabo de Palos - Islas Hormigas y Tabarca, pueden estar menos vinculadas por la dispersión de propágulos de lo esperable. Las simulaciones de dispersión mostraron que la dinámica oceanográfica alrededor del cabo de Palos, caracterizada por fuertes corrientes que fluyen predominantemente hacia el sur, puede desplazar rápidamente propágulos lejos del AMP, incluso en especies con una duración de la vida pelágica relativamente corta, como es la que nos ocupa. Por el contrario, la circulación de las aguas alrededor de la isla de Tabarca parece promover un alto nivel de retención local, principalmente atribuible a la presencia de remolinos costeros. Este resultado fue corroborado por la diferente distribución de los orígenes natales entre los sitios alrededor de ambas reservas, destacando que fuentes distintas de larvas suministraron a las dos zonas en el período considerado. La posible reducción del nivel de exportación demográfica de Tabarca se ve aún más agravada por la disposición espacial del AMP de Cabo de Palos, en la cual la superficie de los potenciales hábitats adecuados para el asentamiento de peces costeros (básicamente, fondos rocosos poco profundos y praderas de *Posidonia oceanica*) que se encuentra bajo protección es muy pequeña. Como consecuencia de ello, incluso con un nivel de aportación de propágulos suficiente, el AMP de Cabo de Palos - Islas Hormigas podria no ser capaz de proporcionar las condiciones adecuadas para la supervivencia de los juveniles de muchas especies costeras.

Además, las informaciones extraídas a partir de la aplicación de las diferentes metodologías empleadas en el presente estudio son coherentes entre sí, y concuerdan en que la posible conexión ecológica entre zonas durante la fase de propágulos sería muy baja. Nuestros resultados proporcionan indicios de que la parte sur del litoral de la región de Murcia, donde se ha previsto el establecimiento de al menos una reserva marina en el futuro próximo, podría estar separada de la parte norte en términos de flujos de propágulos, con Cabo de Palos representando el límite entre ambos sistemas geográficos. La composición de las orígenes natales de los juveniles muestreados fue muy diferente entre el norte y el sur del área de estudio. Junto con este resultado se registró una diferencia en la duración de la fase larvaria pelágica de *O. melanura* entre las dos zonas. Dada la homogeneidad genética global observada en la región, que permite suponer la ausencia de una separación evolutiva en curso, tanto la diferencia biológica como la ecológica podrían ser una consecuencia de las características ambientales que se dan en el área de estudio, especialmente la circulación de las masas de agua. La dinámica oceanográfica a meso-escala que caracteriza el Mediterráneo suroccidental podría ser responsable de una regionalización natural de las masas de agua a lo largo del sistema hidrodinámico que conecta el golfo de Orán (Argelia) con la costa española en los alrededores del cabo de Palos.

Las diferentes piezas de conocimiento obtenidas de este estudio proporcionan información para apoyar el diseño de nuevas AMPs a lo largo de la costa sureste de la península Ibérica. Los resultados indican que la disposición espacial y la posición de las AMPs existentes no parecen generar el óptimo intercambio de propágulos, esperable en redes eficientes de AMPs. Además, las reservas marinas de Cabo de Palos - Islas Hormigas y Tabarca, a pesar de su potencial para la producción de propágulos, no parecen garantizar un suministro suficiente de huevos y larvas hacia las zonas en el sur de la región. Desde esta perspectiva, el establecimiento de nuevas AMPs en el sector sur de la región es recomendable para mejorar el estado de conservación actual de la zona. El posicionamiento de otras AMPs en puntos estratégicos de la costa, no solamente puede aumentar la extensión de los ecosistemas protegidos, sino permitiría ampliar la superficie de hábitat protegido para el asentamiento de las larvas exportadas desde la AMP de Cabo de Palos. En cuanto al sector norte, la escasez de conexión registrada entre Tabarca y Cabo de Palos podría mejorarse aumentando la extensión de los hábitat de aguas poco profundas sometidas a protección entre las dos zonas marinas protegidas. Estas áreas se caracterizan principalmente por extensas praderas de *Posidonia oceanica* y representarían los lugares de asentamiento perfecto para una gran variedad de peces costeros. Desde este punto de vista, podrían actuar como hábitat de cría de larvas procedentes de Tabarca antes que se agreguen como juveniles y sub-adultos en las poblaciones de adultos que viven en la reserva marina de Cabo de Palos.

Es importante destacar que nuestros resultados representan sólo una parte de la información necesaria para el correcto diseño de una red de AMPs. El análisis de una sola especie, aunque representativa de otras especies con características biológicas y ecológicas similares, no es suficiente para obtener una idea adecuada de los patrones de dispersión y de conectividad. Por lo tanto, sería importante aplicar el mismo procedimiento de investigación incluyendo especies con diferentes rasgos biológicos y ecológicos y considerando un dominio temporal más largo. Sin embargo, como ya se ha señalado en otros trabajos, el presente estudio demuestra la eficacia de la combinación de herramientas complementarias para la investigación de los patrones de dispersión de propágulos y la conectividad de las poblaciones.

Chapter I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The need for marine resources conservation

Coastal marine ecosystems are subject to a multitude of anthropogenic impacts (Halpern *et al.* 2008). Intense fishing is considered one of the most serious threats, leading to a worldwide overexploitation of fish populations (Lotze *et al.* 2006). Fishing pressure in many seas exceeds sustainable levels and coastal marine fisheries are under ever increasing risks of collapse (FAO 2014). Fishing effort keeps increasing, the yields remain stable or decline and other human impacts degrade habitats and ecological systems which commercial species depend on (Sale *et al*. 2005, FAO 2014). In this context, it is clear that different conservation strategies and management measures are needed to preserve and restore the health of marine ecosystems (Grafton *et al*. 2010). Marine protected areas (MPAs) are sectors of the coastline and/or sea where human activities, particularly fishing, are regulated, but not necessarily banned. MPAs are among the most essential strategies for protecting the integrity and restoring the health of marine ecosystems from multiple stressors (Sobel and Dahlgren 2004, Claudet 2011). MPAs are considered a powerful tool for marine resources management and biodiversity conservation (Sobel and Dahlgren 2004, Lubchenco and Grorud-Colvert 2015) able to produce a wide array of benefits within their borders (Halpern and Warner 2002, Micheli *et al*. 2004, Micheli *et al*. 2005, Guidetti *et al*. 2008). In spite of the global call for increasing the extent of marine and coastal areas under protection, up to 10% by 2020 (Aichi Biodiversity Target 11 in the Convention on Biological Diversity, www.cbd.int), currently the 2.2% of the seas is included within MPAs and only the 1% is formally protected from all forms of fishing (i.e. in 'no-take marine reserves'; MPAtlas.org 2015). In addition to this an important proportion of MPAs declared are 'paper parks', i.e. areas where conservation laws are nonexistent or transgressed, consequently missing their ecological objectives (Mora *et al*. 2006). Such negative/neutral results, that are often underreported in the literature (Guidetti *et al*. 2008), are mostly due to inappropriate design or ineffective enforcement (Mora *et al*. 2006, Guidetti *et al*. 2008). Moreover, despite an increasing number of MPAs established throughout the world (Deguignet *et al.* 2014, Lubchenco and Grorud-Colvert 2015), there are many issues still unresolved, particularly regarding optimal size, number and placement.

Well enforced MPAs are demonstrated to prevent overfishing in specific areas and conserve biodiversity. Effects of protection are both direct (i.e. restoring populations of target fishes; Micheli *et al*. 2005, Claudet *et al*. 2006, Guidetti and Sala 2007) and indirect (i.e. driving whole communities towards an unfished state; Shears and Babcock 2002, Micheli *et al*. 2004). A well documented effect of MPAs is the increase in density of large-sized individuals of exploited fish within reserve boundaries (Sobel and Dahlgren 2004, Hackradt *et al.* 2014), with species targeted by fishing generally responding to protection in a clearer way compared to non-target species (Claudet *et al*. 2006, Floeter *et al*. 2006, Guidetti and Sala 2007). This 'reserve effect' mirrors in a higher biomass of target species under protected conditions (García-Charton *et al.* 2008, Guidetti *et al*. 2008, Di Franco *et al*. 2009, Gaines *et al*. 2010). From this perspective, an increase in abundance/biomass within the reserve can produce a 'spillover', i.e. adults and juveniles fish may actively move from the reserve to outer areas if the increase in abundance/biomass within the reserve would result in a decrease in the *per capita* availability of resources, such as food or refuges (Sobel and Dahlgren 2004). In addition, an increase of female body size and abundance, due to the 'reserve effect', would determine a greater production of propagules (eggs and larvae) within MPA borders, potentially able to produce benefits also to outer areas through a process named 'recruitment subsidy' (Gell and Roberts 2003). This process is particularly important for species that have a pelagic larval phase, during which eggs and larvae can be transported by sea currents and may concern unprotected areas hundreds of kilometres from MPAs (Gaines *et al*. 2007). Therefore, the study of MPA effects and the biological and ecological processes that drive them is fundamental for assessing the extent of protection benefits on unfished areas and finally enhance the effectiveness of MPAs.

Dispersal, connectivity and networks of MPAs

The life cycle of many coastal fish is composed by two phases: a pelagic propagule (egg + larva) phase and a necto-benthic juvenile/adult phase. The duration of both egg and larval stages is species-specific and can range from hours to several months (Helfman *et al*. 2009). These are critical periods in particular for fishes with a protracted propagule phase, during which mortality rates can exceed the 99,99% of mortality of the initial offspring (Houde 1989). The eggs of many coastal fishes are planktonic and transported passively by sea currents. Although few information are available about larva movement capabilities, currents are thought to play a major role also in the transport of larvae, at least during the early developmental stages of the larval life (Leis 2015). Different studies have demonstrated that during the last moments of the pelagic phase, larvae develop systems that allow them to perceive the surrounding environment and move actively (Leis *et al*. 2006). The perception of different environmental stimuli allow them to choose the most advantageous habitat for settlement, i.e. when the transition from the pelagic to the benthic life takes place and larvae metamorphosise into juvenile fishes.

Dispersal is the process by which living organisms expand actively or passively the space or range where they live and is one of the fundamental life-history traits affecting the dynamics of spatially structured populations (Cote *et al*. 2010). Coastal fish dispersal is generally divided on the base of fish life cycle in: propagule dispersal, due to the transport/movement of eggs and larvae, and post-settlement dispersal due to the movements of juveniles and adults. Even though recently evidences have highlighted that post-settlement dispersal distances could be comparable to the once produced during the pelagic phase (Di Franco *et al*. 2015), there is a general consensus that the scale of dispersal in coastal fish is mainly driven by the propagule phase (Leis 2015). Dispersal determines the level of 'connectivity' between populations, i.e. the direction and magnitude of demographic links between populations by the exchange of individuals at whatever life stage eggs/larvae, juveniles and adults (Cowen *et al*. 2006). As a consequence of individual dispersal, an exchange of genes between populations is produced, so that two 'types' of connectivity can be distinguished: ecological (or demographic) connectivity and evolutionary (or genetic) connectivity. Although there is a continuum between both types of connectivity, it is fundamental to distinguish these two concepts, because patterns of evolutionary connectivity typically develop over larger spatial scales and evolutionary time periods compared to demographic connectivity that, on the contrary, depends on more contemporary processes taking place at more local spatial scales (Leis *et al*. 2011). The existence of this dual nature has to be taken into account for the adequate design of connectivity studies, the choice of the right spatial scale to investigate and the proper analytical approach to use (Leis *et al*. 2011).

The degree to which fish populations are connected has important implications on their persistence, how they respond to natural and anthropogenic threats, and how they should be managed: highly connected populations are more able to withstand local disturbances that degrade or remove habitats, and in the long term less prone to local extinction (Morrison and Sandin 2011, Sale *et al*. 2005). In this context, knowledge on dispersal and connectivity is crucial for optimizing conservation strategies, for example improving guidelines for the establishment of more effective MPAs (Almany *et al*. 2009, Gaines *et al*. 2010). Although MPA effectiveness depends on their goals, a common and expected objective is the recovery of fishery resources within their boundaries to a level that would allow benefits in outer areas through the export of individuals (Palumbi 2003). The gathering of information on dispersal and connectivity is thus fundamental to assess the spatial scale over which the export of propagules from a MPA can sustain outer fished locations or other MPAs within a network. As defined by the IUCN, a MPA network is 'a collection of individual MPAs operating cooperatively and synergistically, at various spatial scales, and with a range of protection levels, in order to fulfil ecological aims more effectively and comprehensively than individual sites could alone' (WCPA/IUCN 2007). MPAs networks are currently considered the best strategy for achieving marine conservation goals and increasing emphasis has been placed on establishing ecologically connected networks worldwide (Jones *et al*. 2007). The correct design of network of MPAs relies on a series of criteria including the proper size of single MPAs, their number and the distance among them (Almany *et al*. 2009). This latter concept refers to the relative position between MPAs within the network and strictly depends on the spatial scale of dispersal and connectivity among local populations of protected species (Jones *et al*. 2007). In this context, in order to establish more efficient networks of MPAs there is the need to improve our knowledge on the biological and ecological traits of species, especially during the pelagic phase, their dispersal capacity and patterns of connectivity considering different spatial and temporal scales.

Aim of the study

Considering the urgent call for protecting marine ecosystems towards the achievement of the Convention of Biological Diversity (CBD) targets, it is fundamental to create the optimal scientific background for the establishment of effective MPAs and networks in the Mediterranean Sea in the next future. The south-eastern sector of the Spanish coast (Murcia Region and south Alicante province) currently hosts two effective MPAs: Tabarca MPA (established in 1986) and Cabo de Palos e Islas Hormigas MPA (established in 1995). In the context of the CBD and other EU-driven conservation initiatives (e.g. Natura 2000 Network, Maritime Strategy, see Pipitone *et al.* 2014), there is the intention both at national and regional level to establish new MPAs along the stretch of coast mentioned, potentially generating an efficient network of MPAs together with the ones already present. For this reason, information on dispersal and connectivity patterns between local populations (both protected and unprotected) are needed for the proper geographical arrangement of future MPAs within the network and maximize their protection benefits.

The aim of the present thesis is the assessment of dispersal and connectivity patterns in a Mediterranean coastal fish using a multidisciplinary approach that encompassed the available methodologies used in the Mediterranean context so far. To achieve this goal a multi-scale approach was implemented: 1) at the scale of North-Western Mediterranean Sea, we investigated patterns of evolutionary connectivity; 2) along the South-Eastern Spanish peninsular coast we assessed patterns of ecological connectivity. Specifically we focused on connectivity patterns as results of propagule dispersal, as this life phase is considered the main responsible of species movements in coastal fishes. The saddled sea bream (*Oblada melanura* [Linnaeus, 1758]) was selected as a model species due to its wide distribution in Mediterranean coastal ecosystems. It is also a species with a relatively high value both for artisanal and recreational fishery.

The thesis is structured in chapters/papers, concerning different perspectives and approaches for assessing connectivity. Firstly, in Chapter II we reviewed the available literature on all the different methodologies implemented in the Mediterranean Sea so far for assessing connectivity and dispersal between fish populations. In Chapter III we analysed patterns of genetic connectivity at the regional scale, considering MPAs and unprotected sites in the North-Western Mediterranean Sea. Then, we scaled-down the geographic context, focusing on the South-Eastern Spanish coast and using a series of methodologies (otolith micro-structural and chemical analyses and propagule dispersal simulations) for investigating patterns of dispersal and ecological connectivity at this spatial scale. In Chapter IV we investigated the spatial variability of early life traits (ELTs), important biological traits involved in shaping propagule dispersal along the coast. In Chapter V, we assessed the magnitude of propagule dispersal and the number of potential natal origins that contribute to settler replenishment along the coast. In Chapter VI the information on ELTs and natal origins was used to develop a propagule dispersal model and identify the putative geographical position of fish natal origins. Finally, in the discussion section (Chapter VII) we combined all the pieces of knowledge obtained from the complementary approaches used, providing insights for the future design of MPAs in the region.

Chapter II

A REVIEW OF METHODS TO ASSESS CONNECTIVITY AND DISPERSAL BETWEEN FISH POPULATIONS IN THE MEDITERRANEAN SEA

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A REVIEW OF METHODS TO ASSESS CONNECTIVITY AND DISPERSAL BETWEEN FISH POPULATIONS IN THE MEDITERRANEAN SEA

Abstract

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. Knowledge on connectivity and fish movements is considered fundamental toward the correct design of marine protected area (MPA) networks for the achievement of the benefits of protection. Connectivity patterns are still largely unknown worldwide. A general lack of knowledge is particularly evident for the Mediterranean Sea where few studies dealing with this topic have been carried out and some methods, currently available for assessing connectivity, have not been used yet. In this review we present the methods used for studying connectivity patterns and fish movements at different life history stages and the main results achieved until now in the Mediterranean Sea. 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 (Giller *et al*. 2004) 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. Connectivity can be defined as the movement of individuals among local or sub-populations, provided that the level of exchange is sufficient to impact the demographic rates of the local population(s) (Cowen and Sponaugle 2009). Thus, connectivity in marine populations results from the dispersal of eggs and larvae, and from the movement (daily, seasonal and ontogenetic) of juveniles and adults (Jones *et al*. 2007, Jones *et al*. 2009, Botsford *et al*. 2009a, Botsford *et al*. 2009b). 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 (Sheaves 2009). The concept of connectivity is critical to understand the functioning of marine populations and communities.

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) (Jones *et al*. 2009, Botsford *et al*. 2009a, Botsford *et al*. 2009b, Almany *et al*. 2009), where dispersal of either larvae, juveniles or/and adults is crucial to maintain populations outside the protected areas (Cowen and Sponaugle 2009). A number of recent reviews have highlighted the importance and utility of knowing connectivity patterns (Fogarty and

Botsford 2007, Jones *et al*. 2007, Jones *et al*. 2009, Botsford *et al*. 2009a, Botsford *et al*. 2009b), as well as the methods available to estimate connectivity (Cowen *et al*. 2000, Palumbi 2004, Cowen *et al*. 2006, Leis *et al*. 2011, Leis *et al*. 2013).

In spite of the above considerations, all over the world the degree of knowledge on species dispersal and connectivity patterns is still scarce (Leis *et al*. 2013), with the most of the studies carried out on tropical species (Burgess *et al*. 2014). Concerning temperate ecosystems, few measure of connectivity are currently available. This is especially true in the case of the Mediterranean Sea, despite being an ideal region for such kind of studies, considering its well studied oceanographic patterns (Schunter *et al*. 2011a). Moreover, the great importance of Mediterranean Sea as biodiversity hotspot (Coll *et al*. 2010) and the cumulative human threats it suffers (Coll *et al*. 2012, Sala *et al*. 2012) make the research on connectivity to be an urgent endeavour (Narayanaswamy *et al*. 2013).

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 (Sabatés *et al*. 2007, Sala *et al*. 2012, Fenberg *et al*. 2012, Unep-MAP 2012). 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 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 the 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) that determine the observed spatial and temporal patterns (Sabatés *et al*. 2007). 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 (Sabatés *et al*. 2007, López-Sanz *et al*. 2009).

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 Pelc *et al*. 2010). In a pioneering study, Vidal-Peñas *et al*. (2001) 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*. (2001) 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*. (2003) 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, Catalonia, NE Spain). Their main findings were that the abundance and species richness of the larvae of some resident species was high in the MPA but also in the adjacent unprotected coastal area. 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*. (2009) 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.

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 (Crec'Hriou *et al*. 2010), Medes islands marine reserve (Lopez-Sanz *et al*. 2011), Cerbère-Banyuls natural marine reserve (Lawson 2006), Côte Bleue marine park (Crec'hriou *et al*. 2008) and Cabo de Palos – Islas Hormigas marine fisheries reserve (Lawson 2006, Crec'hriou *et al*. 2008). 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 (Leis and McCormick 2002) – by means of light traps (Milicich and Doherty 1994, Sponaugle and Cowen 1996). This approach is new in the Mediterranean context, with the exception of one study from the '90s in which an experimental purse seine coupled to a light device was used to capture young fishes (mostly juveniles of *Mullus surmuletus*) (Deudero 2002). In a seminal study performed installing light-traps in the vicinity of the Cabo de Palos – Islas Hormigas marine reserve (Félix-Hackradt 2012, Félix-Hackradt *et al*. 2013), 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.

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 (Sabates 1990a). Even so, the usefulness of such mesoscale ichthyoplankton studies to determine connectivity models is limited, due to their weak predictive value. 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. However, despite these limitations, ichthyoplankton studies can not be neglected, since in many cases they represent an efficient way to validate the results of other methods (especially models).

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 (Leis *et al*. 2011). 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 (Soto-Mendoza *et al*. 2012); to assess the potential impact of the global warming on larval dispersal (Lett *et al*. 2010); or to help in the design of MPA networks and in their further management (Werner *et al*. 2007).

Although models have been used worldwide (Leis *et al*. 2011), 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*. (2010) 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. In the Catalan Sea, Mariani *et al*. (2010), modelled the dispersion of bluefin tuna eggs and larvae, finding a good resemblance with field data on larval distribution in the same sector. They hypothesized mesoscale oceanographic processes, for example coastal eddies, can be responsible for the aggregation and dispersion mechanisms in the area.

The need to improve the efficiency of the protection strategies has led to the development of dispersion models to predict the best combination of size, position and spacing of the MPAs inside a network, in order to maximize the benefits of protection. Basterretxea *et al*. (2012), 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*. (2012a) 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. Recently, Carlson *et al*. (2016) used Lagrangian simulations and field observations to understand the transport of particles between coastal regions in the Adraitic Sea, suggesting possible implications for the establishment of MPAs.

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 (Paris *et al*. 2007) and that settlement could be highly affected by the stochasticity of biological processes (Siegel *et al*. 2008). This has recently led to the development of more accurate “biophysical” 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 (Botsford *et al*. 2009b). An example for the Mediterranean Sea is available in Nicolle *et al*. (2009) 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 Lagrangian tool Ichthyop and the MARS hydrodynamic model. Then they implemented dial vertical migration for larvae > 7 days, getting not fundamentally different results from the passive dispersal model. A remarkable work was made in the last years by Andrello and colleagues that used individual-based simulations to investigate causes and consequences of fish larval transport in the Mediterranean Sea and their implications for conservation strategies. The used a biophysical model to investigate the level of connectivity between the MPAs of the Mediterranean Sea, using the dusky grouper *Epinephelus marginatus* as model species (Andrello *et al*. 2013). Their outcomes showed an overall low level of connectivity between all the MPAs considered and suggested the need to increase MPAs number to enhance the connections between protected populations. Recently, they used an individual-based mechanistic model of larval transport, to explore the effects of future climate change on connectivity patterns among MPAs.

They showed that changes in the hydrodynamic, adult reproductive timing and larval dispersal duration, due to climate change, will influence larval transport and connectivity between fish populations and in turn affect the effectiveness of MPAs (Andrello *et al*. 2015).

Whereas modelling represent a valuable 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 case of biophysical models for example, in order to obtain reliable dispersal simulation, it is fundamental to parameterize the model with accurate information about species life history traits, possibly gathered in the same spatial and temporal context of the oceanographic data (Di Franco and Guidetti 2011, Di Franco *et al*. 2013). Moreover, the spatial resolution of the circulation data can strongly influence the output of the model. In most cases, circulation models do not take into account small-scale physical processes, particularly in the nearshore, despite the study of small-scale (from few kilometers to meters) interactions of water and larvae is considered a fundamental research need (Werner *et al*. 2007). The difficulty to increase model resolution is due to the fact that fine-scale marine circulation is still largely unknown. In fact, although a big effort is currently made to increase the resolution of oceanographic data (Malanotte-Rizzoli 2012), even powerful measurement methods (e.g. fleets of underwater gliders) are not sufficient, as measurements remain scarce both in space and time for many small scale processes (Malanotte-Rizzoli 2012).

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. Coupling models with field and laboratory studies is necessary to better parametrize the model and to test the predictions and the assumptions (Werner *et al*. 2007, Gaines *et al*. 2010). Moreover, the combination with other methods could be useful for corroborating the results of the models (Berry *et al*. 2012, Soria *et al*. 2012).

Genetics

Genetic approaches have become widely used in ecological studies due to the great advance in genetic research and the development of new technologies and laboratory equipments. Moreover, the possibility to adopt a non-lethal sampling protocol (depending on the species and the life stage considered) 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 units, or understand population dynamics, among others (Frankham *et al*. 2011). 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 (Palumbi 2003, Puebla *et al*. 2009). 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.

Allozymes had been the primary method to quantify genetic diversity worldwide as well as in Mediterranean Sea. Allozymes are the direct product of a particular allele (Bader 1998) and assumed to be neutral to selection (Hedrick 1999). The primary advantages to this method are its simplicity, quickness and its unlimited applicability, as any kind of organism produces some type of enzyme (Pujolar *et al*. 2001). Nevertheless, a high number of enzyme loci must be employed (minimum of 20) to get a representative sample of population variability (Bader 1998). 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 ºC). In the Mediterranean, allozymes have been used to reveal the existence of significant genetic structure among *Sarda sarda* (Pujolar *et al*. 2001) and *D. sargus* (Lenfant and Planes 1996) populations and also to detect temporal variability among *D. sargus* cohorts (González-Wangüemert *et al*. 2007). Some studies, failed to reveal any genetic effect from fishing prohibition on *D. sargus* populations other than demographic ones (Lenfant 2003). Other studies found a significantly higher allelic richness in *D. sargus* individuals coming from MPAs compared to unprotected sites (Pérez-Ruzafa *et al*. 2006), 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 (*Allegrucci *et al.* 1997*)*.

With the development of restriction enzymes and universal primers, and the advent of PCR methodology for amplification of genetic material (Kocher *et al*. 1989), 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 (Féral and Féral 2002). 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 (Féral and Féral 2002). 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* (Griffiths *et al.* 2011), populations of other species such *D. sargus* (Gonzalez-Wanguemert *et al*. 2011) were considered panmitic. However many other species displayed a West-East segregation inside Mediterranean basin as was the case of *Pomatochisthus marmoratus* (Mejri *et al*. 2011) and *Pomatomus saltatrix* (Pardiñas *et al.* 2010), in which the Siculo-Tunisia Strait (STS) was identified as the main geographic barrier determining this genetic distinctness, probably due to historical hydrographical regimes.

To resolve spatial scale restrictions, sequence variants in nuclear DNA (nDNA) were developed to enhanced genetic resolution to fine scales (Selkoe and Toonen 2006). Microsatellite markers are the most widely used owing to their high polymorphism, neutrality to selection, and relative low cost (if primers libraries are already available) (Féral and Féral 2002, Selkoe and Toonen 2006). 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. For example, different connectivity patterns were found for closely related species such as *Mullus barbatus* and *M. surmuletus* (Félix-Hackradt 2012, Galarza *et al*. 2009a), while significant heterogeneity was found in *M. barbatus* (Maggio *et al*. 2009). 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 (Schunter *et al*. 2011b). 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 (Riccioni *et al*. 2010).

Although microsatellites are extremely powerful for resolving population structure (Narum *et al*. 2008), they present some limitations (e.g. high potential error rate and low genotyping throughput (Hoffman and Amos 2005, Miller *et al*. 2002). In the recent years, single nucleotide polymorfisms (SNPs) are increasingly being used thanks to their great abundance and wide distribution in the genome of most organisms (Narum *et al*. 2008), that make them particularly appropriate for large-scale genetic studies (Anderson and Garza 2006). These markers have low error rates and require minimal human interaction making them handy, especially in the case of high throughput genotyping protocols (Anderson and Garza 2006). Nevertheless, because of their recent application, few studies have used SNPs worldwide, and only Zarraonaindia *et al*. (2012) considered some locations in the Mediterranean Sea to investigate the population structure of European anchovy (*E.*

encrasicolus). Recently, a combination of microsatellites and SNPs was used to investigate the populations structure of *Dicentrarchus labrax* between the Atlantic ocean and the European coast of the Mediterranean Sea (Souche *et al*. 2015). Authors found that the two regions host two distinct lineages of the species, with SNPs that also revealed a genetic structuring among Mediterranean samples (Souche *et al*. 2015).

Genetic methods present 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 has been demonstrated for the majority of marine species (see Hedgecock *et al*. 2003 for a 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) (Hedgecock *et al*. 2003).

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,* Mamuris *et al*. 2001; *D. sargus,* Gonzalez-Wanguemert *et al*. 2011) or discordant results (e.g. *E. marginatus,* Maggio *et al*. 2006; *D. sargus,* Kaoueche *et al*. 2011). 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 (Maggio *et al*. 2009, González-Wangüemert and Pérez-Ruzafa 2012). Although parentage analysis has been emerging worldwide, mainly in rocky reef environments (Jones *et al*. 2005, Planes *et al*. 2009), 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 (Castro *et al*. 2006, Castro *et al*. 2007).

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 Selkoe *et al*. 2008, Selkoe *et al*. 2010, Meirmans *et al*. 2015).

Otoliths

Otoliths are mineralised concretions that grow throughout the entire lifespan of fishes (Campana 1999); 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 (Green *et al*. 2009). The analysis of an otolith section allows researchers to determine growth and ageing (Green *et al*. 2009), and to estimate the duration of early life stages. In the case of juveniles, it is possible, by back-calculation, to determine the date of spawning, hatching and settlement (Stevenson and Campana 1992) that are fundamental information for larval dispersal modelling (Watson *et al*. 2010) and for investigating connectivity patterns (Almany *et al*. 2009). 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 (Gillanders *et al*. 2003 for a review, (Campana 2005), 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 (Campana 1999).

This important property allowed the development of a series of methodologies for assessing connectivity by using otolith chemistry, based on the identification of natural tags (fingerprint).

Otoliths can be analysed in solution after acid dissolution (Gillanders *et al*. 2001) or directly observing the precise point to be analysed. This second technique requires the use of instruments, as laser ablators, able to sample specific and extremely small portions of the otolith. The analysis of the elemental composition is generally performed by ICP-MS (inductively coupled plasma mass spectrometry) thanks to its high capability to detect elements in low concentrations.

The methods based on the chemical analyses of otoliths 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) (Green *et al*. 2009). 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) (Campana 1999). 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 (Gillanders *et al*. 2003; Elsdon *et al*. 2008). 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 (Gillanders *et al*. 2003). Although few, there are very recent papers that have used the analysis of otolith natural fingerprint in Mediterranean Sea for evaluating connectivity (Table 2.1). Correia *et al* (2011) used oxygen isotopic ratios ($^{18}O/^{16}O$) and carbon isotopic ratios ($^{13}C/^{12}C$) in the otoliths of European conger eel (*Conger conger*) to discriminate fishes from Mediterranean Sea and Atlantic Ocean. Djerking *et al* (2012) 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) (Morat *et al*. 2012). However, although otoliths are preferable for measuring isotopic ratios, other tissues can be used for the same task (Dierking *et al*. 2012), in fact, a number of works, performed in the Mediterranean Sea, have analysed dermal and muscle tissues of sharks (Carlisle *et al*. 2012) and muscle tissues of the yellowfin tuna (*Thunnus albacares*) and the swordfish (*Xiphias gladius*) (Ménard *et al*. 2007).

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 (Mercier *et al*. 2012, Morales-Nin *et al*. 2012). Using this technique Mercier *et al*. (2012) assessed connectivity between adult and juvenile habitats for the gilthead sea bream (*S. aurata*). For their part, Di Franco *et al*. (2012b) 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 fish coming from sites far few kilometres from each other. In a similar study, Di Franco *et al*. (2015) investigated the possible implications for Mediterranean MPAs of larval and post-settlement dispersal of the two banded sea bream (*Diplods vulgaris*). They showed, for the first time in the Mediterranean context, that juvenile dispersal can occur at the same spatial scale of larval dispersal, highlighting the role of dispersal during the juvenile life stages as an important mechanism for connecting populations and its implications in extending the benefits of MPAs.

Table 2.1. Studies that have analysed otolith chemistry for assessing connectivity and fish movements in the Mediterranean Sea.

Method	**Species (Family)**	**Location**	**Spatial scale**	**Results**	**References**
Stable isotopes	*Conger conger* (Congridae)	Mallorca (Spain) and other 3 locations in NE Atlantic Ocean	2500 km (max distance between locations)	Strong differences in isotopic ratios among locations. Low level of connectivity suggested.	Correia *et al*. 2011
Stable isotopes and elemental signatures (also stable isotopes in muscles)	*Solea solea* (Soleidae)	Gulf of Lions (France)	~32 km² (lagoon area)	Lack of migrations between the sea and a coastal lagoon during summer.	Dierking *et al*.2012
Stable isotopes and elemental signatures	*Solea solea* (Soleidae)	Gulf of Lions (France)	100 km (max distance between locations)	Ontogenetic shift between lagoons and sea.	Morat *et al*. 2012
Elemental signatures	*Sparus aurata* (Sparidae)	Gulf of Lions (France)	32-75 km² (area of lagoons investigated)	Migrations between the lagoon and the sea until at least age 4 yr.	Mercier *et al*. 2012
Elemental signatures	*Diplodus sargus* *Diplodus vulgaris* (Sparidae)	Apulian Adriatic coast (Italy)	200 km (max distance between locations)	Larval dispersal up to 100-200 km. Post-settlement dispersal can occur at up 165 km.	Di Franco *et al*. 2012b Di Franco *et al*. 2015

More recently a series of direct otolith marking techniques have been developed. These methods are based on the inoculation of a chemical tag in the otolith that can be recognized after fish catch. Artificial tagging techniques of otoliths include the incorporation of fluorescent compounds (e.g. tetracycline; Jones *et al*. 1999), rare elements and isotopes (Thorrold *et al*. 2006). This allows the direct estimation of movements made by individuals, even from their birth (Elsdon *et al*. 2008) 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; Planes *et al*. 2009). 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.

