UNIVERSIDAD DE
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"Ecología poblacional y patrones de movilidad de los meros (Serranidae: Epinephelinae) en arrecifes rocosos templados del sudoeste del Mediterráneo: Implicaciones para su conservación"

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Thesis submited by Carlos Werner Hackradt for the degree of Doctor of Philosophy within Department of Ecology and Hidrology, University of Murcia.

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Resumen

Resumen

Hace tiempo que se ha ido perdiendo la antigua idea de que los océanos son una fuente inagotable de recursos. Actualmente el medio marino sufre una erosión de su biodiversidad debido al efecto sinérgico de las actividades humanas. La pérdida del hábitat, la contaminación, la sobrepesca y las bioinvasiones, así como los efectos del calentamiento global son las fuerzas principales que confluyen para degradar el ecosistema marino. En los últimos años se han desarrollado numerosos estudios que evalúan las medidas a tomar para una mejor gestión y conservación de la biodiversidad marina. La medida que más atención ha recibido en los últimos años es la implantación y mantenimiento de áreas marinas protegidas (AMPs). Para proponer medidas efectivas de conservación y gestión de especies marinas amenazadas es necesario generar conocimiento científico que abarque los principios de la historia de vida de los organismos, como ecología, comportamiento, movilidad, etc. En este trabajo, nos hemos centrado en comprender algunos patrones ecológicos y de movilidad de varias especies de meros comunes en la zona costera de la cuenca occidental del Mediterráneo, centrándonos, en gran parte, en la reserva marina de Cabo de Palos – Islas Hormigas y la zona costera de su entorno.

Los meros son especies emblemáticas de los arrecifes rocosos mediterráneos, que presentan gran valor ecológico, económico y social. Son especies clave en la estructura de la comunidad arrecifal y presentan gran valor para la pesca artesanal y recreativa a lo largo de toda costa española y en el mundo. Son especies en general de crecimiento lento, que pueden alcanzar más de 40 años de edad, según la especie, son hermafroditas protogínicos y viven siempre muy cerca del fondo. Pueden ser especies gregarias o solitarias o aún vivir en estructura de harenes. En el periodo de la freza se pueden juntar en grupos de centenares a millares de individuos, o bien solamente en parejas, según la especie.

En general los meros presentan una gran variabilidad espacial en su rango de distribución, siendo los principales factores que afectan su abundancia la complejidad del hábitat, la profundidad y las características fisonómicas del ambiente. Las especies de meros son conocidas por ser muy sedentarias o moverse muy poco, con algunas excepciones, como *Epinephelus striatus* y *E. itajara*, que se sabe emprenden movimientos estacionales entre sus áreas de alimentación y los sitios de reproducción.

Actualmente se reconocen más de 160 especies de meros en todo el mundo. En aguas Mediterráneas está documentada la existencia de ocho especies, seis nativas (*Epinephelus marginatus, E. costae, E. aeneus, E. haifensys, E caninus* y *Mycteroperca rubra*) y 2 emigradas del mar rojo (*E. coicoides* y *E. malabaricus*). Hay poca información disponible sobre casi todas las especies de meros del Mediterráneo. Únicamente para *E. marginatus* se ha realizado un conjunto de trabajos que aportan considerable información sobre su biología, ecología y estado de conservación de sus poblaciones.Una serie de trabajos científicos recientes, basados en datos de ecología molecular, cambió la clasificación biológica de estas especies. La problemática ligada a la taxonomía de este grupo se debe a que la familia a la cual pertenecen los meros, Serranidae, en la forma actual que se presenta, no es monofilética. Así, para adecuar su clasificación se propusieron una serie de cambios, dentro los cuales cabe resaltar la elevación de la sub-familia Epinephelinae al nivel de familia y la reclasificación de un gran número especies en otros géneros. Dichos cambios, sin embargo, aún no se han llevado a cabo en la literatura debido a un desacuerdo entre las dos ramas de la taxonomía (clásica y filogenética) pues es casi imposible seguir la nueva clasificación propuesta debido a que no se han encontrado caracteres mensurables que soporten la nueva clasificación filogenética. En lo que se refiere al Mediterráneo, de las seis especies nativas, solamente una se ve afectada por los cambios, *Epinephelus aeneus* se ve readecuado en un nuevo género *Hyporthodus aeneus*. El único que si esta acepto por la academia actualmente.

Este trabajo está dividido en cuatro partes. Primeramente evaluamos los procesos ecológicos de selectividad del hábitat de meros y pequeños serranos en zonas de arrecifes rocosos mediante censos visuales. Se tomaron datos de abundancia de cada una de las especies de Serranidae encontradas. Además, se apuntaron datos ambientales como tipo de fondo (roca, arena, pradera de *Posidonia oceanica* y grava), la cantidad de bloques y agujeros presentes en las unidades muestrales y la cobertura del fondo por el empleo de foto-cuadrados. Estos datos fueron utilizados para determinar cuáles son los factores abióticos que influyen en la distribución de las especies censadas y de qué modo se dan las relaciones entre los depredadores apicales (meros) y los meso-predadores (serranos). Seguidamente, en el segundo y tercer capítulos, la presente tesis se centra en evaluar los aspectos de comportamiento y los patrones de movilidad de dos especies simpátricas de meros (*Epinephelus marginatus* y *E. costae*) utilizando la técnica de seguimiento acústico. En septiembre del 2011 se instaló una red de receptores fijos, modelo VR2W (VEMCO Inc.) a lo repartidos por toda la reserva marina de Cabo de Palos – Islas Hormigas. En octubre del mismo año empezamos a marcar ejemplares de ambas especies de meros con marcas acústicas en ejemplares de ambas especies. Además, con periodicidad mensual se realizaron rastreos activos, mediante transectos a lo largo y ancho de toda la reserva y zonas desprotegidas alrededor, desde una embarcación con el empleo de un receptor móvil (VR100) acoplado a un hidrófono unidireccional (VH100) (también pertenecientes a VEMCO Inc.).

Finalmente, presentamos una evaluación del efecto de la protección en seis reservas marinas de las costas española y francesa, sobre las tres especies más comúnmente encontradas en estas costas (*E. marginatus, E. costae* y *M. rubra*) y la capacidad de dichas reservas en proporcionar ejemplares para las zonas de pesca adyacentes por el proceso conocido como "spillover", es decir la exportación de biomasa, debida probablemente a efectos de denso-dependencia creados por el aumento de las densidades de población dentro de las áreas protegidas.

Los objetivos de este trabajo son los siguientes:

Capítulo II

1. Estudiar las relaciones ecológicas entre los depredadores apicales y meso-depredadores en zonas rocosas costeras del Mediterráneo sur-occidental;

1.1 Determinar la abundancia y distribución espacial de tres especies de meros y dos serranos en una zona protegida y una desprotegida;

1.2 Entender si la densidad poblacional de estas especies puede afectar a la distribución de estas especies;

1.3 Evaluar las relaciones intra e interespecifica de estas especies.

Capítulo III

2. Entender los patrones de actividad y distribución por profundidad para* E. marginatus *y* E. costae *en los arrecifes rocoso del Mediterráneo;

2.1 Determinar los patrones de actividad y distribución por profundidad, diaria, estacional y relacionados con las fases lunares;

2.2 Determinar qué variables ambientales afectan los patrones de actividad y la profundidad de los meros estudiados.

Capítulo IV

3. Estudiar las pautas de movilidad de* E. marginatus *y* E. costae *dentro de la reserva marina de Cabo de Palos - Islas Hormigas;

3.1 Determinar el área de vida y los patrones de movilidad de *E. marginatus* y *E. costae*;

3.2 Comparar el patrón encontrado de ambas especies;

3.3 Evaluar la respuesta de ambas especies frente la protección proporcionada por la reserva marina.

Capítulo V

4. ***Evaluar la eficacia de las medidas de protección para la recuperación de las poblaciones de tres especies de meros dentro y alrededor de varias AMPs en el Mediterráneo occidental;***

4.1 Determinar la capacidad de las AMPs para incrementar la biomasa de meros en el Mediterráneo;

4.2 Identificar gradientes de biomasa de meros a través de los límites de las AMPs, como un indicativo indirecto de exportación de biomasa hacia fondos adyacentes no protegidos;

4.3 Explorar las interferencias de la estructura del hábitat con los efectos de las AMPs sobre la abundancia de meros.

Los resultados obtenidos indican una gran influencia de la densidad en el uso y la partición del hábitat por parte de los meros y serranos. Su distribución se ve afectada principalmente por la profundidad y la complejidad del hábitat. De forma general los meros presentan un gran solapamiento espacial con relación a los microhábitats utilizados, aunque evitan en lo posible la competencia por el espacio utilizando distintas zonas del arrecife (distintas distancias al fondo) y teniendo dietas diferenciadas entre las diferentes especies. Por otra parte, los serranos segregan el uso del hábitat entre sí ya que se alimentan básicamente de los mismos items. Por lo tanto, parecen existir mecanismos que permiten la convivencia de las distintas especies en el mismo hábitat; se comprueba asimismo necesidades papel de los grandes depredadores como estructuradores de la comunidad arrecifal.

En cuanto al comportamiento de los meros, en general *E. marginatus* y *E. costae* son especies con un comportamiento muy similar. Ambas son especies de actividad diurna, típico para las especies de meros al tratarse de depredadores que se guían básicamente por la visión para obtener el alimento. Ambas especies se ven afectados sus patrones de actividad y profundidad por variaciones en las condiciones ambientales tales como presión atmosférica, intensidad del oleaje y las corrientes, y la temperatura del agua. En periodos de borrasca se detecta una menor actividad de ambas especies, las cuales suelen moverse hacia lugares más profundos del arrecife, metiéndose en agujeros, al igual que ocurre en períodos de temperatura muy baja. En lo que respecta a la movilidad, se da una gran superposición del área vital tanto entre individuos de la misma especie como entre especies de meros. Estos peces pasan largos periodos de tiempo en un mismo sitio, pero esporádicamente realizan movimientos amplios a lo largo de la reserva marina. En este trabajo se pudo comprobar que ambas especies realizan incursiones en la zona desprotegida, lo cual constituye una prueba de que existe spillover para ambas especies. Las tasas de salida ("spillover") y entrada ("spill-in") de *E. marginatus* entre la reserva integral y la parcial son las mismas, lo cual puede ser una consecuencia de que la reserva aún no haya alcanzado su capacidad de carga para esta especie. Hemos identificado que para *E. marginatus* la fase potencialmente dispersora, en la que más se mueven, es en el caso de los ejemplares más pequeños, que acaban moviéndose más para poder encontrar un sitio donde fijarse. Por otro lado, para *E. costae* se mostró que los individuos adultos de mayor tamaño son los más capaces de desplazarse largas distancias.

Estos datos son congruentes con los encontrados en el último capítulo. La evaluación de las reservas marinas nos indica que estas especies responden muy bien a la protección con un incremento de la abundancia y talla de los individuos. Además, se ha comprobado que las reservas marinas mantienen un efecto de exportación para estos animales, que puede llegar a los 1000 m alrededor de las AMPs. Este movimiento neto de adentro hacia fuera podría ser consecuencia de que estas especies responden de forma denso-dependiente al uso del hábitat, de modo que les obliga a moverse buscando un sitio para establecerse y consecuentemente saliendo de la protección promovida por la reserva marina a las zonas de pesca de alrededor.

Este trabajo presenta un conjunto de datos de base que son fundamentales para el uso y gestión de áreas marinas protegidas y para medidas de conservación de las especies de meros en el Mediterráneo, y por extensión a las zonas costeras de todo el mundo. Está claro que estos animales responden muy bien a protección, por lo que establecer zonas cerradas para proteger el hábitat óptimo de estas especies a buen seguro que conseguirá incrementar la abundancia de las poblaciones de meros. Además, el establecimiento de AMPs no solamente es una medida efectiva de protección para las especies sino que se muestra como una medida eficaz de gestión pesquera gracias al efecto de exportación de biomasa comprobada para estas especies. Además, la mejor estructuración poblacional dentro de los límites de la reserva es en términos de clases de talla, les confiere mayor fecundidad, por la presencia de individuos más grandes y la resistencia a posibles eventos catastróficos.

Los meros son especies emblemáticas del ambiente de arrecifes, sea rocoso o de coral, de modo que la protección de esas especies, además de garantizar la estructura ecológica de esos ambientes, puede generar una forma de rentabilización no cruenta para las localidades donde estos animales viven, visto que son un atractivo muy importante para el buceo recreativo.

Chapter I

General Introduction

Introducción General

1. General Introduction

The name ‘grouper’ defines a characteristic group of reef fishes belonging to the family Serranidae; this word probably has its origin from the Portuguese “garoupa”, which in turn derives from the word used by Indians from Brazil to name this fish. Groupers are not only biologically diverse and fascinating in their own right, but are also among the most commercially important and highly regarded of all fish species associated with reef fisheries around the world. While representing a small percentage of world fisheries, they constitute a significant component of coastal rocky and reef fisheries in much of the tropical and subtropical regions.

Given the ecological and economic importance of this group in many parts of the world, it is surprising how little is known about their biology and ecology. At present, with the development of recreational SCUBA diving and the increasing number of marine biologists and underwater photographers, knowledge about their depth and geographical distribution is growing exponentially. Most information until now, however, remains inaccessible because it is buried in grey literature and unpublished or restricted-circulation reports; the fast decline of populations due to overfishing and habitat loss, however, requests to acquire rapidly updated scientific information to provide appropriate management and conservation measures for this species.