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 (Lombarte and Lleonart 1993). Otolith shape seems to reflect the local environmental conditions affecting a group of conspecifics at the same location (Campana and Casselman 1993), and differs among groups of fish that live in distinct geographical areas. Thus, otolith morphometry can be considered as a natural mark and can be used as an indirect way to infer connectivity among fish stocks that occupy distinct geographical regions.

Few studies have used otolith morphometrics and shape to identify fish stocks in the Mediterranean Sea, and none of them has inferred connectivity. Some of these studies aimed to discriminate Mediterranean fish stocks from Atlantic ones for different species, e.g. *Scomberesox saurus* (Aguera and Brophy 2011), *Trachurus trachurus* (Stransky *et al*. 2008), *Merluccius merluccius* (Torres *et al.* 2000). 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. In particular, considering the positive relationship between water temperature and otolith growth rates (Lombarte and Lleonart 1993), the warmer water of the Mediterranean Sea could determine increased otolith sizes.

Otolith shape appears to be an efficient tool to distinguish populations at local scales (<200km) in the Mediterranean Sea as demonstrated for *E. encrasicolus* (Kristoffersen and Magoulas 2008) and *S. solea* (Mérigot *et al*. 2007). Morat *et al*. (2012) identified several local populations of *M. barbatus* in the Gulf of Lions (France). Turan (2006) 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 (Begg and Brown 2011), it remains still difficult to establish which between these two determinants contribute the most to otolith shape (Begg and Brown 2011), 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.

Parasite identification method takes advantage of the fact that some species of parasites live in defined geographical areas and fish can become infected only when they come within the area suitable for the transmission of that specific parasite. Thanks to this, parasites can be used as a natural tag to discriminate different stocks and to evaluate dispersion and migratory routes (Durieux *et al*. 2010). 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 (Lester and MacKenzie 2009). 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 (MacKenzie 1999). However, 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*) (Abaunza *et al*. 2008) and the wild albacore (*Thunnus alalunga*) (Mele *et al*. 2010).

Meristic and morphometric variability have been applied for stock identification and connectivity studies due to its favourable cost-effectiveness ratio (Dwivedi and Dubey 2012). 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; Dwivedi and Dubey 2012). In the Mediterranean Sea, this technique have been used for the study of populations of horse mackerels (*T. trachurus*) (Bektas and Belduz 2009), anchovies (*E. encrasicolus)* (Kristoffersen and Magoulas 2008), sardines (*Sardina pilchardus*) (Silva *et al*. 2008) bluefish (*P. saltatrix*) (Turan *et al*.

2006) and the sparids *D. sargus, D. puntazzo* and *Lithognathus mormyrus* (Palma and Andrade 2002).

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 (Wall and Herler 2008). 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 (Marshall and Pierce 2012). 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 (Marshall and Pierce 2012).

In the Mediterranean, this procedure has not been applied to the study of bony fishes, except in one case (Lelong 1999), 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 finding permanent and identifiable marks (Wall and Herler 2008).

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 (Chapman and Kramer 2000).

Analysis and interpretation of data generated by these studies may have strong influence on the development of fisheries (Uriarte and Lucio 2001). The validity of their conclusions relies on the following assumptions: (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 (Attwood and Swart 2000) and can affect swimming performance (Serafy *et al*. 1995) is calling such assumptions into question.

The main techniques used in mark and recapture studies are Passive Integrated Transponder (PIT), visibly deployed fluorescent elastomers (VIFE) and wire tags, dart barbed and T-bars (Thorsteinsson 2012). In Mediterranean waters T-bar technique was applied principally to evaluate migration and connexion between stocks of bluefin tuna (*T. thynnus*) (Fromentin 2002) and albacore (*T. alalunga*) within and between Mediterranean Sea and Atlantic Ocean (Arrizabalaga and Costas 2003). 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 (Hackradt 2012).

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 a intermediate spatial scale during a long time period (Afonso *et al*. 2008) 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* (De Metrio *et al*. 2005).

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 (Block *et al*. 1998). This methodology has helped researchers to better understand the population dynamics of pelagic species in the Mediterranean Sea such as bluefin tuna (De Metrio *et al*. 2005) and swordfish (*X. gladius*) (Canese *et al*. 2008).

Acoustic telemetry techniques are used to study fish behaviour and movements . They are efficient tools to obtain information about fish home range (Lowe *et al*. 2003), site fidelity (Pastor *et al*. 2009) and homing behaviour (Jadot *et al*. 2006). 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 (La Mesa *et al*. 2012). Mobile receivers (hydrophones) allow to follow tagged fish continuously but in a short period of time (<24h) (Jadot *et al*. 2006). 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) (Welsh *et al*. 2012). The acoustic transmitter is a miniature tag (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 (March *et al*. 2010).

Studies of movement patterns of adult fish using acoustic tags in the Mediterranean Sea are relatively scarce. Five studies have been conducted in the Palma Bay MPA (Mallorca, Balearic Islands) on five different species: *Serranus cabrilla* (Alós *et al*. 2011), *Diplodus vulgaris* (Alós *et al*. 2012) and *Diplodus annularis* (March *et al*. 2010). Their results showed that fishes present high site fidelity and spend 95% of their time within an area smaller than 1 km^2. D'Anna *et al*. (2011) found that *D. sargus* seems to have a very small home range (0.01-0.17 km^2). A similar home range was found for *E.*

marginatus (Hackradt 2012), *E. costae* (Hackradt 2012), *Sparisoma cretense* (La Mesa *et al*. 2012). Remarkable work was recently carried out by Di Lorenzo and colleagues, that used acoustic telemetry for assessing the movement pattern (Di Lorenzo *et al*. 2014) and the diel activity (Di Lorenzo *et al*. 2016) of *D. sargus sargus* in a MPA of the South-Western Adriatic Sea.

All this work 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 by the size and functionality of the transmitters. Larger tags can provide increased reception range and more enduring batteries, but limit the size of the individuals and life stages that can be studied. 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 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 (Leis *et al*. 2011). 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 (Leis *et al*. 2011). 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*. (2011a), 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*. (2012a), in a study conducted along the South-Western Adriatic coast, found that the potential dispersal of *D. sargus* propagules up to 100 km from the MPA is supported by both Langragrian simulations model and genetic homogeneity at the same spatial scale. In the same sector of the Mediterranean Sea, Pujolar *et al*. (2013) used genetic tools and Lagrangian simulations to investigate patterns of connectivity between marine protected areas (MPAs) and neighbouring non-protected areas. They showed that effectively enforced MPAs can provide benefits to neighbouring or relatively distant non-protected fishing areas. Recently, Koeck *et al*. (2015) coupled the outputs of Lagrangian simulations and field observations on ichthyoplankton distributions for assessing the effects of different dispersal scenarios on the connectivity patterns in a transboundary MPA located between Corsica (France) and Sardinia (Italy). The results of all these works highlight the potential ability of integrated methods to increase the level of certainty on connectivity patterns resulting from model simulations.

Less common, not only in the Mediterranean Sea, is the work done by Correia *et al* (2012) 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. More recently, Karahan *et al*. (2014) combined geometric morphometrics of body shape, Fourier analysis of otolith shape and genetic markers to distinguish two anchovy species in the Eastern Mediterranean Sea. The difference in the “type” of connectivity estimated by genetic methods and otolith methods (see Leis *et al*. 2011) and a different spatial and temporal scale at which they should be properly applied (Jones *et al.* 2009), make the results of these two methods hardly comparable in most of the cases. Selkoe *et al*. (2008) 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*. (2008) which integrated the use of mtDNa 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 above-mentioned genetic parentage analyses and the TRAIL (trans-generational isotopic labelling of otoliths), which has never been 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*. (2009) and Almany *et al*. (2007). 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. This kind of behaviour makes “easy” the use of parentage analyses or TRAIL, because it allows to know the precise point in which the eggs will be spawned. However, this behaviour is hard to find in Mediterranean fish species. 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 (Selkoe *et al*. 2008).

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 (Shanks 2009). In this perspective, juveniles and adult movements could have a crucial role in determining connectivity patterns among populations, as recently pointed out for the first time also in the Mediterranean context (Di Franco *et al*. 2015).

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 oceanographic 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. From this perspective it is desirable an increased use of multidisciplinary approaches for assessing connectivity in the Mediterranean Sea, trying to implement those methods that have not been used yet in the Mediterranean context, but taking into account that each method has its own spatio-temporal resolution range and focuses on distinct life stages, and thus leads to results that should be compared cautiously with the outcomes of other methods.

In recent years a big effort was made for the establishment of new MPAs throughout the Mediterranean Sea (Gabrié *et al*. 2012), but, as in other parts of the world, many protected areas have not showed the expected results (Mora *et al*. 2006, García-Charton *et al*. 2008). 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 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 (Botsford *et al*. 2001, Lockwood *et al*. 2002). For the Mediterranean Sea few works are available on these important topics and almost all were conducted in the NW sector (Fig. 2.1), 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.

Figure 2.1. Areas of the Mediterranean Sea (dark grey) where most of the studies on connectivity and fish movements were carried out until now.

Chapter III

SPATIAL GENETIC STRUCTURE IN THE SADDLED SEA BREAM (*Oblada melanura* [Linnaeus, 1758]) SUGGESTS MULTI-SCALED PATTERNS OF CONNECTIVITY BETWEEN PROTECTED AND UNPROTECTED AREAS IN THE WESTERN MEDITERRANEAN SEA

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SPATIAL GENETIC STRUCTURE IN THE SADDLED SEA BREAM (*Oblada melanura* [Linnaeus, 1758]) SUGGESTS MULTI-SCALED PATTERNS OF CONNECTIVITY BETWEEN PROTECTED AND UNPROTECTED AREAS IN THE WESTERN MEDITERRANEAN SEA

Abstract

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

Keywords: genetic connectivity; marine protected areas; spatial scale; sea bream

Introduction

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

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

2003, Jones *et al*. 2007, Almany *et al*. 2009, Green *et al*. 2014). At a local spatial scale, the assessment of the level of connectivity between protected and unprotected locations allows to estimate the effectiveness of a MPA to sustain outer non-protected areas or other MPAs within the same network; at a broader scale, it permits the delineation of environmental or anthropogenic barriers to population connections, and allows the characterization of distinct management units.

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

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

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

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

Materials and methods

Study area and sample collection

Sampling of *O. melanura* was carried out between September and October 2013. Three sectors (i.e. stretches of coastline of ca. 80-100 km) spaced about 400-600 km from each other were selected along the European coast of the Western Mediterranean Sea (Central France, Northern Spain and Southern Spain) (Fig. 3.1). In each sector 3 locations were selected (see Fig. 3.1 for location names and abbreviations). The central location of each sector corresponded to an MPA, respectively: Porquerolles (which became part of the National park of Port-Cros in 2012), Cap de Creus natural park (established in 1998) and Cabo de Palos marine reserve (established in 1995). The other 2 locations of each sector were unprotected and located about 40-50 km northwards and southwards of each MPA (Fig. 3.1).

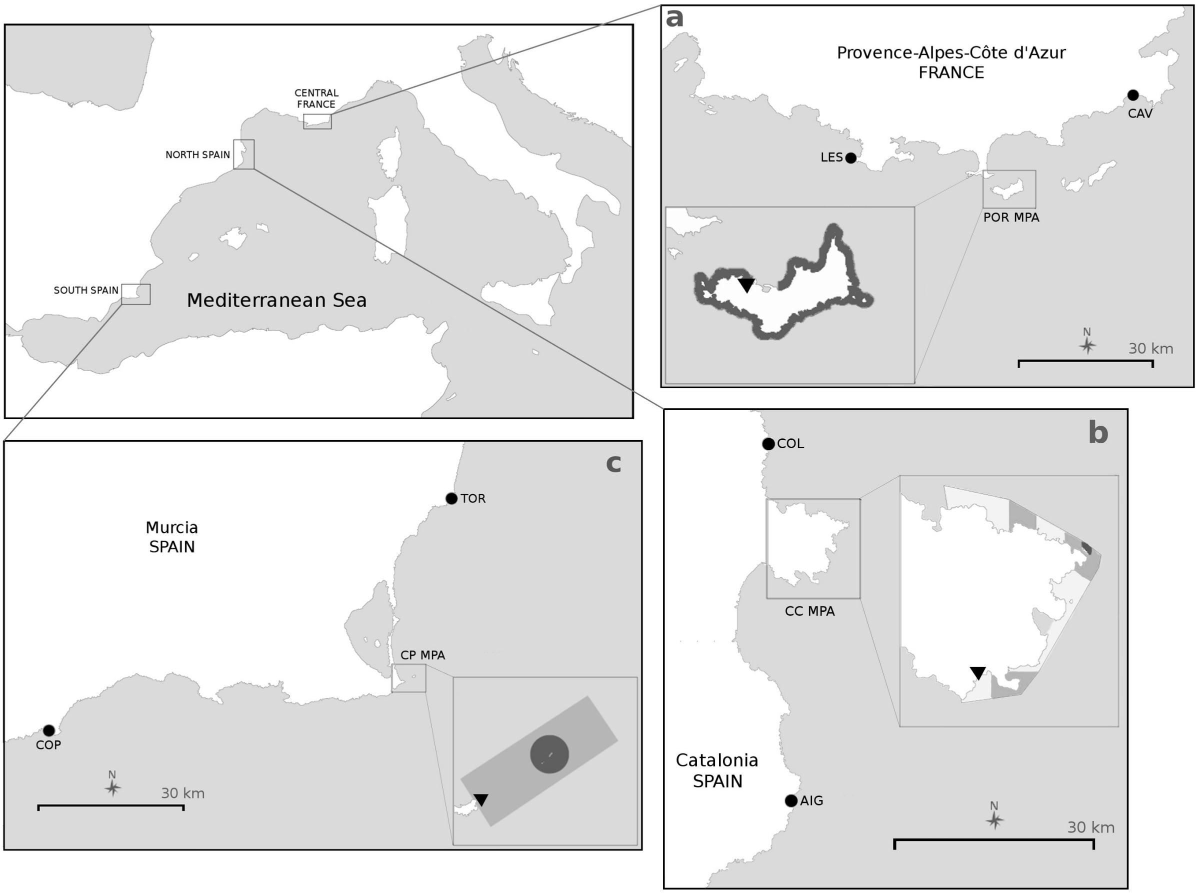

Figure 3.1. Study area. **a**. Central France (Cav= Cavalaire, Por MPA = Porquerolles MPA, Les = Les Embiez); **b** Northern Spain (Col = Colera, CC MPA = Cap Creus MPA, Aig = Aiguablava); **c**. Southern Spain (Tor = Torrevieja, CP MPA = Cabo de Palos MPA, Cop = Cabo Cope). In each sector (a, b and c): black dots represent unprotected locations; the MPA is highlighted and the black triangle indicates the protected sampling location. Grey scale (light, medium and dark), where present, in each MPA represents the different levels of protection (low, medium and high, respectively).

In each location, both protected and unprotected, 25-32 juveniles (i.e. individuals of 3-4 months of age), for a total of 258 individuals, were sampled during the night, by snorkeling, using a hand net and a torch. Specimens were firstly euthanized immersing them in a water solution with few drops of 95% alcohol for minimizing their suffering (Leary *et al*. 2013) and, after cessation of opercular movements, preserved in absolute ethanol used for genetic analysis. In the laboratory, caudal fins were dissected from each specimen and stored in absolute ethanol at -20ºC.

DNA extraction and PCR amplification

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

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

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

DNA® before being viewed under UV light and were visualized by capillary electrophoresis using ABI Prism 3730 automated genetic analyser (Applied Biosystems). Allele scoring was done using GeneMapper v.3.5 software (Applied Biosystems, Foster City, California).

Data analysis

All loci were tested for the presence of null alleles using the software MICRO-CHECKER v.2.2.3 (Van Oosterhout *et al*. 2004). The software POWSIM (Ryman and Palm 2006) was used to assess the statistical power of the markers used in the study using Chi-squared and Fisher's exact tests. A range of predefined levels of expected divergence (Fst=0.001, 0.005, 0.01, 0.05) was tested using an Ne (effective population size) of 1000 and t (time of divergence) of 10. The total number of alleles (N), the number of private alleles (PA), observed (Ho) and expected (He) heterozygosities for each locus and location were obtained with GenAlex v.6. (Peakall and Smouse 2006). Standardized allelic richness (AR) and inbreeding coefficient (Fis) and their estimated probabilities were calculated via 10^4 random permutations using FSTAT version 2.3.9.2 (Goudet 2002). A Student's t test was used among all pairs of locations to test for potential differences in the genetic diversity.

Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were tested using GENEPOP version 3.4 (Raymond and Rousset 1995). Where multiple comparisons were tested, the sequential Bonferroni procedure (Rice 1989) was used to adjust the statistical significance.

Genetic differentiation was investigated by a series of statistical approaches.

Firstly, we used the Bayesian approach implemented in the software STRUCTURE (Pritchard *et al*. 2000), a model-based clustering algorithm that infers the most likely number of clusters in the data. The statistical procedure organizes individuals into a predefined number of clusters (K), with a given likelihood. Ten runs of 10^6 Markov Chain Monte Carlo (MCMC) and 10^5 burn-in-length were carried out using admixture model with no prior local information and $1<K<9$. The most likely K was determined with the criterion of Evanno *et al*. (2005) using the web tool Structure Harvester v0.6.94 (http://taylor0.biology.ucla.edu/structureHarvester/). The method is based on the rate of change of mean posterior probability between 2 different clusters (ΔK) in function of the number of cluster (K), and the optimal number of clusters is the one that corresponds to the higher value of ΔK.

Genetic differentiation was also investigated through the Discriminant Analysis of Principal Components (DAPC; Jombart *et al*. 2010) as implemented in ADEGENET version 1.3-6 (package used in R software, version 3.1.1; R Development Core Team 2011). DAPC does not rely on explicit population genetics models, and is useful when the structure is subtle (Jombart 2008, Jombart *et al*. 2010, Vander Wal *et al*. 2013). The *dapc* function is based on 2 steps: data are firstly transformed using a PCA, then a discriminant analysis (DA) is performed. As recommended in Jombart *et al*. (2010), for the *dapc* we retained a number of principal components that corresponded to more than ~90 % of cumulated variance and all the linear discriminants. Two methods for DAPC are available, depending on whether the number of origin samples (K) is known or not, and both were used in this study. In the first case (no information on K), before performing *dapc,* the function *find.clusters* was implemented to identify the optimal number of clusters. This function runs a k-means algorithm after transforming the data through a PCA (a step done in order to reduce the number of variables and to speed up the clustering algorithm; Jombart 2013). K-means is run sequentially with increasing values of K, and different clustering solutions are compared using Bayesian Information Criterion (BIC). The optimal clustering solution is the one that corresponds to the lowest BIC, indicated by an elbow in the curve of BIC values as a function of K. In our case all the PCs were retained for the analysis (Jombart *et al*. 2010) and the maximum number of possible clusters was set to K=9. In the second case, the function *dapc* was directly used considering the information on the local samples and the outcomes were visualized through a scatterplot.

Finally genetic differences among sampling locations were assessed by a hierarchical AMOVA in ARLEQUIN 3.5.1.2 (Excoffier *et al*. 2005), partitioning genetic differentiation among sectors and among locations within each sector. ARLEQUIN was also used to test for Isolation-by-distance (IBD) using a Mantel test, correlating genetic distance versus linear coastal distance between locations and to calculate pairwise Fst between locations. Fst values were then visualised through a principal coordinate analysis (PCoA) constructed from the genetic distance matrix. Pairwise genetic differentiations were also calculated using the more recent heterozygosity-independent Jost's D (Jost 2008), which is thought to be a more appropriate statistic for highly variable markers such as microsatellites (Meirmans and Hedrick 2011). This statistic, based on a bootstrap method (1000 bootstrap repeats) to estimate p-values, was calculated with DEMEtics (Gerlach *et al*. 2010) (package used in R software, version 3.1.1; R Development Core Team 2011).

Results

All 11 loci considered were polymorphic, with the total number of alleles ranging from 2 to 22. The most of loci showed a high level of polymorphism (Table 3.1). The lowest mean number of alleles (2.8) was recorded for locus 'Omel20', while the highest (18.8) for locus 'Dvul84'. The software MICROCHECKER detected no evidence for null alleles or genotyping errors due to stuttering or large allele dropout. Simulations using recorded allelic frequencies in POWSIM showed that the markers used in this study have enough statistical power to reveal genetic differentiation at Fst values ranging from 0.001 to 0.05 (p = 1.000).

Values of Ar, PA, Ho, He and Fis for each combination of locus × location are shown in (Table 3.1). Observed values of genetic diversity did not differ significantly between protected and unprotected locations in each sector (Student's t test, p>0.05). Observed heterozygosity values were relatively high across all loci, apart from locus Omel20 (for which the lowest number of alleles was recorded). Differences in mean values of Ho (range 0.724-0.794) were not significant among locations (p>0.05 for all pairwise Student's t test) similarly to what observed for mean values of He (range 0.752-0.788) and Ar (range 10.54-11.36) (p>0.05 for all pairwise Student's t test). Only 4 out of 99 tests departed significantly from HWE after Bonferroni correction (locus Omel02 at Les and Tor; locus Omel61 at Cav and CC MPA, see Fig. 3.1 for abbreviations of location names). No LD was observed between any pair of loci after Bonferroni correction.

Table 3.1. Summary statistics of 11 microsatellite loci and overall mean among the 9 locations considered. Ar = allelic richness, PA = number private of alleles, Ho = observed heterozygosity, He = expected heterozygosity, Fis = inbreeding coefficient.

Locus	**Cav**	**Por MPA**	**Les**	**Col**	**CC MPA**	**Aig**	**Tor**	**CP MPA**	**Cop**
Dvul2									
Ar	8.68	8.66	9.45	10.80	9.00	9.71	10.46	10.00	11.00
PA	0	0	0	0	0	0	0	0	0
Ho	0.929	0.800	0.600	0.719	0.760	0.759	0.767	0.600	0.880
He	0.816	0.767	0.746	0.766	0.847	0.849	0.810	0.816	0.859
Fis	-0.120	-0.008	0.212	0.078	0.128	0.128	0.071	0.284	-0.004
Dvul33									
Ar	4.69	2.95	3.83	2.79	2.00	2.84	2.97	3.00	2.00
PA	2	0	0	0	0	0	0	0	0
Ho	0.643	0.750	0.633	0.656	0.654	0.533	0.700	0.680	0.560
He	0.551	0.531	0.561	0.513	0.499	0.516	0.531	0.519	0.499
Fis	-0.227	-0.402	-0.111	-0.267	-0.292	-0.016	-0.303	-0.291	-0.102
Dvul4									
Ar	6.00	6.66	6.83	6.79	6.85	7.64	6.97	6.95	5.00
PA	0.000	0.000	0.000	0.000	0.000	2.000	0.000	0.000	0.000
Ho	0.741	0.719	0.828	0.800	0.731	0.724	0.793	0.840	0.792
He	0.818	0.706	0.789	0.780	0.741	0.763	0.814	0.806	0.794
Fis	0.120	-0.001	-0.023	0.007	-0.018	0.077	0.050	-0.021	0.034
Dvul84									
Ar	19.41	18.94	19.54	18.20	17.00	19.39	17.16	17.00	19.00
PA	0	1	0	0	0	0	0	0	0
Ho	0.857	0.867	0.963	0.867	0.840	0.857	0.800	0.920	0.960
He	0.926	0.908	0.932	0.922	0.917	0.935	0.927	0.916	0.933
Fis	0.092	0.068	-0.009	0.081	0.067	0.104	0.154	0.016	-0.009
Omel02									
Ar	11.85	12.66	13.29	13.21	13.84	11.84	13.26	12.00	14.00
PA	0	0	0	0	0	0	0	0	0
Ho	0.893	0.875	0.900	0.935	0.846	0.828	0.897	0.720	0.880
He	0.879	0.901	0.886	0.898	0.890	0.892	0.881	0.886	0.906
Fis	0.002	0.044	0.001	-0.022	0.069	0.092	0.003	0.133	0.049
Omel20									
Ar	2.00	3.54	2.00	2.79	2.96	2.98	2.85	3.00	3.00
PA	0	1	0	1	0	0	0	0	0
Ho	0.179	0.250	0.300	0.312	0.269	0.267	0.200	0.240	0.120
He	0.162	0.225	0.255	0.268	0.292	0.309	0.183	0.215	0.311
Fis	-0.080	-0.095	-0.160	-0.150	0.098	0.155	-0.077	-0.095	0.627
Omel27									
Ar	14.94	15.16	20.49	19.04	17.81	14.44	18.30	14.00	18.00
PA	3	0	1	0	0	0	0	0	0
Ho	0.885	0.774	0.733	0.938	0.846	0.828	0.833	0.880	0.720
He	0.910	0.912	0.937	0.917	0.918	0.899	0.927	0.912	0.931
Fis	0.053	0.170	0.176	-0.006	0.098	0.100	0.117	0.055	0.246
Omel38									
Ar	9.76	8.70	8.76	7.52	6.00	8.65	7.62	7.00	7.00
PA	1	0	0	0	0	0	0	0	0
Ho	0.821	0.938	0.700	0.594	0.731	0.667	0.767	0.760	0.840
He	0.813	0.805	0.789	0.798	0.757	0.815	0.776	0.804	0.781
Fis	0.009	-0.149	0.129	0.242	0.054	0.164	0.029	0.075	-0.055
Omel54									
Ar	17.42	17.15	16.21	15.86	17.00	16.38	16.58	18.00	16.00
PA	0	0	0	0	0	0	0	0	0
Ho	0.964	0.906	0.933	0.812	0.880	0.800	0.900	0.920	0.800
He	0.925	0.905	0.911	0.913	0.904	0.926	0.928	0.931	0.916
Fis	-0.025	0.015	-0.007	0.125	0.007	0.121	0.047	0.032	0.147
Omel58									
Ar	7.80	6.60	6.68	6.60	7.96	7.64	6.97	8.00	8.00
PA	0	2	0	0	0	0	0	0	0
Ho	0.889	0.656	0.700	0.844	0.885	0.900	0.733	0.760	0.880
He	0.795	0.704	0.696	0.745	0.791	0.785	0.723	0.805	0.814
Fis	-0.090	0.083	0.011	-0.117	-0.098	-0.131	0.002	0.076	-0.060
Omel61									
Ar	18.22	16.40	17.88	16.96	19.69	15.39	17.11	17.00	16.00
PA	0	1	0	0	0	0	0	0	0
Ho	0.929	0.844	0.867	0.906	0.885	0.800	0.900	0.960	0.840
He	0.923	0.911	0.913	0.924	0.926	0.909	0.925	0.918	0.923
Fis	0.012	0.058	0.067	0.035	0.064	0.136	0.008	-0.026	0.110
All									
Ar	10.979	10.675	11.360	10.960	10.919	10.627	10.932	10.541	10.818
Ho	0.794	0.762	0.742	0.762	0.757	0.724	0.754	0.753	0.752
He	0.774	0.752	0.765	0.768	0.771	0.782	0.766	0.775	0.788

The results from the Bayesian approach implemented in STRUCTURE, applying the Evanno's method, are shown in Table 3.2. When we considered all the 9 locations together, the higher value of ΔK was recorded for K=3. Evanno's method needs at least 2 clusters in order to calculate a ΔK and there is no possibility for a single homogeneous cluster to be selected. This must be the case in our study, although the software suggests a K=3, the mean posterior probability indicates that individuals belong to a unique homogeneous group with each individual having approximately the 33% of probability to come from one of the 3 clusters (Fig. 3.2). The same outcome was obtained when the analysis was performed on each sectors separately, revealing in the three cases a higher value of ΔK for K=2, but with no clear evidences for supporting the presence of this clustering pattern.

Table 3.2. Bayesian clustering results of STRUCTURE analysis. K represents the number of genetic clusters.

K	Mean LnP(K)	Stdev LnP(K)	Delta K	Mean posterior probability for cluster: 1	2	3
1	-11597.300	0.100	—			
2	-12360.460	73.533	0.234			
3	**-13106.420**	**383.837**	**0.497**	0.333	0.333	0.334
4	-13661.480	581.308	0.070			
5	-14175.440	504.356	0.284			
6	-14425.000	377.529	0.180			
7	-14610.380	494.721	0.210			
8	-15045.420	696.567	0.083			
9	-14865.720	549.576	—			

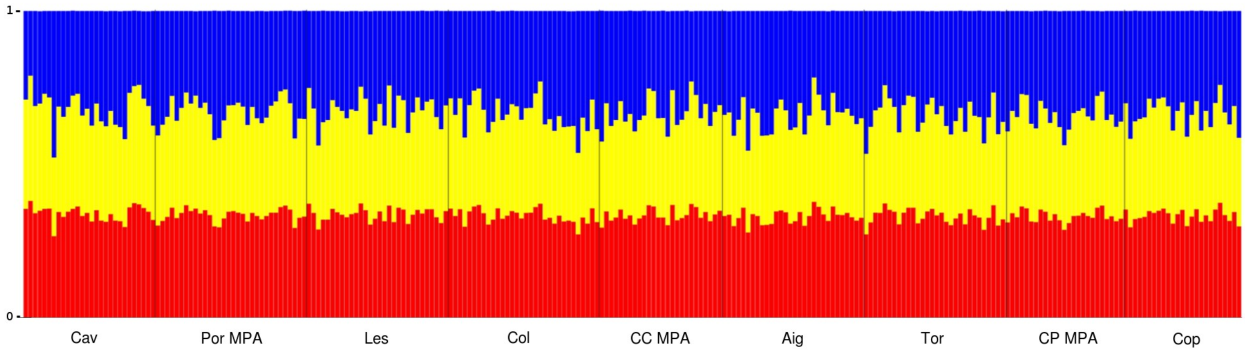

Figure 3.2. Averaged barplot of STRUCTURE results for *K* = 3 (number of clusters). Vertical bars represent individuals. Each colour represents one of the 3 inferred clusters (K) with lengths proportional to the probability of each inferred cluster assignment. Black vertical lines separate sampling locations. The meaning of abbreviations to name each location is specified in Figure 3.1.

DAPC analysis was firstly performed without any *a priori* group information. Using the function *find.cluster*, the lowest BIC value (381.115) corresponded to K=3 (Fig. 3.3). For DAPC analysis, 75 PCA axes and two discriminant functions were retained.

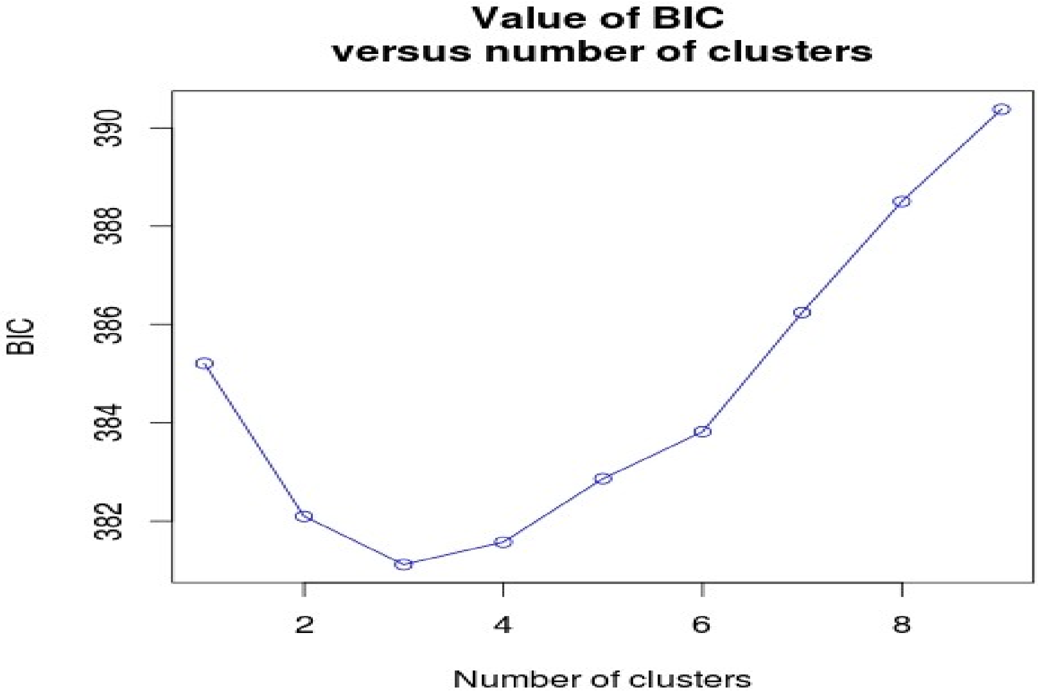

Figure 3.3. Bayesian Information Criterion (BIC) in relation to the number of clusters identified by the *find.cluster.*

One cluster (C2 in Fig. 3.4 and Fig. 3.5) included mainly individuals from Por MPA, Les and Col, which together account for ~50.0% of the total membership probability of the cluster. In a second cluster (C1) the highest membership probability was recorded for individuals from Cav and Aig (14.9% and 13.6% respectively). In a third cluster (C3) the highest membership probability was recorded for individuals from Aig and CC MPA. Individuals from the Southern Spain sector showed similar values of individual membership probability for the three clusters.