1.1 Taxonomy remarks on groupers

The family Serranidae is distributed worldwide and comprises around 449 species (Nelson *et al.* 2006). The taxonomy and classification of Serranidae has been investigated for a long time and is subject to continuous change. The first approach to solve the relations within the family was provided by Jordan & Eigenmann (1890), who defined six subfamilies:

Serraninae, Epinephelinae, Anthiinae, Grammistinae, Latinae, and Percichthyinae. After this, a lot of works changed the classification of the family in an attempt to find phylogenetic relationships within species (Katayama 1959, Gosline 1966, Kendall 1979, see Smith & Craig 2007 for further details). Johnson (1983) diagnosed the monophyletic group proposing a subdivision of the family into three subfamilies: Serraninae, Anthiinae and Epinephelinae. This author further divided the Epinephelinae subfamily into 5 tribes: Niphonini, Epinephelini, Diploprionini, Liopropomini, and Grammistini (Johnson 1988). Recently, based on molecular evidences, Craig *et al.* (2001) could observe that the Epinephelinae group is, in reality, paraphyletic. Afterwards, Smith & Craig (2007) documented that family Serranidae is paraphyletic and proposed a new classification separating Serranidae from Epinephelidae (Smith & Craig 2007).

The true groupers are included into the sub-family Epinephelinae which comprises 163 species (Craig & Hasting 2007, Craig *et al.* 2011). But many of the problems indicating that Serranidae would be a paraphyletic group were encountered within the Epinephelinae (Craig *et al.* 2001). Therefore, Craig & Hasting (2007) promoted a re-evaluation of the tribe based on analysis of 155 species (Serranidae and Acanthomorph fishes) and provided a new insight on the classification of the Epinephelini tribe. The main changes proposed by these authors to assume monophyletics groups were to remove genera *Achanthistius* and *Niphon* (distantly related percomorph lineages), to propose 11 valid monophyletic genera (one of them, the resurrected genera *Hyporthodus*, and to reclassify a number of species on different genera.

On the particular case of Mediterranean groupers, many of the taxonomic changes proposed affect these species. According to Craig & Hasting (2007) *Epinephelus marginatus*, *E. costae* and *E. caninus* would be relocated into the genera *Mycteroperca*. *Epinephelus hayfensis* would be relocated into the resurrected genera *Hyporthodus*, and only *Epinephelus* *aeneus* would not be affected by changes. However, because this new classification of Serranidae was exclusively based on molecular evidences, and they did not include strong morphologically diagnosis of characters permitting people to identify species without molecular tools, the proposed changes have not been completely accepted by the international scientific ichthyological community (Craig *et al.* 2011). As no consensus has been reached between the two principal taxonomic schools, the one based on phylogenetic relationships and the other on morphological measurable characters, I adopted the old classification until new scientific information is not available. This decision is based on most recent overview about groupers (Craig *et al.* 2011) in which the necessity to undertake further studies was highlighted to allow full application of the above molecular results.

1.2 Ecology and biology of groupers

Grouper species are generally broad-spectrum carnivorous fish (Heemstra & Randall 1993). Some species are primarily piscivorous (*Mycteroperca fusca*, *Epinephelus costae*, *M. acutirostris*) and others eat on macro- and meso-invertebrates (e.g., *Epinephelus itajara*, *E. marginatus*, *Cephalopholis fulva*). Their feeding habits make groupers to be the main predators on reef habitats, which means that they can be considered as key to maintain the structure of reef communities (Goeden 1982, Parrish 1987). This concern gives them a great ecological importance to maintain food webs, through top-down cascading effects on marine ecosystems (Braum & Worm 2009).

Groupers are distributed throughout the tropical and temperate oceans of the world, and live either in coastal zones and in oceanic islands, distributed from shallow subtidal zones to depths down to 500 m and more; also, they occur in many different habitats, usually closely associated to consolidated substrates such as rocky or coral reefs (Hemstra & Randall 1993). Some species can also be found in estuaries and mangroves, at least during some stage of their development, as is the case of *E. itajara* (Koening *et al.* 2007, Félix-Hackradt & Hackradt 2008), experiencing important movements between shelter and reproductive places (Starr *et al.* 2007, Félix-Hackradt & Hackradt 2008, Pina-Amargós & Sanzón 2009).

Individual sizes are extremely variable in groupers. The family comprises from small fishes to species reaching large sizes, sometimes measuring more than 200 cm, such as *Epinephelus itajara* (Bullock *et al.*, 1992), *E. lanceolatus* (Gomon *et al.* 1994) and *E. quinquefasciatus* (Craig *et al.* 2009). These animals generally have slow growth, and sexual maturity is reached late on the development, sometimes more than 10 years (Craig *et al.* 2011). They are long-lived species, including the smaller ones, often with a lifespan exceeding 40 years.

Much information is available in the scientific literature regarding reproductive patterns of groupers. Most species are hermaphrodites (Sadovy & Liu 2008, Craig *et al.* 2001) with a great proportion of sequential protogynous species (Sadovy & Liu 2008). But in some cases fish can act as gonochoristic species, as found on *Mycteroperca rosacea*, probably dependent on the social context (Erisman *et al.* 2008). The reproductive behaviour is very diverse in the group. Some species form spawning aggregations, gathering hundreds or thousands of fishes in the same place (e.g., *Epinephelus striatus*) (Sadovy de Mitcheson *et al.* 2008). Other species reproduce on pairs, male and female, such as *Epinephelus akaara* (Heemstra & Randall, 1993) or *E. marginatus* (Zabala *et al.* 1997). They could be a solitary territorial or living in small social groups comprising one male and several females as the case of *Cephalopholis boenak* (Liu & Sadovy 2005). After reproduction, eggs are released in the water column where they hatch. Larvae can stay four to nine weeks in the pelagic environment (Koenig *et al.* 2007), depending on the species. As a consequence, species display large spatial distribution with high degree of connectivity between populations (Zatcoff *et al.* 2004, Schunter *et al.* 2011).

Grouper species generally present a heterogeneous distribution and even show considerable plasticity to occupy different habitats (Shpigel & Fishelson 1989, Alevizon *et al.* 1985, Sluka & Sullivan 1996) and can shift even within the same type of habitat (Sluka *et al.* 2001). Among the main factors affecting the distribution of groupers are habitat complexity (Jones, 1991), habitat type (Sluka *et al.* 2001, Liu & Sadovy 2005) and topographic features of the bottom (La Mesa *et al.* 2002). Habitat selection in groupers is likely to be density-dependent and may affect the demographic parameters such as growth, survival and reproduction (Lindberg *et al.* 2006).

1.3 Fish mobility and their implications

Fish movements have considerable implications on the understanding of fish life history and behaviour, but unfortunately the aquatic environment supposes a hurdle to make studies on movement, as in many cases direct observation is not possible. Large-scale patterns of fish migration can be inferred from the catch frequency (Francis 2001, McBride *et al.* 2001, Nielsen *et al.* 2001, Griffiths *et al.* 2002; Hartgers & Buijse 2002), but most studies performed with fish handling relies on methods which involve some kind of marks or tags (dye, plastic label, implant visible, integrated passive transponder, etc.) allowing the visual identification of individuals or the use of a detection device (Matthews & Reavis 1990, Appeldoorn 1997, Zerbi *et al.* 1999, Munro 2000, Jiménez & Fernández 2001, Patterson *et al.* 2001, Brouwer *et al.* 2004). More recently, researchers have used transmitters capable of emitting an acoustic or electromagnetic signal that can be received with a radio, satellite or an acoustic receiver (Matthews *et al.* 1990, Matthews 1992, Bagley *et al.* 1994, Almeida *et al.* 1999, Ledgerwood *et al.* 1999, Thorstad *et al.* 2000, 2001, Bolden 2002, Connolly *et al.* 2002, Block *et al.* 2004). This kind of work have revealed that many species of grouper are really reef associated fishes, with strong site fidelity (Lembo *et al.* 2002). In general, they establish over a small and suitable place (their home range) where finding adequate shelter and food and all other basic requirements for their development. In theory the home range is the mechanistic basis for explaining the occurrence of biomass export from within to outside an MPA via a density-dependent diffusion process (Rodwel *et al.* 2003, Kerwath *et al.* 2009, Kellner *et al.* 2008, Zeller *et al.* 2003, Pérez-Ruzafa *et al*. 2008, Amargós *et al.* 2010), thus causing spillover.

The importance of understanding the spatial scales of movement and home range size of a target species is fundamental to help design and implementation of marine protected areas, and therefore for effective protection. Many studies have related the fish life history to protection efficiency (Claudet *et al.* 2010, Grüss *et al.* 2011), and more recently there has been a large increase in the number of studies that attempted to relate the size of the home range and movement patterns of the species with design of marine protected areas (Claudet *et al.* 2010, Langebrake *et al.* 2012).

We know that for migratory species, such as tuna and mackerel, protected areas may have little or no effect as these species spend much of their time in unprotected areas (Grüss *et al.* 2011). Even in the case of very mobile species, such as many carangid species, effect of protection can be applied to only part of the time which is spent within the boundaries of the protected area (Wetherbee *et al.* 2004, Kerwath *et al.* 2009). However, species with low mobility rate, normally site-attached with small home range, spend much of their time within the boundaries of the protected area and end up by being positively affected by protection (Kramer & Chapman 1999, Grüss *et al.* 2011).

1.4 The general conservation status of groupers

Among the most common species favoured by the reduction in fishing pressure are top predators (Shears & Babcok 2002, McClanahan *et al.* 2007, Stevenson *et al.* 2007, DeMartini *et al.* 2008, Sandin *et al.* 2008, Stallings 2009), and especially groupers (Sluka *et al.* 1997, Chiappone *et al.* 2000, Unsworth *et al.* 2007). Groupers are emblematic species around the world, as they are of great importance for both recreational and artisanal fisheries (Harper *et al.* 2000, Coll *et al.* 2004, Morales-Nin *et al.* 2005, Sadovy de Mitcheson *et al.* 2012), and also they have a great appeal on divers, whose choice of diving sites has been demonstrated to be directly related to the abundance and size of grouper species (Rudd & Tupper 2002, Félix-Hackradt & Hackradt 2008). Most species of Epinephelinae are endangered, and about half the species of groupers found throughout the oceans of the world are under some level of threat (Morris *et al.* 2000, Aguilar-Perera 2006, Sadovy de Mitcheson *et al.* 2012). The high susceptibility of grouper species to overfishing and habitat loss is likely due to their biology and life style, which promote a synergetic effect with anthropogenic activities (Eklund & Shufle 2001). High site fidelity, high longevity, late maturity, formation of spawning aggregations, slow growth rate and low resilience (5 to 14 years to minimum population doubling time) are some of the characteristics that determine a high to very high level of vulnerability of these species (Cornish & Harmelin-Vivien 2004, De Almeida Rodrigues Filho *et al.* 2009, Sadovy de Mitcheson *et al.* 2012).

During the last decades, a notable decline of their populations was observed worldwide, and particularly in the Mediterranean Sea (Chauvet 1991; Gracia 1996; Sadovy de Mitcheson *et al.* 2012). But marine protected areas are notable in replenishing and restoring endangered grouper populations (Bouchereau *et al.* 1999, Russ & Alcala 1996a, Russ & Alcala 2004, Unsworth *et al.* 2007, Harmelin-Vivien *et al.* 2007). Recent works demonstrated that groupers generally respond well to protection by increasing their abundance, biomass and mean size within their boundaries (Russ & Alcala *et al.* 1996a, Russ & Alcala 2004), including MPAs harbouring small no-take zones (Unsworth *et al.* 2007). But only in few MPAs grouper spillover has been documented away their boundaries (Russ & Alcala 1996b).

1.5 An overview of groupers in the Mediterranean Sea

The status of grouper species in the Mediterranean Sea is not different from other oceans of the world. According to Craig *et al.* (2011), six native grouper species exist in the Mediterranean: *Epinephelus marginatus*, *Epinephelus costae*, *Epinephelus caninus*, *Epinephelus aeneus*, *Hyporthodus haifensis* and *Mycteroperca rubra*. Most of the Mediterranean grouper species are under some degree of threat or species are remarked as data deficient (Craig *et al.* 2011).

The main studies on groupers in the Mediterranean Sea are primary centred on *E. marginatus*. The principal objectives of these works have focused on the estimation of population parameters (Reñones *et al.* 2007, Aronov & Goren 2008, Reñones *et al.* 2010), reproductive behaviour (Zabala *et al.* 1997a,b, Marino *et al.* 2001, Hereu *et al.* 2006), relationships with habitat variables (Harmelin & Harmelin-Vivien 1999, La Mesa *et al.* 2002), mobility (Lembo *et al.* 2002, Pastor *et al.* 2009), and the effect of marine protected areas (Reñones *et al.* 1999).

Epinephelus marginatus **(Lowe, 1834); Dusky grouper:**

This species have a large geographical range of distribution, so that they can be found in the Mediterranean Sea and Eastern Atlantic Ocean, from British islands to South Africa entering on the Indian Ocean to Madagascar, and in the Western Atlantic coast, from Southern Brazil to Uruguay and Northern Argentina (Froese & Pauly 2012). It is a necto-benthic species which is highly associated to coastal rocky bottoms (Heemstra & Randall 1993, Harmelin & Harmelin-Vivien 1999). They have a depth range distribution by size classes (Harmelin & Harmelin-Vivien 1999, Reñones *et al.* 1999, La Mesa *et al.* 2002) with habitat ontogenetic changes (Harmelin & Harmelin-Vivien 1999).