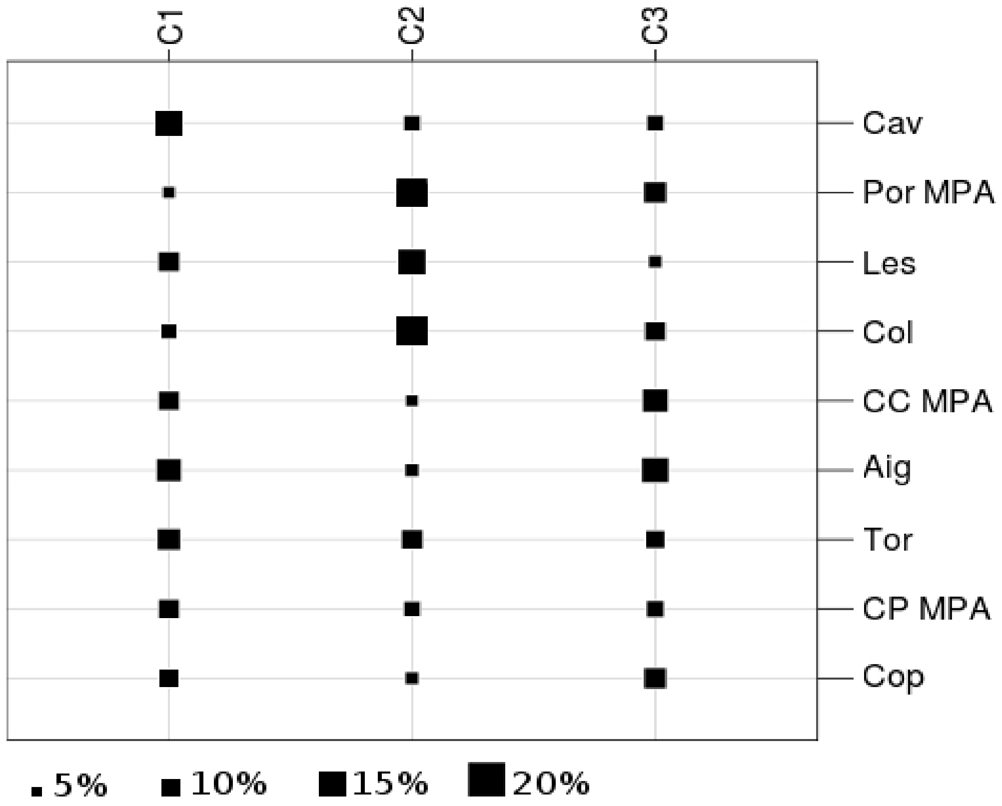

Figure 3.4. Repartition of the individual mean posterior probability of the 9 locations considered in the 3 clusters identified by the DAPC without *a priori* information on the origin samples. The size of the squares is proportional to the values of probability. The meaning of abbreviations to name each location is specified in Fig. 3.1.

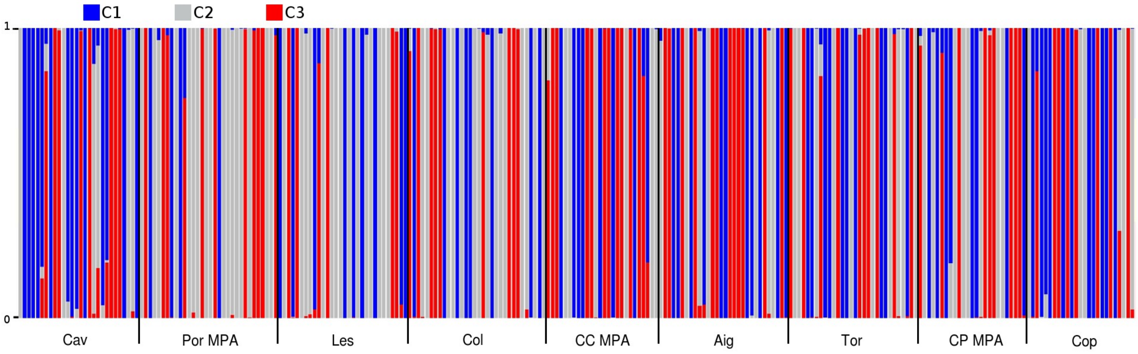

Figure 3.5. STRUCTURE-like barplot of DAPC analysis. Each individual is represented by a vertical bar. Each colour represents one of the 3 inferred clusters (C1, C2, C3) with lengths proportional to the probability of each inferred cluster assignment. Number of clusters K = 3, as obtained with *find.clusters*. The meaning of abbreviations to name each location is specified in Figure 3.1.

In the second *dapc* analysis, clusters were defined *a priori,* retaining 85 principal components and the maximum available number of discriminant functions. In a first *dapc* all the 9 locations sampled were considered separately: the first axis in the scatterplot separated southern locations (Southern Spain sector) from northern ones (Central France and North Spain sector), with Cav, CC MPA and Aig partially separated from Por MPA, Les and Col (Fig. 3.6a). The second axis grouped Cav and Aig, separating them from CC MPA and the remaining locations. The main groupings identified by the first axis of the scatterplot were used for running other 2 *dapc* for a better visualization of the outcomes (Fig. 3.6b and 3.6c).

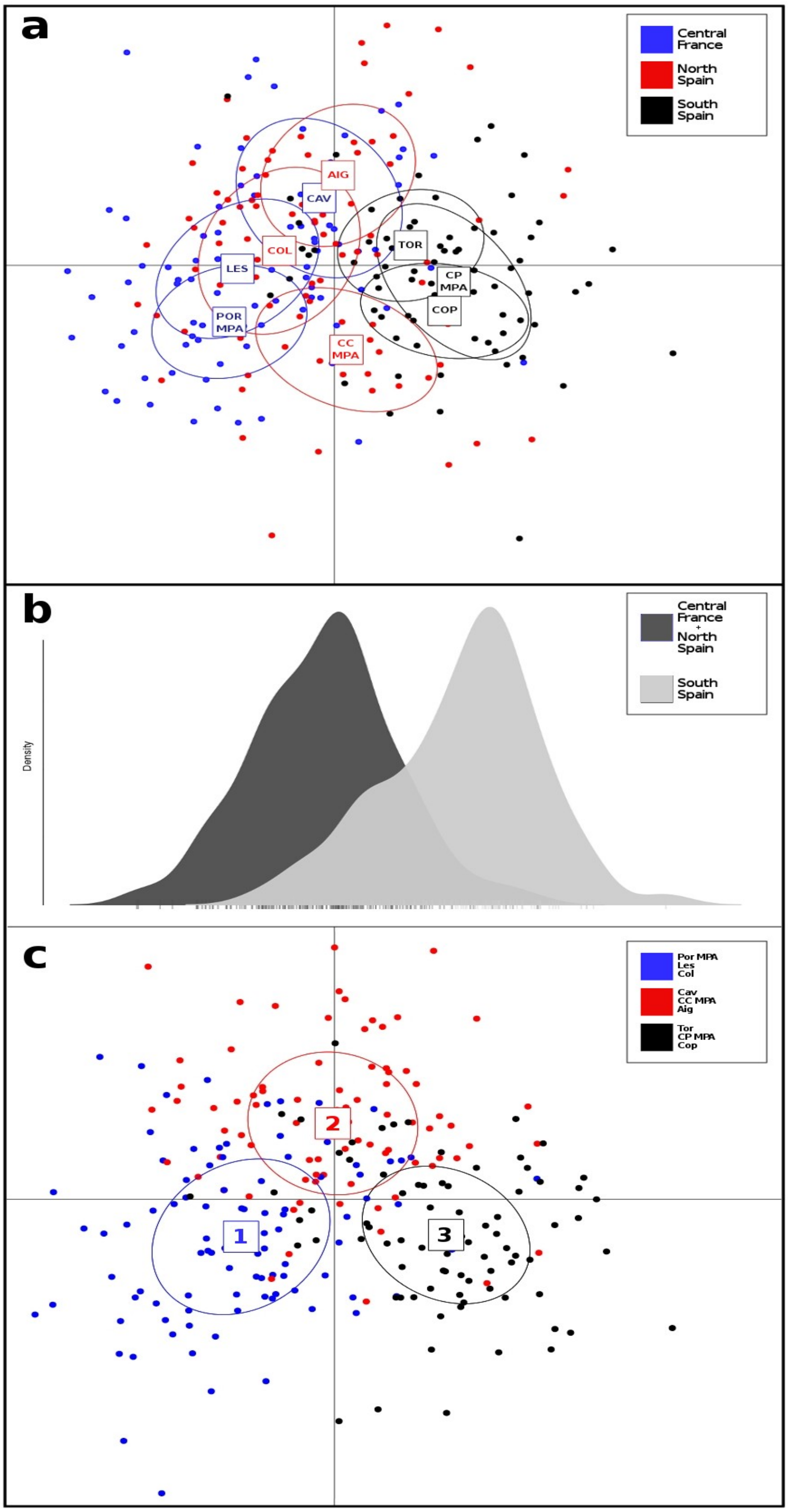

Figure 3.6. Scatter plot of the DAPC with *a priori* information on the origin samples: a) groups corresponding to the 9 locations sampled (the 2 axes represent the 2 major discriminant analysis eigenvalues); b) two groups (1 discriminant analysis eigenvalue); c) three groups (the 2 axes represent the 2 major discriminant analysis eigenvalues). See Fig. 3.1 for location names.

Analysis of molecular variance, performed considering the geographical groupings, suggested no genetic structuring, with no significant differences among sectors and within sectors (Table 3.3). No Fst comparison resulted significant after Bonferroni correction. Overall, values of Fst, were low with an average value, considering all the 9 locations, of 0.002. The highest value were recorded between Cav-Por MPA and CP MPA-Por MPA (0.0083 for both comparisons) (Table 3.4).

Table 3.3. Hierarchical analysis of molecular variance (AMOVA). d.f. (degrees of freedom), SS (sum of square).

Grouping	Source of variation	d.f.	SS	Variance components	Percentage of variation	P-value
Sectors 1.France 2.North Spain 3.South Spain	Among groups	2	9.183	0.002	0.05	0.303
	Among populations within groups	6	25.281	0.000	0.00	0.493
	Within populations	507	2139.98	4.221	99.95	0.364
	Total	515	2174.44	4.223		

Table 3.4. Pairwise comparison between locations. Fst below diagonal, Jost's D above diagonal. See Figure 3.1 for location names.

	Cav	Por MPA	Les	Col	CC MPA	Aig	Tor	CP MPA	Cop
Cav		0.0337	-0.0007	-0.0187	-0.0044	-0.0515	-0.0080	0.0027	-0.0195
Por MPA	0.0083		0.0119	-0.0192	0.0004	0.0117	0.0154	0.0450	0.0327
Les	0.0018	-0.0006		-0.0143	0.0231	-0.0157	0.0065	0.0386	-0.0059
Col	0.0008	-0.0036	-0.0035		0.0074	-0.0141	-0.0176	0.0065	-0.0048
CC MPA	0.0014	0.0004	0.0041	0.0011		-0.0062	-0.0025	-0.0018	-0.0212
Aig	-0.0046	0.0015	-0.0005	-0.0004	-0.0007		-0.0245	0.0079	-0.0195
Tor	0.0019	0.0017	-0.003	-0.003	0.0012	-0.0018		-0.0370	-0.0416
CP MPA	0.0026	0.0083	0.0036	0.0009	0.0024	0.0022	-0.0043		-0.0493
Cop	0.0020	0.0056	0.0012	-0.0002	-0.0004	-0.0018	-0.0026	-0.0040	

PcoA graphically described the genetic divergence computed in pairwise Fst. Although non-significant, a segregation between the group formed by Por MPA, Les and Col and all the other locations was observed along axis 1 of the ordination plot, while axis 2 segregated Cav, Aig and CC MPA from the locations of the Southern Spain sector (Fig. 3.7), corroborating the results from DAPC analysis. The Jost's D values showed similar results to the Fst values (Table 3.4). Mantel test also resulted not significant, indicating that no relation between geographical and genetic distances occurs.

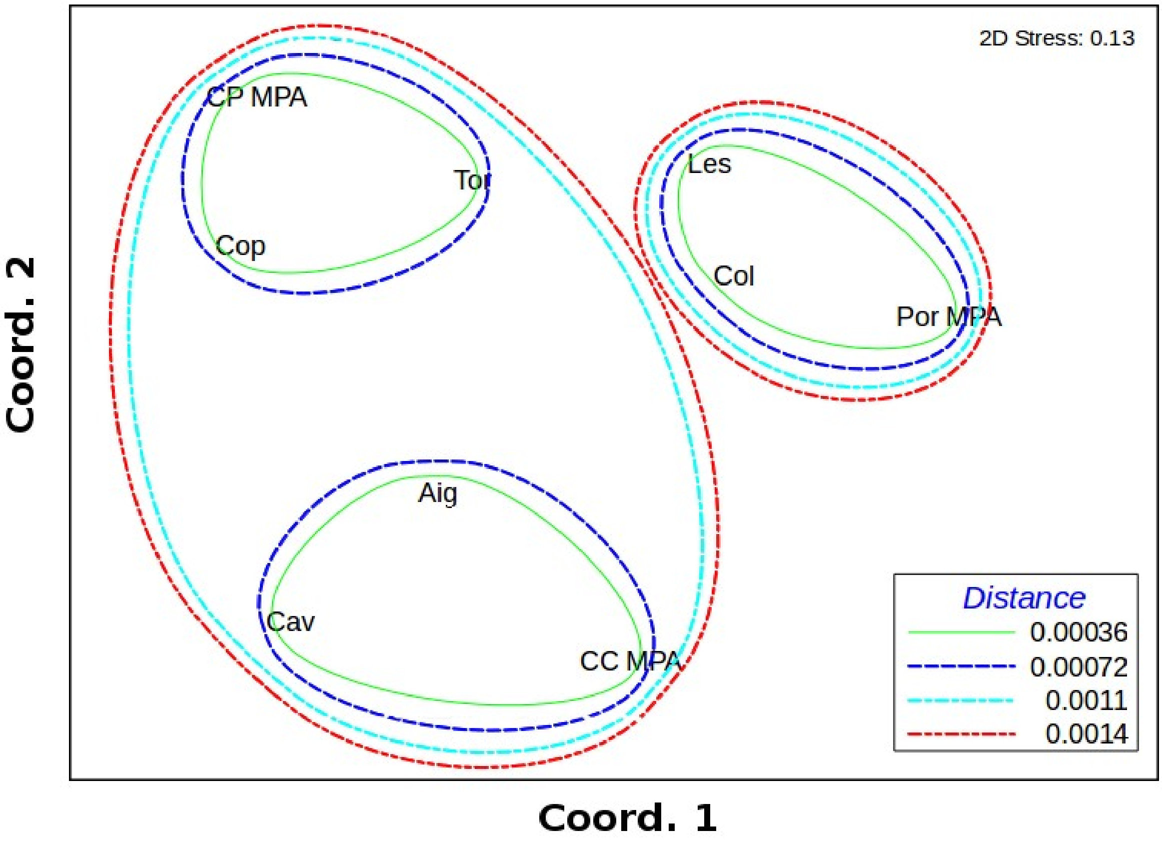

Figure 3.7. Principal Coordinates Analysis for *O. melanura* samples. See Figure 3.1 for location names.

Discussion

The results of the study revealed a high level of genetic diversity in *O. melanura*, expressed as both expected heterozygosity and allelic richness.

Similar results were recorded, for the same species, using a different set of microsatellite primers (Gkafas *et al*. 2013), and in other sparid fish in the Mediterranean Sea (Franchini *et al*. 2011). High genetic diversity is fundamental for maintaining the adaptability of natural fish populations and sustainable yields in fisheries (Kenchington *et al*. 2003).

At a local spatial scale (within sectors, 50-100 km) protected samples of *O. melanura* were in general well connected with non-protected ones. The lowest values of Fst and Jost's D were mainly recorded for pairwise comparisons between locations within the same sector, suggesting a high genetic flow between MPAs and locations outside their borders, within each sector considered. Moreover, no differences were found between protected and unprotected samples in terms of genetic variability. This latter result contrasts with the work of Pérez-Ruzafa el al. (2006) that found higher values of both total and standardized allelic richness in protected populations of *Diplodus sargus sargus* than in unprotected ones, considering two MPAs of the Western Mediterranean Sea (two within the Southern Spain sector and one within the Northern Spain sector of this study). Genetic diversity loss in commercial important species can be a consequence of fishing selection (Pérez-Ruzafa *et al*. 2006). However, the inference about benefits due to protection from fishing was not an aim of the present study. In fact, the choice to analyse juveniles and consider recently established MPAs (e.g. Por MPA) have to be seen in the light of a study mainly focused on recent genetic connectivity patterns, among MPAs and unprotected areas, rather than MPA effects.

The low level of genetic differentiation, considering all the sampling locations, suggested that local samples of saddled sea bream form a unique, continuous unite along the coast of the Western Mediterranean Sea.

On the other hand, at the regional scale (considering the three sectors, distant 500-1000 km apart), further analyses revealed the presence of a genetic clustering that has geographic consistency despite the high gene flow. The results from DAPC analysis, performed without *a priori* information, showed the occurrence of three genetic clusters, whose composition can provide information on the spatial genetic pattern of the species in the study area. The same pattern was corroborated by the ordination plot based on the pairwise Fst between sampling locations, while, although the Bayesian approach indicated the existence of the same number of clusters, there were no evidences for supporting this clustering pattern by this method, as a great homogeneity among the three clusters resulted from the posterior membership probability.

It is difficult to establish with certainty which are the main factors that contributed to the formation of this clustering pattern. The occurrence of the three clusters could be related to an historical isolation of differentiated groups that are currently admixed by ongoing gene flows in the Western Mediterranean Sea. We cannot exclude temporal variation in contributing to the identified clustering pattern. Our sampling design was mainly focused to assess spatial genetic differences and did not include any temporal replicate. This aspect would be certainly worth further investigation. Additional samplings in the study area or over a larger spatial scale would permit a better understanding of the genetic structure of the species in the region.

Although the overall high level of admixture, the cluster C2 shows a composition that seems to reflect the geographical features of the region, being mainly composed by individuals from neighbouring locations, all located in the Gulf of Lions. That could suggest a past isolation of the groups of *O. melanura* inside the gulf or more limited gene flow with the locations off the gulf. Interestingly, although CC MPA and Aig are geographically close to the samples mainly grouped in cluster C2, individuals from these two locations were the least present in the cluster C2, while a higher membership probability to the same cluster was found for the individuals of the Southern Spain sector. This outcome could be a consequence of the dispersal characteristics of the saddled sea bream that has a sedentary behaviour during the juvenile/adult phase and its dispersal capability is mainly due to propagules (eggs and larvae) dispersal. Relatively closer locations, separated by habitat discontinuities that do not permit the movement of juveniles and adults, could be less connected than distant ones by propagule dispersal, that for *O. melanura* can take place up to ~100 km (Calò *et al*. 2016, see Chapter V). The lower genetic purity of group dominance of clusters 1 and 3 could be due to the fact that the study areas correspond to marginal areas. The origin of the cluster 1 could be shifted to the Gulf of Genoa while the cluster 3 might be to the Alboran Sea.

The DAPC analysis with *a priori* information revealed a separation between the northern part of the study area (Central France and Northern Spain) and the Southern Spain sector. The genetic differentiation between the northern and the southern part of the Western Mediterranean Sea was already pointed out in several recent studies focused on the genetic structure of different coastal fish. Schunter *et al*. (2011a) specifically addressed the role of the

Ibiza channel, that in our study separated the Southern Spain sector from the other two sectors considered, by acting as an important barrier for the circulation of the Western Mediterranean Sea, thus determining genetic differentiation across this boundary (but see also Schunter *et al*. 2011b). Similar results were found for other commercial species of the Sparidae family (Pérez-Ruzafa *et al*. 2006, Galarza *et al*. 2009b for an example with *O. melanura*) and for the red mullet *Mullus barbatus* (Galarza *et al*. 2009a), along the same stretch of Mediterranean coastline.

Within the northern sectors of our study area (France and Northern Spain) a separation was found between a group formed by Cavalaire, Cap de Creus natural park and Aiguablava and a second group composed by Porquerolles national park, Les Embiez and Colera. This pattern, although subtle, is concordant with the results previously discussed and is likely to be related to geographical arrangement of the sampling locations and the oceanographic circulation of the Western Mediterranean Sea. In particular, Porquerolles, Les Embiez and Colera, could be genetically more similar due to the circulation of the Liguro-Provençal current along the gulf of Lion, separating them from locations outside the gulf. This oceanographic system is one of the most important current in the Mediterranean Sea and follows a South-Western trajectory skimming the coasts of France and Spain (Mounier *et al*. 2005). The Liguro-Provençal current could be responsible of a high dispersal of larvae directly towards the Catalan coast and a low dispersal towards locations inside the gulf of Lion. This could explain the relatively high values of Fst between Porquerolles MPA and Cavalaire, and the high genetic similarity between Cavalaire and the two southern locations of the Northern

Spain sector (south of Cap de Creus peninsula). A similar conclusion was reached by Lenfant and Planes (1996), that found a genetic distinction between populations of *Diplodus sargus*, relatively close to each other, across the Western edge of the gulf of Lion.

The detected complex pattern of population connectivity suggests that restrictions to gene flow could be located within the sectors investigated rather than between them. Moreover, the results indicate that the gene flow in the study region may not be related to geographical distances; certain locations showed limited connectivity with their surroundings, whereas other locations showed long distance genetic exchange, as suggested for other fish species (Evans *et al*. 2010, Villegas Sánchez *et al*. 2014). In this context, reduced levels of genetic differentiation may be promoted in marine organisms with large effective population sizes complemented with high levels of population admixture of distinct genotypes among locations; however, both conditions could contribute favouring subtle but complex genetic structures, depending on the balance between current-mediated larval dispersal and adult active homing behaviour over small and large geographic scales (Baeza and Fuentes 2013, Lemer and Planes 2014, Vergara-Chen *et al*. 2014).

The microsatellite DNA analysis of saddled sea bream over multiple spatial scales indicated a non-significant genetic differentiation between samples and a subtle pattern of genetic heterogeneity at the regional scale, providing an evidence of different levels of gene flow and population connectivity as result of genetic admixture along the locations studied in the Western Mediterranean Sea. The outcome of this study showed that the analysis of genetic differentiation over multiple spatial scales can reveal the presence of different genetic patterns that should be considered for the management of marine resources. Thereby, our study can have important implications for the conservation biology and fisheries management of the saddled sea bream and species harbouring similar biological attributes, and provide useful information for genetic population studies of other ecologically and commercially important coastal fishes. To conclude, it is fundamental to take into account different spatial scales in the genetic analysis of samples representative of marine species for the improvement and/or design of future conservations strategies in the Western Mediterranean Sea. From this perspective, the differences in local and regional patterns of genetic differentiation recorded in this study emphasize the need to consider ecological units, rather than political subdivisions, for the management of marine resources.

Chapter IV

PATTERNS OF EARLY LIFE TRAITS SPATIAL VARIABILITY OVER THE COMPETENCY PHASE: A CASE STUDY FROM A MEDITERRANEAN COASTAL FISH

This chapter has been submitted to *Marine Environmental Research* as follows: Calò A, Di Franco A, Guidetti P, Muntoni M, Pérez-Ruzafa Á, García-Charton JA. Patterns of early life traits spatial variability over the competency phase: a case study from a Mediterranean coastal fish.

PATTERNS OF EARLY LIFE TRAITS SPATIAL VARIABILITY OVER THE COMPETENCY PHASE: A CASE STUDY FROM A MEDITERRANEAN COASTAL FISH

Abstract

Multi-scale patterns of pelagic larval duration (PLD) and spawning dates (SpDs) spatial variability were investigated in the saddled sea bream *Oblada melanura*. The role of the competency phase in influencing PLD spatial variability was also assessed considering settlement-competent larvae and post-settlers. PLD of settlement-competent larvae was indicated as days after hatching (DAH). Overall, variability in post-settler PLD was found to be statistically higher than settlement-competent larvae DAH variability. No significant variability in DAH was detected at large spatial scale (~100 km), whereas PLD was found to vary significantly. No significant variability in PLD was found at fine spatial scales (~10 km and ~1 km). An opposite pattern was found for post-settler spawning dates, found to be highly variable at small spatial scale (~1 km), but not at larger ones. The outcomes of the study suggest that: 1. ELTs can vary with different magnitude at different spatial scales; 2. the competency phase can influence PLD variability more than earlier larval stages. This information, beside its ecological relevance, can have important applied implications for the development of more accurate larval dispersal models.

Keywords: early life traits, spatial variability, competency phase, Mediterranean Sea, saddled sea bream

Introduction

Most coastal fishes have a bipartite life cycle composed by a pelagic egg-larval phase followed by a demersal, juvenile-adult phase (Vigliola 1998, Sale 2004). These two phases are “separated” by a transition stage named 'settlement', that is the process by which larvae leave the open water and establish contact with the benthic habitat. It generally coincides with the metamorphosis into juveniles (Searcy and Sponaugle 2000). The pelagic larval duration (PLD) is the stint larvae spend in the open water (i.e. the time between hatching and settlement). Since Panella (1971), PLD has been estimated through the reading of otoliths and more specifically by counting the number of daily increments (i.e. rings) between the otolith nucleus and the settlement mark, a discontinuity in the otolith micro-structure indicating the moment in which the metamorphosis took place (Wilson and Mccormick 1997). Counting daily increments on the otoliths of juvenile fish may also allow to identify spawning dates (SpDs) through a process of back-calculation (Green *et al.* 2009).

PLD and SpDs, recognised as early life traits (ELTs), have a major influence on the degree to which propagules (i.e. eggs and larvae) are subjected to transport by physical processes such as, sea currents, gyres, etc (Sponaugle and Cowen 1997). Thus, ELTs can crucially affect species dispersal (i.e. the process by which living organisms expand actively or passively the space or range where they live) (Shanks *et al.* 2003, Shanks 2009) and consequently connectivity (Treml *et al.* 2012), with this latter defined as the demographic link between populations through the exchange of individuals at whatever life stage (Beldade *et al.* 2007, Gaines *et al.* 2007, Jones *et al.* 2007).

In this context, ELTs are largely used to feed biophysical larval dispersal models, in which oceanographic dynamics are coupled together with fish biological traits, allowing both to hindcast and forecast dispersal trajectories and to estimate larval dispersal (Werner *et al.* 2007, Leis *et al.* 2011, Calò *et al.* 2013, see Chapter II). Although ELTs are recognised to be key parameters for simulating dispersal patterns for several marine species (Watson *et al.* 2010), they are generally assigned to models on the basis of a single estimation in space and/or time, without taking into account their possible spatial and temporal variability, thus potentially impairing the output of larval dispersal models or affecting their accuracy when applied to other geographical or temporal contexts. For these reasons it is fundamental to gather information on ELTs and their variability. The limited available studies, from this perspective, highlighted that significant intra-specific ELT variability can occur both in time (Bay *et al.* 2006) and space (Bay *et al.* 2006, Kingsford *et al.* 2011, Di Franco and Guidetti 2011, Di Franco *et al.* 2013).

Settlement competent larvae (i.e. larvae that have all requisite characters prior to settlement and are “ready” to undergo metamorphosis; Sale 1991) can display the ability to extend their larval phase and delay the settlement until optimal settlement and metamorphosis conditions are met (Victor 1987, Pollux *et al.* 2007). Thus, competency phase could contribute, more than previous larval stages, to influence patterns of PLD spatial variability. The analysis of larval phase duration over the competency phase (i.e. from its beginning to its end) could shed a light on this issue, and provide further information to refine larval dispersal models during the last phase of the larval life, during which dispersal could be less influenced by sea currents dynamics than earlier larval stages (Leis 2007). This knowledge may also provide an indirect indication on the ability of pre-settlement larvae to extend their larval phase immediately before settlement.

With the aim to gain further information on fish ELTs and their variability, the spatial variability in PLD and SpDs of a Mediterranean coastal fish, the saddled sea bream *Oblada melanura,* was investigated at different spatial scales (from hundreds of metres to hundreds of kilometres) in the South-Western Mediterranean Sea. The role of the competency phase in influencing PLD spatial variability was also assessed, by considering both late pre-settlement (settlement-competent) larvae and early post-settlement individuals, and comparing PLD variability of these two life stages. The results of this study, apart their ecological meaning, shall provide important insight for developing more accurate biophysical larval dispersal models.

Material and methods

Sampling design and data collection

The saddled sea bream *Oblada melanura* (Linnaeus 1758) (Perciformes: Sparidae) was chosen as a model species as it is widely distributed in Mediterranean coastal ecosystems (Bauchot and Hureau 1986, García-Charton *et al.* 2004). It is generally abundant and has a relatively high commercial value for artisanal and recreational fisheries (Harmelin-Vivien *et al.* 1995, Lloret *et al.* 2008, Claudet *et al.* 2008, Félix-Hackradt *et al.* 2014).

Pre-settlement (settlement competent) larvae of *O. melanura* were collected using light-traps (Ecoceàn, Care®), which have been previously used to effectively sample several families of reef fish immediately prior to settlement both in the Mediterranean and elsewhere (Carassou and Ponton 2006, Félix-Hackradt *et al.* 2013, Catalán *et al.* 2014). In their pioneering study in the Mediterranean Sea, Felix-Hackradt *et al.* (2013) detected a supply of pre-settlers of *O. melanura* between June and July both in 2010 and 2011 along the coast of the Murcia region (Southern Spain, Western Mediterranean Sea). Based on this finding light-traps were deployed, in the same geographical area, in June, July and August 2013, in order to entirely cover the putative period of larval supply to the coast. For each month, sampling operations were carried out during 9 nights (for a total of 27 sampling nights) around the new moon. This choice was made because coastal pre-settler supply of *O. melanura* is strictly related to this lunar phase, with the peak of supply occurring in a narrow window of 3-4 days around the new moon (Faillettaz, 2015). No pre-settlers were sampled by light-traps in June nor in August. The same timing of larval supply (i.e. only around the new moon of July) was recorded, in 2013, in a northern sector of the West Mediterranean Sea using the same sampling method (Faillettaz, 2015).

Three zones separated by ~60 km were selected along the coast of SE Spain: Southern Alicante province (SAP), Central Murcia region (CMR) and Western Murcia region (WMR), (Fig. 4.1). In each zone 6 light-traps were installed at sunset and retrieved at sunrise during three consecutive nights, for a total of 18 light-trap samples per zone. Light-traps were deployed on an imaginary line parallel to the coast using a buoy moored at 20 –30 m depth, depending on how close the sampling site was to the coast (distance ranging 0.3 –0.8 km) maintaining a minimum distance of 300 m apart from each other (Fig.

4.1). Nevertheless, no pre-settlers were sampled in the CMR zone during the three sampling nights of July.

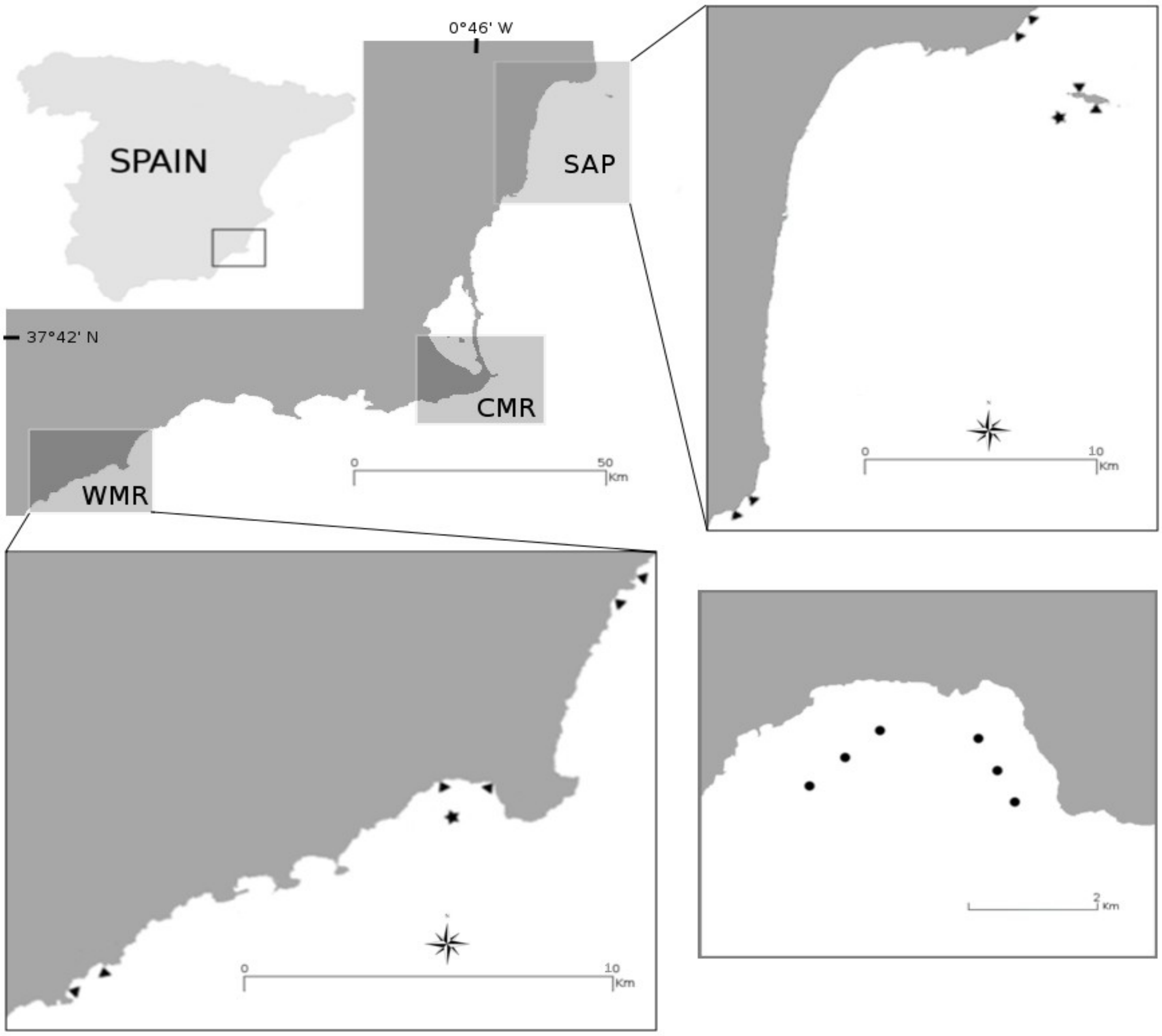

Figure 4.1. Map of the study area: southern Alicante province (SAP), central Murcia region (CMR), Western Murcia region (WMR). In SAP and WMR (highlighted), stars indicate pre-settlers sampling sites (each representing a group of 6 light-traps). Triangles indicate post-settlers sampling sites. Bottom right, example of how light-traps (black dots) were deployed in WMR zone. CMR zone not highlighted.