Epinephelus costae **(Steindachner, 1878); Goldblotch grouper:**

It is a demersal coastal species distributed throughout the Mediterranean Sea and Eastern Atlantic (Froese & Pauly 2012) from coast of Africa. It feed basically on fish and invertebrates (Lopez & Oilav 2005). Usually this species occurs in hard bottom and surrounding areas (Heemtra & Randall 1993) such as seagrasses (*Posidonia oceanica*). Some works report their occurrence in sandy and muddy bottoms (Craig *et al.* 2011). The IUCN red list categorizes this species as data-deficient (Heemstra *et al.* 2008) and Craig *et al.* (2011) report that this species was common in the Mediterranean and Africa in the ‘70s. Actually there is no work evaluating abundance and distribution of this species, despite being considered a common species inside Italians MPAs (La Mesa *et al.* 2006), and also being target of many artisanal and recreational fisheries.

Mycteroperca rubra **(Bloch, 1793); Mottled grouper:**

It is also a demersal species with a similar distribution of *E. costae* and inhabiting the same habitats. This is probably the less known species of the three cited here. It is generally an uncommon species, feeding primarily on fish and invertebrate (Aronov & Goren 2008). Some recent works suggest that the species form spawning aggregations on Turkey (Aronov & Goren 2008).

2. Objectives

Taking into account the majors gap on knowledge in the biology, ecology and behaviour of groupers, it is urgent to collect information which could be useful to propose management and conservation measures for the conservation of this important fish group. The present work is structured in 6 chapters, each dealing with a specific aspect. This Chapter I provide a remarks on groupers biology and a general overview of the thesis. Chapter II covers ecological aspects of the life history of groupers and combers in one marine protected area (Cabo de Palos – Islas Hormigas marine reserve) and one unprotected zone (Cabo Tiñoso). On this chapter II investigate the habitat use and habitat selection of groupers, and the effect of grouper density on these relationships, furthermore I evaluate the relations between and within species. In Chapter III I tackle the behaviour patterns of two grouper species, *E. marginatus* and *E. costae*, decoupling their patterns of activity and depth occupancy and relating the described behaviour to a set of environmental variables. Chapter IV is dedicated to investigate the home-range and mobility patterns of two species on relation to marine reserve and the relations between them. Finally, in Chapter V I explore the capability of marine protected areas to export biomass of grouper across their boundaries to surrounding, unprotected areas. In which 4 Chapters that comprise a specific study I related to each one objective described below.

Chapter II

1 Study the ecological relationships between top predators of South-Western Mediterranean rocky reefs;

1.1 Determine the abundance and spatial distribution of tree species of grouper and two species of combers in one protected and one unprotected zones;

1.2 Understand whether density can affect the distribution of those species;

1.3 To evaluate the intra and interspecific relationship of groupers and combers species.

Chapter III

2 Understand activity patterns and depth-related behaviour for* E. marginatus *and* E. costae *in Mediterranean rocky reefs;

2.1 Determine diel, seasonal and moon phase patterns of activity and depth strata occupancy;

2.2 Determine what environmental variables can affect patterns of activity and depth distribution.

Chapter IV

3 To evaluate movement of **E. marginatus** ***and*** **E. costae** ***inside a Cabo de Palos - Hormigas Islands marine reserve;***

3.1 Determine the home range and movement patterns for *E. marginatus* and *E. costae*;

3.2 Compare mobility patterns of both species;

3.3 Evaluate the responses of both species face of the MPA.

Chapter V

2.4 To evaluate the efficiency of protection measures to promote the recovery of populations of three groupers species both within and around MPAs in Western Mediterranean Sea;

2.4.1 Determine capacity of MPAs to enhance groupers biomass in the Mediterranean Sea;

2.4.2 Identify gradients on groupers biomass across MPAs boundaries;

2.4.3 Explore interference of structural habitat on grouper abundance within MPAs.

Chapter II

Habitat use by predator fishes (Serranidae) in South-Western Mediterranean rocky reefs: can density affect their distribution?

El uso del hábitat por peces depredadores (Serranidae) en arrecifes rocosos del Sudoeste del Mediterráneo: ¿Puede la densidad afectar su distribución?.

Habitat use by predator fishes (Serranidae) in South-western Mediterranean rocky reefs: can density affect their distribution?

ABSTRACT

Groupers are key species to maintain the structure of reef communities. One of the most intriguing problems related to the increase in fish density due to protection measures is to what extent density-dependent habitat selection occurs. The interest of characterizing habitat use by different grouper species in natural reef sites with distinct density levels becomes evident in order to estimate the distribution of reef habitat quality across coastal areas as a way to optimise MPA network design. We evaluated the distribution of groupers and combers in a marine reserve and an unprotected area in South-western Mediterranean Sea through underwater visual censuses, and we determined their fine-scale spatial variability. Abundance of groupers was heterogeneously distributed among habitats, occupying preferentially deep, complex and coralligenous microhabitats. Size-selective depth distribution was found in groupers, in which larger individuals occupied deeper zones and smaller ones at shallower and coastal sites. However, all these relationships are lost or relaxed under low density levels. Additionally, niche-breadth response was density-dependent, indicating that fish occupy available habitats in function of population densities. In the presence of low density of groupers, combers are more abundant and more widespread over the available microhabitats, and are better size-structured. More studies focusing on *in situ* evaluation of interspecific relationships are necessary to better understand population dynamics, for a correct fishery management and conservation of these over-exploited species.

Key-words: groupers, combers, density-dependence, spatial distribution, niche partitioning.

1. Introduction

Understanding the distribution of marine fishes relative to habitat features and availability is one of the most important concerns of marine coastal ecology in the face of climate change, in which sea warming may drastically affect habitats (West & Salm 2003). This issue has been exhaustively studied worldwide in a variety of distinct habitats such as coral reefs (Nanami *et al.* 2005, Brokovich *et al* 2006) and temperate rocky reefs (García-Charton & Pérez-Ruzafa 1998, 2001, Aburto-Oropeza & Balart 2001, Ferreira *et al.* 2001). Most studies concluded that fish species occurrence and distribution is directly related to habitat configuration (García-Charton & Pérez-Ruzafa 2001, La Mesa *et al.* 2002) and that abundance and richness can be predicted by habitat diversity or the number of micro-habitats available on a given location (Eagle *et al.* 2001, Wilson *et al* 2008).

It is known that differential habitat selection is one of the principal relationships which permit species to coexist (Rosenzweig, 1981). By selecting different shelter or food resources provided by habitats, species are able to share the same reef zones and thus to optimize reef space. However, as the reef environment is normally patchy there will be a physical limit for individuals to occupy such space, thus likely creating density-dependent relationships. One of the most intriguing problems related to the increase in fish density due to fishing protection measures is to what extent density-dependent habitat selection (DDHS) occurs (Lindberg *et al.* 2006). In their study on gag (*Mycteroperca microlepis*), which combined field observations and manipulative experiments, both using artificial reefs in the North-eastern Gulf of Mexico, Lindberg *et al.* (2006) found that this species experienced density-dependent habitat selection. By identifying natural density-dependent processes, we can understand what aspects of a population's biotic and abiotic environment are crucial candidates for protection (Hixon & Webster 2001).

Groupers are among the main predators on reef habitats, so that these species are considered as key to maintain the structure of reef communities (Goeden 1982, Parrish 1987). There are distributed throughout the tropical and temperate oceans of the world, and registered in coastal zones as well as in oceanic islands, and they are distributed from shallow subtidal zones to depths down to 500 m and more; also, they occur in many different habitats, usually closely associated to environments with consolidated substrate such as rocky or coral reefs (Hemstra & Randall 1993). Some species can also be found in estuaries and mangroves, at least during one stage of its development, as is the case of *E. itajara* (Koening *et al.* 2007).

Nevertheless, even showing a plasticity to occupy different habitats, grouper species generally have a heterogeneous distribution (Shpigel & Fishelson 1989, Alevizon *et al.* 1985, Sluka & Sullivan 1996) and can vary even within the same type of habitat (Sluka *et al.* 2001). Among the main factors affecting the distribution of groupers are habitat complexity (Jones, 1991), habitat type (Sluka *et al.* 2001, Liu & Sadovy 2005) and topographic features of the bottom (La Mesa *et al.* 2002). Habitat selection in groupers is likely to be density-dependent and may affect the demographic parameters such as growth, survival and reproduction (Lindberg *et al.* 2006). The interest of characterizing habitat use by different grouper species in natural reef sites with distinct density levels (as caused by different levels of fishing pressure) becomes evident in order to estimate the distribution of reef habitat quality across coastal areas as a way to optimise MPA network design.

The status of grouper species in the Mediterranean Sea is not different from other oceans of the world. Six native grouper species exist in the Mediterranean, four from the genus *Epinephelus* (*Epinephelus marginatus*, *Epinephelus costae*, *Epinephelus aeneus* and *Epinephelus caninus*), one from the genus *Mycteroperca* (*Mycteroperca rubra*) and one from the resurrect genus *Hyporthodus* (*Hyporthodus haifensis* synonym of *Epinephelus haifensis*) (for more details see Craig *et al.* 2011). Fortunately, grouper species are extremely favoured by protection measures in Western Mediterranean MPAs (especially the dusky grouper, and to a lesser extent, goldblotch and mottled groupers), so that average abundance, biomass and individual weight of groupers are generally much higher within the protected areas than immediately outside (Hackradt *et al.* in prep.-Chapter V).

The main studies on groupers at Mediterranean Sea are primary centred on *E. marginatus*. The other grouper species are far less studied regarding these and other aspects of their ecology. The principal objectives of these works have focused on the estimation of population parameters (Reñones *et al.* 2007, Aronov & Goren 2008), reproductive behaviour (Zabala *et al.* 1997a, Marino *et al.* 2001, Hereu *et al.* 2006), relationships with habitat variables (Harmelin & Harmelin-Vivien 1999, La Mesa *et al.* 2002), mobility (Lembo *et al.* 2002, Pastor *et al.* 2009), and the effect of marine protected areas (Reñones *et al.* 1999, Hackradt *et al.* in prep.-Chapter V).

Thus, the aims of this study were (1) to describe general patterns of spatial distribution of the three species of groupers (Epinephelinae) and two species of combers (Serraninae) in a marine reserve and an unprotected area along a vertical (depth) gradient, and (2) to characterise the habitat use of groupers and combers in the two areas with distinct levels of fish density (high *vs*. low).

2. Materials & Methods

2.1 Study organisms

Dusky groupers (*E. marginatus*) have a large geographical range of distribution, so that they can be found in the Mediterranean Sea and Eastern Atlantic Ocean, from British islands to South Africa entering on the Indian Ocean to Madagascar, and in the Western Atlantic coast, from Southern Brazil to Uruguay and Northern Argentina (Craig *et al.* 2011). It is a necto-benthic species which is highly associated to coastal rocky bottoms (Heemstra & Randall

1993, Harmelin & Harmelin-Vivien 1999). They have a depth range distribution by size classes (Harmelin & Harmelin-Vivien 1999, Reñones *et al.* 1999, La Mesa *et al.* 2002) with habitat ontogenetic changes (Harmelin & Harmelin-Vivien 1999). Goldblotch grouper (*E. costae*) is a demersal coastal species distributed throughout the Mediterranean Sea and Eastern Atlantic (Craig *et al.* 2011). It feed basically on fish (Lopez & Orvay 2005) and usually occurs in hard bottom and surrounding areas (Heemstra & Randall 1993). The IUCN red list categorizes this species as data deficient (Craig *et al.* 2011). For its part, mottled grouper (*M. rubra*) is also a demersal species with the same distribution of *E. costae* and inhabiting the same habitats, however is by far the less known species of the three (Heemstra *et al.* 2008). The other grouper species are rarer in the Western Mediterranean Sea, and very few data on abundance and ecology are provided in the literature. For their part, combers are small Serranidae predators. The painted comber (*Serranus scriba*) is a demersal, which can be found down to 150 m depth (Louisy 2006). This species is distributed on Eastern Atlantic from bay of Biscay until Mauritania, including Canary, Azores and Madeira Islands. It is also found in the Mediterranean and Black Sea (Tortonese 1986). The maximum size recorded is 36 cm. It is a synchronic hermaphrodite species, commonly found on rocky bottoms and *Posidonia* meadow (Louisy 2006). The comber (*Serranus cabrilla*) is a small, solitary and territorial fish distributed in Eastern Atlantic from English Channel to South Africa, including Cabo Verde, Canary, Azores and Madeira Islands and also the Mediterranean and Black Sea (Tortonese 1986). It is typically found on rocky bottoms surrounding crevices (Louisy 2006).

2.2 Study area

This study was conducted on rocky reefs of Cabo de Palos – Islas Hormigas marine reserve (CP) and in Cabo Tiñoso (CT), an unprotected area, both located in SE Spain (Fig. 2.1).

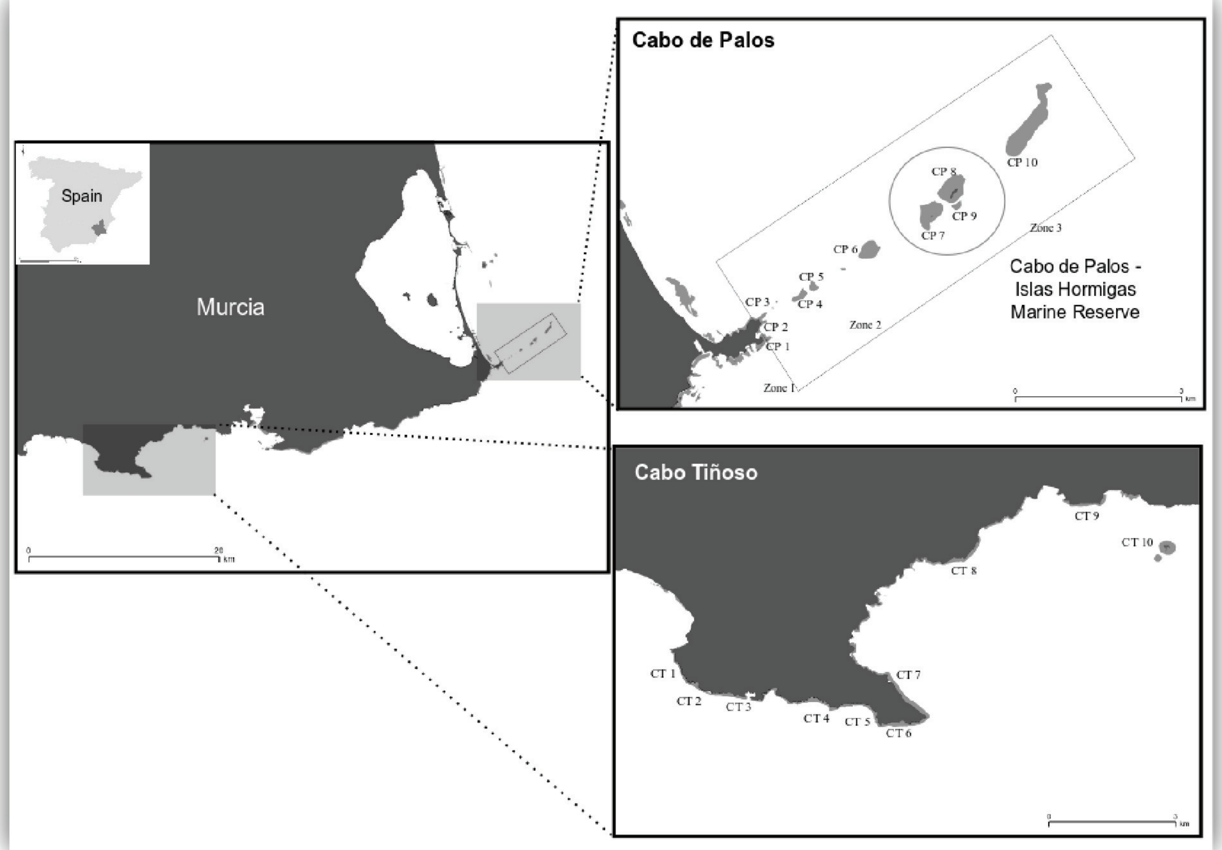


Figure 2.1: Map showing both sampling localities (Cabo de Palos and Cabo Tiñoso) ad each sampling point (10) in each locality. Sampling points were divided into 3 zones namely, zone 1: points 1 to 3, zone 2: points 4 to 6, and zone 3: points 7 to 10.

The marine reserve (37º38’ N, 0º42’ W), which was established in 1995, is rectangular in shape and occupies 1898 ha. It is divided into a no-take zone (NT) with 270 ha around the Hormigas islands where all activity is prohibited (except scientific research). The remaining area is a buffer zone (BZ), in which some local artisanal fisheries and recreational diving are allowed. The bottom in this area is formed in the shallower areas by rocky boulders of various sizes interspersed with extensive patches of *Posidonia oceanica* forming a narrow belt following the coast, while at deepest portions (>16 m) detritic formations predominate, after which a series of steep rocky shoals and small islands are aligned seaward from the cape to the north-east, where extensive algal communities cover the infra-littoral zone, while the circalittoral zone is dominated by coralligenous habitats dominated by the gorgonians

Paramuricea clavata and *Eunicella singularis* (Calvín *et al.* 1999, García-Charton *et al.* 2010). Cabo Tiñoso (37º32’ N, 1º06’ W) is located approximately 50 km to the West of Cabo de Palos. It consists of a vertical rocky reef which is the projection into the sea of a coastal cliff, with *P. oceanica* patches interspersed with small-to-medium sized rocky boulders down to the first 20 m of depth, from which large blocks covered primarily by photophylic algae dominate the submarine seascape, attaining depths > 40 m, where coralligenous habitat appears.

2.3 Sampling design

Sampling was done during spring and summer of 2009, 2010 and 2011 in Cabo de Palos and during 2010 and 2011 in Cabo Tiñoso. Within each combination of location and year, three zones separated by thousands of meters were haphazardly selected, and in each zone, three sites (S) separated by hundreds of meters were randomly located, within which six replicated 50×5-m^2 transects were performed. The spatial allocation of sampling effort was scattered in such a way that the environmental (habitat types) and depth (5-35 m) spectra covered in each locality were as wide as possible. In each transect all fish species belonging to the subfamilies Serraninae and Epinephelinae were visually censused. For each fish we visually measured its size in classes of 2 cm. In addition, for each individual we recorded its position along the transect and we estimated its distance above the bottom (in cm). Each transect was divided into five 10-m long segments in which the following environmental variables were recorded: depth (in meters); number of rocky boulders; number of holes; reef slope (in degrees, estimated visually considering three levels: 1: 0-30º; 2: 30-60º, 3: 60-90º), rugosity (adopting a visual scale ranging from 1 to 5, where 1 is a plate profile and 5 is a bottom of maximum complexity, based on Gratwick & Speight 2005); percentage substrate cover by rock, *Posidonia oceanica*, sand and gravel (estimated visually); and percentage cover by photophylic algal, calcareous algae and sessile invertebrates on rocky substrate. The latter variables was assessed taking four 25×25 cm photoquadrats haphazardly placed in each 10-m long segment of the transect; to calculate cover values, each image was subdivided into nine regular portions by positioning a grid, and in each portion the percentage of algal cover (photophylic or coralline) and benthic invertebrates was evaluated. Each transect was then characterized and classified into one of eight micro-habitats as defined by Calvín (2000), whose spatial arrangement roughly follows a depth distribution (Table 2.1).

Table 2.1: Micro-habitats available on sampled localities, Cabo de Palos and Cabo Tiñoso with code reference applied and depth range (in m) of distribution.

Micro-habitat description	Code	Depth Range
Sand or gravels bottom mixed with rock reef and boulders	ICAGI	5-20m
Posidonia oceanica meadow mixed with rock reef and boulders	ICPOS	5-20m
Shallow reef dominated by photophylic algal cover	RISAF	0-15m
Shallow reef dominated by red and coralligenous algal and sea urchins	RISCIE	0-15m
Pre-coralligenous rock reef slope dominated by photophylic algal cover	PRECORF	15-25m
Pre-coralligenous rock reef slope dominated by coralligenous algal cover	PRECORAL	15-25m
Coralligenous rock reef	CORAL	25-35m
Coralligenous rock reef dominated by gorgonian cover	CORAG	25-35m

2.4 Data analysis

To test for spatial and temporal differences in species density an analysis of variance (ANOVA) was done on data from 2010 and 2011 only. The field experimental design consisted of four factors: year of sampling (Y, random, 2 levels – excluding 2009 from the analyses in order to achieve a balanced design), locality (L, fixed, comparing the marine reserve with the unprotected area), and the spatial random factors zone (Z, 3 levels, nested in the Y×L interaction) and site (S, 3 levels, nested in Z) (Underwood 1997). All analyses were done using R software, package “gad”.

A multivariate permutational analysis of variance (PERMANOVA) (Anderson 2001) based on Bray-Curtis dissimilarities (Bray & Curtis 1957) on log-transformed data was run in univariate mode, using density of the target species as response variables to compare densities between micro-habitats and localities. We used PERMANOVA because it is a statistical procedure which is robust to unbalanced designs as this. A pair-wise comparison was done to compare means of the levels of those factors which turned out to be significant (Clarke & Gorley 2006). For these analyses, 9999 permutations were applied under a full model, using PRIMER v.6 package.

Multiple linear regressions were applied to evaluate the relationship of each species density with environmental variables separately for each location. Fish density values descriptors were exhaustively regressed to all possible combinations of habitat variables and selected using information theoretical approach. Linear models were tested using the identity link function and Gaussian distribution. Models were compared using the Akaike's information criterion (AICc), and ranked by Akaike weights (w_i) and AICc differences (ΔAICc) (using MuMIn package, R software). ΔAICc is the difference between the AICc of each model and the AICc of the best model, and Akaike weights are the probability of each model to be the best one (Burnham & Anderson 2004).

General additive models (GAM) where used to explore the relationship between mean size of each species and depth for Cabo de Palos and Cabo Tiñoso data separately. Depth was introduced as a continuous smooth variable modelled as a non-parametrically variable using a loess smoother (lo(Depth)). Gaussian distribution error and identity link functions were applied based on Hastie & Tibshirani (1990) and Venables & Ripley (2000). We use GAM because it performs well when the relationship between predictor variables is expected to be complex, not easily fitted by standard linear or non-linear models.

We determined the frequency of occurrence of each species at each microhabitat and the frequency of each microhabitat available in the environment. Differences on microhabitat occupancy were tested by homogeneity chi-square test (χ^2) separately for each location.

To measure the uniformity in the distribution of individuals among the microhabitats we calculated the niche breadths for each species using Levins' (1968) niche breadth index *B*:

$$B = 1/\left(\sum p_i^2\right)$$

where p_i is the proportion of individuals found using microhabitats, this parameter ranging from 1 (when all individuals occur in only one microhabitat) to *n*. To facilitate comparisons among species, Levins' niche breadth was standardised in accordance with Hurlbert (1978):

$$BA = (B - 1)/(n - 1)$$

where *BA* is Levins' standardised niche breadth, which is expressed on a scale from 0 to 1, where a value close to 0 represents a narrow niche breadth and high specialisation and 1 indicates a broad niche breadth and low specialization.

An electivity coefficient (*E*) was calculated separately for each species for every microhabitat category using the following formula (adapted from Vanderploeg & Scavia 1979, Eagle *et al.* 2001):

$$E = [W - (1/n)]/[W + (1/n)]$$

where *n* is the number of microhabitats, and $W = (r/p_\mathrm{k})/\Sigma(r/p_\mathrm{k})$, being *r* the proportion of microhabitats utilised by the species, and p_k the proportion of microhabitat *k* available in the environment. Positive values represent greater use of a habitat type than was available and negative represent use of a habitat category less than would be expected from its availability.

To ameliorate the interpretation of fish relationship we calculated niche overlap using Pianka index (1986):

$$O_{jk} = \sum p_{ij} p_{ik} / \sqrt{\sum p_{ij}^2 p_{ik}^2}$$

where O_{jk} is Pianka's measure of overlap between species *j* and species *k*, p_{ij} is the proportion that microhabitat *i* is of all microhabitats used by species *j*, and p_{ik} is the proportion that microhabitat *i* is of all microhabitats used by species *k*. This measure ranges from 0 (no resources used in common) to 1 (complete overlap). In contrast to niche breadth, niche overlap measures the degree to which two different species overlap in their use of a particular resource.

To understand fish relationship in function their density, we performed a linear regression of fish density on niche breadth. This analysis was performed to try to evaluate whether the uniformity in distribution along micro-habitats can be affected by the density of the species. Additionally we performed a Pearson's correlation among abundance values of species pairs, in order to evaluate the relationship between each species. Correlations coefficient (*r*) is a measure of intensity of association between two variables (Zar 1984). In this study was considered a strong correlation with *r* values range between 0.71 to 1, moderate correlation between 0.31 to 0.70 and weak correlation between 0 to 0.30. Each scatter-plot of mutual abundance was fitted using a smooth line function in R statistical package.

3. Results

3.1 Spatial distribution and habitat relationships

The density of dusky and goldblotch groupers (*E. marginatus* and *E. costae*, respectively) differed among locations (L) (Table 2.2), so that these species always occurred in higher densities at Cabo de Palos than in Cabo Tiñoso, regardless of the year (Fig. 2.2). Regarding mottled groupers (*M. rubra*), a significant Y×L interaction means that the levels of one factor are not independent of the levels of the other one; in this case, high abundances are found on 2010 in C. Palos than in 2011, on the other hand in C. Tiñoso higher abundances are found at 2011 (Fig. 2.2). We also found a significant heterogeneous horizontal variability in fish density among zones within locations [Z(Y×L)] and/or at the finer spatial scale [S(Z(Y×L)] for all grouper and comber species studied (Table 2.2).

Table 2.2: Analysis of variance (ANOVA) of density by each grouper and comber species found in Cabo de Palos marine reserve and Cabo Tiñoso. MAR: *E. marginatus*; ECO: *E. costae*; RUB: *M. rubra*; SCR: *S. scriba*; CAB: *S. cabrilla*. Factors studied: Y= year; L= Locality; Z= zone; S= site. Pseudo-F= F. Significance level: *** P < 0.001; ** P < 0.01; * P < 0.05; ns: no significance.