Samples were collected each morning and all the pre-settlers of *O. melanura* were recognised directly on board, while all the other organisms incidentally collected by the light-traps were released alive. All *O. melanura* specimens were firstly euthanized, immersing them in a water solution with few drops of 95% alcohol for minimizing their suffering (Leary *et al.* 2013) and, after cessation of opercular movements, preserved in 70% ethanol. No direct proof for identifying the onset of the competency phase is available for *O. melanura*. However, considering the sampling method adopted in this study (competent fish larvae are strongly attracted to light at night; Sale 1991), pre-settlement individuals caught by light-traps were initially assumed to be near- or just-competent individuals. This assumption was then verified in the laboratory by assessing fish morphology and size and later through otolith analysis.

Post-settlers (i.e. individuals already metamorphosed and settled in the benthic habitat) of *O. melanura* were collected 10-15 days after pre-settlers collection (i.e. 20th-26th July 2013), in the two zones where pre-settler individuals were previously collected (SAP and WMR). No sampling of post-settlers was carried out in June and August, given that no pre-settlers had been caught in these two months. Specimens were sampled in shallow water habitats (i.e. <2m of depth) characterized by shadowed overhangs and steep rocks, as these are considered to be the main habitat requirements of *O. melanura* early post-settlers (Harmelin-Vivien *et al.* 1995, Bussotti and Guidetti 2011, see Félix-Hackradt *et al.* 2014 for evidence from the study area). In each of the 2 zones where pre-settler supply was recorded, 3 locations (spaced 6-15 km) were randomly selected and in each location 2 sites, separated by ~1 km, were randomly chosen for a total of 6 sites per zone. In each site a small hand-net was used to capture 20-25 post-settlers of *O. melanura*. The number of post-settlers was chosen in accordance with recent studies dealing with spatial variability of ELT in other sparid species (Di Franco and Guidetti 2011, Di Franco *et al.* 2013, Di Franco *et al.* 2014). After collection post-settlers were euthanized and preserved following the same protocol used for pre-settlement specimens. The sampling operations of the present research have been approved by the research ethics committee at the University of Murcia (www.um.es/comisioneticainvestigacion), which is taking all relevant national, European (e.g. Directive 2010/63/EU) and international legislation and guidelines into account. The sampling activity did not involve endangered or protected species.

Otoliths preparation and analyses

Before extracting the otoliths, total length (TL) for each pre- and post-settler individual was measured to the nearest 0.1 mm. Due to alcohol preservation, the reported TL may be smaller than the actual size at collection, due to shrinkage (Sponaugle and Cowen 1997). However, measurement bias can be reasonably excluded because methods of preservation were consistent among sites for both pre-settlers and post-settlers. All the pre-settlers sampled by light-traps had well-formed fish-shaped bodies and similar sizes, perfectly matching the description of this late larval stage provided by several authors (Fig. 4.2) (Raffaele 1888, Lo Bianco 1909, Ranzi 1933).

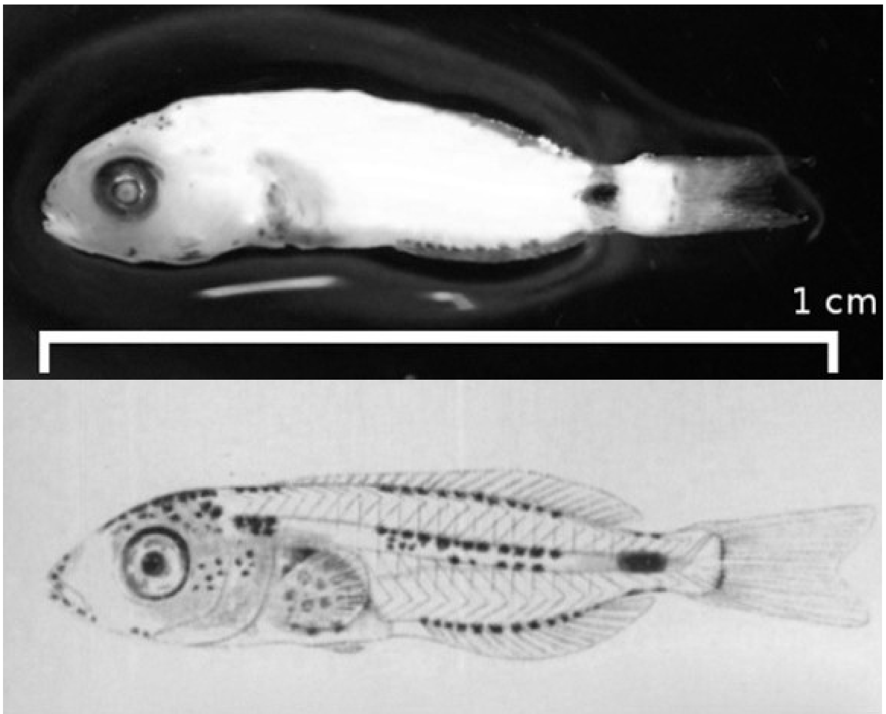

Figure 4.2. Upper image: pre-settler of *O. melanura* (9.4 mm) sampled by light trap. Lower image: larval individual of 10 mm indicated as “small fish” (Ranzi 1933).

Two specimens collected during the same night in a light-trap deployed in zone SAP, showed different morphological characters and larger sizes compared to the other pre-settlement individuals. These two individuals were morphologically comparable to early post-settlers and could have been attracted by the light-trap after their settlement. For this reason these two specimens were excluded from pre-settler sample.

Standard otolith ageing by analysis of daily micro-increment formation (growth rings) (Green *et al.* 2009) was used to determine PLD and SpDs. One sagittal otolith was removed from each specimen (pre- and post-settlers) and processed following a standard procedure (Green *et al.* 2009). The deposition of daily increments in the otoliths of juvenile sparids fish of the genus

Diplodus was pointed out by Vigliola (1997) and already assumed for *O. melanura* by Raventós and Macpherson (2001). The transition-centred method, described by Wilson and Mccormick (1997), was used to identify the settlement mark. In the present study a decrease in daily-increments width associated to an optical contrast between pre- and post-settlement zones in the otoliths of post-settlers (observed in the otoliths of the first 20 post-settlers analysed) was identified as the settlement mark (Fig. 4.3), with the settlement transition completed within one increment. No settlement mark was identified on the otoliths of pre-settlers (this evidence supporting the assumption about pre-settlement competency of these specimens). The daily rings were read using a microscope with magnification 400×. Sub-daily increments (i.e. faint increments occurring between well-defined increments; Raventós and Macpherson 2001) were found on the otoliths and not considered on the basis of the method described by Raventós and Macpherson (2001).

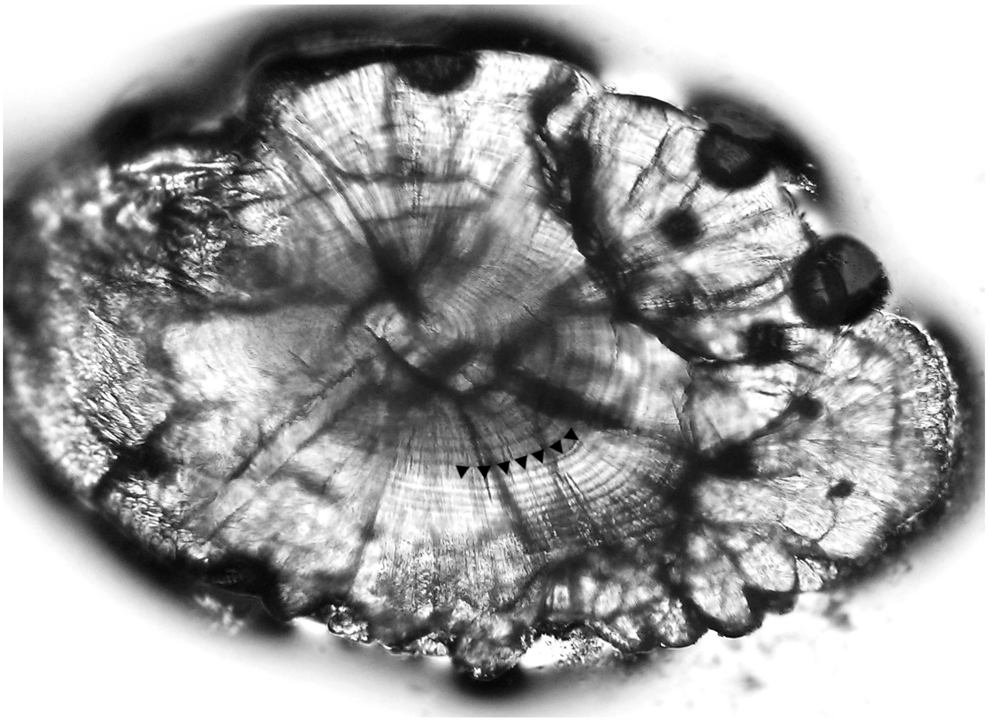

Figure 4.3. Otolith section of a post-settler of *Oblada melanura* (200x magnification). Black triangles indicate the settlement mark (image set to highlight the settlement mark).

Each otolith was read twice through two independent readings (according to Searcy and Sponaugle 2001) and only the otoliths for which the two readings matched perfectly were considered in data analysis. The time of initiation of daily increments deposition in the otolith is unknown for *O. melanura*, so this information was assumed from findings in other sparid fishes for which daily increments initiation has been validated (Vigliola 1997), considering that the first daily increment formed at hatching (Tsuji and Aoyama 1982). Thus, counts could slightly under- or overestimate actual ELT values. However, any potential bias in comparing pre- and post-settlers larval duration can be excluded, because the method of measurement was consistent among pre- and post-settlers.

For each specimen, the hatching date was back-calculated by subtracting the total number of growth increments from the sampling date (Di Franco *et al.* 2011, Di Franco and Guidetti 2011). Pelagic larval duration in pre-settlers was named “days after hatching” (DAH) to avoid possible confusion with post-settlers PLD, i.e. the duration estimated on post-settlers, which encompasses the whole larval phase finishing with the settlement event. The SpD was then calculated by subtracting 2 days (corresponding to the approximate time between spawning and hatching in *O.melanura*; Antolović *et al.* 2010) from the previously estimated hatching date. For each post settler, settlement date was calculated by subtracting the number of growth increments after the settlement mark from the sampling date. The frequency distribution of settlement dates, assumed as a proxy of the magnitude of larval supply, was compared to the frequency distribution of pre-settlers collected with light-traps each night. In this way, an estimate of the efficacy of the pre-settler sampling in covering the actual larval supply was obtained.

Data analysis

To test for spatial variability in pre-settler DAH, a univariate PERMANOVA was run, where Zone (Zn) was treated as a random factor with 2 levels. TL was considered as a covariate to prevent fish size effects on spatial comparisons. Thirty-two and thirty otoliths (replicates), from zone SAP and WMR respectively, perfectly matched the two readings and were considered in the analysis.

To test for spatial variability in post-settlers PLD and SpDs, two univariate PERMANOVAs were run, where Zone (Zn) was treated as a random factor with 2 levels, Location (Loc) was treated as a random factor (nested into Zn) with 3 levels and Site (Si) was treated as a random factor (nested into Loc) with 2 levels. As for pre-settlers, TL was set as covariate. Between 14 and 17 otoliths from each site (for a total of 182 otoliths) perfectly matched the two readings and were considered in the analysis. Following (Di Franco *et al.*, 2013), in order to run PERMANOVA, the SpD of each fish was converted into an integer between 1 (indicating 16th June 2013, first SpD recorded) and 19 (indicating 4th July 2013, last SpD recorded). Linear regression analysis (DISTLM, distance based linear model) was used to assess the relationship between SpD and PLD for all post-settlers.

Before performing PERMANOVAs, data were tested for homogeneity of dispersion using Permutational Analysis of Multivariate Dispersions (PERMDISP) based on Euclidean distance, which is equivalent to Levene's test for heterogeneity of variances when used on univariate data (Anderson *et al.*, 2008). No evidence of heterogeneity of variance was highlighted for DAH, PLD or SpD data ($p > 0.05$ for all the tests).

To test for potential differences between the variability of DAH and PLD, pre-settler and post-settler data were combined together in a new dataset. For each zone, only post-settlers spawned in the same time window as pre-settlers were considered in the analysis (39 from SAP and 53 from WMR, for a total of 92 individuals, corresponding to the 50% of all post-settlers), to reduce as much as possible the bias due to fish spawned during different spawning events (the range of post-settlers SpDs completely included SpD range of pre-settlers, see Results). PERMDISP was used to calculate the deviation of each DAH and PLD value from the centroids of the combined factor Zone-Life Stage. The individual deviation values were then analysed using PERMANOVA where Zone was treated as random factor with 2 levels and Life Stage ('LS', i.e. pre-settler and post-settler) was treated as random factor with 2 levels, crossed with Zone. Individual TL was set as covariate. This approach has been developed to test variability in dispersion in multi-factorial designs (Di Franco *et al.* 2014).

All statistical analyses were performed using Primer 6 PERMANOVA+ software package (University of Plymouth) and R statistical software.

Results

Pre-settlers sizes ranged from 8.6 to 10.7 mm with a mean value of 9.5 ± 0.1 mm (mean ± SE) (Fig. 4.4). Pre-settlers SpDs and DAH ranged from 22nd to 28th June 2013 (Fig. 4.5a) and 10-13 days, respectively (Fig. 4.6). The overall mean DAH (± SE) was 11.5 ± 0.1 days. Mean values for DAH in zone SAP and WMR were 11.6 ± 0.1 and 11.4 ± 0.1 days (Fig 4.6), respectively, this difference not being statistically significant (Table 4.1). A significant effect of the covariate TL on DAH was detected (Table 4.1), with an increment in DAH with increasing TL. Almost 80% of pre-settlers captured in both zones shared the same SpDs, although with different frequency in each zone (Fig. 4.5a).

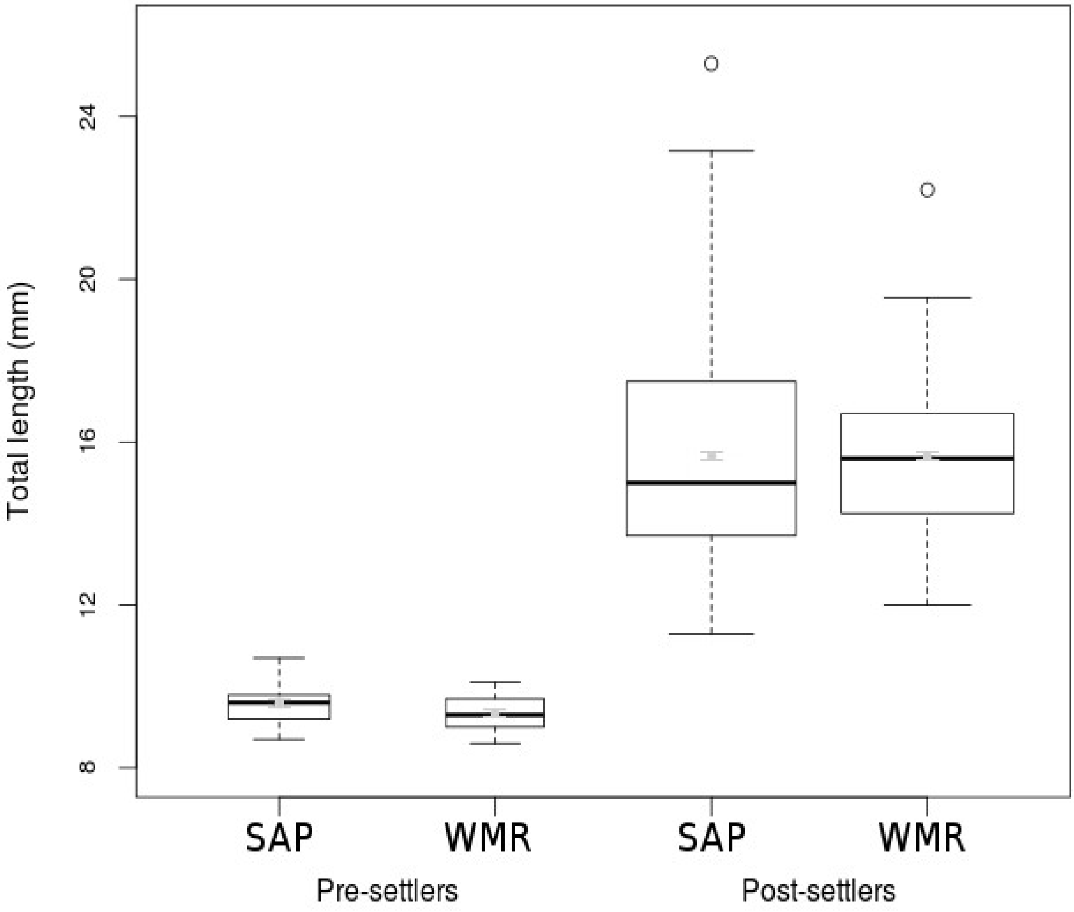

Figure 4.4. Box-plot of TL for pre- and post-settlers in SAP and WMR. Box-plot width proportional to the square root of the number of samples. Grey dots and bars indicate means and standard errors respectively.

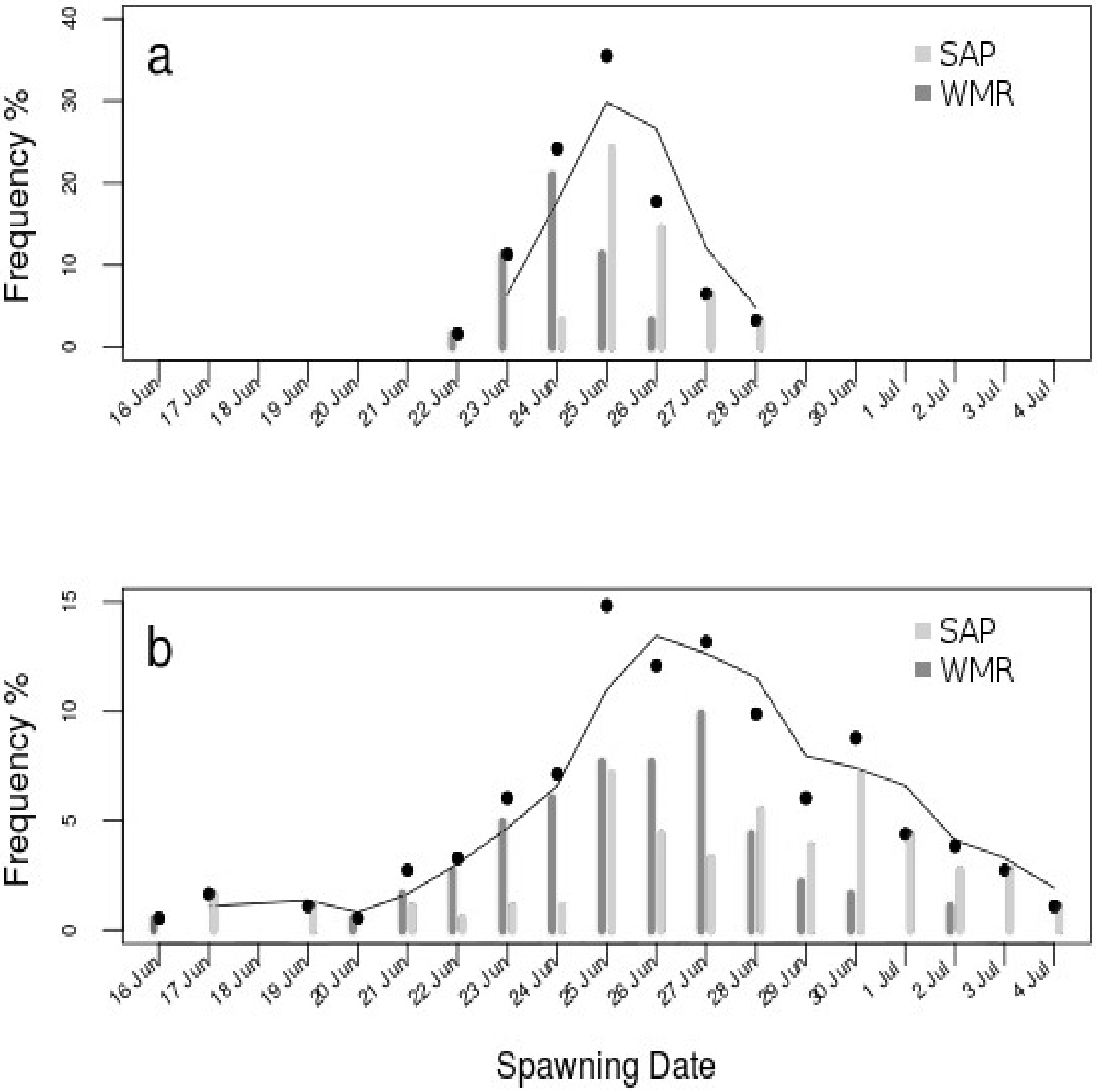

Figure 4.5. Pre-settlers spawning dates (a). Post-settlers spawning dates (b). Black dots represent total frequency per each day. Continuous lines represent moving averages. Dark grey and light grey bars indicate the frequency of spawning dates for each zone.

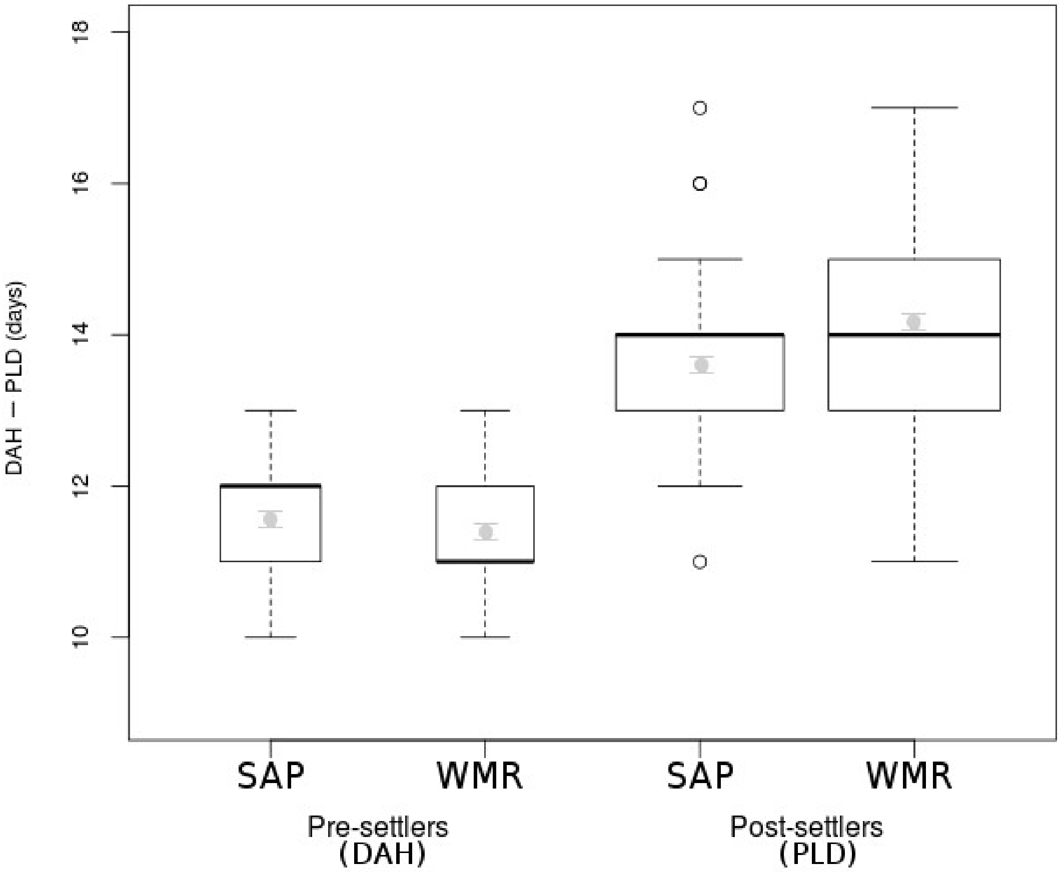

Figure 4.6. Box-plot of DAH and PLD for pre- and post-settlers respectively. Box-plot width proportional to the square root of the number of samples. Grey dots and bars indicate means and standard errors respectively.

Table 4.1. PERMANOVA on pre-settlers DAH (upper table) and post-settlers PLD and SpDs (lower table). TL=total length, Zn=zone, Loc=location, Si=site, ns=non significant, *=p<0.05, **=p<0.01.

Source		DAH		
	df	MS	Pseudo-F	
TL	1	6.3892	15.27	**
Zn	1	5.82E-003	1.39E-002	ns
Res	59	0.4184		
Total	61			

Source		PLD			Spawning Dates		
	df	MS	Pseudo-F		MS	Pseudo-F	
TL	1	0.75279	0.5473	ns	619.86	54.526	**
Zn	1	12.859	9.161	*	201.86	1.4688	ns
Loc(Zn)	4	1.3884	0.60752	ns	135.81	6.7683	*
Si(Loc(Zn))	6	2.2786	1.9206	ns	19.674	5.3955	**
Res	169	1.1864			3.6463		
Total	181						

Post-settlers sizes ranged from 11.3 to 25.3 mm with a mean (± SE) of 15.6 ± 0.2 mm (Fig. 4.4). PLD averaged 13.9 ± 0.1 days, ranging from 11 to 17 days (Fig. 4.6). Post-settler SpDs ranged from 16th June to 4th July 2013 (Fig. 4.5b). A significant difference at the scale of 'Zone' on PLD was recorded (PERMANOVA, $p<0.05$), while factors 'Location' and 'Site' resulted not statistically significant (Table 4.1). PLD recorded in zone WMR (14.1 ± 0.1 days) was higher than that recorded in zone SAP (13.6 ± 0.1 days) (Fig. 4.6). Post-settler SpDs were found to vary significantly both at the scale of Location ($p<0.05$) and Site ($p<0.01$), but not between Zones (Table 4.1). A significant effect of TL on SpDs was evidenced (Table 4.1), with larger sizes corresponding to fish that have been spawned earlier. A significant negative relationship between PLD and SpDs was detected among post-settlers ($p<0.01$) with longer PLD associated to post-settler spawned earlier (Fig. 4.7).

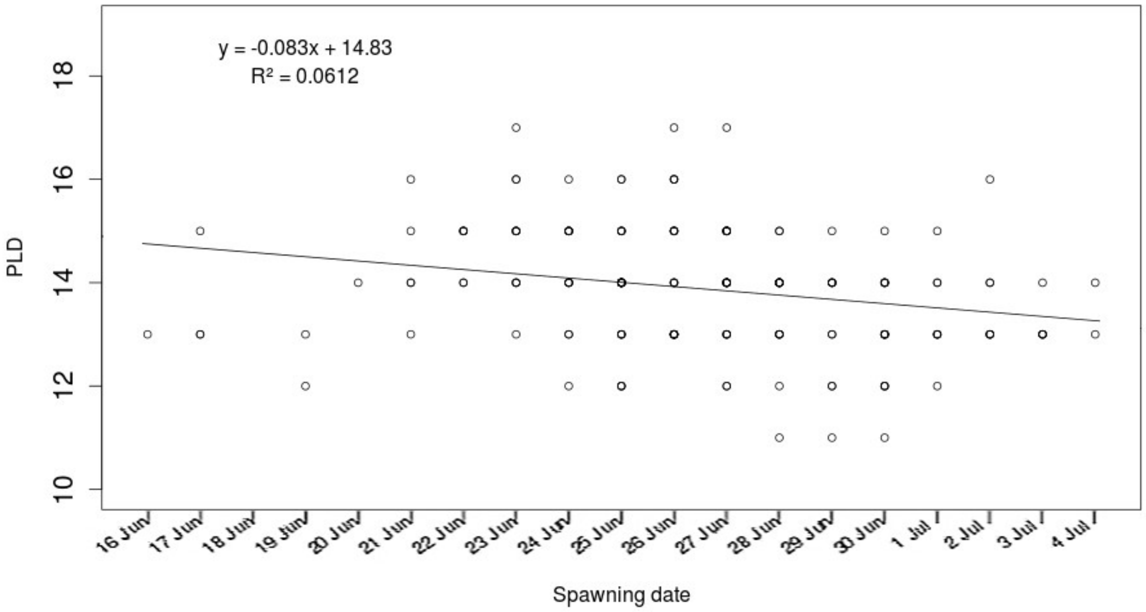

Figure 4.7. Linear correlation of post-settler PLD versus spawning date.

Post-settler settlement dates ranged from 1st to 20th July with a peak of settlement between 11th and 14th (Fig. 4.8). Less than the 4% of post-settlers recorded in SAP and WMR settled before the first day of pre-settler sampling campaign in these zones.

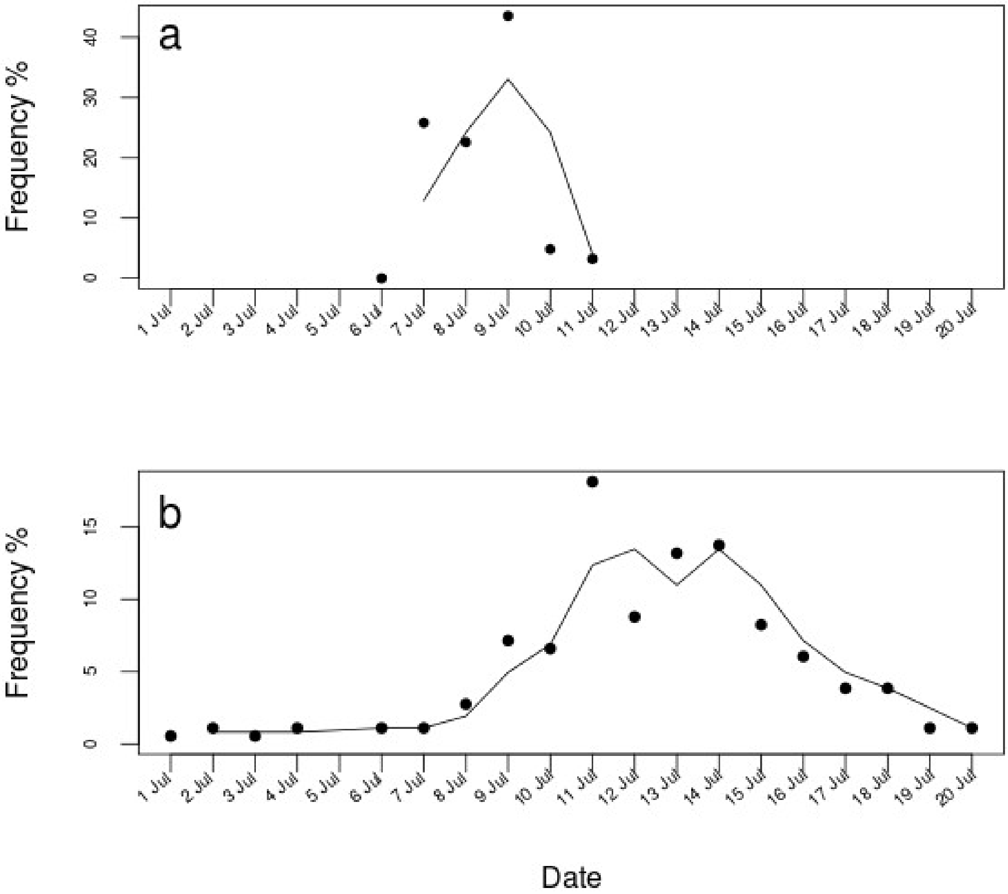

Figure 4.8. Pre-settlers sampling dates (a). Post-settlers settlement dates (b). Black dots represent total frequency per day. Continuous lines represent moving averages.

Analysis of PERMANOVA on PERMDISP deviation values showed a significant difference for the factor Life Stage (LS) between DAH and PLD, with PLD (coefficient of variation = 8.2%) more variable than DAH (coefficient of variation = 6.2%). All the other source of variation included in the analyses (Zone, Zone*LS and TL) resulted not significant (Table 4.2).

Table 4.2. PERMANOVA on DAH and PLD distance from group centroid obtained from PERMDISP. TL=total length, Zn=zone, LS=life stage, ns=non significant, *=p<0.05.

Source		Distance from Centroid		
	df	MS	Pseudo-F	
TL	1	1.17	0.278	ns
Zn	1	0.16369	1.0127	ns
LS	1	1.7881	6.1859	*
ZnxLS	1	0.17261	0.54735	ns
Res	149	0.31536		
Total	153			

Discussion

ELTs can have crucial implications on fish larval dispersal and settlers distribution along coastal habitats (Sponaugle 2010). Information on ELTs variability is thus fundamental for better understanding patterns of dispersal and connectivity and for the correct parametrization of biophysical larval dispersal models (Di Franco *et al.* 2013). Post-settlers PLD and SpDs of *O. melanura* recorded in this study varied in space depending on the spatial scale considered. This outcome matches the hypothesis formulated by Di Franco and Guidetti (2011) for which patterns of variability in fish ELT could depend on the spatial scale of analysis (Di Franco and Guidetti 2011, Di Franco *et al.* 2013). Variability in ELT could be a consequence of a number of processes, both physical and biological in nature, acting alone or in synergy, concerning adults and/or larvae, and working with different magnitudes at different spatial scales (Sponaugle and Cowen 1997). PLDs were found to be significantly variable at the scale of 'zone' (~100 km) but not at smaller spatial scales ('location', ~10 km and 'site', <1km). Geographic spatial differences (i.e. at the scale of hundreds of km) in PLD could be determined by distinct oceanographic patterns between distant areas (Thresher and

Brothers 1989, Wellington and Victor 1992) that may also potentially lead to intra-specific genetic differences, determining a possible divergence in the duration of the larval period among populations (Wellington and Victor 1989, Thresher and Brothers 1989, Chambers and Leggett 1992). In this context, the lower value of PLD recorded in SAP (north of the study area) could be a consequence of the physical characteristics of water masses in the region. Data of sea surface temperature (SST) recorded in the Western Mediterranean Sea indicated that the north of the study area (hosting the SAP zone) was constantly characterised by higher temperature compared to the southern sector (where the WMR is located), during the entire pelagic phase of *O. melanura* in 2013 (www.socib.eu; Fig. 4.9). From this perspective, higher values of SST in zone SAP could have promoted a faster larval growth, shortening the time needed for developing all the characteristics necessary to settle (Green and Fisher, 2004).

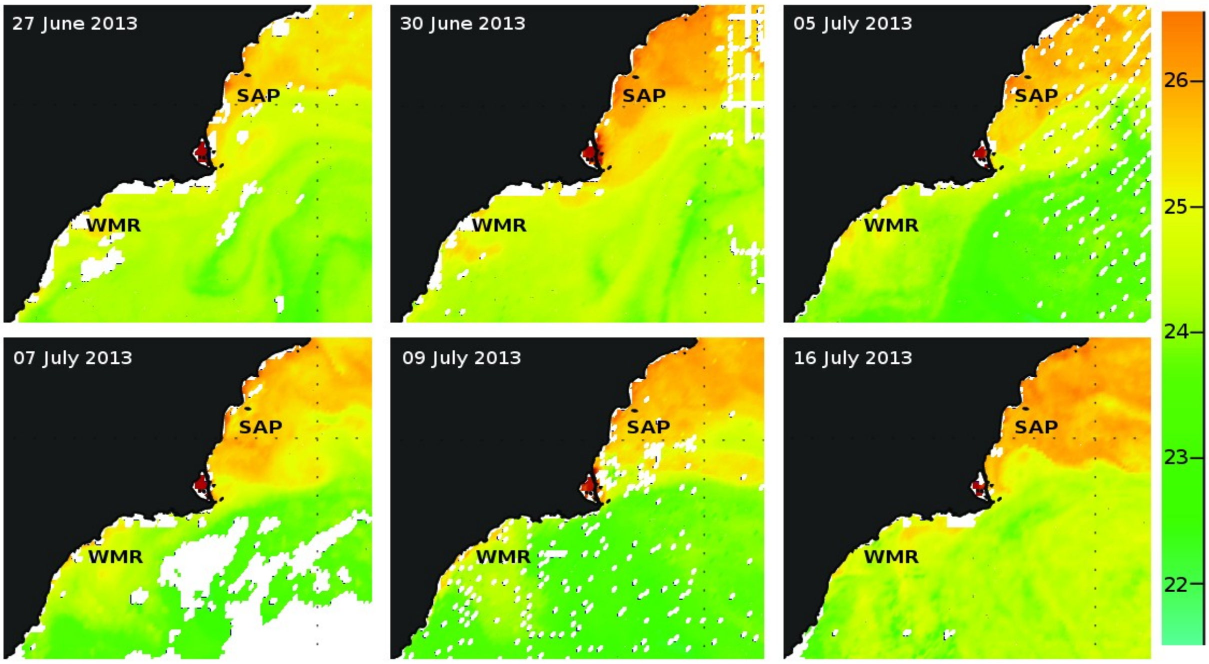

Figure 4.9. Sea surface temperature (°C) in the study area. Satellite images show the profile of sea surface temperature of 6 days over the time window in which the pelagic phase of *O. melanura* occurred in 2013 (www.socib.eu). 'SAP' and 'WMR' indicate the position of the 2 zones considered in the study.

Behaviour could indirectly influence local scale pattern of variability in PLD also after the settlement. From this perspective, post-settlers of *O. melanura* exhibit a particular ontogeny-dependent post-settlement residence-persistence strategy (Kiparissis *et al.* 2008). In particular, early post-settlers arrive in small shoals and then actively aggregate in larger shoals with increasing fish size (Harmelin-Vivien *et al.* 1995, Kiparissis *et al.* 2008). Such behaviour is very different from that described for other sparid species, which start settlement in large shoals that gradually decrease in size as the fish grow (Harmelin-Vivien *et al.* 1995). In this study, post-settlers, although sampled a few days after settlement, showed sizes generally larger than those indicated by Kiparissis *et al.* (2008), corresponding to fish that may have already passed several stages of aggregation. Multiple aggregation steps could mix several shoals that had settled independently from each other and may have experienced different early life histories, progressively smoothing the differences and leading to a low variability in PLD among relatively close sites.

A different pattern from the one observed in PLD was recorded for SpDs for which the level of significance of spatial differences increased with increasing spatial resolution from zone, to location and then to site. Patterns of SpDs recorded here could be influenced by spawners reproductive behaviour. The absence of differences in SpD between zones could suggest that at a regional scale the physical-chemical characteristics of water masses (e.g. temperature, considered one of the main factors in determining the timing of spawning; Wootton 1990) could be relatively similar leading to a concurrent time of gonad maturation and successive spawning period among groups of fish living in areas spaced out ~100 km from each other. At a more local scale (<10 km), environmentally cued spawning events may differ as a consequence of differences in current dynamics among locations (Sponaugle and Cowen 1997). Different groups of spawners could select different times for releasing gametes on the basis of local current flows to maximize offspring supplies to the coast. The formation and maintenance of larval patches, due to passive accumulation or behaviour-mediated aggregation (Paris and Cowen 2004), could also influence the variability observed at local spatial scale. Different patches, assumed to be horizontally sized from 1-2 up to 6 km (depending on the species and the geographic area considered; Paris and Cowen 2004), could replenish different nearby coastal sites (Di Franco *et al.* 2013).

Settlement-competent larvae are known to be behaviourally active and to have quite remarkable swimming capabilities (Montgomery *et al.* 2006, Leis 2006). This ability can allow pre-settlement larvae to extend their PLD and potentially affect its variability more than previous larval stages. The significant difference in the pattern of statistical dispersion between DAH and PLD (with PLD more variable than DAH) recorded in this work could be an indication that larvae of *O. melanura* can extend their pelagic phase immediately before settlement. The analysis of individuals coming from different breeding events could have led to biased comparisons between the ELTs of the two life stages investigated in the study. From this perspective, the consideration of the same spawning window for both pre- and post-settlers (i.e. removing post-settlers spawned outside the pre-settlement SpDs period) allowed to draw a reliable comparison between the two life stages, that additionally were found to share the same spawning peak. The ability of *O. melanura* larvae to extend their larval phase seems to be corroborated by the negative relationship between post-settlers' SpDs and PLD. This result indicates that fish spawned earlier were more likely to prolong their PLD than recently spawned ones and provides insights on the potential ability of larvae to anticipate or delay settlement and synchronize its timing with specific environmental conditions.

The role of competency phase in shaping patterns of ELTs is also suggested by the different spatial variability of DAH and PLD observed between the two zones investigated. While no significant differences between zones in terms of pre-settler DAH were highlighted, PLD of post-settlers was found to vary significantly between the two zones. These distinct patterns indicate that the observed variability in PLD presumably took place during the competency phase and could be influenced by processes affecting the duration of the larval phase immediately before settlement. A similar pattern was recently found in a laboratory work on a coral reef species, with a PLD similar to the one of *O. melanura,* for which a metamorphosis delay was recorded in individuals that had just reached the competency phase (Wenger *et al.* 2014). Although in this study it was not possible to ensure *a priori* that all pre-settler individuals were competent when sampled, successive laboratory analyses indicated that pre-settlers were just-competent individuals, based on the fact that: 1) all the specimens had a fish shaped body and no individuals belonging to earlier ontogenetic stages were found in the light-traps; 2) the minimum value of post-settler PLD, that can be assumed as the minimum age at competency recorded in the study, was 1 day higher than the minimum values of pre-settler DAH; 3) no settlement mark was found on pre-settler otoliths.

Finally, PLD was on average more than 2 days longer than DAH. Although a larger mean value of PLD was expected, as PLD is a measure of the length of the whole larval phase while DAH represent the duration of the larval phase before its completion, the implications might not be trivial. Light-traps were deployed generally at 400–500 m from the coast; even considering the possibility that light-traps have captured pre-settlers that were a tens of metres away from them (due to photo-attraction), it is possible that pre-settlers of *O. melanura* were already relatively close to the coast 2 days before their settlement. This outcome is particularly relevant taking into account the offshore distribution of early stage larvae of *O. melanura* in the West Mediterranean Sea (Sabatés 1990b) and could have important implications for the development of more precise larval dispersal models of this species. The observed distribution of DAH (corrected for the duration of the egg phase) could be used as the time over which dispersal models could be run instead of PLD, as currently used in the majority of biophysical models. The shortening of dispersal time from PLD to DAH would allow models to be run over a time period in which larval dispersal is putatively less influenced by behaviour than the one that includes the competency phase, and thus more driven by current dynamics, on which dispersal models are mainly based.

In conclusion, based on the available literature, fish larval dispersal is generally thought to depend on a series of biological and physical factors (e.g. ELTs, fish sensory capabilities and current dynamics; Wolanski and

Kingsford 2014), whose variability in space and/or time could have crucial implications on patterns of dispersal and consequently connectivity. From this perspective, the outcomes of the present study suggest that ELTs (i.e. PLD and SpDs) can vary with different magnitude at different spatial scales and that the competency phase can influence, more than earlier larval stages, PLD spatial variability. Larval dispersal models should thus take into account the potential spatial variability of PLD and SpDs, as for other fundamental ELTs, whenever models are applied to a range of spatial scales, or whenever extrapolated to different geographic contexts. From this perspective, the findings of this study, beside their ecological meaning, can provide 1) interesting information for developing more accurate larval dispersal models of *O. melanura* in the studied region and 2) useful insights (e.g. the use of DAH) to refine models for other fish species and regions.

Chapter V

PROPAGULE DISPERSAL AND LARVAL PATCH COHESIVENESS IN A MEDITERRANEAN COASTAL FISH

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PROPAGULE DISPERSAL AND LARVAL PATCH COHESIVENESS IN A MEDITERRANEAN COASTAL FISH

Abstract

The assessment of fish dispersal is fundamental for both conservation and management of fishery resources as it provides crucial information for the establishment of more effective marine protected areas (MPAs) and networks of MPAs. In this study we investigated otolith elemental composition in early life stages of the saddled sea bream *Oblada melanura* (Linnaeus, 1758) (Perciformes: Sparidae) in order to obtain information on its propagule (egg and larva) dispersal in the South-Western Mediterranean Sea. Specifically, considering pre-settlement individuals we investigated (1) larval patch cohesiveness during the last phase of the larval life; considering early post-settlement individuals we investigated (2) the number of potential natal sources, and (3) propagule dispersal distances. Results showed that different larval patches can merge in the pelagic environment after having travelled separately for some days. Seven natal sources were found to replenish, with different proportions, almost all the sampling sites along a stretch of coastline of ~180 km, suggesting that propagule dispersal can take place, at least, up to ~90 km. This information provides important insights for understanding fish dispersal processes and supports the correct establishment of spatially explicit conservation strategies such as MPAs and MPA networks in the South-Western Mediterranean Sea.

Keywords: propagule dispersal, natal origins, patch cohesiveness, saddled sea bream, Mediterranean sea

Introduction

Dispersal is the process by which organisms distribute themselves actively or passively affecting patterns of connectivity (i.e. the link between sub-populations through the exchange of individuals), meta-population dynamics and ultimately species persistence (Cote *et al*. 2010, Shima and Swearer 2010). The study of dispersal patterns is of paramount importance for marine biodiversity conservation and fisheries management as it provides vital information for the establishment of effective marine protected areas (MPAs) and networks of MPAs (Grüss *et al*. 2011b, Green *et al*. 2014). In many coastal fish with a bipartite life cycle (i.e. composed by a larval and a juvenile/adult phase), the life stages after settlement (i.e. when the pelagic larval phase ends with the metamorphosis into juvenile) are usually considered relatively sedentary, so that species dispersal potential is mostly determined by the propagule (i.e. egg and larva) phase (Leis 2015, but see Di Franco *et al*. 2015). Despite the huge effort being currently undertaken, the empirical measure of propagule dispersal is still extremely challenging for fish ecologists. This is due to several issues, for example, the difficulty to mark small planktonic larvae and/or to recapture them (given the high mortality rates) (Barbee and Swearer 2007, Fontes *et al*. 2009).

Recently, a number of studies have been conducted using natural environmental markers for investigating fish natal origins and evaluate species dispersal capacity (Leis *et al*. 2011, Calò *et al*. 2013 for reviews). Otoliths are particularly useful as they record the chemical characteristics of the surrounding environment experienced by fishes during all life phases (Barbee and Swearer 2007). Otoliths grow by a continuous deposition of layers of calcium carbonate, mainly aragonite, in a protein matrix (Campana 1999). During the deposition, trace-elements from the ambient water can substitute for calcium remaining permanently in the matrix (Campana 1999). The physical and chemical characteristics of the environment (primarily temperature, salinity and trace-elements concentration) influence the incorporation rates of some specific elements (e.g. Sr and Ba) into the otolith matrix (Campana 1999, Walther and Thorrold 2006, Barnett-Johnson *et al*. 2008, Muhlfeld *et al*. 2012). Variations in otolith elemental composition can be used to discriminate larval groups that experienced different environmental conditions and obtain information on important early life history traits such as the number of potential natal sources, larval dispersal distances and pathways (Hamilton *et al*. 2008, Fontes *et al*. 2009, Shima and Swearer 2010, Di Franco *et al*. 2012b, Miller *et al*. 2014, Shima *et al*. 2015, but see also Berumen *et al*. 2010).

Analysis of the chemical profile from the otolith edge toward its core provides information on the different environmental conditions previously experienced by a larva (Sandin *et al*. 2005). The comparison of otolith chemical profiles of larvae originating from the same patch (i.e. an aggregation of larvae) can be used to investigate the consistency of the patch during the pelagic phase (i.e. cohesiveness), that is, if larvae travelled together to the sampling site or experienced different dispersal pathways, that would result in different individual otolith chemical profiles (Shima and Swearer 2009, Ben-Tzvi *et al*. 2012). The knowledge of the ontogenetic cohesiveness of a larval patch can provide important insights on post-settlement demographic dynamics (Shima *et al*. 2015) as different larval trajectories, potentially shaped by a variable dispersal environment, can have important implications on the post-settlement persistence of the larval patch (Shima and Swearer 2009, Shima and Swearer 2010, Shima *et al*. 2015).

Analysis of the chemical composition of otolith cores (i.e. the inner portion of otoliths that forms during the embryogenesis) allows the assessment of the number of potential sources of propagules that supply different settlement sites along a stretch of coastline (Ruttenberg *et al*. 2008). From this information it is possible to infer the spatial scale over which propagule dispersal takes place, considering the distance among sites replenished by a single natal source and the number of potential sources within the sampled area (Standish *et al*. 2008, Di Franco *et al*. 2012b, Di Franco *et al*. 2015).

In this study we investigated geochemical signatures in the otoliths of early life stages of the saddled sea bream *Oblada melanura* (Linnaeus, 1758) (Perciformes: Sparidae) in order to obtain information on its propagule dispersal characteristics in the South-Western Mediterranean Sea. Specifically, considering pre-settlement individuals of *O. melanura* we investigated (1) the cohesiveness of larval patches during the last phase of the larval life; considering early post-settlement individuals, we investigated: (2) the number of potential natal sources and post-settler replenishment pattern of our study area; and (3) propagule dispersal distances. This information can provide important insights for understanding fish dispersal processes and supports the proper establishment of spatially explicit conservation tools such as MPAs and MPA networks.

Materials and methods

Study area and sampling design

The study was conducted along the south-east Spanish coast (SW Mediterranean Sea). This area is particularly relevant from a conservation perspective as it hosts an important biodiversity hotspot in the Western Mediterranean Sea (Calvin Calvo *et al*. 1999). *Oblada melanura* was chosen as a model species as it is widely distributed and generally abundant in Mediterranean coastal habitats (Bauchot and Hureau 1986, García-Charton *et al*. 2004) and for its relatively high commercial value for artisanal and recreational fisheries (Harmelin-Vivien *et al*. 1995, Lloret *et al*. 2008, Félix-Hackradt *et al*. 2014). This sedentary fish forms big shoals that inhabit littoral waters above rocky bottoms and *Posidonia oceanica* beds up to 30m deep (Bauchot and Hureau 1986). Along the south-eastern Spanish coast the reproduction takes place between June and July with the majority of spawning output released during a narrow temporal window of 2-3 weeks (Félix-Hackradt *et al*. 2013). Larvae after having travelled in the pelagic environment for ~2 weeks (Raventós and Macpherson 2001), settle in shallow water habitats (2 m or less in depth) characterized by shadowed overhangs and steep rocks (Bussotti and Guidetti 2011, Félix-Hackradt *et al*. 2014). After settlement, small groups of juveniles actively aggregate in larger shoals, finally recruiting to the adult population (Kiparissis *et al*. 2008). Larval patch cohesiveness was investigated considering late stage larvae (pre-settlers) of *O. melanura*. Specimens were caught using light-traps in 2013 during July, the month with the highest larval supply recorded along the coast in the study area (Félix-Hackradt *et al*. 2013). Six light-traps were deployed in each of 2 sites (S1 and S2, Fig. 5.1), 150 km apart. Light-traps were deployed along a transect parallel to the coast, ~200m apart, to avoid interference due to photo-attraction, and individually moored with a buoy at 20 –30 m depth, depending on how close the sampling site was to the coast (distance ranging 0.3 –0.8 km). Three light-trap samples from the northernmost site S1 (here named T1, T2 and T3), sampled during the same night, and one light-trap sample from the southernmost site S2 (T4, collected during the following sampling night) contained from 8 to 11 pre-settlers each, and thus were processed in the laboratory. All the other light-trap samples were not included in the successive analyses, because they contained only 1-2 individuals and thus it was impossible to investigate any inter-individual variability in dispersal to assess patch cohesiveness.

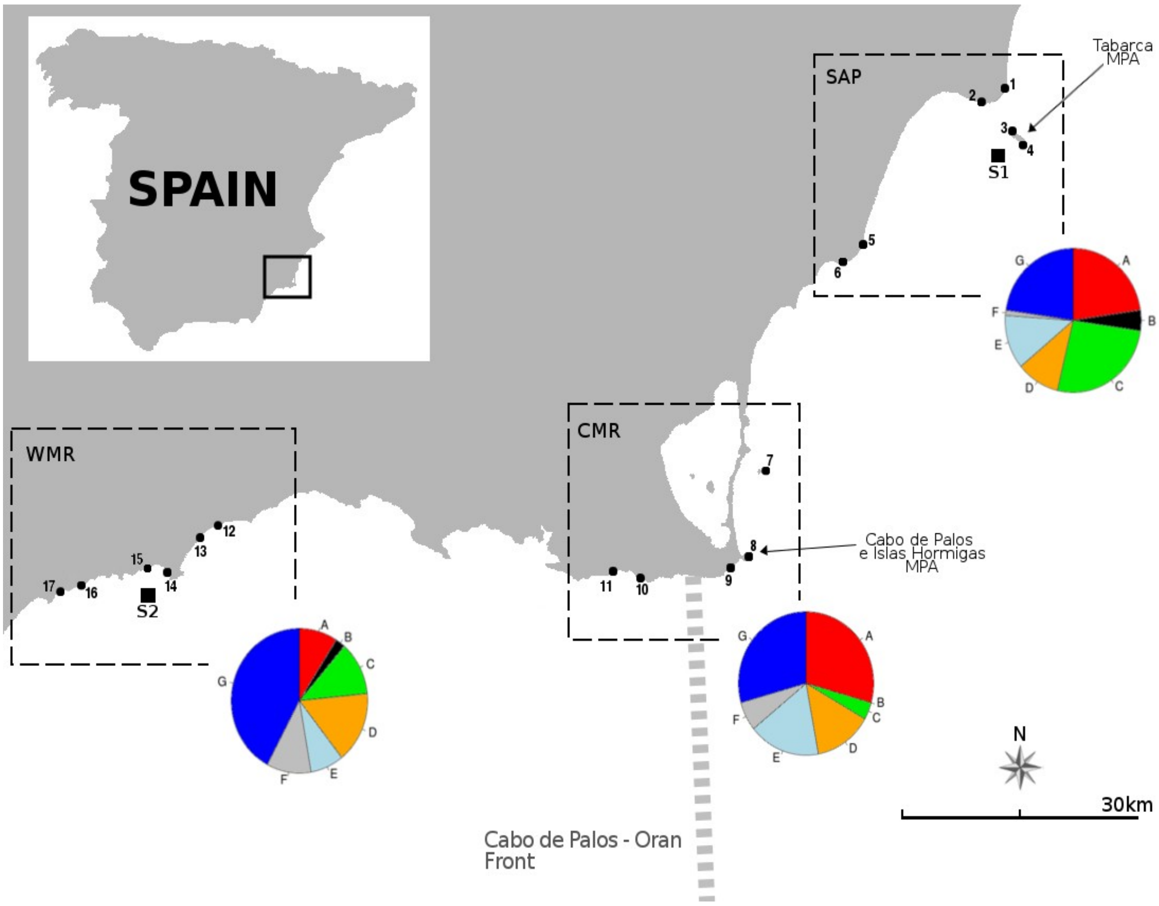

Figure 5.1. Map of the study area. Black dots indicate post-settlers sampling sites (numbered progressively from the northernmost to the southernmost); dashed line rectangles represents zones: south Alicante province (SAP), central Murcia region (CMR) and west Murcia region (WMR). Black squares represent pre-settlers sampling sites where light traps have been deployed. Pie charts indicate, for each zone, the proportion of post-settlers belonging to the each natal source clustered (see results). Highlighted the supposed position of the Cabo de Palos-Oran front (see discussion).

With the aim to investigate the number of potential natal sources and propagule dispersal distances of *O. melanura* in the study area, early post-settlement individuals were sampled in July 2013 after the peak of settlement. Post-settlers were collected in 17 sites (stretches of 1 km of coastline), scattered within 3 zones (stretches of 30 km of coastline), as follows, from North to South: Southern Alicante province (SAP, 6 sites), central Murcia region (CMR, 5 sites) and Western Murcia region (WMR, 6 sites) (Fig 5.1). SAP and CMR host 2 effective MPAs: Tabarca MPA and Cabo de Palos e Islas Hormigas MPA (Fig 5.1). In each site 11 to 15 post-settlers were collected in shallow waters (i.e. <2m of depth), using a hand net during snorkelling. All the specimens were firstly euthanized, by immersing them in a sea water solution with few drops of 96% alcohol to minimize suffering (Leary *et al*. 2013) and, after cessation of opercula movements, preserved in 70% ethanol.

Otolith preparation and analysis

In the laboratory, total length (TL) for each pre- and post-settler individual was measured to the nearest 0.1 mm. The right sagittal otolith was removed from each specimen, cleaned of soft tissue using plastic pins and rinsed with ultra-pure water ($18.2 M\Omega cm^{-1}$). Then, otoliths were mounted sulcus side up onto a glass slide using Crystalbond™ thermoplastic adhesive. Otoliths were polished with 3 µm and 1 µm Imperial lapping film through the nucleus. Individual age for each pre-settler and post-settler was determined by analysing daily micro-increment formation (growth rings) (Green *et al.* 2009). Otoliths were then rinsed with an ultrasonic bath for 10 minutes in ultra-pure water in order to remove surface contamination.

Elemental analysis was performed using a Thermo Elemental X series II inductively coupled plasma mass spectrometer (ICP-MS) coupled to a New-Wave Research UP213 with aperture imaging laser ablation (LA) system. Otolith sections were viewed remotely on a computer screen where the area for ablation was selected. The laser was focused on the sample surface and fired through the microscope objective lens. Helium gas was flushed into the ablation cell to reduce the deposition of ablated aerosols and to improve signal intensities (see Giannossa *et al*. 2015 for further details on system setup). The ablated aerosol was then mixed with Argon before entering the ICP torch.

Otoliths of pre-settlers (8.6-10.7 mm individual TL range and 11.4 days mean age) were analysed to investigate larval patch cohesiveness. Specifically, we compared the variability of otolith chemical profiles among individuals coming from the same light-trap sample (here considered as a larval patch). Pre-settlers otoliths were analysed through a series of 7 laser spots, following the major axis of the sagitta, and positioning each spot on a different daily growth ring from the edge of the otolith (corresponding to the otolith ring laid down during the sampling day) to the seventh last growth ring. In this way, for each pre-settler, we obtained the chemical profile of the last 7 days of larval life. The earliest days of life were not considered since they could have added an additional source of variation (ontogenetic) masking or confounding the spatial variability of otolith chemical signatures, on which the patch cohesiveness analysis is based (see *Data analysis* for the complete rationale of the statistical method). The mean age of *O. melanura* individuals sampled by light traps is ~11 days, thus we confidently excluded the first 3-4 days after hatching from the analysis, corresponding to the yolk sack phase of the species (Antolović *et al*. 2010). Spot size was set to 15 µm in order to fit it with the daily rings width. Each spot run consisted of 42 s acquisition: 10 s blank to correct for background, 2 s of pre-ablation to remove surface contamination (laser at 30% power), 10 s ablation and 20 s for washout.

Otoliths of post-settlers (11.3-25.3 mm individual TL range and 29.2 days mean age) were used for obtaining information on the number of potential natal origins and larval dispersal distances of *O. melanura*. Post-settler otoliths were analysed in 2 different regions: the core and the edge. The core elemental composition was investigated to obtain information about natal origins and was analysed through three vertical pits with a diameter of 30 µm (approximate size of the core) and 10 µm deep. The otolith edge was investigated to quantify recent elemental incorporation (i.e. material laid down just before capture) that was later tested for site discrimination (see *Data analysis*) and to account for within otolith variability (Di Franco *et al*. 2012b, Di Franco *et al*. 2014). Edge portion was analysed by three horizontal pits using the same laser spot dimensions as previously specified. Each spot run consisted of 62 s acquisition: 25 s blank to correct for background which was subtracted from each sample, 2 s of pre-ablation to remove surface contamination (laser at 30% power), 10 s ablation and 25 s for washout. Otoliths were placed in the ablation chamber in groups of 6, randomly selected from the 17 sampling sites, to prevent sample batch bias.

For both pre-settlers' and post-settlers' otoliths, instrumental precision was maintained by analysing solid glass standard material from the National Institute of Standards and Technology (NIST 610 and NIST 612) every 6 samples, carrying out a linear interpolation between the 2 consecutive sets of standards. Calcium was used as an internal standard to take into account variation in ablation and aerosol efficiency. All the 7 elements analysed (^{7}Li, ^{24}Mg, ^{45}Mn, ^{66}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb) were expressed as ratios relative to ^{44}Ca. Detection limits were calculated from the concentration of analyte yielding a signal equivalent to 3× the standard deviation of the blank signal for each of the elements (Table 5.1). Recoded values of Li, Mn, Zn and Pb were consistently below the detection limits and thus were excluded from the analysis.

Table 5.1. Estimates of precision, accuracy and limits of detection (LOD) for post settlers' otoliths (upper table) and pre settlers' otoliths (lower table). Values for %RSD (% relative standard deviation) and % accuracy are dimensionless.

Element ratio	NIST 610 %RSD	NIST 612 %RSD	NIST 610 %Accuracy	NIST 612 %Accuracy	LOD mmol/mol
Mg:Ca	9.8	11.1	84	92	0.03852
Sr:Ca	7.8	8.3	115	98	0.00148
Ba:Ca	7.1	9.4	91	89	0.00066

Element ratio	NIST 610 %RSD	NIST 612 %RSD	NIST 610 %Accuracy	NIST 612 %Accuracy	LOD mmol/mol
Mg:Ca	6.6	14.4	98	106	0.04568
Sr:Ca	7	9.3	98	93	0.01441
Ba:Ca	6.1	7	98	82	0.00076

Data analysis

Otolith elemental concentration data were converted to molar concentrations and log (x+1) transformed.

Larval patch cohesiveness was investigated analysing within patch (i.e. light-trap) variability of pre-settler otolith chemical profiles. In particular we compared the chemical composition of otoliths day by day, grouping together chemical data from chronologically homologous rings of different otoliths (i.e. rings corresponding to the same day of life for all the fish). Based on the proviso that the chemical composition of otoliths is similar for individuals of the same age and population that experienced the same environmental conditions, we hypothesised that for individuals caught in the same light-trap, the variability of the chemical composition would have increased from the first ring analysed (i.e. most recent ring, laid down immediately before the sampling) toward the last ring analysed (i.e. 6 days before sampling), in the case in which fish travelled different pathways (i.e. starting from different sites and then reaching the same light-trap). Otherwise, if pre-settlers travelled together to the site where we collected them (light-trap), a similar variability would be found between edge and the previous rings (days of larval life) of the otolith. In order to perform the analysis, otolith chemical data were normalised and grouped by ring analysed (i.e. day of life) from the last (1, the edge of the otolith) to the seventh last (7) and considering the different light-traps samples separately. Then, individual deviations from the centroids for each otolith ring, across individuals coming from the same light-trap, were calculated using PERMDISP. Centroid distances were finally analysed through a PERMANOVA in which 'light-trap' (LT) was treated as a random factor with 4 levels, 'specimen' (SP) was treated as random factor with 8-11 levels, nested within LT and 'otolith ring' (OR) was treated as a fixed factor with 7 levels, crossed to SP. This model, considering OR as a factor crossed to SP, was adopted as a solution for repeated measures (the repeated measure effect is removed by fitting it as a factor). For each light-trap, a means plot of distance from centroid per otolith ring was used to show the variation in fingerprints variability moving from day 1 to day 7.

The number of post-settler potential natal origins was investigated analysing otolith core elemental concentrations (as a proxy for identifying the existence of single or multiple areas of origin (Di Franco *et al*. 2012b) for a total of 230 individuals. No Mn:Ca spike, generally considered as an indicator of the core location (Brophy *et al*. 2004, Ruttenberg *et al*. 2005), was recorded in almost all otolith cores analysed. For this reason the centroid of the three core pits for each otolith was considered for subsequent analysis, as it has been done in other species where no Mn:Ca spike seems to be an ineffective “core localizer” (Papetti *et al*. 2013, Guidetti *et al*. 2013). The similarity profile permutation test (SIMPROF, 'clustsig' package, R software) procedure was carried out on the output of a cluster analysis (based on Ward’s minimum variance hierarchical approach with Euclidean distance) to determine which clusters were significantly different at the 5% level. Similarity percentage (SIMPER, 'vegan' package, R software) was used to assess which Elemental/Ca ratios mainly contributed to the differences among the significant clusters identified by the SIMPROF. The edge portion of post-settler otoliths (i.e. post-settlement portion laid down just before capture) was analysed to assess the ability of the method to discriminate spatially distinct chemical signatures and validate post-settler otolith core analysis (Di Franco *et al*. 2012b). Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences between the 17 sampling sites, both considering the multi-elemental dataset and considering each Elemental:Ca ratio separately. In the two cases, 'Site' (Si) was treated as a random factor (17 levels), ‘Otolith’ (Ot) was treated as a random factor nested in (Si) (11-15 levels). There were three replicate ablations for each otolith (total n=690). Canonical analysis of principal coordinates (CAP, Anderson and Willis, 2003) and jackknife cross validation (% of correct classification) were also performed on the edge portion of the elemental data of post-settlers to assess how accurately post-settlers were classified to sites where they were collected. A specific randomization test (White and Ruttenberg, 2007) was used to estimate the probability that reclassification success (% of correct classification) was better than random. For these analyses, for each specimen the centroids of the three replicate sample pits were calculated and used for CAP analysis.

Once different natal origins were identified (arbitrarily numbered from 1 to 7, see Results), we investigated post-settler replenishment pattern in the study area, testing for possible differences in zone and site replenishment for each identified natal source. With this aim we ran a univariate two-way PERMANOVA using natal origin number as a variable. 'Zone' (Zn) was treated as a random factor (3 levels); 'Site' (Si) was treated as a random factor (5 to 6 levels) nested in (Zn). Finally, a Mantel test (based on 10^4 permutations, 'ade4' package, R software) was performed to assess if closer sites were more likely to be replenished by the same natal origin. Thus, we measure the correlation between the abundance of post-settlers coming from the same natal origin and the geographic distance between pairs of sites. The distance matrix of abundances was created considering the 17 sites as observations and the major natal sources identified as variables. Thus, each site was associated to n values of abundance, corresponding to the number of post-settlers coming from each of the n major natal sources.

PERMANOVA and PERMDISP analyses were run using Primer 6 PERMANOVA+ software package. All the other analyses were run using R software (R core development team).

Results

For the analysis of pre-settlers' patch cohesiveness, a significant difference in multivariate chemical composition variability between otolith rings (i.e. days of life) was recorded (PERMANOVA, $p<0.01$), with an increasing variability moving from day 1 to day 7 (Fig. 5.2) for all the 4 light-trap samples (indicating with 'day 1' the sampling day and 'day 7' the 6th day before sampling). Pairwise comparisons between days indicated that statistically significant increments in chemical fingerprint variability were not simultaneous across light-traps samples (i.e. did not happen between the same pair of days across light-traps), resulting in a significant interaction 'light-trap' x 'otolith ring' ($p<0.05$): for pre-settlers from light-trap T4 the first significant increment was recorded between the 2nd and the 3rd ring, in T1 between the 4th and the 5th ring, while in T2 and T3 a significant increment was recorded between the 1st and the 2nd ring (indicating with '1st ring' the most recent ring formed in the otolith) (Fig. 5.2).