		MAR		ECO		RUB		SCR		CAB	
	df	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Y	2	1.03	ns	1.47	ns	2.56	ns	0.49	ns	1.15	ns
L	1	11.5	*	6.23	*	1.77	ns	5.01	ns	5.27	ns
YxL	1	1.79	ns	1.99	ns	3.95	*	1.47	ns	0.21	ns
Z(YxL)	10	3.13	***	1.86	ns	4.01	***	4.13	***	3.21	***
S(Z(YxL))	34	1.26	*	2.15	***	1.17	ns	1.22	ns	1.08	ns

On the other hand, combers showed two distinct patterns. While *S. cabrilla* followed the same pattern of groupers, greater densities of *S. scriba* were found at coastal zone (zone 1). Regarding Cabo Tiñoso location we observed the higher densities of *E. marginatus* associated to zones one and two; *E. costae* occurred mainly on zone three. Conversely comber species showed similar densities between zones in Cabo Tiñoso location. Both *E. costae* and *M. rubra* were more abundant at Cabo de Palos location, being the latter more abundant in the first year (Fig. 2.2).

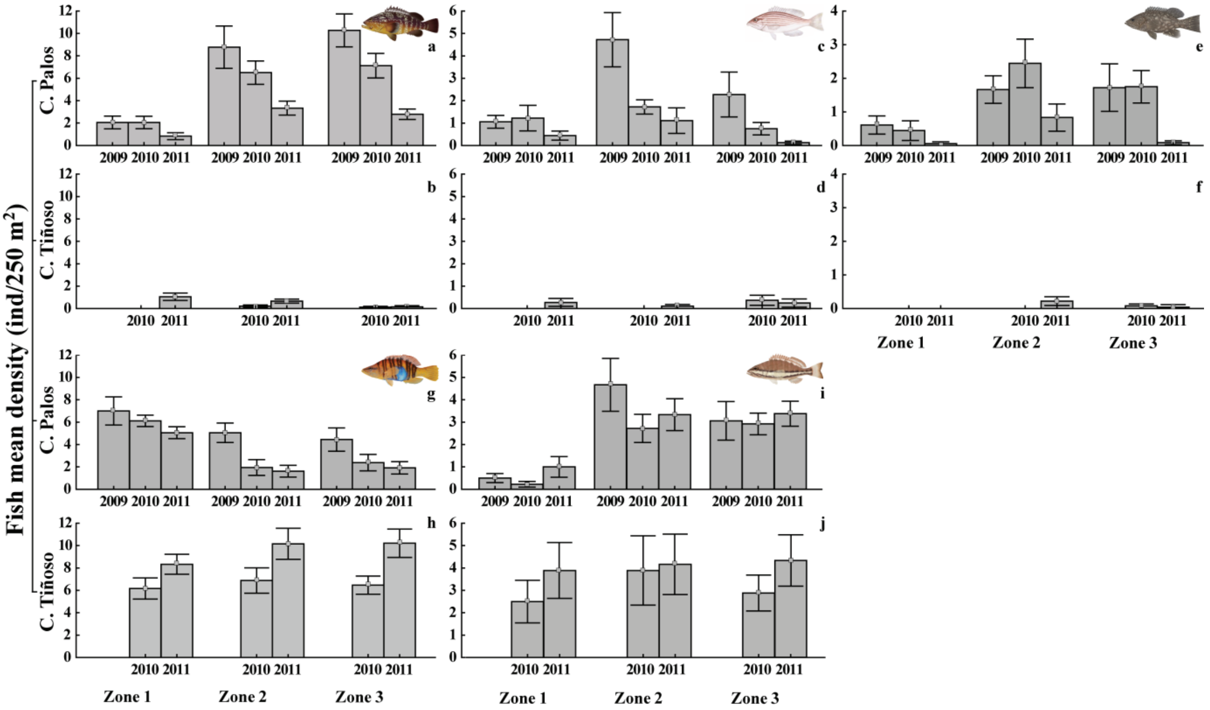


Figure2.2: Mean abundance (± SE) of each grouper and comber species, at C. Palos (Cabo de Palos - Hormigas Islands marine reserve) and C. Tiñoso (Cabo Tiñoso) in each year sampled

and zone (Zones are described on Fig. 2.1).

Depth was the most important variable explaining spatial distribution of studied species, as it appeared in the majority of models selected (Table2.3). In Cabo de Palos, *E. maginatus* distribution was related to deeper, steep rocky bottoms covered by photophylic algal with high rugosity, while *E. costae* was also related to greater depths but low coralline algal cover. Yet, *M. rubra* distribution was associated to greater depths in rocky bottoms with high rugosity. For their part, *S. scriba* abundance was higher in shallow flat habitats with low number of boulders and little sessile invertebrate cover, but with many holes. For its part, abundance of *S. cabrilla* was related to deep and steep rocky habitats with holes and also at the rock-sand interface (pers. observ.). In Cabo Tiñoso, however, no model was selected for *E. marginatus* (Table 2.3). Abundance of *E. costae* was related to gentle slopes, and that of *M. rubra* to coralline algal cover. For its part, *S. scriba* in C. Tiñoso was related to shallow habitats with important cover by sessile invertebrates, while *S. cabrilla* was more related to deeper rough habitats recovered by coralline or photophylic algae, and at sand rocky interface (Table 2.3).

Table 2.3: Habitat feature model selected using AIC (Akaike information criteria) by species determined by multiple regression. CP: Cabo de Palos marine reserve; CT: Cabo Tiñoso; AIC: value of AIC; wi: Akaike weights; Intercept and β cofficient: linear model and symbols "+" and "–" indicate slope. (B: boulders, H: holes, Rug: rugosity, Slo: slope, R: rocky, P: *P. oceanica*, S: sand, Pb: gravel, CAF: photophylic algal, CC: coralline algal, CIB: sessile invertebrate, D: depth)

	SP	**Best model**	**df**	**AIC**	**wi**	**Intercept**	**β coefficient**
CP	*E. marginatus*	CAF+D+R+Rug+Slo	7	1002.1	0.05	-20.1	0.08+0.31+0.06+1.39+2.82
	E. costae	-CC+D	4	844.1	0.01	0.6	-0.04+0.12
	M. rubra	D+R+Rug	5	739.8	0.01	-2.67	0.03+0.02+0.31
	S. scriba	-B-CIB-D+H-Slo	7	899.6	0.02	10.24	-0.03-0.9-0.19+0.05-0.97
	S. cabrilla	D+H-P-Rug+S+Slo	8	758.2	0.02	-0.60	0.23+0.04-0.04-0.82+0.08+0.55
CT	*E. marginatus*	Null	2	283.1	0.01	0.367	
	E. costae	-Slo	3	260.2	0.01	0.51	-0.19
	M. rubra	CC	3	26.50	0.01	0.0005	0.002
	S. scriba	CIB-D	4	712.9	0.01	11.34	0.08-0.31
	S. cabrilla	CAF+CC+D+Rug+S	7	681.6	0.01	-11.93	0.09+0.14+0.30+0.67+0.28

Regarding vertical distributions of species mean size (Fig. 2.3), in general we found a positive relationship of mean size with depth, evidencing that bigger individuals occupied the deeper sites, however this relationship was only significant for *E. marginatus* (F: 2.87, df: 2.8, $p<0.05$) and *S. scriba* (F: 2.96,df: 3.3, $p<0.05$), both in C. Palos (Fig. 2.3).

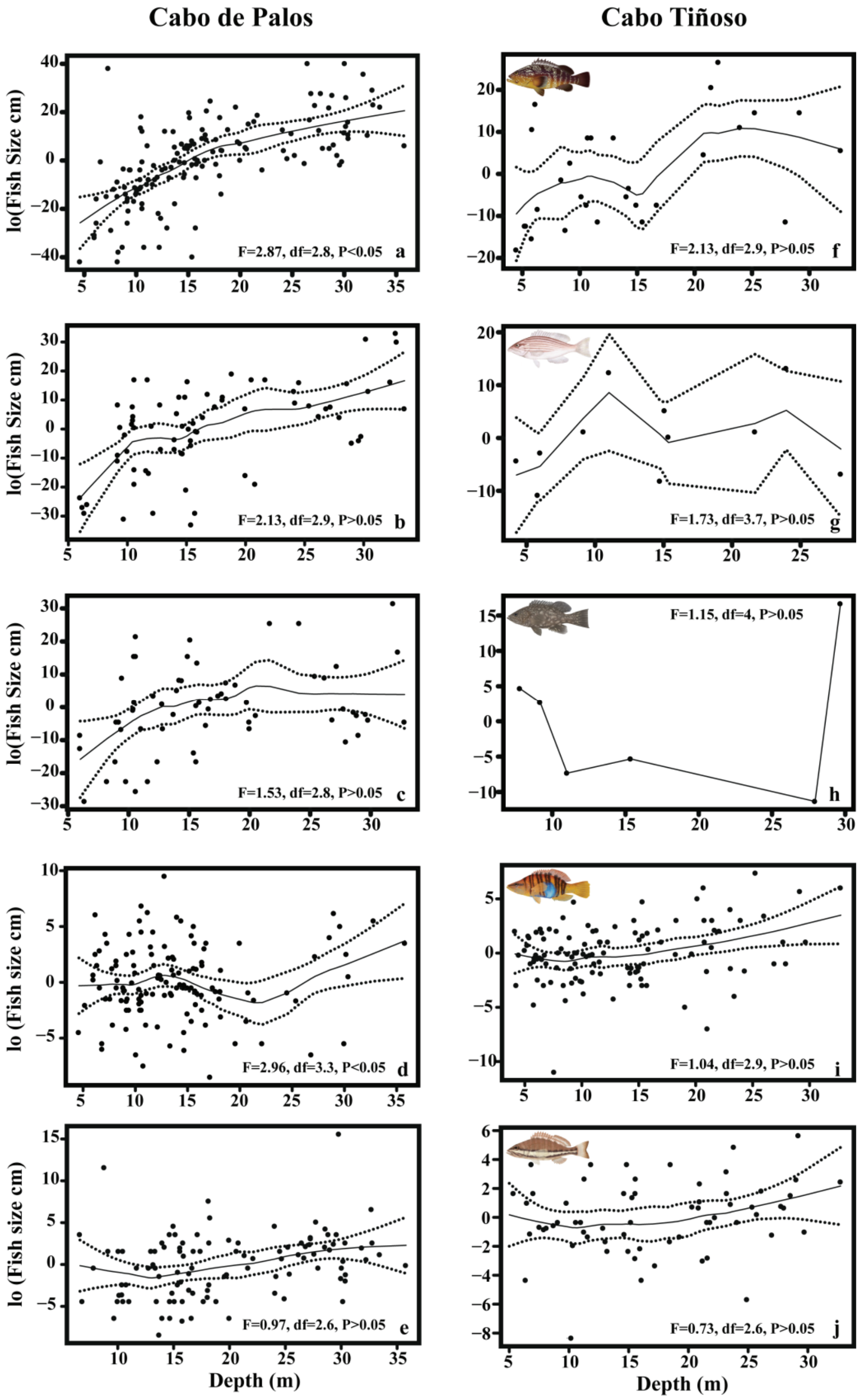


Figure 2.3: Results of generalized additive model (GAM) fitted for mean size of grouper species as a function of the smooth variable depth (m). The y-axis is scaled to zero correspond to the mean in log scale.

PERMANOVA results showed an unequal microhabitat use among locations, as highlighted by the statistical significance of the interaction between factors Location and Microhabitat (L×MH) (Table 2.4). Abundance of dusky groupers in C. Palos was higher in pre-coralligenous and coralligenous microhabitats, while in C. Tiñoso a more homogeneous distribution was found among microhabitats (Table 2.5). A similar tendency for microhabitat distribution could also be recorded for *E. costae* and *M. rubra* species. *E. costae* were evenly distributed among microhabitats in C. Palos, with a peak of abundance in CORAL microhabitat. *M. rubra* was more abundant on RISCIE, PRECORF, PRECORC and CORAL microhabitats (Table 2.5). On C. Tiñoso, both species was absent on *PRECORC* and *M. rubra* could not be registered on RISAF and PRECORF microhabitats additionally. For its part, *S. scriba* was proportionally more abundant at shallower microhabitats with *Posidonia* and sand, while higher densities of *S. cabrilla* were related to deeper zones, and at minor extent also to shallow microhabitats such as ICAGI and ICPOS (Table 2.5).

Table 2.4: PERMANOVA results for density of each, grouper and comber species found on micro-habitats (MH) studied at each locality (L) Cabo de Palos and Cabo Tiñoso, and on the interaction of orthogonal factors (LxMH). MAR: *E. marginatus*; ECO: *E. costae*; RUB: *M. rubra*; SCR: *S. scriba*; CAB: *S. cabrilla*. df: degrees of freedom; pF: Pseudo-F from PERMANOVA; Significance level: *** P < 0.001; ** P < 0.01; * P < 0.05; ns: no significance.

		MAR		**ECO**		**RUB**		**SCR**		**CAB**	
Biomass	**df**	**pF**	**P**	**pF**	**P**	**pF**	**P**	**pF**	**P**	**pF**	**P**
L	1	57.93	***	22.67	***	24.9	***	771.2	***	199.9	***
MH	7	2.36	**	1.65	ns	1.09	ns	1.10	ns	2.15	*
LxMH	6	5.85	***	1.95	*	1.95	*	2.99	***	9.6	***

3.2 Habitat distribution and niche use

In general, niche breadth (BA) values in C. Palos were very similar across species, the exception being *S. cabrilla* which presented the lower niche breadth value (Table 2.5). In C. Tiñoso, there was a higher variability of niche breadth among species; *E. marginatus*, *S. scriba* and *S. cabrilla* presented a wider niche breadth than in C. Palos, while the opposite occurred with *M. rubra*, while *E. costae* did not showed differences between locations (Table 2.5).