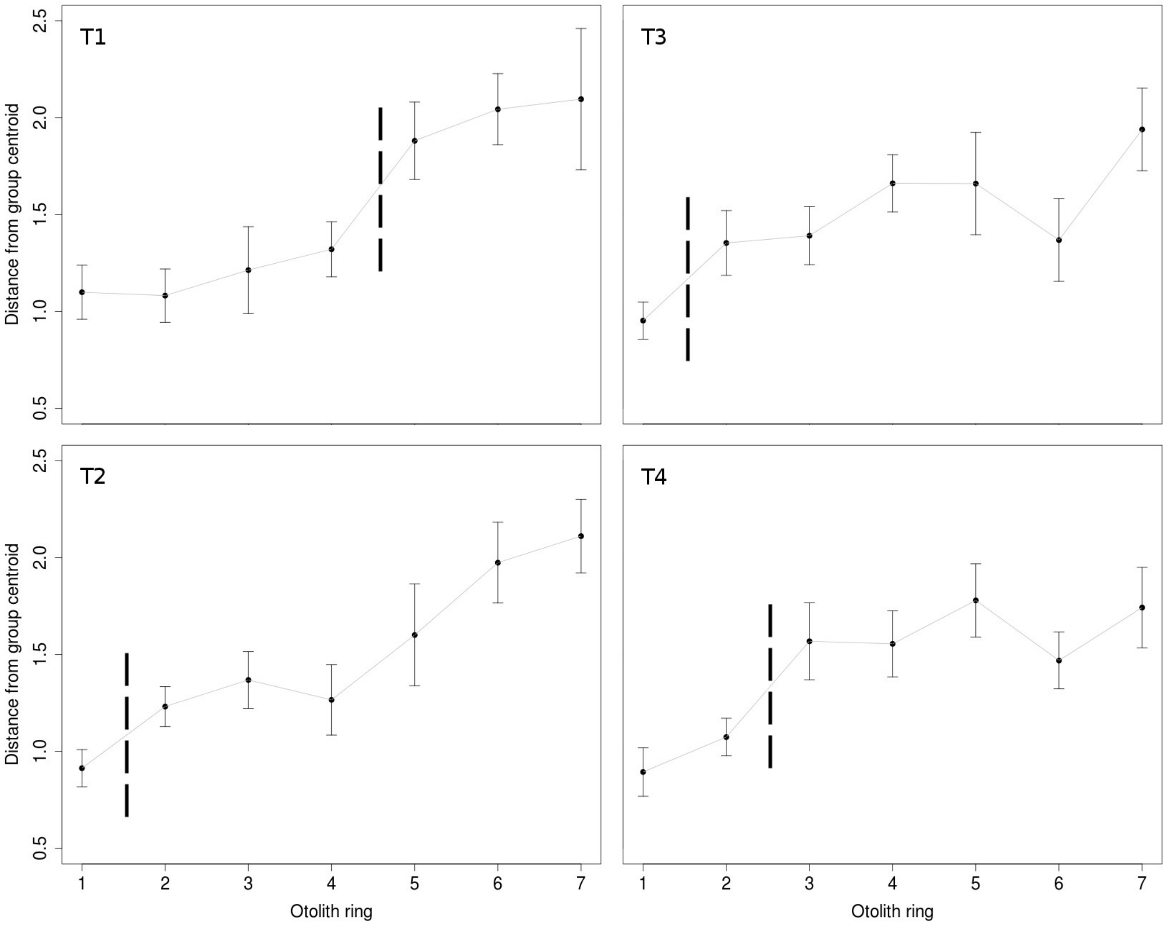

Figure 5.2. Plots of average distance (± standard error) from group centroid per each otolith ring (day of life) analysed, as calculated by PERMDISP. Each plot represent a light-trap sample: T1 (10 specimens), T2 (8 specimens), T3 (11 specimens) and T4 (11 specimens). Vertical dashed segments identify significant differences in chemical fingerprint variability between pairs of consecutive rings.

Regarding the number of natal origins, SIMPROF test segregated 7 statistically different clusters, i.e. core chemical 'fingerprints' potentially corresponding to seven different post-settler natal sources (Fig. 5.3). The five major natal sources (A, C, D, E and G) accounted together for 92.3% (212 individuals) of all the post-settlers with single contribution ranging from 12.2% to 32.3%. The remaining natal sources, B and F, consisted, respectively of 5 (2.1%) and 13 (5.6%) individuals.

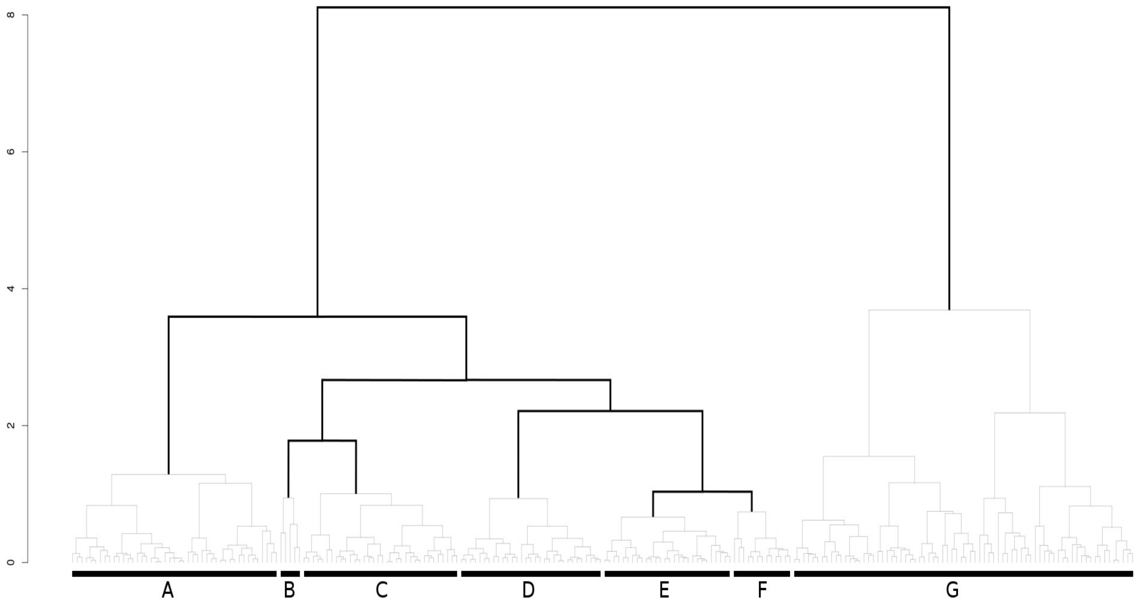

Figure 5.3. Cluster (based on Ward's approach ad Euclidean distances) of post-settlers otolith cores into groups considering multivariate (i.e. Mg:Ca, Sr:Ca and Ba:Ca) elemental concentrations. Black branches indicate statistically significant groups resulted from SIMPROF (arbitrarily named from left to right). Grey branches indicate non-significant differences.

SIMPER analysis indicated that Mg:Ca ratio contributed, on average, for ~50% of the total dissimilarity in pairwise comparisons among five major natal sources identified, while Ba:Ca and Sr:Ca contributed with similar proportion to the remaining 50% of dissimilarity. Cluster 'G' (that included 1/3 of all the post-settlers) was characterized by a twofold concentration of Mg:Ca compared to the remaining clusters. Cluster 'A' and 'D' showed lower values of Ba:Ca than C, E and G. Sr:Ca ratios were different in all 5 groups (Fig. 5.4).

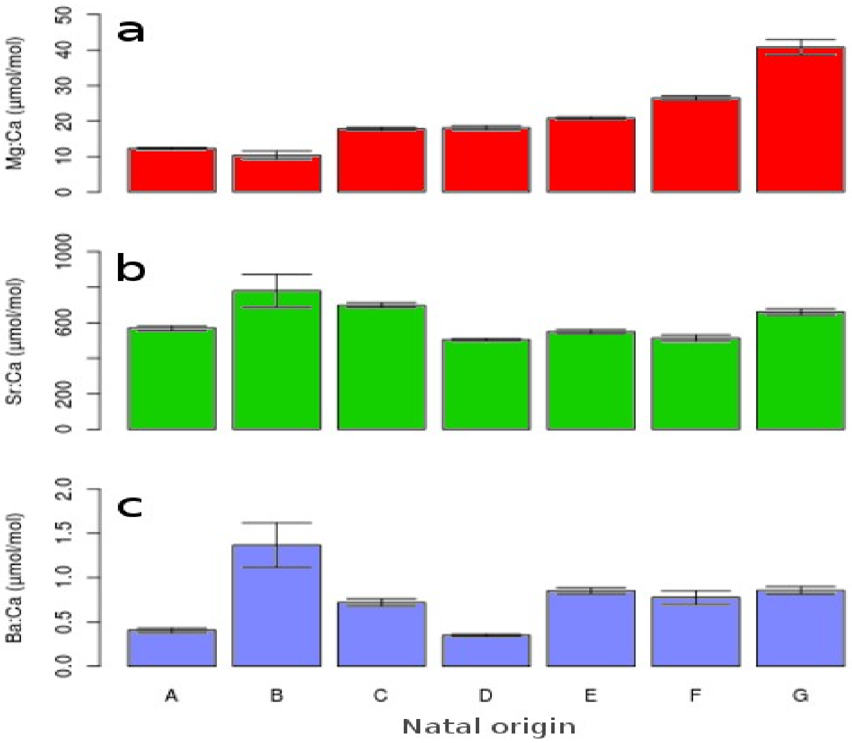

Figure 5.4. Average (± standard error) a) Mg:Ca, b) Sr:Ca and c) Ba:Ca ratios in the core region of the clusters identified by SIMPROF (see Figure 3).

The chemical composition of the juvenile portion (edge) of post-settlers was significantly different among the sampling sites in multi-elemental analysis (PERMANOVA, $p<0.01$). Considering each Elemental:Ca ratio separately, 2 of the 3 elements investigated (Mg and Ba) showed significant differences among sites (PERMANOVA, Mg:Ca $p<0.01$, Ba:Ca $p<0.001$), while the Sr:Ca ratio didn't differ significantly between sites (Fig. 5.5). Significant differences between otoliths were also found in both multi-elemental analysis and the three univariate analyses ($p<0.01$, for all tests), suggesting within site differences among individuals. A significant jackknife reclassification success was found (randomization test $p=0.002$) with 11.3% of post-settlers correctly classified to collection site in cross-validation of CAP analysis (i.e. 5.8% correct classification to one of 17 sites due to chance alone) (Fig. 5.6).

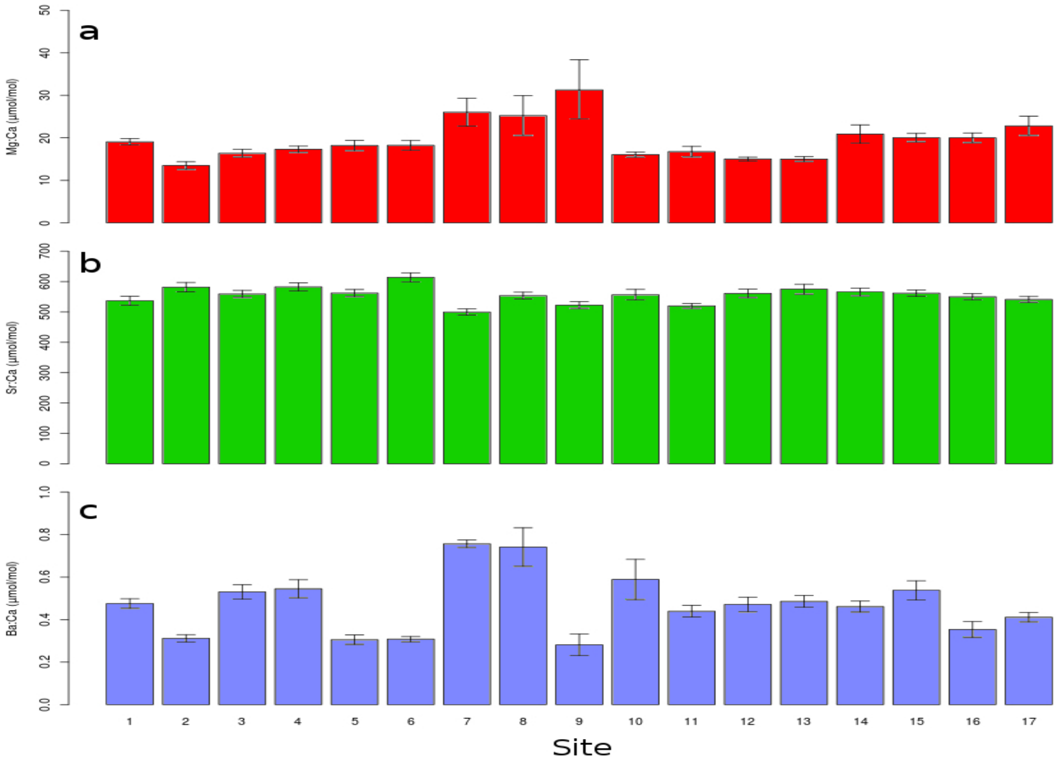

Figure 5.5. Average (± standard error) a) Mg:Ca, b) Sr:Ca and c) Ba:Ca ratios in the edge portion of post-settler otoliths per sampling site.

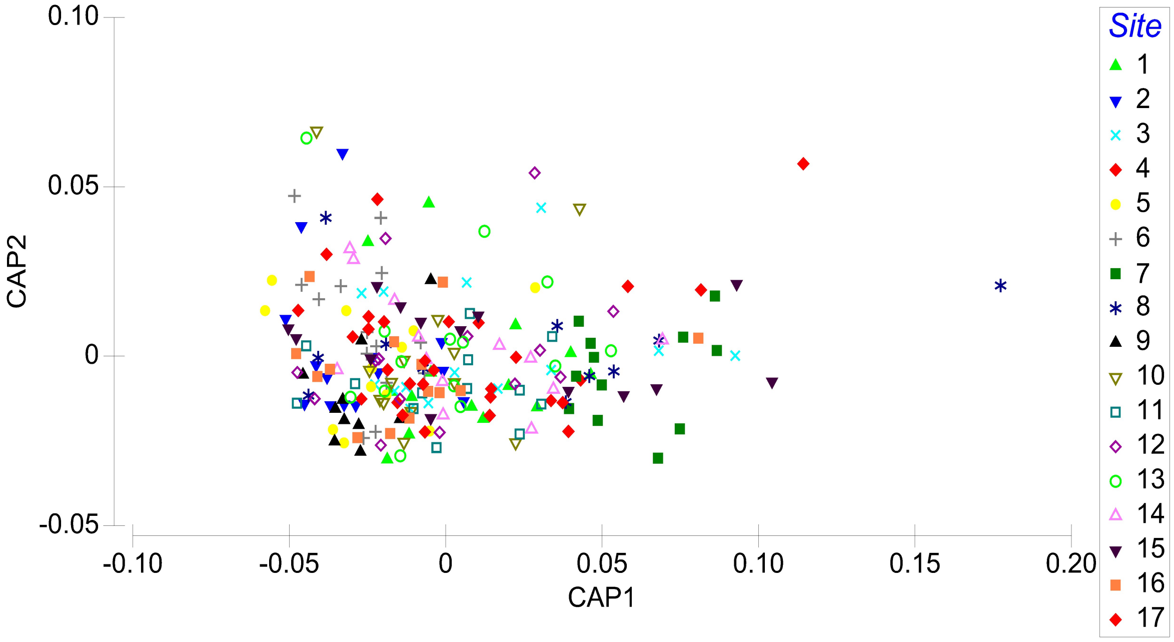

Figure 5.6. CAP analysis of multivariate elemental fingerprint from post-settler otolith edge portion. Each symbol represent a different sampling site (see legend on the right).

Post-settlement replenishment from the natal origins identified was found to be statistically different among the 3 zones (PERMANOVA $p<0.01$), with the southernmost zone (WMR) highly different from the northernmost zone (SAP) (pairwise comparison $p<0.01$) and weakly different from the central zone (CMR) (pairwise comparison $p<0.05$). No significant differences were found between SAP and CMR. No difference was detected at site scale. The 5 major natal sources replenished, with different proportions, almost all the sampling sites. Post-settlers from source 'G' were mainly found in the WMR zone, with similar, lower, abundances recorded in the other two zones. Source 'A' similarly replenished north (SAP) and central (CMR) sites. Source 'C' was mainly composed by individuals from SAP sites (north of the study area). Individuals from source 'E' were mainly sampled in CMR, while a comparable number of post-settlers coming from the source 'D' was found in each site (Fig. 5.1 and 5.7). Regarding the last two natal sources, individuals from 'F' were found in WMR, CMR and the southernmost site of SAP. Source 'B' replenished five sites (1, 2, 5, 13 and 14) with one individual per site. The abundance of post-settlers sharing the same natal origin and the geographic distance between sites were significantly positively correlated (Mantel test, $r=0.38$ and $p=0.0001$), indicating that geographically closer sites had, on average, higher abundances of post-settlers coming from the same natal origin, than distant sites. Focusing on the natal sources G, A and C (the three major natal sources clustered, representing more than 2/3 of all post-settlers), the two higher values of post-settler abundance, coming from each source, were recorded in pairs of adjacent and nearest sites (17-16 for source

'G', 7-8 for source 'A' and 3-4 for source 'C'), while lower values were recorded in all the other sites (Fig. 5.7).

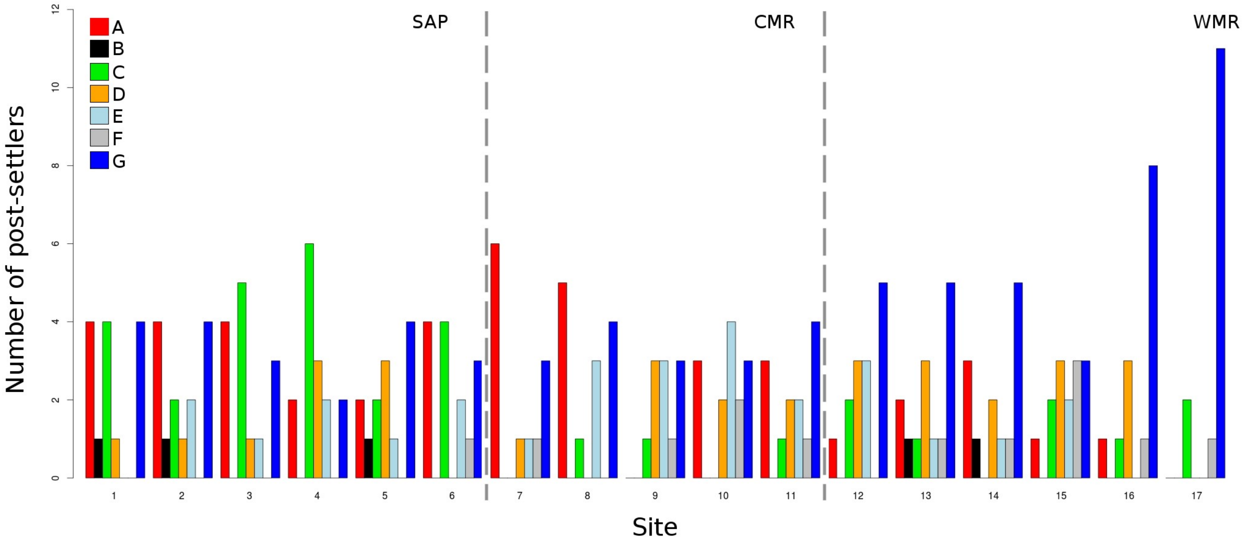

Figure 5.7. Total number of post-settlers, for each sampling site, coming from each natal source identified by SIMPROF and represented by a colour (see legend up left). Sampling sites are numbered progressively from the northernmost to the southernmost (see Fig. 5.1). Vertical dashed lines separate the three zones considered (SAP, CMR and WMR, see text for details).

Discussion

For most marine fish, the pelagic phase before settlement represents a critical period of dispersion shaping connectivity patterns between populations (Burgess *et al*. 2014, but see Di Franco *et al*. 2015). Knowledge on species dispersal characteristics (e.g. number of natal origins, dispersal distances) is crucial for understanding fish population dynamics and connectivity. This knowledge can ultimately aid the design of more effective marine protection strategies (Pujolar *et al*. 2013, Green *et al*. 2014, Di Franco *et al*. 2015). In this study we analysed otolith chemical composition of early life stages of *O. melanura* in order to provide information on the dispersal characteristics of its propagule pelagic phase in the South-Western Mediterranean Sea.

The analysis of larval patch cohesiveness suggests that groups of larvae can merge in the pelagic environment after having travelled separately for some days. Recent work demonstrated a similar larval patch formation in a small triplefin fish from New Zealand reefs, using otolith chemical analysis (Shima and Swearer 2009, Shima *et al*. 2015). However, cases of protracted larval patch cohesiveness were also documented, with larvae experiencing the same dispersal pathway during the entire pelagic phase (Ben-Tzvi *et al*. 2012, Bernardi *et al*. 2012). In our study, in two light-traps, increased otolith chemical variability was recorded between the sampling day and the day immediately before, suggesting that, during the sampling night two or more discrete groups of larvae were attracted toward the same trap, after travelling different dispersal pathways. In the other two light-traps, a stable chemical composition between sampling day and the previous ones was observed, suggesting that the aggregation of distinct patches can take place at different times of the larval phase. It is difficult to establish if this aggregation tendency is a result of larval behaviour or particular oceanic features (e.g. gyres) that can mix together patches previously separated. Kiparissis *et al* (2008) found evidences of aggregation behaviour in post-settlement individual of *O. melanura*, which differed from other Mediterranean sparid fishes (Harmelin-Vivien *et al*. 1995). In particular, post-settlers arrive in small shoals and then actively aggregate in larger shoals with increasing fish size after various aggregation steps (Kiparissis *et al*.

2008). From this perspective, the same aggregation behaviour could characterize the larval phase, determining a mix of distinct larval patches, potentially originating from different natal sources, during the pelagic phase. This patch mixing tendency could be an adaptive strategy that would guarantee a higher genetic diversity, and thus higher survival probability, when faced with environmental uncertainty of the settlement habitat and its associated high mortality rates (Nanninga and Berumen 2014, Shima *et al*. 2015). In the light of these considerations, it is evident that diversity in post-settler natal sources recorded in each sampling site could be a consequence of multiple aggregation steps that can occur both during the propagule dispersal phase and soon after the settlement process. These would contribute together to demographic heterogeneity, fundamental in driving meta-population dynamics and the persistence of species (Kritzer and Sale 2004).

The analysis of post-settler core elemental composition indicate the presence of multiple sources of propagules, replenishing several sampling sites with different proportions along the south-eastern coast of Spain. Recent work carried out in the Eastern Atlantic and in the Mediterranean Sea produced similar results with high diversity of natal sources in early post-settler individuals sampled in coastal sites (Fontes *et al*. 2009, Di Franco *et al*. 2012b, Di Franco *et al*. 2015). The significant spatial variability in the chemical composition of the juvenile portion (i.e. otolith edge) of post-settler otoliths was corroborated by the reclassification success (significantly higher than random) of post-settlers to their sampling sites. These outcomes highlight the ability of the method to discriminate spatially distinct chemical signatures, at the analysed spatio-temporal scale, allowing us to reasonably draw inferences on the core portion. The segregation of different core chemical signatures is likely to be related to spatial differences in chemical-physical characteristics of the water masses where spawning took place. Different chemical fingerprints in otolith cores could also be associated to a temporal variability in the characteristics of water masses associated to temporally distinct spawning events. From this perspective, otolith micro-structural analysis carried out on the same specimens analysed in this study, showed that in 2013 the study area was replenished by simultaneous spawning events that took place in a temporal window of 2 weeks (see Chapter IV), so we can confidently assume that the different chemical fingerprints recorded are unlikely to be a consequence of a temporal variation.

The methodology used does not allow to geographically locate the natal origins identified, impeding knowledge of the exact range and frequency distribution (i.e. kernel) of larval dispersal distances. However, it allows estimation of the spatial scale over which dispersal can take place in the area. All the sites, located along ~180 km of coast, were supplied by multiple natal sources. This information allows us to infer that the most conservative value of maximum dispersal distance is ~90 km (i.e. half of the study area). In fact, supposing the case in which two of the natal sources identified were adjacent and in the centre of the study area, propagules would have travelled at least 90 km. In all the other cases, the dispersal distance would be greater. This estimate of propagule dispersal distance is in accordance with the results of other studies conducted in the Mediterranean Sea on other sparid species, using otolith natural tags (Di Franco *et al*. 2012b, Di Franco *et al*. 2015) and, in general, within the range of propagule dispersal suggested for other temperate fish species (Anadón *et al*. 2013). Additional information coming from complementary approaches, for example biophysical larval dispersal models or larval behaviour studies, are needed to corroborate the estimate of dispersal distance found for *O. melanura* in the study area. From this perspective, recent work conducted in the North-Western Mediterranean Sea, found that larval stages of *O. melanura* can have surprisingly high swimming capabilities, with critical velocities higher than 13 cm s^{-1} (that is the average current speed recorded for the same sector of the Mediterranean Sea, Faillettaz 2015). Thus, although the pelagic larval duration (PLD) of the saddled sea bream is relatively short, compared to other Mediterranean coastal fishes (Macpherson and Raventos 2006, Di Franco *et al*. 2013), the swimming capacity of the species could be a possible explanation of the rapid propagule dispersal from the spawning grounds to their settlement habitats.

Even though it is not possible to locate the natal sources, the results suggest that the pattern of larval supply to the sampling sites has a certain spatial dependence. Post-settlers from the same natal origin were more frequently found in closer sites, with the highest abundances of post-settlers coming from the same origin recorded in pairs of adjacent sites and lower values in all the other sites of the study area. This output was consistent among almost all the major natal origins identified. Moreover, although the major natal origins supply almost all the sampling sites, significant differences in post-settlers replenishment was recorded at the scale of zone suggesting that post-settler demographic composition can change at the scale of 50-100 km. A similar result was found by Fontes *et al*. (2009) who analysed otolith core composition of the temperate wrasse *Coris julis* in the Azores archipelago. The spatial difference in natal source composition between the northernmost (SAP) and the southernmost (WMR) zones of the study is also in accordance with a significant difference in PLD of *O. melanura* between the same zones considering the specimens of this study, resulted in a mean value of PLD statistically lower in SAP zone than in WMR zone (see Chapter IV). This concordant result could indicate that the geographic origin of propagules and early life history traits (e.g. PLD) could have a common source of variability or be non-independent. Patterns of post-settlers distribution along the coast are likely to be a consequence of pre- and/or post-settlement processes as well as both biological (e.g. behaviour, inter- and intra-specific competition for food and space, mortality) and physical (i.e. current dynamics during the larval dispersal phase). From this perspective, the high difference in natal origin composition found between the southernmost zone (WMR) and the northernmost zone (SAP) and the weak, but significant, difference found between the WMR zone and the central zone (CMR) could be a consequence of the mesoscale oceanographic pattern that characterizes the region. Rossi *et al* (2014) pointed out that the separation between the Alboran Sea and the rest of the Mediterranean Sea, generally thought to be along the Almeria-Oran front, could actually have its Spanish coastal extreme around Cabo de Palos, that in our study area is located in the CMR zone (Fig. 5.1). The presence of this oceanographic boundary, already suggested to be a biogeographical barrier in the Western

Mediterranean Sea (Pérez-Ruzafa 2010), could reduce the dispersal of propagules from the WMR zone toward the northernmost zones and vice versa. However, it is very hard to draw conclusions on the forces that determined the spatially dependent outcomes recorded in this study, as the necessary information is unavailable to elucidate them, especially considering the lack of information on fish spawning grounds for the studied species. From this perspective, further investigations are to be conducted using other methodologies that would permit direct tracking of propagules movements from source to settlement site. These methods (e.g. otolith artificial tagging or genetic parentage analysis), although undoubtedly powerful, have been rarely or never applied so far in the Mediterranean context given the lack of spawning ground location data and the huge sampling effort needed for obtaining representative results (Calò *et al*. 2013, see Chapter II).

To conclude, the present study provided insights on the propagule dispersal traits of a temperate coastal fish. This is the first study aimed to estimate dispersal distances in the South-Western Mediterranean Sea and, to our knowledge, the first to provide information on larval patch dispersal cohesiveness during the pelagic phase of a Mediterranean coastal fish. Information on propagule dispersal patterns is vital to support planning of MPAs and especially for the implementation of effective MPA networks (Planes *et al*. 2009, Gaines *et al*. 2010). From this perspective, our results are particularly relevant considering the environmental values of the region and the strong effort that is being accomplished to build an effective network of MPAs in the sector of Mediterranean Sea considered. Although the study of one species is insufficient to make generalisations on the spatial arrangement for effective MPAs, it does provide a valid contribution within this field. Together with information on other species and/or using different approaches, this knowledge can provide the necessary background for correct marine spatial conservation actions.

Chapter VI

USE OF LAGRANGIAN SIMULATIONS TO HINDCAST THE GEOGRAPHICAL POSITION OF PROPAGULE RELEASE ZONES IN A MEDITERRANEAN COASTAL FISH

USE OF LAGRANGIAN SIMULATIONS TO HINDCAST THE GEOGRAPHICAL POSITION OF PROPAGULE RELEASE ZONES IN A MEDITERRANEAN COASTAL FISH

Abstract

The study of organism dispersal is fundamental for elucidating patterns of connectivity between populations, thus crucial for the design of effective protection and management strategies. This is especially challenging in the case of coastal fish, for which information on egg release zones (i.e. spawning grounds) is often lacking. Here we assessed the putative position of egg release zones of the saddled sea bream (*Oblada melanura)* along the south-eastern coast of Spain in 2013*,* using the information on fish natal origins obtained from otolith chemical analysis. To this aim, we hindcast propagule (egg and larva) dispersal using Lagrangian simulations using two approaches: 1) a back-tracking model and 2) the comparison of settler distribution between simulation output and the homologous distribution resulting from otolith chemical analysis. Simulations were also used to assess which factors mainly contributed to dispersal distances. In back-tracking simulations, we found that a small amount of propagules was moved back toward zones that can potentially host reproductive events of the considered species. From the second approach a correlation between simulation outputs and field results (otolith analysis) was found, suggesting that the oceanographic characteristics of the study area could have determined the pattern of settler distribution recorded with otolith analysis. Dispersal distance was significantly affected by the geographical position and the depth range of propagule release zones, while no effect was ascribed to dispersal duration in the range of tested values. The method used was the first attempt to assess the geographical position of propagule release zones in the Mediterranean Sea, and can represent a valuable approach for elucidating dispersal patterns in other coastal species.

Keywords: Lagrangian simulations; propagule release zone; dispersal distance; fish; Mediterranean sea

Introduction

The study of causes and consequences of organism dispersal is crucial from both the ecological and evolutionary perspectives (Burgess *et al.* 2015). It provides vital information on demographic processes, species' responses to environmental variability and anthropogenic stresses and on gene flow among populations, which, in turn, affect meta-population dynamics and species local adaptation (Burgess *et al*. 2015). In the case of fish, dispersal plays a major role in determining the spatial scale over which populations interact genetically and ecologically (i.e. connectivity) and how should be managed (Grüss *et al.* 2011b, Green *et al*. 2014). In spite of its great importance, the accurate quantification of dispersal is still a challenging issue. Direct measures of dispersal are made hard by the difficulty to track individuals throughout their life cycle, especially during early developmental stages (Barbee and Swearer 2007, Cowen 2007, Calò *et al*. 2013, see Chapter II). Most marine coastal fish species have a complex life cycle composed by a pelagic propagule (egg and/or larva) phase, that ends with the settlement in benthic habitats, followed by a demersal juvenile/adult phase (Leis *et al.* 2011). Post-settlement stages are considered relatively site attached, so it is the propagule phase that mostly contributes to species dispersal capacity (Leis 2015, but see Di Franco *et al*. 2015). In this context, a major issue for fish ecologists is represented by the lack of knowledge on the locations where eggs are released (i.e. spawning grounds) (Thorrold *et al.* 2007). This, together with the minuscule dimension of eggs and larvae, practically impedes to track propagules from their origins to their destination (i.e. settlement sites) and obtain an exhaustive measure of connectivity during the pelagic phase (Thorrold *et al.* 2001). The location of egg release zones is only possible through direct observations of spawning events or using acoustic methods, or indirectly through traditional ecological knowledge (e.g. fisherman knowledge about zones of fish massive catches) (Boomhower *et al.* 2007, Heyman *et al*. 2004).

In the last decade, different modelling tools based on outputs of water circulation models were developed for simulating particle dispersal, assuming that propagules are advected and diffused similarly to water particles (Cowen 2007). Lagrangian-based, spatially-explicit individual-based models (IBMs) have been recognized as powerful tools to track pelagic particles from potential release zones to settlement habitats (Werner *et al.* 2007, Watson *et al*. 2010). IBMs have been used both to hindcast and forecast patterns of propagule transport and address challenging ecological questions such as: the assessment of the potential impact of climate change on propagule dispersal (Lett *et al.* Irisson, 2010, Andrello *et al.* 2015) or to help in the design of MPA networks and in their further management (Andrello *et al.* 2015). IBMs have been also used to understand how dispersal and connectivity can be influenced by spatial and temporal variability of different physical and biological factors (Andrello *et al*. 2013, Ospina-Álvarez *et al*. 2013, Ospina-Alvarez *et al.* 2015), providing crucial information on the factors that drive fish settlement variability and helping the development of effective fishery management strategies (Ospina-Alvarez *et al*. 2015). Model simulations were also used to corroborate results or test hypotheses on propagule dispersal based on complementary methodologies such as genetic analysis or chemical analysis of calcified structures (Calò *et al*. 2013, see

Chapter II).

In 2013, otolith of juveniles individuals of the saddled sea bream (*Oblada melanura*) were chemically analysed in order to identify the number of potential natal origins sources of propagules along the Mediterranean south-eastern coast of Spain (Murcia region). These propagule release zones were found to supply a series of coastal sites spread along ~180 km of coastline (Calò *et al*. 2016, see Chapter V). In this context, dispersal simulations could be used to hindcast the geographical position of the propagules' source identified, determining which source spatial arrangement along the coast was more likely to produce the distribution of settlers recorded with otolith analysis. This would represent an important step for the conservation of the species, allowing to gather information on source–sink dynamics and helping to understand of the processes that determine settler distribution along coastal habitats.