Table 2.5: Mean density (250 m^{-2}) and (±) standard error and niche breadth for each species for both localities. A low niche value represents a narrow niche breadth and high specialisation. Likewise, a high value represents a wide niche breadth and low specialisation

		E. marginatus	*E. costae*	*M. rubra*	*S. scriba*	*S. cabrilla*
Cabo de Palos		4.9 ±0.40	1.4 ±0.21	1.1 ±0.15	3.8 ±0.29	2.5 ±0.23
Cabo Tiñoso		0.4 ±0.07	0.9 ±0.06	0.1 ±0.02	8.1 ±0.46	3.6±0.47
ICAGI	**CP**	1.0 ±0.42	0.1 ±0.13	0.1 ±0.13	4.4 ±0.80	1.4 ±0.84
	CT	0.2 ±0.16	0.2 ±0.25	0.1 ±0.13	6.0 ±1.30	6.8 ±2.43
ICPOS	**CP**	1.5 ±0.53	0.6 ±0.27	0.5 ±0.27	5.9 ±0.55	0.5 ±0.23
	CT	0.8 ±0.32	0.5 ±0.39	0.2 ±0.10	8.7 ±1.28	3.1 ±1.30
RISAF	**CP**	2.9 ±0.55	1.6 ±0.44	0.6 ±0.22	4.7 ±0.58	0.6 ±0.23
	CT	0.1 ±0.06	0.2 ±0.14	0	8.8 ±0.85	1.3 ±050
RISCIE	**CP**	2.5 ±0.39	0.8 ±0.31	1.4 ±0.53	5.7 ±0.96	0.5 ±0.18
	CT	0.6 ±0.21	0.1 ±0.05	0.1 ±0.05	10.2 ±0.80	0.6 ±0.45
PRECORF	**CP**	7.6 ±1.07	1.4 ±0.27	1.5 ±0.37	3.9 ±0.81	3.1 ±0.54
	CT	0.2 ±0.14	0.2 ±0.19	0	8.0 ±1.30	3.8 ±1.59
PRECORC	**CP**	7.0 ±1.28	1.5 ±0.51	1.4 ±0.46	2.6 ±0.53	4.0 ±0.62
	CT	0.3 ±0.12	0	0	9.3 ±1.71	5.0 ±1.34
CORAL	**CP**	8.4 ±1.43	3.1 ±1.26	1.5 ±0.59	1.1 ±0.41	6.1 ±0.72
	CT	0.6 ±0.29	0.2 ±0.10	0.2 ±0.14	3.2 ±0.92	9.5 ±1.03
CORAG	**CP**	6.6 ±1.78	1.4 ±0.85	0.6 ±0.25	0.6 ±0.37	5.4 ±0.92
Niche breadth (BA)	**CP**	0.63	0.64	0.64	0.64	0.55
	CT	0.71	0.62	0.38	0.72	0.64

The occurrence of grouper individuals among microhabitats clearly differed between locations. A preference for deep microhabitats such as pre-coralligenous and coralligenous was recorded for *E. marginatus* in C. Palos (χ^2 = 47.8, $P<0.001$, Fig. 2.4a), while in C. Tiñoso other microhabitats apart from coralligenous were preferred, such as ICPOS and RISCIE (χ^2=32.3, $P<0.001$). Individuals of *E. costae* in Cabo de Palos preferentially occurred on microhabitats on rocky bottom (χ^2=45.8, P<0.001), while in C. Tiñoso they were more related to *Posidonia* mixed beds (ICPOS) (χ^2=47.5, P<0.001) (Figure 2.4b). For its part, *M. rubra* occurred in C. Palos in higher frequencies on intermediate-depth microhabitats (such as PRECORF and PRECORC) (χ^2=45.1, P<0.001), decreasing in abundance for both shallower and deeper habitats; instead, in C. Tiñoso an opposite pattern of occurrence was observed, with preferential occurrence for both deeper and shallower microhabitats (χ^2=114.3, p<0.001) (Fig. 2.4c). Painted combers (*S. scriba*) showed the same density distribution among microhabitats, being more frequently encountered in shallow rocky reefs and pre-coralligenous formations, and no significant differences among microhabitats between both locations (Fig. 2.4d). Combers (*S. cabrilla*), for their part, clearly preferred deeper rocky microhabitats such as (CORAL) in both localities (CP: χ^2=65.4, p<0.001; CT: χ^2=44.3, p<0.001), though ICAGI and ICPOS were also selected by this species in C. Tiñoso (Fig. 2.4e).

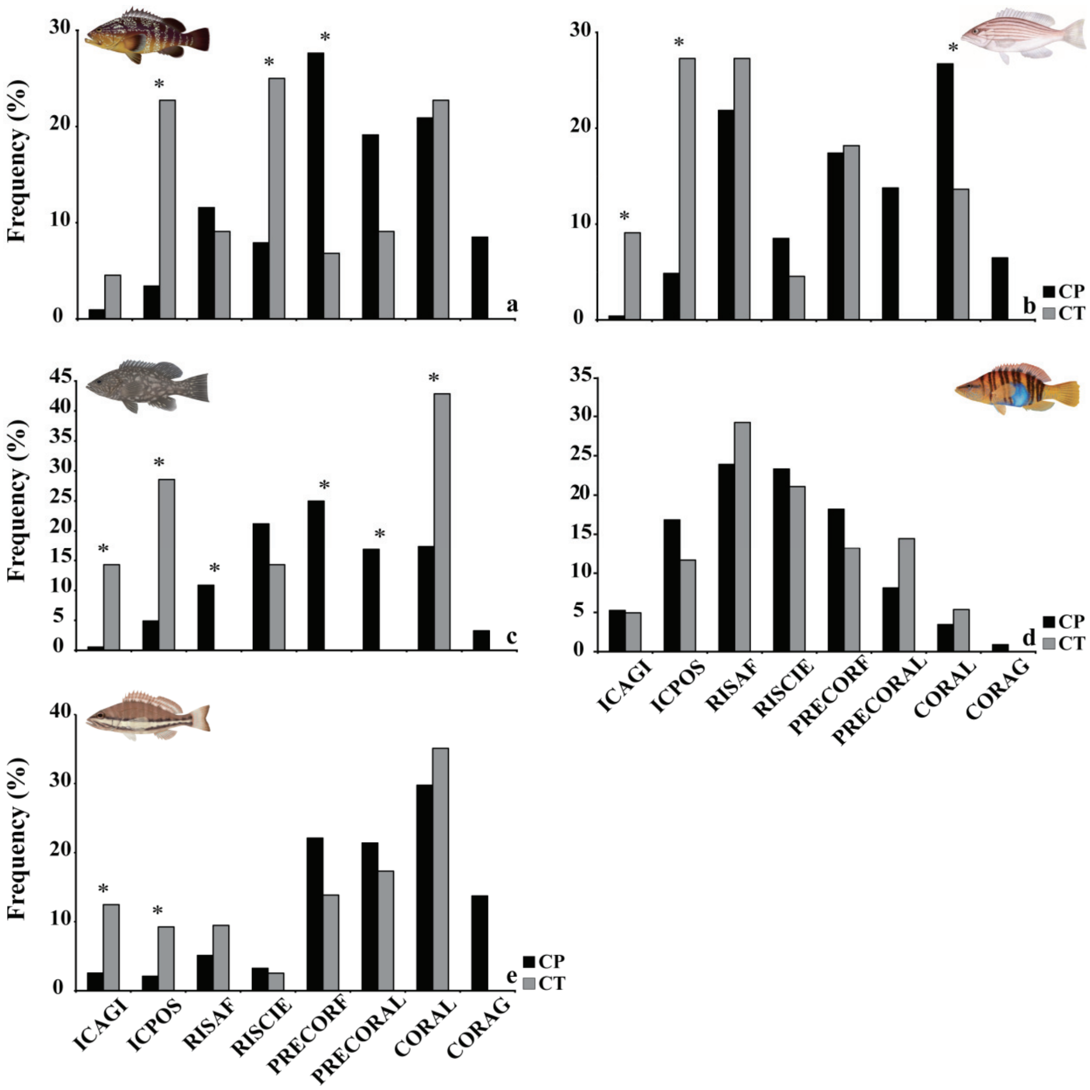


Figure 2.4: Frequency of occurrence of each specie on microhabitat available on Cabo de Palos (black bars) and Cabo Tiñoso (gray bars). * show significance difference on microhabitat preference between locality revealed by χ^2 test. a: *E. maginatus*, b: *E. costae*, c: *M. rubra*, d: *S. scriba*, e: *S. cabrilla*.

Considering the size distribution of small serranids across microhabitats (Fig. 2.5), we did not find significant differences of size-related occupancy for both locations. But in the case of the three species of groupers living in C. Palos, there was a strong size-related distribution within microhabitats in which shallow mixed bottoms (ICPOS, ICAGI) and rocky shallow microhabitats (RISAF, RISCIE) were preferentially occupied by small sized individuals, while on deeper rocky reef habitats (PRECORF, PRECORC, CORAL, CORAG) larger sized individuals predominated (Fig. 2.5a). In C. Tiñoso, groupers were generally smaller in size than in the case of C. Palos population, and a small increase in size in the deeper rocky microhabitats (PRECORAL and CORAL) could be seen only for *E. marginatus* (Fig. 2.5).

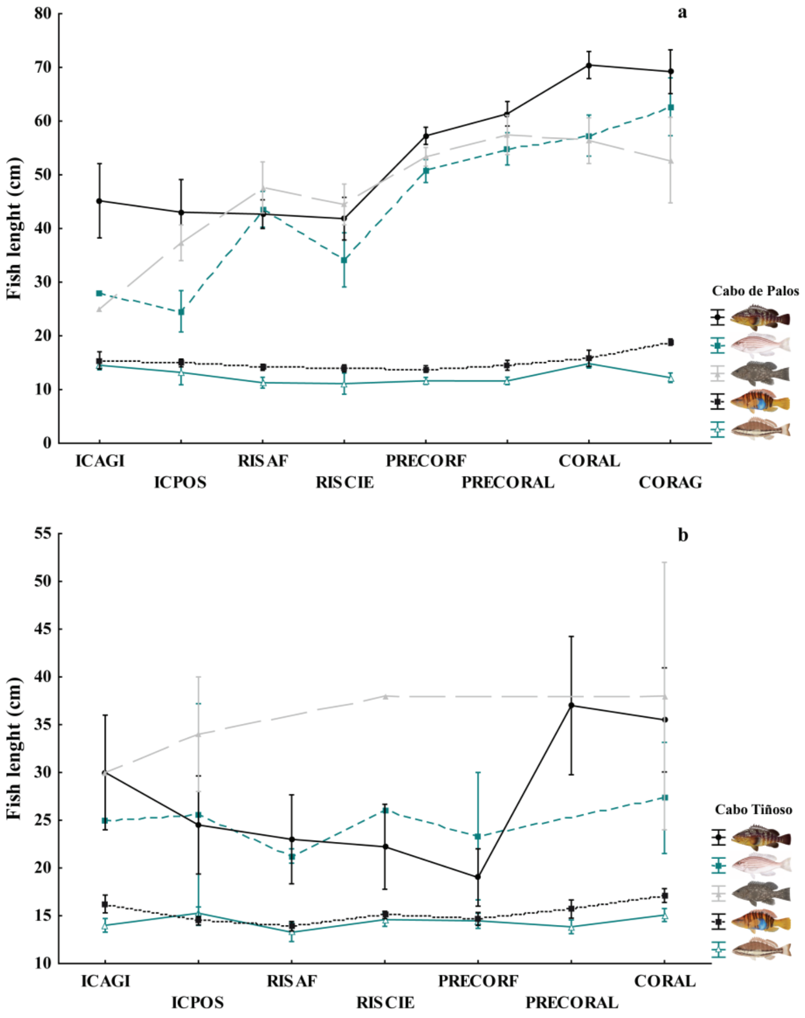


Figure 2.5: Mean fish length (TL in cm) (±SE) of each species on microhabitats available at each locality. a: Cabo de Palos Islas Hormigas marine reserve and b: Cabo Tiñoso.

The electivity index summarizes well the patterns of habitat selection for the species studied (Fig. 2.6). Microhabitats are given in order of relevance for each species and locality: *E. marginatus* showed preferences for CORAG, CORAL and PRECORAL at C. Palos and ICPOS, CORAL and ICAGI in C. Tiñoso; *E. costae* selected PRECORC and CORAL in C. Palos and ICAGI and ICPOS in C. Tiñoso; *M. rubra* selected preferentially all rocky microhabitats (RISCIE, RISAF, PRECORF, PRECORC, CORAL) at C. Palos and for ICAGI, ICPOS and CORAL in C. Tiñoso; *S. scriba* preferred ICAGI, ICPOS for both localities and additionally RISCIE in C. Palos, and PRECORF in C. Tiñoso; finally, *S. cabrilla* shared preference for deeper rocky microhabitats (CORAL and CORAG specifically in C. Palos) in both localities and ICAGI was also selected in C. Tiñoso (Fig. 2.6).