In the present study we implemented a biophysical IBM to investigate the putative geographical position of propagule release zones of *Oblada melanura* identified with otolith chemical analysis, along the south-eastern coast of Spain in 2013. Dispersal simulations were also used to analyse the pattern of settler distribution along the coast and assess the factors that mainly influenced propagule dispersal distances. We used species-specific information on early life history traits (ELTs), i.e. spawning dates, pelagic larval duration and settlement dates, of the selected species. This information was gathered in the same spatial and temporal context of the oceanographic data implemented for the simulations. Apart from their ecological importance in the geographic context considered, the results of the study can provide useful insights for the development of new approaches to investigate the position of fish spawning areas.

Material and methods

Hydrodynamic model

The Western Mediterranean OPerational forecasting system (WMOP; Juza *et al*. 2016) is based on a regional ocean configuration of the ROMS model implemented over the Western Mediterranean Sea (www.socib.es). The ROMS is a free-surface split-explicit model, solving the hydrostatic primitive equations using terrain-following curvilinear vertical coordinates, employing the Arakawa-C horizontal and vertical grid staggering (Shchepetkin and McWilliams 2005). The WMOP has a resolution from 1.8 to 2.2 km and 32 sigma-levels, with a spatial coverage from Gibraltar strait to Sardinia Channel (6ºW, 9ºE, 35ºN, 44.5ºN). The model is forced by high-resolution winds (5 km, 3 hours) from the Spanish Meteorological Agency. The simulation used in this study is a sample over the period 2013-2014 of a 6.5-year long simulation of WMOP starting in September 2008. Initial and boundary conditions were provided by the CMEMS MED-MFC model.

Larval dispersal model

Daily outputs of three-dimensional velocities fields simulated by WMOP were used to simulate *Oblada melanura* larval dispersal using the software Ichthyop 3.2 (C Lett *et al*. 2008). The time step of iteration was set to 100 s in order to keep it lower than the ratio of cell size to maximum current velocity, so that propagules do not cross more than one cell boundary in a single time step (Courant–Friedrichs–Lewy condition). Given that no information on egg buoyancy nor on larvae active swimming and vertical migration are available for *O. melanura*, in all the simulations a neutral buoyancy was assigned to eggs and larvae were subjected only to current transport (i.e. passive dispersal) without considering any active swimming.

Putative natal origins and settler distribution of Oblada melanura

To locate the major natal origins of *O. melaura* identified along the study area in 2013, two different approaches were used: 1) a backtracking propagule dispersal simulation and 2) the comparison between settler distributions obtained from model simulations and the pattern recorded from post-settler otolith chemistry by (Calò *et al*. 2016, see Chapter V).

Running the larval dispersal model in backtracking mode allowed to explore those areas where it is hydrodynamically possible for propagules to be transported toward the sampled coastal sites. The dispersal duration used for the backtracking simulation was 13.5 days, i.e. the sum of the mean days after hatching (DAH=11.5 days) (Chapter IV) plus 2 days of egg phase duration. The choice to use the DAH instead of the mean pelagic larval duration (PLD=14 days) was made in order to consider a dispersing period that does not include the last days of larval life (i.e. the competency phase) in which behavioural and movement capabilities are thought to be more developed (Leis 2007). Nine release zones where distributed along the coast, approximately in correspondence to the sites where settlers were sampled in 2013 for otolith chemical analysis (Figs. 6.1a and 6.1b). Particles were released following the settlement dates of *O. melanura* recorded in 2013, but subtracting 2.5 days (i.e. the mean difference in days between DAH and PLD). Eight simulations were run covering all dates moving backward in time from 14th July to 7th July. In each simulation 1,000 particles were released from each zone, a number which was initially tested as being large enough to cover all possible origins of the particles.

For the second approach, the recorded settler distribution along the coast in 2013 was tested against a series of settler distributions resulting from different Ichthyop runs. In each model run, release zones were randomly selected from a set of potential release zones distributed along the coast. Result from otolith chemical analysis showed the presence of 5 major natal origins (here considered as a proxy of egg release zones) that supplied 17 coastal sites in 2013. In order to properly compare these results with Ichthyop simulation outputs, settler distribution data from the 17 sites were pooled, by couple of neighbouring sites, in 9 locations (apart from site 7, Figs. 6.1a and 6.1b). This choice was made because, due to model requirements, it was not possible to consider all the 17 sites as release/settlement zones for propagule simulations. In Ichthyop, 13 potential release zones were created: 9 were located in correspondence to the 9 'pooled locations' of the otolith study (named from L1 to L9, Fig. 6.1b), 2 zones were located outside the study area (O1 and O2) and 2 other zones were positioned inside the study area (M1 and M2). These last 4 zones were created for better covering the whole domain. The zones L1-L9 (Fig. 6.1b) were used as settlement zones (the same 9 zones being used in the backtracking experiment). All the release and settlement zones had approximately the same surface. Fifty Ichthyop simulations were run, each with 5 release zones randomly chosen from the set of 13 potential release zones. The dispersal duration used for running the model was the mean DAH of *O. melanura* plus 2 days of egg phase duration, for a total of 13.5 days. The spawning depth range considered was 0-20m in all simulations, being the depth range in which *O. melanura* larvae are more commonly found (Sabatés *et al.* 2007). The spawning dates recorded for *O. melanura* in 2013, from the 21thJune to the 2nd July, were used as release dates. These dates accounted together for 95% of all the spawning dates recorded in 2013. Two hundred particles were released from each zone and per each date, for a total of 12,000 particles per simulation (200 particles * 5 zones * 12 dates). In Ichthyop the 'stop when recruited' option was turned on, i.e. particles were assumed to settle and stopped moving when passing over a settlement zone, and considering a minimum DAH for settling of 10 days, being this the minimum value of DAH recorded for the species (Chapter IV). After each model run, the distribution of propagules that settled in the 9 settlement zones was used to generate a data frame with 5 variables (release zones) and 9 replicates (settlement zones). Each value of the data frame represented the ratio of propagules settled in one of the 9 settlement zones, released from each of the 5 random release zones, divided by the total number of settling propagules from each release zone. A Mantel's test (based on 10^6 permutations, 'ade4' package, R software) was used to investigate which model-generated data frames were significantly correlated to the homologous data frame obtained from otolith analysis results (built with the 5 major natal origins and the 9 'pooled locations'). Before performing Mantel's tests, a distance matrix (based on Euclidean distance) was created from each data frame. Finally patterns of settler distribution generated from simulations that produced statistically significant Mantel's tests were analysed and compared to the pattern recorded from otolith chemical analysis. For model-generated settler distributions, the local retention, defined as the fraction of propagules released from a zone that settled back to the same zone, was also assessed.

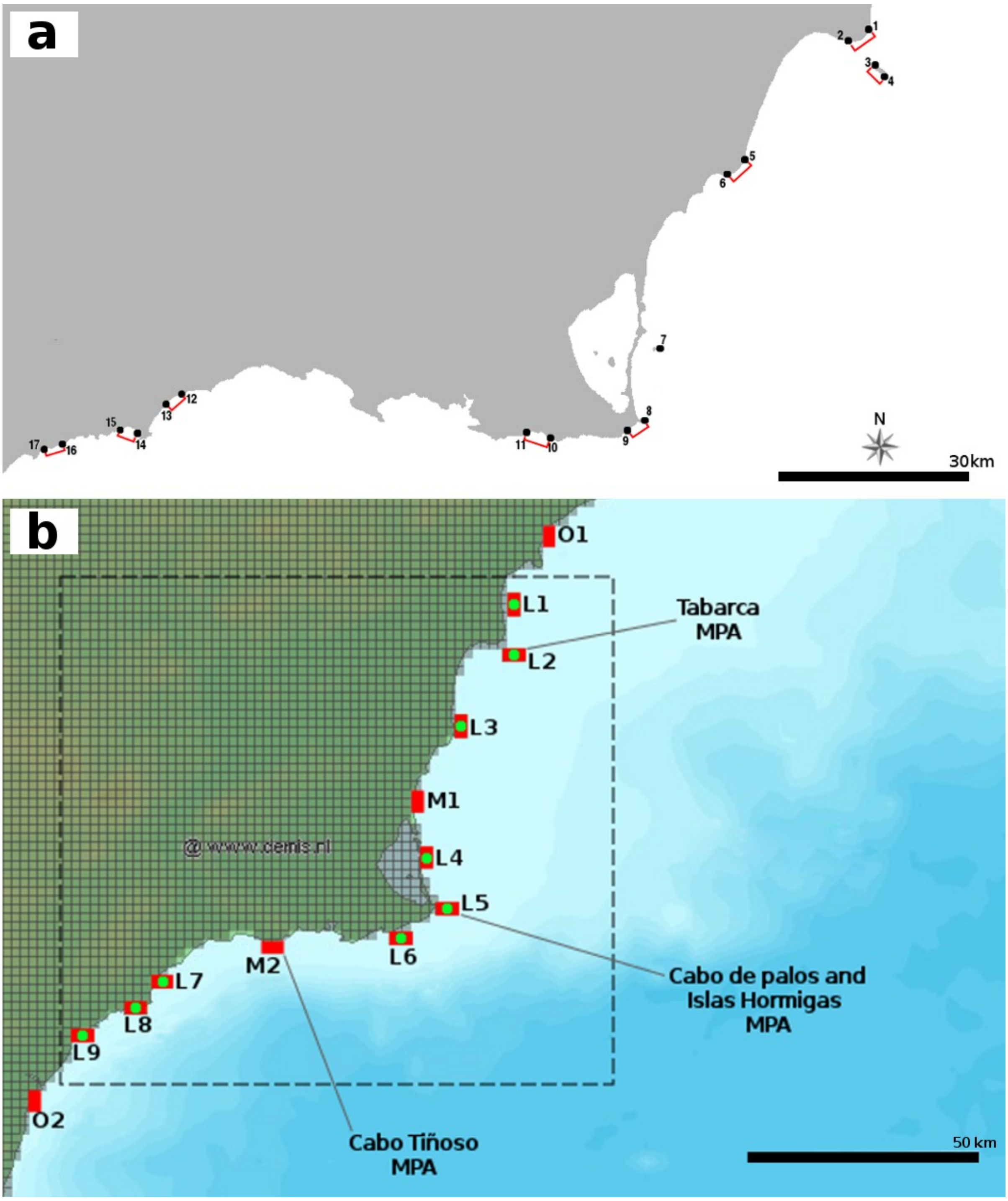

Figure 6.1. Study area: a) study area considered in the work on otolith chemical analysis (Calò *et al*. 2016, see Chapter V): black dots represent the 17 sites considered, red segments link the pairs of sites pooled for creating the 'pooled locations'; b) modelling area considered in Ichthyop: red rectangles represent the 13 potential release zones, green dots mark the 9 settlement zones, the dashed rectangles encloses the study area in (a).

Dispersal distance of Oblada melanura

To investigate the factors contributing to propagule dispersal of *O. melanura* along the coast of the Murcia region, the distance from the release point to the final point at the end of larval transport was measured for each simulated larva as the great-circle distance (i.e. the shortest distance between two points on a sphere). Three release zones were selected from the set of 13 release zones previously considered: L1 , L5 and M2 respectively in the north, centre and south of the study area. These zones host three established/planned MPAs of the region, respectively from North to South: Tabarca MPA (established in 1986), Cabo de Palos MPA (established in 1995) and Cabo Tiñoso MPA (planned to be declared in 2016). Given that no information on the exact spawning depth range of *O. melanura* is available, we considered 4 different release depth ranges: 0-5 m, 5-10m, 10-15m and 15-20m. Two different propagule dispersal durations were considered as resulted from otolith micro-structural analysis: the mean DAH (11.5 days) and the mean PLD (14 days). An Ichthyop simulation was run for each combination of depth ranges (4) and dispersal durations (2), resulting in a total of 8 runs. In each run 30 particles were released in each of the 3 release zones and following the spawning period recorded in *O. melanura* in 2013, i.e. from the 21thJune to the 2nd July for a total of 12 releasing dates. A high consistency between simulation runs repeated under the same software configuration and parameter values was observed during preliminary analyses, indicating that the number of released particles was sufficient to provide robust simulated patterns. Model outputs were merged together creating a data frame containing 720 dispersal distance values (4 depth ranges * 2 dispersal durations * 3 zones * 30 particles). To test for potential differences in dispersal distances related to spawning zone position, depth and propagule dispersal duration, analysis of variance (ANOVA) was performed, considering the factors 'Release zone' (Z) (random, with 3 levels), 'Depth range' (D) (fixed, with 4 levels crossed to Z), and 'Dispersal duration time' (T) (fixed, with 2 levels, PLD *vs*. DAH, crossed to Z and D). There were 30 replicate distance values (one for each simulated propagule) per each combination of levels of the three factors considered. Before performing ANOVA, data were tested for homogeneity of variance using Chocran's test, finding no evidence of heterogeneity of variance in all cases ($p>0.05$). ANOVA was run using the package in R software (R Development Core Team, 2013).

Results

Putative natal origins and settler distribution of Oblada melanura

Backward simulations showed that most particles were transported offshore, northward or toward the centre of the Alboran Sea (Fig. 6.2). Nevertheless, in two simulations, corresponding to the release dates 14th and 13th of July, an inshore accumulation of propagules was recorded along a stripe between release zones 2 and 4, in the north of the study area (Figs. 6.2a and 6.2b). In both cases these stripes of particles originated from release zone 3. In two other simulations (release dates 10th and 9th of July, Figs. 6.2e and 6.2f), an inshore accumulation of propagules was observed in the north of the study area in the proximity of release zone 2 (corresponding to Tabarca MPA), also originated from release zone 3.

Concerning forward simulations from randomly selected sets of release zones, the 5 random release zones selected for each of 50 model runs and the associated Mantel's tests are reported in Table 6.1. Among all Mantel's tests between the settler distribution pattern based on otolith chemical analysis and the Ichthyop-generated settler distribution patterns, two tests (Ichthyop runs #3 and #28) resulted statistically significant ($p<0.05$), indicating a correlation between field data and model simulation outputs, while other two tests (Ichthyop runs #4 and #26) were marginally significant ($p<0.1$) (Table 6.1). Settler distribution in the 9 'pooled locations' from the 5 major natal origins resulted from the study on otolith chemical analysis are shown in Fig. 6.3a. In the 4 simulations for which propagule distribution was correlated to the homologous based on otolith chemical analysis, release zone L2 was always present, release zones L5, M2, L9 and O2 were present 3 times while M1, L4, L6, L7 and L8 were never present (Fig. 6.3). Considering these 4 simulations, the mean percentage of local retention was 32% (average from release zones L1 to L9), with the highest value (52%) recorded in L9 and the minimum (0%) recorded in L5. Propagules released from L2 supplied almost all the settlement zones considered. The fraction of local retention from L2 was the highest fraction recorded among all settlement zones, while the others zones were supplied with ever-decreasing fractions of settling propagules with increasing distance from L2. The same pattern resulted for L3 and L9 (Fig. 6.3), with the release zone hosting the highest value of settling propagules and lower values with increasing distance from the source. Settled propagule distribution was clearly different between the northern and the southern sectors of the study area, with release zones in the north only supplying the northern and the central settlement zones while the opposite was observed for propagules released from the southern zones (Fig. 6.3).

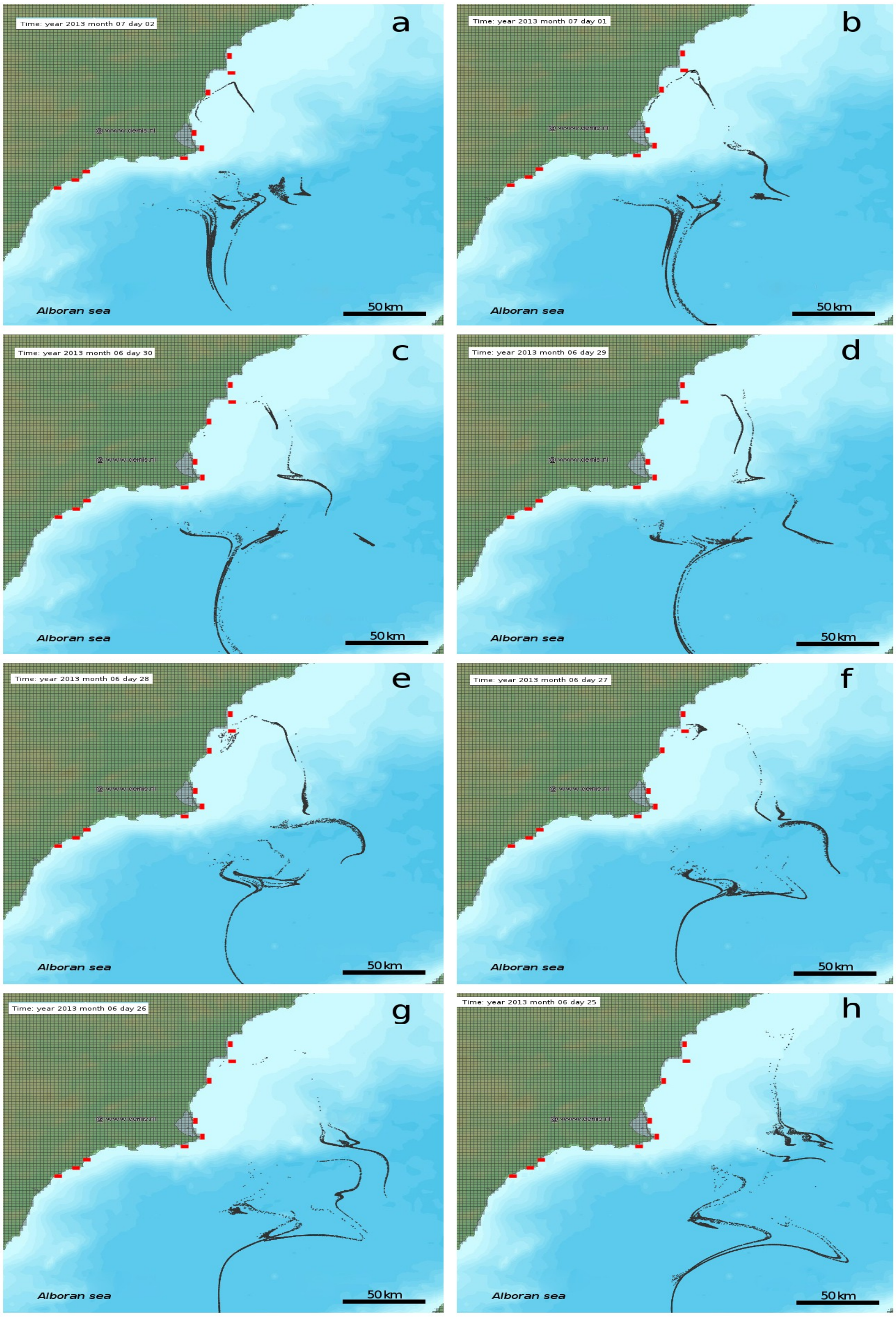

Figure 6.2. Outputs from backtracking simulations. Each image represents the final step of each of the 8 simulations run backward with a different release date: from 14th of July (a) to 7th of July (h). Black dots represent propagules. Red rectangles represent release zones.

Table 6.1. Results from Mantel's correlations between the settler distribution obtained from otolith chemical analysis and from Ichthyop simulations. Rows highlighted in red indicate significant Mantel's correlation. In grey, tests that showed tendency to statistical significance.

Ichthyop run	**Release zones**					**Mantel's test**	**pvalue**
	1	**2**	**3**	**4**	**5**		
1	O1	L2	M1	M2	O2	0.3436	0.1191
2	L3	M1	L6	L9	O2	0.0721	0.4377
3	L1	L2	L5	L9	O2	0.496	0.0381
4	O1	L2	L5	M2	O2	0.4435	0.056
5	L1	M1	L6	L9	O2	0.0932	0.418
6	L2	M1	L5	L6	L9	-0.0785	0.5457
7	O1	L4	M2	L8	O2	0.2379	0.2013
8	L1	L5	L6	M2	L7	-0.1127	0.6623
9	L1	L3	M1	L4	L8	-0.3411	0.9044
10	O1	L1	M1	L4	L5	-0.2326	0.7645
11	O1	L1	L3	L6	M2	-0.081	0.5805
12	O1	L2	L3	L4	L7	-0.1017	0.6317
13	O1	L3	L4	L5	M2	0.0764	0.3799
14	L1	L3	M1	L5	L6	-0.2409	0.8184
15	L1	L4	L5	L9	O2	0.2395	0.1954
16	O1	L1	L4	L7	L8	-0.0748	0.6087
17	L1	L2	M1	L5	L9	-0.0378	0.4597
18	L2	L3	M2	L7	O2	0.3344	0.1346
19	O1	L1	L3	M1	L7	-0.2599	0.7963
20	O1	L2	L6	L7	L8	0.0212	0.4575
21	O1	L2	M1	L4	M2	-0.0833	0.5823
22	L1	L3	L4	L7	L9	-0.1178	0.6695
23	O1	M1	L7	L9	O2	0.0043	0.4801
24	L1	L2	L3	L4	L9	0.116	0.3171
25	L3	L4	L5	M2	L8	-0.0635	0.5914
26	L2	L5	M2	L9	O2	0.455	0.0505
27	L1	M1	L6	L7	L8	-0.3424	0.9102
28	O1	L2	L3	M2	L9	0.428	0.0313
29	L1	L3	M1	L6	L8	-0.3115	0.8969
30	L1	L3	M1	L4	L9	-0.1929	0.7006
31	L1	L2	L5	L8	O2	0.3561	0.1078
32	M1	L5	L6	L7	O2	-0.0554	0.5673
33	L1	L2	L3	M2	L7	0.078	0.3683
34	L5	L6	M2	L7	L9	0.0607	0.3873
35	L1	L3	M1	L7	O2	0.0037	0.4877
36	L3	L4	L7	L9	O2	0.1683	0.2804
37	O1	L2	L3	M1	L6	-0.1062	0.5975
38	O1	L2	L5	L6	L7	-0.0202	0.5068
39	L1	L3	L6	L7	O2	0.2136	0.2255
40	L2	L4	L7	L8	L9	0.1435	0.285
41	L1	L2	L4	L5	M2	0.2217	0.1865
42	L2	M2	L7	L9	O2	0.3952	0.0832
43	O1	L1	M2	L8	O2	0.2395	0.192
44	O1	L4	L5	L6	L8	0.1763	0.2584
45	L1	L2	L3	M1	M2	0.0204	0.386
46	L4	L5	L6	L6	M2	-0.068	0.597
47	L1	L4	L5	L6	O2	0.2415	0.1865
48	L1	L2	L3	L6	M2	0.205	0.1882
49	L1	L2	L3	L4	L8	-0.1286	0.6768
50	L3	M1	L5	L7	L8	-0.3173	0.8755

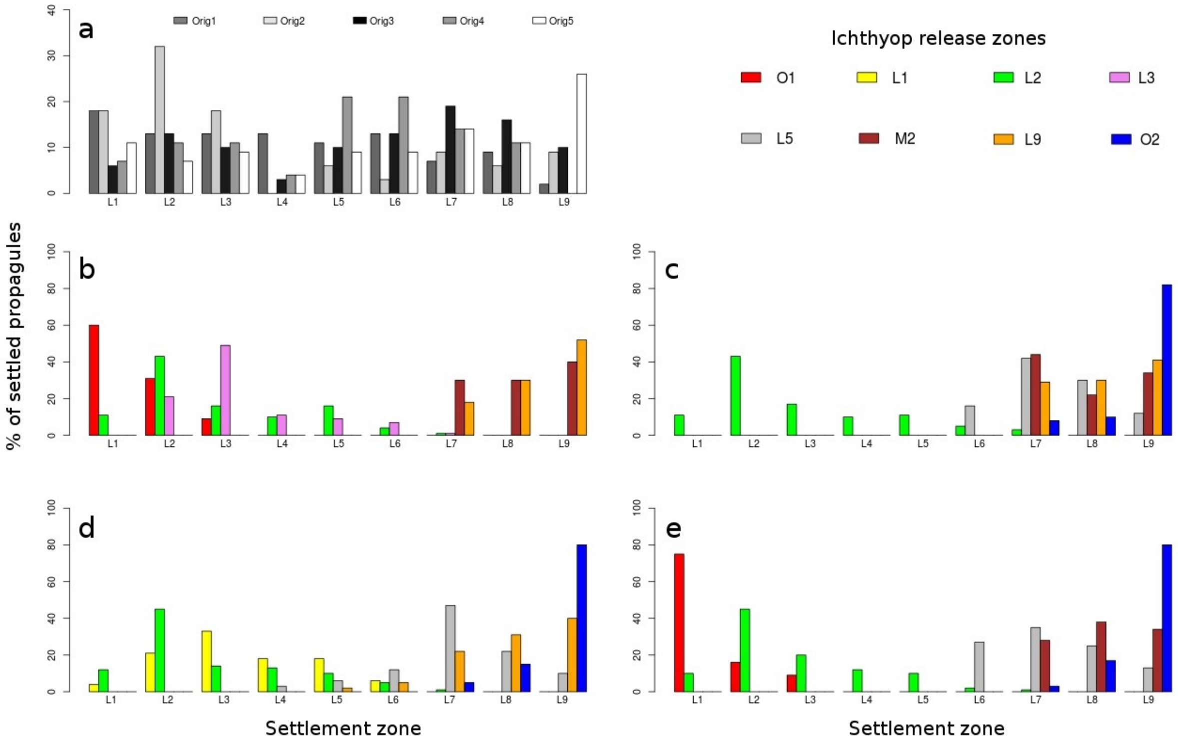

Figure 6.3. Percentage of *O. melanura* settlers from natal origin/release zone. a) Results from otolith chemical analysis: settler distribution in the 9 'pooled locations' from the 5 major natal origins (grey scale) identified in (Calò *et al*. 2016, see Chapter V); b)-d) Propagule distributions as resulted from Ichthyop simulations in the 9 settlement zones from random sets of release zones. Each barplot represents the propagule distribution associated to a different simulation that produced significant (b and c) or tendency to significant (d and e) Mantel's correlation with the distribution obtained from otolith chemical analysis (see Table 6.1 for details).

Dispersal distance of Oblada melanura

Dispersal distances ranged from 1 to 197 km with a mean value of 55.2 km considering all model runs. Short dispersal distances were more common than longer ones considering all particles released: 62% dispersed between 0 and 50 km, 17% between 50 and 100km, 15% between 100 and 150km and 6% between 150 and 200 km (Fig. 6.4).

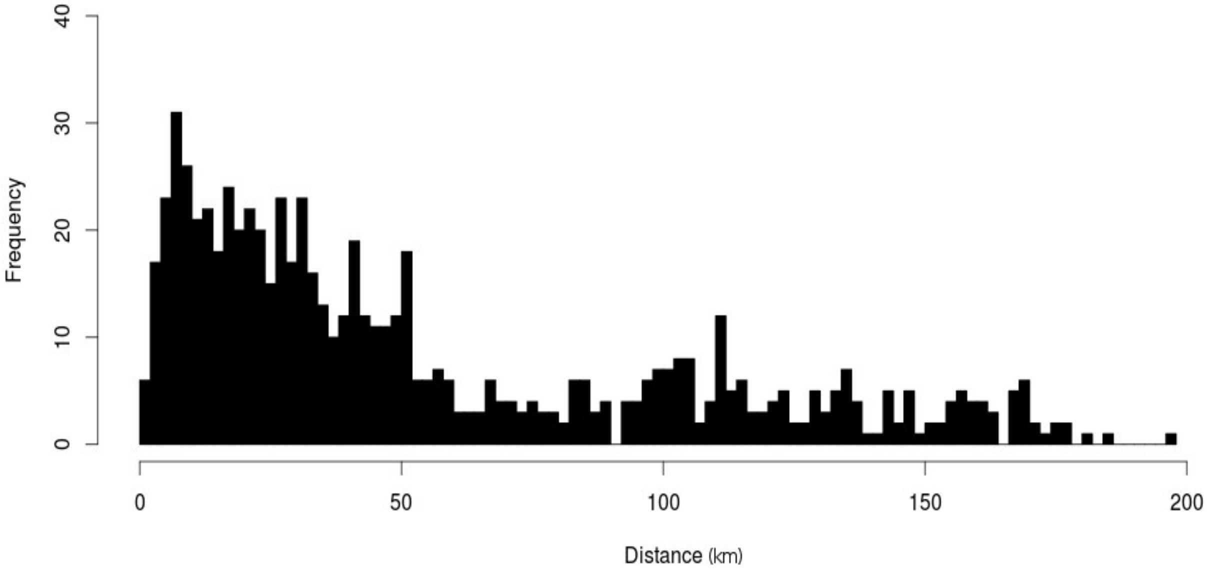

Figure 6.4. Frequency distribution of dispersal distances in *O. melanura* from all release zones.

A significant interaction between factors 'Z' and 'D' was detected (Table 6.2), so that the effect of depth on dispersal distances depended on the release zone and *vice versa*: in the three zones an increase in dispersion distance from shallow to deeper waters was detected, although in Tabarca and Cabo Tiñoso the observed dispersion distances increased gradually with depth, while in the case of Cabo de Palos the difference detected was between the shallowest depth fringe and the other strata (Fig. 6.5). Overall, distances obtained for particles released in Cabo de Palos (88.2 ± 3.1 km, mean ± SE) were greater than for those released from Tabarca (44.2 ± 2.8km) and Cabo Tiñoso (33.1 ± 1.8km) (Fig. 6.5). No effect of dispersal duration was obtained in the range of tested values (Table 6.2).

Table 6.2. ANOVA on dispersal distances: Df=degrees of freedom, SS= sum of squares, T=dispersal duration, D=release depth range, Z=release zone, *=p<0.05, ***=p<0.001.

Source	**Df**	**SS**	**Fvalue**	
T	1	2.63	3.027	
D	3	72.63	8.41	*
Z	2	177.76	146.975	***
T*D	3	2.7	1.64	
T*Z	2	1.73	1.434	
D*Z	6	17.27	4.76	***
T*D*Z	6	3.29	0.908	
Residual	696	420.89		

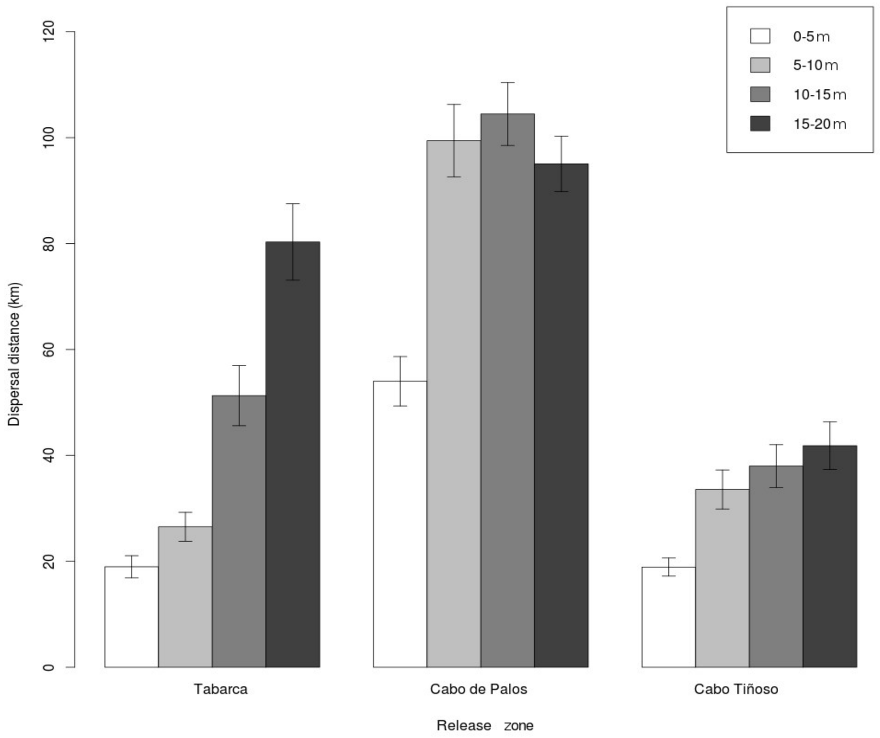

Figure 6.5. Dispersal distance per each combination of release zone and release depth range (legend). Data are mean ± SE.

Discussion

The geographical location of fish release areas is considered as one of the hardest issues to solve for fish ecologists. Here we adopted two approaches to hindcast the putative position of *O. melanura* propagule release zones as assessed in 2013, based on the results of otolith chemical analysis of setter individuals reported in (Calò *et al*. 2016, see Chapter V). Results from the backward simulations showed that the majority of propagules released from settlement areas were moved back toward areas tens to hundred of kilometres far from coastal habitats. Results were consistent among all model runs, each starting from a different release date. No information on the reproductive habits and behaviour of *O. melanura* are available, so that it is difficult to exclude *a priori* any possibility on the position of egg release grounds. Adults of the saddled sea bream are relatively site attached and generally live over seagrass beds and rocky bottoms up to 30 m of depth (Bauchot and Hureau 1986). From this perspective, the areas where most of particles were accumulated in backward simulations were often over sea bottoms more than 100 m deep, thus, based on the available information, likely to be too distant from the coast for representing potential spawning grounds of the considered species. On the other hand, the accumulation of particles in the southern sector of the Alboran Sea suggests that the North-African coast could potentially host spawning grounds of *O. melanura*. The distance between the Algerian coast and the south-eastern Spanish coast (150-200 km), in fact, is comparable to the distance that the saddled sea bream is able to cover during the propagule phase (Calò *et al.* 2016, see Chapter V). A portion of propagules was moved back toward coastal areas that can potentially sustain adult populations of *O. melanura*., in particular the northern sector of the study area, from zone L4 to zone L2 (Tabarca MPA). These zones are characterised by extended patches of sea grass beds (mainly composed by *Posidonia oceanica*) disposed along a bar parallel to the coast (Calvin Calvo *et al.* 1999) that represent optimal habitats for hosting reproductive populations of saddled sea bream.