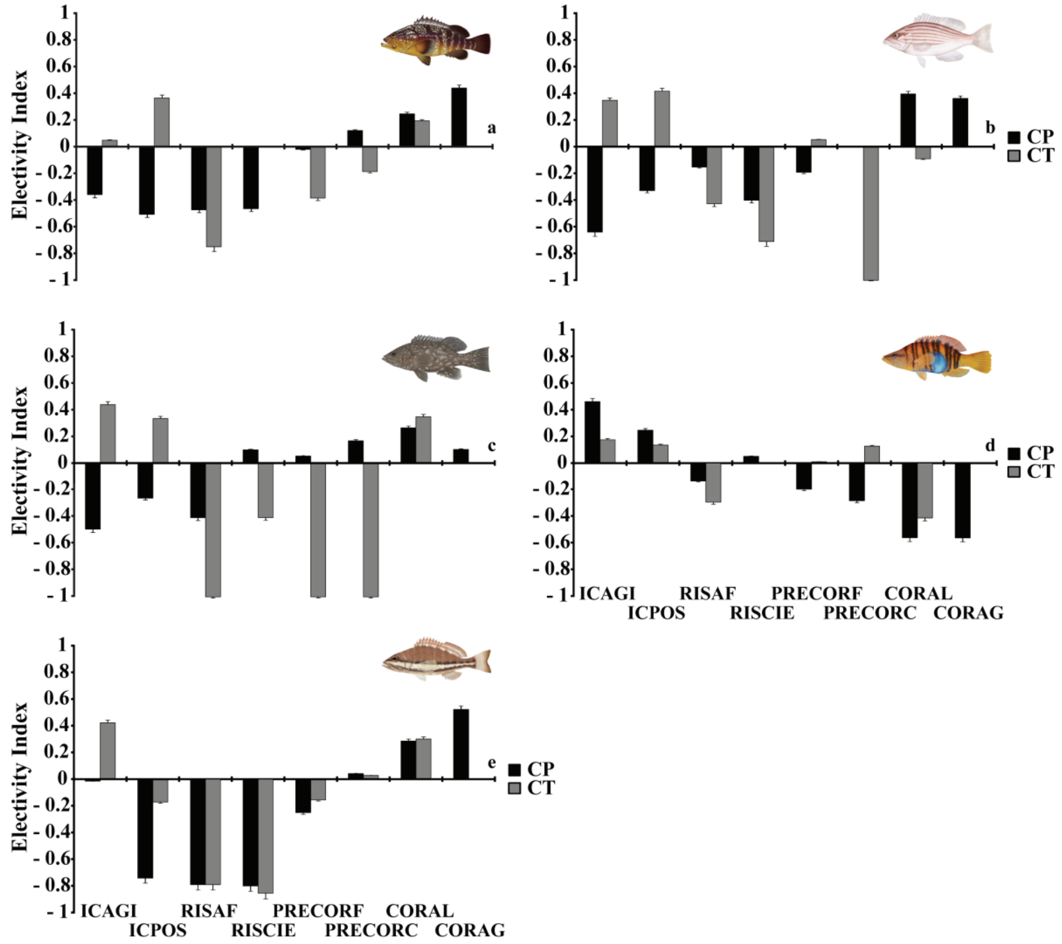


Figure 2.6: Electivity index for microhabitat of each grouper and comber specie *E. marginatus* (a), *E. costae* (b), *M. rubra* (c), *S. scriba* (d), *S. cabrilla* (e). Positive values represent greater use of a habitat type than was available. Negative values represent use of a habitat category less than would be expected from its availability.

Analysing the results of linear regressions identify for all species, except *S. scriba*, a positive relationship between niche breadth and fish density (Fig. 2.7).

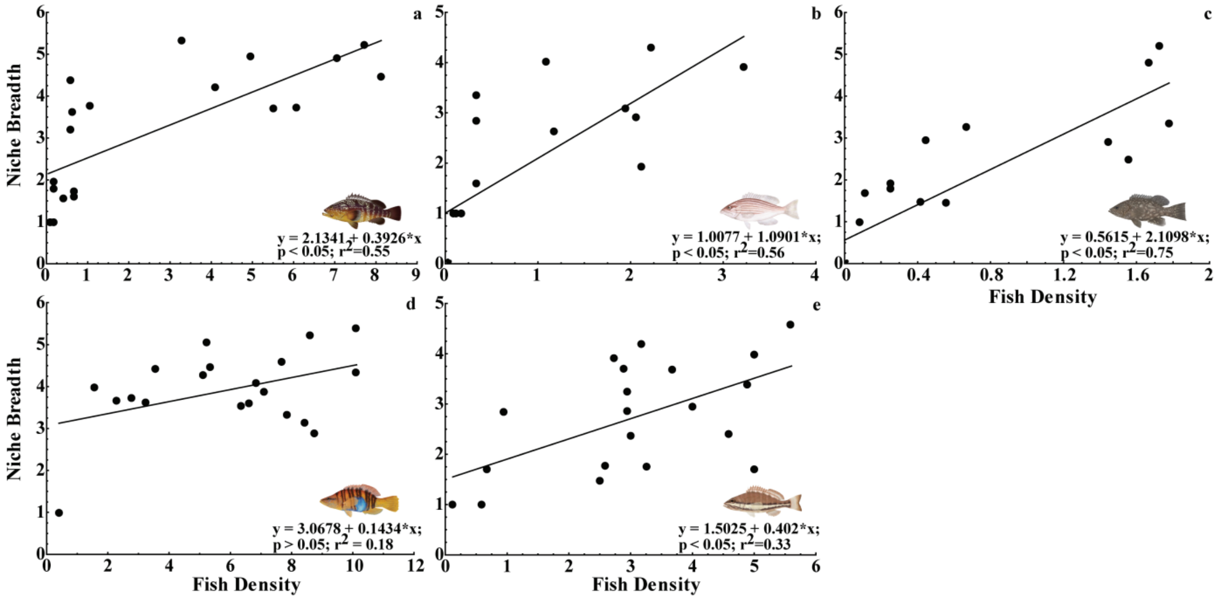


Figure 2.7: Linear regression between niche breadth index and fish density (250 m^{-2}) in each sampling point (10) at two sampling localities, Cabo de Palos-Hormigas Islands marine reserve and Cabo Tiñoso. a: *E. marginatus*; b: *E. costae*; c: *M. rubra*; d: *S. scriba*; e: *S. cabrilla*.

3.3 Inter-specific relationships

Significant correlations were found among abundance values(ind./250m^2) of the studied species. Correlations between grouper species were positive; *E. marginatus vs. E. costae r* = 0.48; *E. marginatus vs. M. rubra r* = 0.47 and *E. costae vs. M. rubra r* = 0.56; and significant in all cases (P < 0.05). (Fig. 2.8). For their part, significant negative correlations (P < 0.05) between each grouper species with the abundance of *S. scriba* were observed; *E. marginatus vs. S. scriba r* = -0.24; *E. costae vs. S. scriba r* = -0.15 and *M. rubra vs. S. scriba r* = -0.13. For *S. cabrilla* we did not identify any significant correlation with groupers species but a negative significance correlation occurs between both combers species; *S. scriba vs. S cabrilla r* = -0.36 (Fig. 2.8).

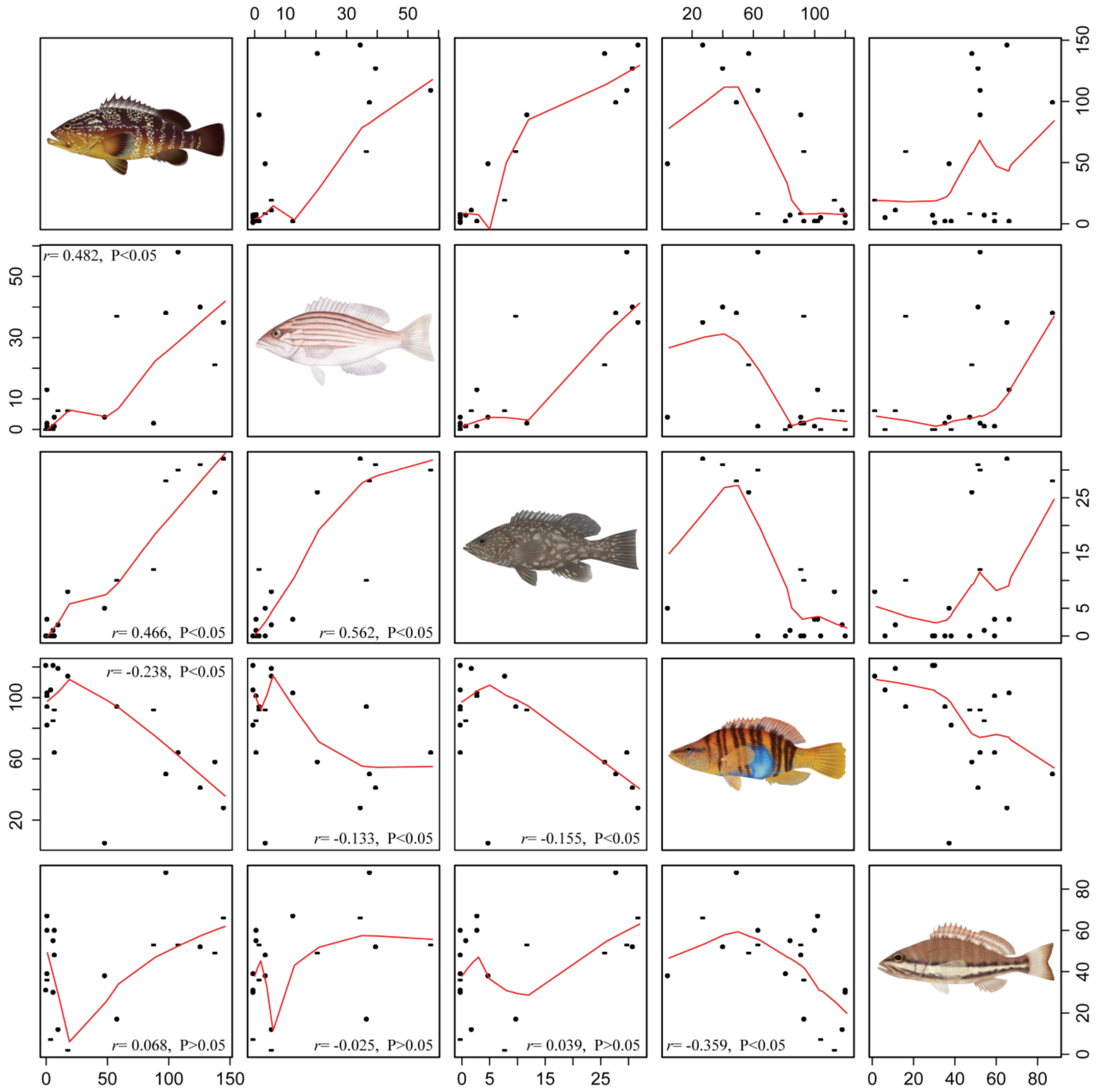


Figure 2.8: Pearson correlation (R) and significance level (p) of fish abundance (ind.250m^{-2}) between Serranidae species pair found on Cabo de Palos Hormigas Islands marine reserve and Cabo Tiñoso. Tendency line was fitted as a smooth line function. R values between 0-0.30 are considered weakly correlated, moderately correlated between 0.31-0.70 and strongly correlated between 0.71 - 1; P value of correlation is also indicated.

In general, the niche overlap between grouper and comber species was higher for C. Palos than C. Tiñoso data, ranging from 0.48 to 0.95 in the former case and 0.39 to 0.85 in the latter one (Fig. 2.9). The higher overlap values were observed between grouper species, although the highest value in C. Palos was between dusky groupers (*E. marginatus*) and combers (*S. cabrilla*), while the lower value in the marine reserve was observed between both comber species. In the case of C. Tiñoso, the lowest overlap value was observed between mottled groupers (*M. rubra*) and painted combers (*S. scriba*) (Fig. 2.9).

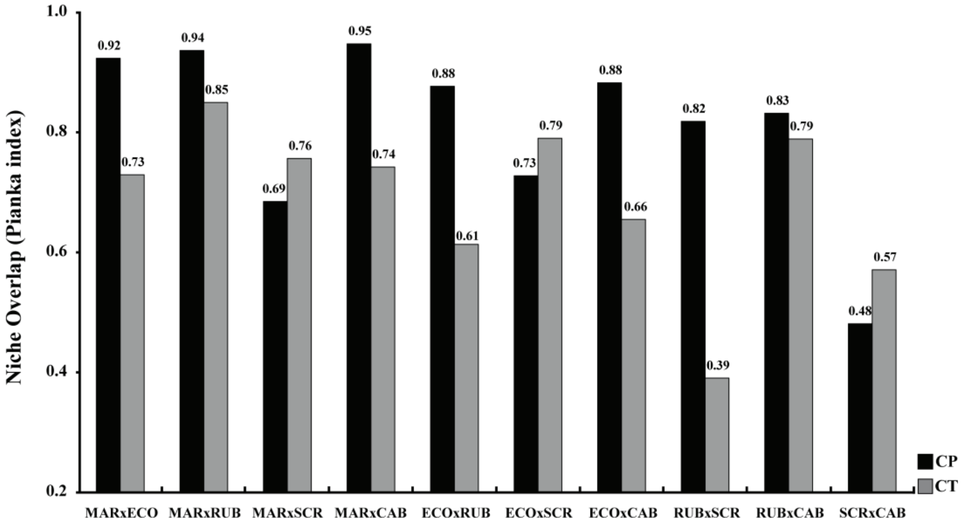


Figure 2.9: Niche overlap (Pianka index) between species pairs on Cabo de Palos Hormigas Islands marine reserve (black bars) and Cabo Tiñoso (Gray bars). MAR: *E. marginatus*; ECO: *E. costae*; RUB: *M. rubra*; SCR: *S. scriba*; CAB: *S. cabrilla*.