In the second approach, we considered the major natal origins identified with otolith chemical analysis in 2013 (Calò *et al*. 2016, see Chapter V) and evaluated their putative geographical position along the study area. The results showed that two simulations, run using random sets of release zones, produced settled propagule distributions significantly correlated to the homologous settler distribution recorded in 2013 using otolith chemical analysis. Other two simulations produced a propagule distribution with marginally significant correlation. These four simulations had similar sets of release zones, sharing from 3 to 4 release zones among a total of 5, while 5 of the 13 possible release zones were never selected, demonstrating a strong consistency among model outputs. In the simulated propagule distributions, for each release zone, the highest fraction of settled propagules was recorded in one settlement zone and ever decreasing fractions were recorded with increasing distance from that zone. This pattern is in agreement with the spatial correlation among frequencies of settlers sharing the same natal origin observed in Calò *et al*. (2016). The identification of the exact geographical position of the natal origins found with otolith chemical analysis is very difficult using currently available methodologies. Thus, it is hard to establish whether the spatial dependency characterising settler frequencies found in Calò *et al*. (2016) is the consequence of a distance-dependent dispersal from the spawning areas. However, the concordance with simulation outputs allows us to hypothesize the putative position of the natal origins identified from otolith analysis. For model-generated distributions, the highest fraction of settled propagules was generally recorded inside or in the proximity of the corresponding release zone: for the release zones L2 (Tabarca MPA), L3 and L9 the highest fraction of settled propagules corresponded to local retention, while in the case of O1, M2 (Cabo Tiñoso MPA) and O2 the highest settled fraction originated from the closest zone to the corresponding release one. Only in the case of L1 and L5 (Cabo de Palos MPA) a partial southward shift was recorded between the release zones and the settlement zones where the highest frequencies of settling propagules were recorded. These results suggest that, in certain sectors of the study area, the current circulation pattern in 2013 could have promoted a distance-dependent dispersal from release zones, with short dispersal distances more likely to occur than longer ones. From these considerations, we speculate that some of the main natal origins identified in 2013, based on otolith micro-chemistry, could have been located inside or in proximity of the zones where the highest fractions of settlers were recorded in 2013. Our result suggest that release zone L2 (Tabarca island) could be of critical importance for this species in the region as it was the only present in all the model simulations that produced significant correlations with otolith results, and its was the zone supplying the majority of settlement zones. Concurrently, in this zone the highest frequency of settlers sharing the same natal origin was recorded (Fig. 6.3a). Zone L2 hosts the marine reserve of Tabarca, an effective MPA (Lozano Quijada and Ramos-Esplá 2015) with coastal habitats mainly characterised by shallow rocky bottoms and *P. oceanica* sea grass beds (Ramos-Esplá, 1985). From this perspective, Tabarca MPA could be likely to host one of the natal origins identified with otolith analysis, also considering results from the back tracking simulations that showed optimal hydrodynamic conditions for propagule dispersal around this MPA in 2013. Propagule distribution from model simulations also highlighted a clear separation between the northern and the southern sector of the study area: propagules released from northern zones rarely reached settlement zones in the south of the study area and *vice versa.* This outcome is concordant with the significant difference in settler natal origin composition recorded between the two sectors of the study area in 2013, using otolith chemical analysis (Calò *et al*. 2016, see Chapter V). The resemblance between simulation outputs and field results from otolith analysis suggest that small and mesoscale oceanographic features, like coastal eddies, could have played a major role in shaping settler distribution of *O. melanura* in 2013 along the study area. The formation of mesoscale eddies (MEs, with a diameter of 50-100 km) is frequent along the the stretch of coastline considered (Millot 1999), and was also observed in the simulations carried out in this study. Recent studies highlighted that MEs may represent important habitats for the larval stages of coastal fishes (Nakata et al. 2000, Sabatés *et al*. 2007, Shulzitski *et al*. 2015, Sabatés *et al.* 2013). There are evidences that larvae entering the MEs grow consistently faster than those outside the eddies, consequently leading to lower mortality rate and higher probability to successfully settle in coastal habitats (Shulzitski *et al*. 2015). MEs could also be responsible for larval patch formation in the pelagic environment, supporting the results on patch cohesiveness recorded in the study area. Indeed, otolith analysis of *O. melanura* larvae indicated that groups of larvae originating from different natal origins had merged during the larval phase (Calò *et al*. 2016, see Chapter V). From this perspective, even in the absence of behavioural traits or active movements, the formation of heterogeneous larval patches can be promoted by water mixing structures in different moments of the pelagic phase. In the model implemented here, we considered larvae as passive particles, including no behavioural or movement capabilities, thus potentially biasing our model outputs (Leis, 2007, Leis *et al*. 2011). On the other hand, by shortening the dispersal duration implemented (i.e. using the DAH instead of the PLD) we prevented running the model during the competency phase of the saddled sea bream (Chapter IV), thus reducing the potential influence of behaviour and active movements that characterise the last moment of the larval phase (Leis, 2007). In addition, the use of proper information on ELTs (i.e. spawning dates, propagule larval duration), gathered at the same spatial and temporal context of the oceanographic data, allowed us to improve the accuracy of the simulated dispersal.

The spatial scale of dispersal recorded here is in accordance with the estimation obtained from otolith chemical analysis of *O. melanura* for the same spatial and temporal context (Calò *et al*. 2016, see Chapter V). Results from dispersal simulations showed that dispersal distance depended mainly on spatial factors. The geographical position of release zones was mainly responsible of distance variability as also highlighted in recent works (Treml *et al.* 2015, Thomas *et al.* 2014). In our study, propagules released in the centre of the study area (Cabo de Palos MPA) dispersed 2-fold further than those released in the northern and southern zones. This result is in accordance with the oceanographic characteristics of the region. The northern half of the study area is characterised by an extended continental shelf (~300 km from the coast) and shallow waters (0 - 200m). In the southern sector the continental shelf is very narrow and the continental slope, cut through by a series of coastal canyons, is only few thousands of meters distant from the coast (Calvin Calvo *et al*., 1999). Cabo de Palos marine reserve is geographically located in the transition between these two morphologically different areas and the oceanographic circulation in its surroundings reflects these geomorphological features, determining strong currents compared to the the northward and southward coastal sectors of the study area. This pattern was clearly highlighted by averaging current velocities in the study area over the time window in which simulations were run (i.e. 21st June 2013 – 15th July 2013) (Fig. 6.6, 1-4). From this point of view, the southward shift in the distribution of settled propagules released from zone L5 (i.e. Cabo e Palos MPA) reported above, could be a consequence of the strong current dynamic characterising the area around Cabo de Palos (Fig 6.6, 1- 4). Dispersal distance variability also depended on the depth range of propagule release, since particles released in shallow waters dispersed less in average that those released deeper in the 3 release zones considered. This outcome is partially in contrast with the general assumption that current velocities tend to decrease with depth. However, the particular oceanographic conditions characterising the study area are responsible for this dispersal pattern, with deeper waters moving faster than shallow once (Fig. 6.6, a and b). This difference is probably due to coastal recirculation characteristics, for example the formation of coastal eddies. These oceanographic features are related to wind forcing and are consequently more pronounced at the surface than in deeper water layers. The analysis of model runs with different release depths shows that the formation of a relatively wide recirculation system trapped a large number of particles close to the surface, while particles released in deeper water, only partially affected by the eddy, dispersed longer paths southward (Fig. 6.7). The significant variability in dispersal distances due to spatial factors could have blurred the effect of dispersal duration, here found non-significant. Although the relation between propagule dispersal and its duration in the pelagic environment is currently a matter of debate (Shanks 2009, Leis *et al*. 2013), it is generally accepted that fish dispersal is positively correlated with dispersal duration. In this work, however, the relatively short time of dispersal and the small difference between the two dispersal durations (2.5 days) considered in this study could have determined the lack of significant variability of the temporal factor. Moreover, the frequent occurrence of small and medium-size coastal eddies along the study area could have contributed to the observed result. Propagules that enter the eddy could remain trapped inside it, blurring the effects of different dispersal times. The variability of propagule dispersal associated to the geographical context and the release depth suggests that even for species with a short propagule dispersal phase (~14 days in the case of *O. melanura*), local differences in oceanographic conditions can lead to a wide range of dispersal outcomes.

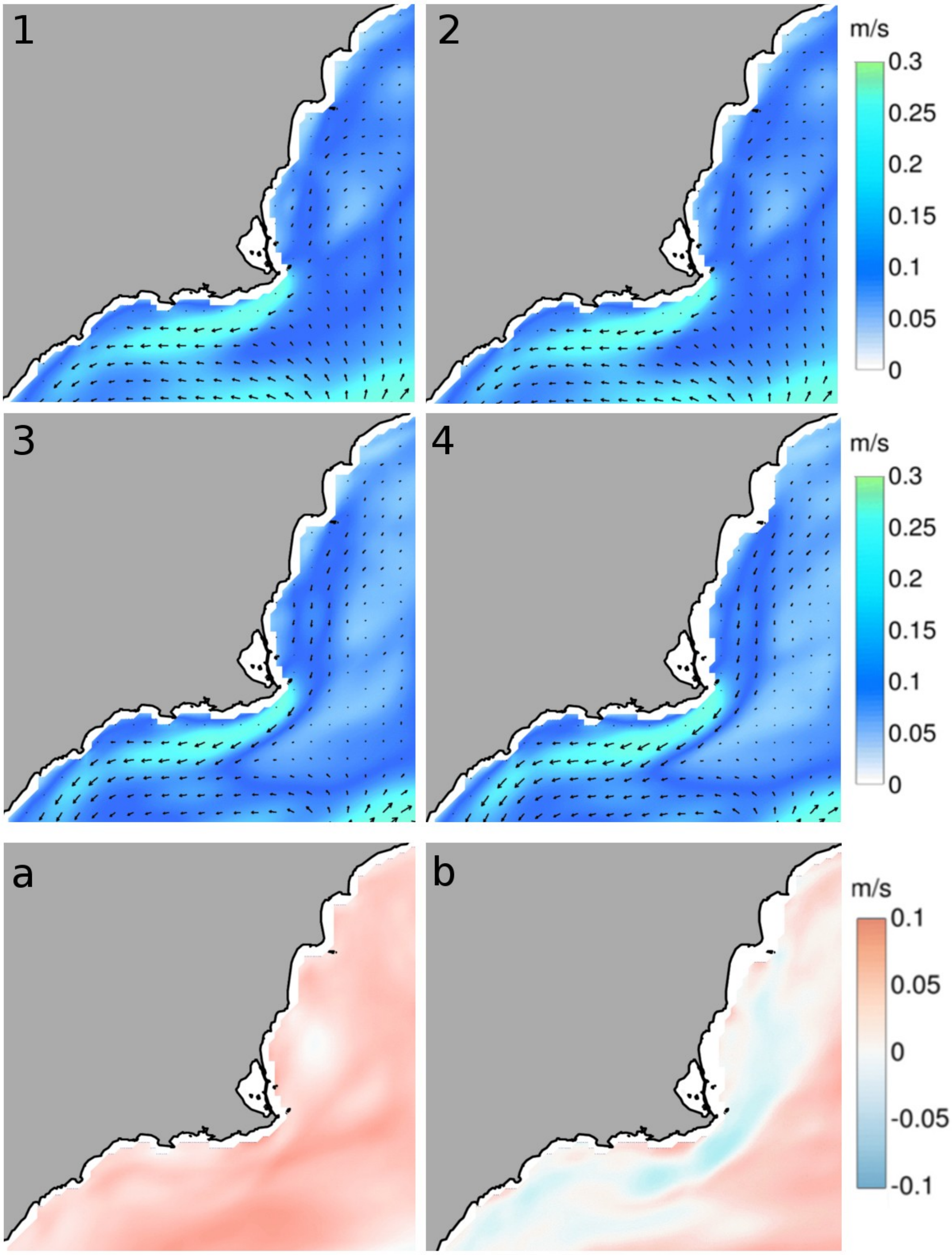

Figure 6.6. (1-4) Maps of average current velocities over the simulation period 21 June – 15 July 2013 at different depth: 5 m (1), 10 m (2), 15 m (3) and 20 m (4). (a and b) Maps of difference in average current velocity between 0 m and 10 m (a) and between 10 m and 20 m (b) over the same time period: positive values (red colours) indicate that currents at the upper layer were faster than deeper currents; negative values (blue colours) indicate that deep currents were faster than currents at the upper layer.

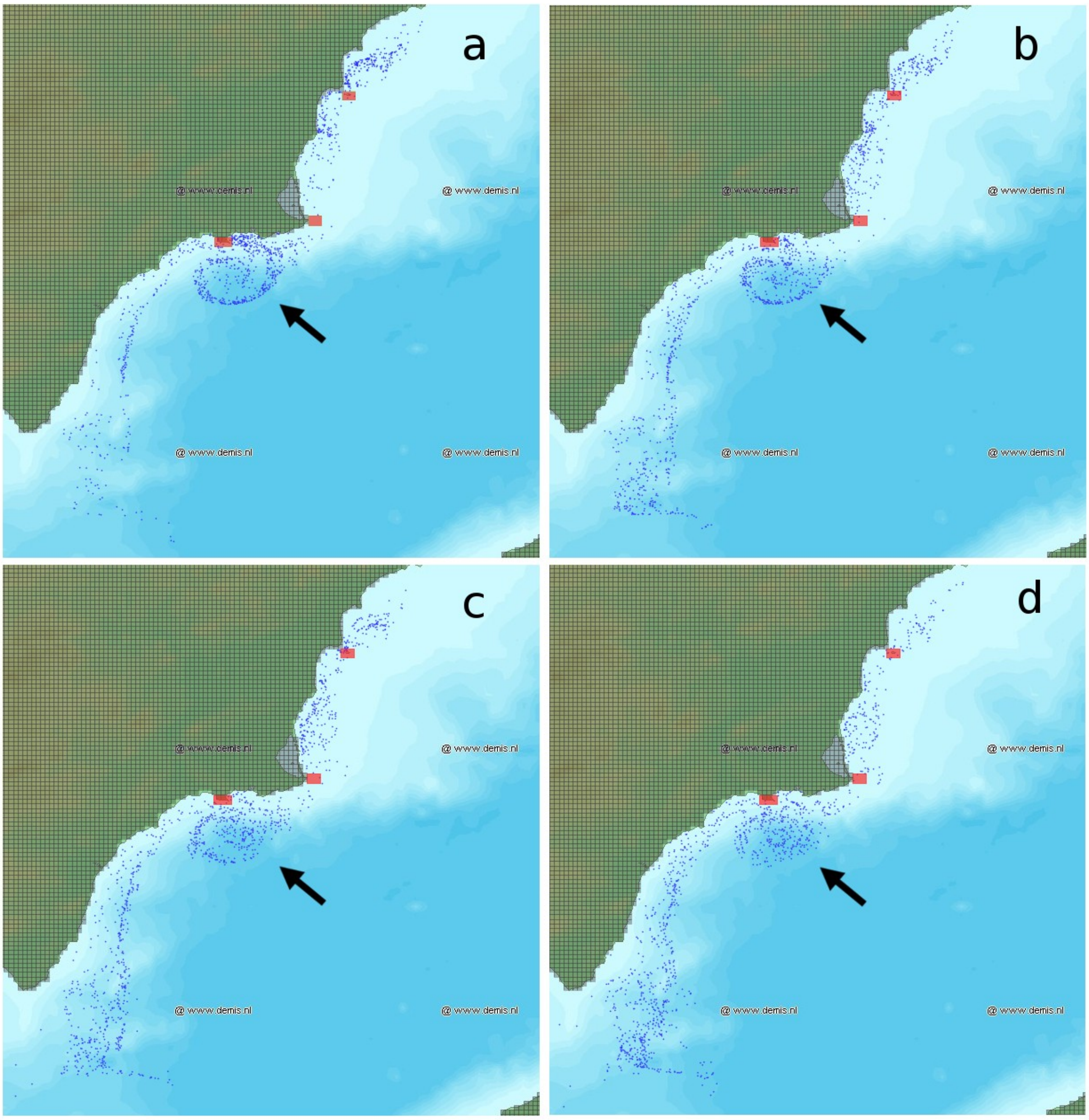

Fig 6.7. Last steps of Ichthyop simulations from the 3 release zones (red rectangles) and daily release from 21st June to 2nd July. Each image shows the last step of a simulation run with a different release depth: 5 m (a), 10 m (b), 15 m (c) and 20 m (d). Black arrows indicate the position of one of the coastal eddies probably responsible of particle trapping in shallower waters (note the decreasing density of particles in the eddy and the increasing abundance of particles in the south of the domain from (a) to (d).

Information on the location of fish spawning grounds and the scale of dispersal is crucially important for elucidating connectivity patterns between populations, and provide the optimal background knowledge for planning efficient protection and management strategies. The method used here is a first attempt to locate fish release zones in the Mediterranean Sea, combining model simulations with information on natal origin composition resulting from otolith chemical analysis, supported by accurate information on fish biological traits. The approach implemented could provide valuable results for the localization of fish spawning grounds for other species, especially considering the current lack of methodological alternatives for addressing this issue worldwide.

Chapter VII

GENERAL DISCUSSION

GENERAL DISCUSSION

The Mediterranean Sea is considered one of the world priority eco-regions representing a hotspot of biodiversity (Coll *et al*. 2010, Gabrié *et al*. 2012). It is an historically exploited basin, with anthropogenic pressures increasing over the last century, which are responsible of habitat loss and degradation, pollution and species depletion (Coll *et al*. 2014). For these reasons, a huge effort is being made to improve the current conservation status of the Mediterranean Sea. Among the available management tools, MPAs are advocated worldwide for achieving conservation goals and their establishment has incremented in the last decade (Lubchenco and Grorud-Colvert 2015). The European Western Mediterranean is the eco-region that received the most of protection effort up to now, hosting more than the 40% of Mediterranean MPAs. Despite this relatively high protection level, the conservation target established both at international (under the CBD) and European level (under the Barcelona convention) has not been reached so far, and an urgent enlargement of marine ecosystems under protection through the establishment of efficient MPAs networks is being increasingly advocated.

Efficiency of MPA networks relies on a series of spatial criteria concerning size and zonation of single MPAs and their relative position. Populations connectivity is one of the fundamental aspects to be considered for the spatial arrangement of MPAs inside a network, since it determines the spatial scale over which protection benefits take place and the scale that should be considered for the optimal management of marine populations. Connectivity is intimately linked to species dispersal capacity, thus, in the framework of marine spatial protection, it is fundamental to study the ecological and biological forces that drive patterns of dispersal and connectivity. Despite the high importance of dispersal and connectivity information, the review work in Chapter II showed that very few studies have dealt with these processes in the Mediterranean sea until now. As a consequence no MPAs or MPA networks have included estimates of dispersal and connectivity in their design, potentially failing to guarantee the exchange of organisms' larvae both toward unfished areas and other MPAs within a network (Gabrié *et al*. 2012) and consequently missing part of their conservation objectives (Almany *et al*. 2009).

From the management perspective assessing patterns of evolutionary connectivity is important for the maintenance of both biodiversity and genetic diversity (Leis *et al*. 2011). Results of this study indicated that the MPAs investigated in the Western Mediterranean Sea are well connected with their neighbouring unprotected sites for the studied species. This is an important result for the maintenance of fish population dynamics, as genetic links are the only proof that guarantees an exchange of breeding adults between populations (Palumbi 2003). On the other hand, the signals of genetic structuring recorded at the regional scale suggest that the presence of oceanographic barriers can affect genetic flows even between close locations, potentially able to generate genetic breaks over the evolutionary time scale (White *et al.* 2010). For this reason it is important to monitor the temporal dynamics of population genetics, including other species with different biological and ecological characteristics (Schwartz *et al.* 2007). This is especially true when patterns of genetic differentiation appear along transboundary coastal regions of different political entities, such as countries or regional communities, that could adopt diverse management strategies for the same management unit (fishing stock, conservation unit, etc.). This case is well represented in our study. The high level of genetic connectivity between the two opposite extremes of the Gulf of Lions suggests that Port-Cros MPA could act as a source of propagules for a stretch of Spanish coastal ecosystems that is currently out of protection. From this perspective, a proper interplay between management authorities involved in fishery resource conservation of the Gulf of Lions would guarantee an optimisation of protection benefits. Current research efforts, such as the project eCATE (http://crem.univ-perp.fr/projet-ecate) is well suited to this transboundary management perspective.

Although information on genetic connectivity can support the implementation and the monitoring of protection strategies at the regional scale, they partially lose appeal when contextualised to a more local spatial scale. Examples of genetic homogeneity even at a relatively large spatial scale, as the one recorded over the Western Mediterranean in this study, are generally common in marine ecosystems. This is due to the fact that the exchange of very few individuals (i.e. less than 10) between populations per generation is enough to maintain a sufficient genetic flow and avoid the development of genetic differences by genetic drift (Kimura and Maruyama 1971, Shulman 1998). However, a so small number of fish interchanged between populations in each generation can barely provide a sufficient demographic contribution for other protected populations and much less in areas where fishing is allowed (Schultz and Cowen 1994, Purcell *et al*. 2006). For this reason, during the design of MPA networks, and in the subsequent evaluation of protection effects, a greater attention should be given to patterns of ecological connectivity.

Well enforced MPAs are able to drive the entire fish communities towards an unfished status and produce increased densities and larger body sizes in fish populations (Guidetti and Sala 2007, Claudet *et al.* 2008, Di Franco *et al.* 2009). Therefore, it is likely that a greater fish biomass would generate a proportionately higher number of propagules, that can be exported beyond reserve borders (Kritzer and Sale 2004).

Cabo de Palos e Islas Hormigas MPA and Tabarca MPA are two of the most ecologically effective MPAs in the Western Mediterranean Sea (Harmelin-Vivien *et al.* 2008, Goñi *et al*. 2008, Hackradt *et al.* 2014). In both MPAs high fish biomass are recorded, especially due to the presence of large apex predators (e.g. groupers) (Bayle-Sempere 1999, García-Charton *et al*. 2004). The evidences of protection benefits and the relatively short distance between them (less than 60 km) allow to hypothesize that the MPAs can mutually act as source/sink of propagules, providing also an important supply for the unprotected areas in the between. Despite this potential scenario, the findings of this study suggest that Cabo de Palos and Tabarca MPAs could be less linked by propagule dispersal than expected. Dispersal simulations showed that the oceanographic dynamic around Cabo de Palos MPA, often characterised by strong southward currents during the study period, can rapidly shift propagules far away from the MPA even in species with a relatively short duration of the pelagic larval phase. On the contrary, water circulation around Tabarca MPA seems to promote a high level of local retention, mainly attributable to the occurrence of coastal recirculation features, with a minor export of propagules toward unprotected sites located 20-30 km northward and southward from the island. A very low export was instead recorded toward Cabo de Palos MPA. This finding was corroborated by the different natal origins found for individuals coming from sites around Cabo de Palos compared to those in the north of the study area, including Tabarca MPA, thus highlighting that distinct sources of settlers supplied the two zones in the period considered. The possible reduced level of demographic connection between Tabarca and Cabo de Palos is even worsened by the spatial arrangement of habitats in Cabo de Palos MPA, in which a very low extent of potential settlement habitats for Mediterranean coastal fish (i.e. shallow rocky bottoms and *Posidonia oceanica* meadows; Bussotti and Guidetti 2011, Félix-Hackradt *et al*. 2014) is under protection. As a consequence, even in the presence of sufficient propagule supply, Cabo de Palos MPA would not be able to provide the proper settlement requirements to a number of species larvae (Fig. 7.1.).

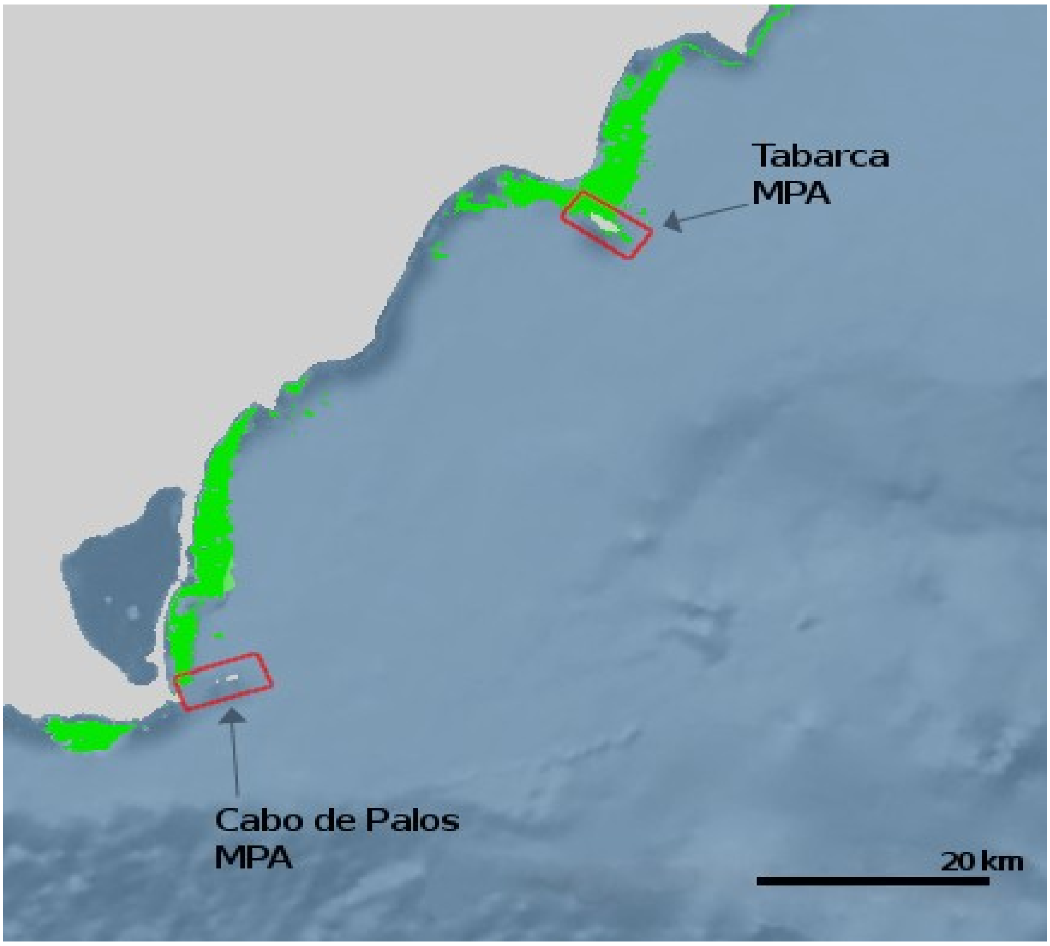

Figure 7.1. Position and extent of the main *Posidonia oceanica* meadows (green) in the north of the study area (http://www.ecocartografias.com). Red rectangles indicate the position and the extent of the 2 MPAs in the area.

In a work carried out in the same area, genetic analysis conducted on the white sea bream (*Diplodus sargus sargus*), a species belonging to the same family of *O. melanura* and with similar ELHTs, evidenced a genetic isolation of populations living in Cabo de Palos and higher genetic flows between Tabarca and the southern sector of the region considered (González-Wangüemert *et al.* 2004). From this perspective, the potential lack of demographic connectivity could lead to consequences at the evolutionary time scale, that in the case of the white sea bream could have resulted in a genetic separation of populations living in Cabo de Palos from those from Tabarca.

The information coming from the different methodologies are concordant on the possible low ecological connection during the propagule phase of *O. melanura* in the area. Our findings provide hints that the southern part of the Murcia region, where new MPAs will be established in the next future, could be demographically separated from the northern one in terms of propagule flows, with Cabo de Palos representing the limit between the two geographic systems. Natal origin composition of settlers highly differed between the north and the south of the study area. Together with the demographic diversity a biological difference was recoded in the PLD of *O. melanura* between the two sectors. Given the overall genetic homogeneity recorded in the area, that allows to assume the absence of an ongoing evolutionary segregation, both the biological and the ecological difference observed could be a consequence of environmental determinants acting in the study area, especially water circulation. The oceanographic dynamic along the coast could be responsible of a natural regionalization of the water masses, probably due to the pronounced geomorphological discontinuity characterising the area, potentially determining a division between the north and the south and impeding an optimal exchange of propagules. Model simulations showed that particles released in the northern sector of the region scarcely reached southern coastal sites of the study area and *vice versa*. In addition the frequent occurrence of coastal eddies could be able to promote the high level of local retention recorded in different locations, with the exception of Cabo de Palos. All this could contribute to a low level of ecological connectivity.

The different pieces of knowledge gathered in this study provide insights for supporting the design of new MPAs along the south-eastern coast of Spain. Results indicate that the spatial arrangement and the position of the existing MPAs could not generate the optimal interchange of propagules expected in efficient networks. In addition, Cabo de Palos MPA and Tabarca MPA could not guarantee a sufficient supply of individuals toward the southern unfished areas of the region, despite their potential propagule production. From this perspective, the establishment of new MPAs in the southern sector of the region is advisable for improving the current conservation status of the area. The positioning of other MPAs in strategic points of the coast, further from increasing the extent of protected ecosystems, would enlarge the surface of protected settlement habitats for propagules exported from Cabo de Palos MPA, thought to be mainly subjected to a southward dispersal. The spatial arrangement of 3-4 MPAs, sized similarly to Cabo de Palos MPA and Tabarca MPA, taking into account the average dispersal distance (i.e. ~50 km) recorded in this study could contribute to the creation of a continuum of connections between Cabo de Palos and Cabo de Gata MPA, in the Andalusia region. In this context, the future establishment of a MPA in Cabo Tiñoso (http://lospiesenlatierra.laverdad.es/blog/3552-la-reserva-de-cabo-tinoso-en--2016) represent a first important step toward the establishment of an ecologically-coherent network of MPAs in the region (Fig. 7.2).

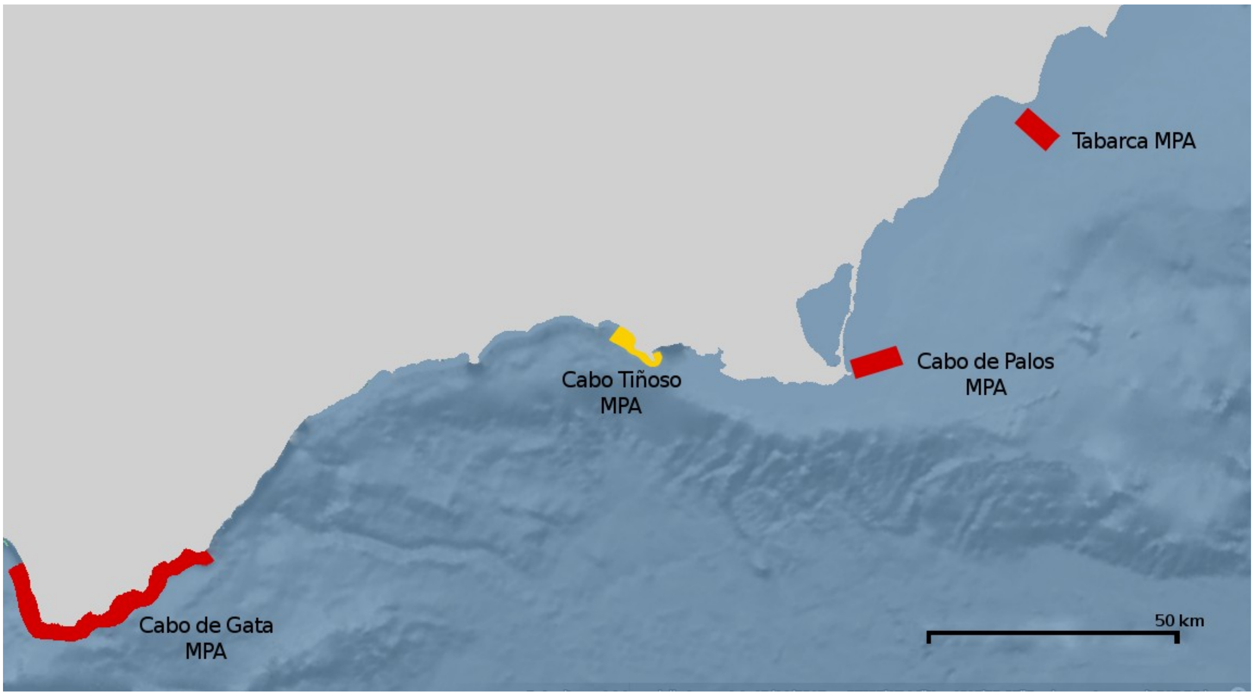

Figure 7.2. Map of the current position and size of MPAs in the South-East coast of Spain. In red the MPAs already established; in yellow the MPA that is going to be established in the next year.

Regarding the northern sector, the recorded weakness of demographic connection between Tabarca and Cabo de Palos could be improved by increasing the extent of shallow water habitats under protection between the two MPAs. These areas are mainly characterised by extended patches of *Posidonia oceanica* meadows (Calvin Calvo *et al*. 1999; Fig. 7.1) and would represent settlement sites suitable for a number of coastal fish (Vigliola *et al*. 1998, Cheminée *et al*. 2011, Félix-Hackradt *et al*. 2014, Ventura *et al*. 2014) From this point of view, they could act as nursery habitats for larvae coming from Tabarca before the recruitment in adult populations living in Cabo de Palos MPA.

It is important to remark that our findings represent only a part of the information needed for the correct design of an MPA network. The analysis of a single species, although representative of other species with similar biological and ecological characteristics, is not enough to draw an adequate picture of dispersal and connectivity patterns. Thus, it would be important to apply the same investigation procedure including species with different biological and ecological traits and over a longer temporal domain. However, as already pointed out in other works, this study demonstrates the effectiveness of combining complementary tools for investigating patterns of propagule dispersal and connectivity.

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