To better understand distribution of fishes, we assessed distance from reef of each animal and performed a one-way ANOVA ($F_{(4,3842)} = 375.5$; $P < 0.05$) to identify significant differences. *E. marginatus*, *S. scriba* and *S. cabrilla* were the most site-attached species while *E. costae* and *M. rubra* showed the greatest range of vertical movements in the water column (Fig. 2.10).

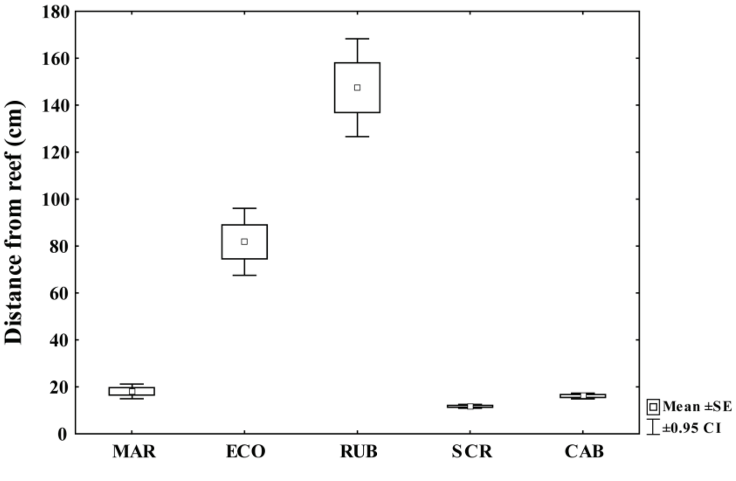


Figure 2.10: A Box-plot of mean distance from groupers and combers in relation to reef slope. MAR: *E. marginatus*; ECO: *E. costae*; RUB: *M. rubra*; SCR: *S. scriba*; CAB: *S. cabrilla*.

4. Discussion

Habitat structural complexity, risk of predation and interspecific competition are important factors affecting habitat selectivity in fish (Jordan *et al.* 1997, Munday *et al.* 2001, Schofield 2003). Nonetheless, intra-specific relationships such as co-specific abundance or density-dependent survivorship can affect the coexistence among individuals (Holbrook & Schmitt 1995), and thus ultimately determine habitat selection (Lindberg *et al.* 2006).

As worldwide groupers populations are under some level of threat (Craig *et al* 2011) owing to their high economic value to fisheries (Sadovy de Michelson *et al.* 2012), stable and high density populations could only be found nowadays inside marine reserves (Hackradt *et al.*, in prep.). Although it is not the purpose of this work, a clear evidence of protection effect could be illustrated for some of the studied species. All groupers (*E. marginatus*, *E. costae* and *M. rubra*) were more abundant in the protected than in the unprotected location; it is noteworthy that the opposite occurred for combers, being more numerous at Cabo Tiñoso. Thus, the spatial closure of a determined amount of area has a direct impact on population density and demography of commercially important species (Garcia-Charton *et al.* 2008) as those of Epinephelinae sub-family. These changes could have huge effects on intra- and interspecific relationships as groupers are important predators on marine ecosystem.

Both studied locations were very similar regarding habitat structure. Still, spatial distribution of groupers was highly heterogeneous. The principal differences between places probably can be attributed to the high level of fisheries on C. Tiñoso. But at C. Palos, a small spatial scale differences on abundance can be found and probably can be attributed to habitats features and microhabitat availability. This seems to be a recurrent pattern in groupers population in different locations and habitats (Sluka *et al.* 2001). Such fine-variation could be explained by a differential response of each species to microhabitat features as suggested by La Mesa *et al.* (2002). We can observe that depth and habitat heterogeneity are important factors explaining Serranidae distribution in our sampling area. But when population density is critically reduced, by fishing activities for example, habitat features probably have lower importance on shaping distribution patterns within species.

We hypothesize that smaller and therefore younger individuals were constrained to shallower areas, and a subsequent displacement to deeper zones is gradually attained as they increment in size. This size-related distribution is supported by the present data only for *E. marginatus* at C. Palos. Mean size of groupers inhabiting the shallow microhabitats (mixed reef with sand or gravels bottom (ICAGI), reef mixed with *Posidonia oceanica* meadows (ICPOS) and shallow reefs (RISAF and RISCIE)) were significantly smaller than those recorded at deeper microhabitats (pre-coralline (PRECORF and PRECORC) and coralline (CORAL and CORAG)). This size related habitat partition suggests the existence of ontogenetic movements (Egglestoon 1995, Sluka *et al.* 2001) in the case of groupers but not of combers. Previous works highlighted the importance of depth on structuring for *E. marginatus* population (Chauvet 1991, Harmelin & Harmelin-Vivien 1999, Reñones *et al.* 1999, 2007, Lenfant *et al.* 2003, La Mesa *et al.* 2006). However, La Mesa *et al.* (2002), based on observation of fishes between 40-80 cm (TL), indicate that dusky grouper does not shift toward deeper waters when increase size, but rather they enlarge their bathymetric range. Conversely, Reñones *et al.* (2007) stated that microhabitat play an important role on structuring size class and age distribution, and suggested that if suitable microhabitat is available, then fish will use it regardless of the depth range. Nevertheless, it is a difficult task to separate effects of microhabitat and depth, because sometimes they co-vary. More investigation is needed to decouple this intrinsic relationship between microhabitat, depth and fish distribution (Berkström *et al.* 2012).

In theory fish tend to select others microhabitats than the "optimal" when in high densities (Sheperd & Litvak 2004). This was the case of combers, which in Cabo Tiñoso selected additional microhabitats avoided in Cabo de Palos. However, the same pattern did not happen to groupers, which occurred in suboptimal microhabitats such as shallow areas, when in lower densities. As groupers displayed size-related depth distribution and that groupers population in Cabo Tiñoso is composed by small sized individuals due to fishing pressure, we believe that selection of suboptimal microhabitats is related to population size structure, as smaller groupers prefers shallower habitats.

According to the ideal free distribution theory, an individual choose the preferred habitat to settle based on habitat suitability, aiming maximizing their fitness (Morris 1987). Habitat suitability is considered density-dependent when at higher densities of individuals the "suitability" of such habitat is reduced, causing individuals to occupy areas "not optimal" with increasing abundance. This statement reflects a positive relationship between geographical area occupied and population abundance. However according Shepherd & Litvak (2004) such evidence cannot be attributed solely to density-dependent habitat selection because they may conceivably arise from environmental mechanisms, and if movement between habitats is limited (not ideal and free) then environmental effects can lead to patterns predicted by density-dependent habitat selection. Differently from other works that related abundance and area to evidence patterns of habitat selection (Sheperd & Litvak 2004), we used a measure of the uniformity of distribution of individuals among microhabitats, like presented to Berkström *et al.* (2012). The niche breath index used here incorporates habitat suitability as it is a measure of species distribution according to microhabitat availability. Thus, the positive relationship found between niche breadth and density of species studied, is a good indicator of the existence of density-dependence, as individuals are free to move between available microhabitats.

Regarding interspecific relationships, we found positive correlation between abundance of all grouper species and negative relationships between groupers and *S. scriba* and between comber species. When data from niche overlap is taken into consideration, the same relationships could be depicted by spatial relationships of the habitat, with high overlap between groupers, and small overlap between *S. scriba* and *E. marginatus* and between comber species. Although niche overlap of groupers is high, it could be observed that 3 species spatially partition their reef environment by occupying distinct strata on the water column. While *E. marginatus* is highly site attached, *E. costae* and *M. rubra* undergo huge vertical movements. Taking into account that *E. marginatus* eats mainly crabs, cephalopods and bony fishes (López & Orvay 2005, Reñones *et al.* 2007) and other groupers (*E. costae* and *M. rubra*) are essentially piscivorous (López & Orvay 2005, Aronov & Goren 2008), it could be asserted that although spatial niche is overlapped, feeding niche may be not. Although species are found in the same kind of habitat and that rocky habitat is very complex providing a lot of refuges, these refuges could potentially harbour greater densities, making space a less limiting factor than food itself. Moreover, the distinct dietary habits associated to a differential occupation of water column can avoid food, shelter or any kind of competition, allowing coexistence.

For their part, *Serranus* species are both strongly site-attached and feed on invertebrates and small fish (Relini *et al.* 2002) belonging to the same trophic level (Vizzini & Mazzola 2009). However, differently from groupers, which segregate their diet to avoid competition, combers displayed distinct depth and microhabitat requirements, and thus segregated spatially their distribution, as observed by Fasola *et al* (1997).

Finally, we were able to observe a negative effect of groupers on abundance of painted combers (*S. scriba*). As the abundance of meso-predators may increase in the absence of top predators (as observed in C. Tiñoso), we suggest the existence of a controlling mechanism on *S. scriba* population through cascading top-down effects (Baum & Worm 2009), reinforcing top-predators key-role on healthy ecosystems.

5. Conclusions

This work provides useful and new information about habitat use and sharing by Serranidae species in rocky reefs of the Southwestern Mediterranean Sea. A positive effect of protection could be evidenced on grouper density inside the MPA. This effect is noticeable by a restoration of the population demography of groupers species and the recovery of a pristine population structure, in which size classes are well represented. Here we demonstrated that abundance of groupers is heterogeneously distributed among habitats, occupying preferentially deep, complex and coralligenous microhabitats. Vertical distribution is size-selective in which larger groupers are found at deeper zones and smaller ones at shallower and coastal sites. All these relationships are lost or relaxed under low density levels, i.e., under fishing pressure. Additionally, niche-breadth response was density-dependent, indicating that fish occupy available habitats in function of population densities. In the presence of low density of groupers, combers are more abundant and more widespread over the available microhabitats, and are better size-structured. More studies focusing on *in situ* evaluation of interspecific relationships are necessary to better understand population dynamics, for a correct fishery management and conservation of these overexploited top predators species.

Chapter III

Activity patterns and depth distribution of groupers (Serranidae; Epinephelinae) revealed by acoustic telemetry in temperate rocky reefs at South-western Mediterranean Sea: A preliminary approach.

Patrones de actividad y la distribución a lo largo de la profundidad de los meros (Serranidae; Epnephelidae) a la luz de la telemetría acústica en los arrecifes rocosos templados del Sudoeste del Mediterráneo: Un análisis preliminar.

Activity patterns and depth distribution of groupers (Serranidae; Epinephelinae) revealed by acoustic telemetry in temperate rocky reefs at South-western Mediterranean Sea: A preliminary approach.

ABSTRACT

Studying and interpreting fish behaviour constitute an essential task to promote an adequate stock management and guarantee effective population conservation. The use of space and habitat is directly related to fish behaviour. To understand how fish behaviour can be affected by spatial measures of management, such as MPAs, we installed an array of VR2W receivers at Cabo de Palos Islas Hormigas marine reserve, South-western Spain and marked a total of 38 groupers (30 dusky groupers, *Epinephelus marginatus* and 8 goldbloch grouper *Epinephelus costae*) with coded acoustic transmitters. For a period of 6 months (Winter and Spring/ 2012) we monitored fishes passively. Seasonal and daily activity patterns were evaluated as regard to environmental data. Both species showed a seasonal influence on activity patterns, moving to deeper waters and diminishing the number of relocations (proxy for activity) on cold months. Also, we identified that storm periods can have great influence in groupers behaviour, reducing the number of relocations number during these events.

Key word: pattern of activity, depth distribution, moon phases, storm influence.

1. Introduction

Marine fish show a surprising variety of behaviours. The behaviour can implicate on a variety of patterns of movement, often affecting fishery and consequently their management. Wide migrations, as shown by many fish groups like salmonids (from sea to the river), anguiliformes (from river to the sea) or engraulids (from marine environment to estuarine) (Brege *et al.* 1996, Beckley & van der Lingen 1999, Dare & Potter 2003), are usually studied in large scales, analyzing the process as a whole, not focusing on small scales movements mediated by behaviour. Large scale movements are more predictable, which facilitates the establishment of a target fisheries and management tools (Dare & Potter 2003).

Movements of nomadic species are stochastic in nature (Gillanders *et al.* 2001, Block *et al.* 2004, Fromentin *et al.* 2004), and typically related to factors such as abundance of prey or water temperature (Davis & Stanley 2002). Demersal fish, particularly reef-associated species, use a small area of a habitat for long periods of time (Matthews *et al.* 1990, Zeller 1997, Griffiths & Wilke 2002). This pattern of use of space can be highly detrimental to a population due to high risk of local extinction by overfishing (i.e. Koening *et al.* 2000). However, the same behaviour make conservation strategy easier to be applied through simply closing area by creating a marine protected area (MPAs).

MPAs have been advocated as an alternative tool for the conservation of endangered species and ecosystems (Polacheck 1990, Bohnsack 1996, Attwood *et al.* 1997, Griffiths 2000). A detailed knowledge of the behaviour of species is necessary to decide what proportion of the habitat must be protected in order to provide a positive effect on the stock exploited. Mixed behavioural strategies within species and populations (Dingle 1996), selection pressure imposed by fishing (Law 2000) and structural differences in habitat and oceanographic conditions, result in large variations in the area used by fish. Quantifying these effects can provide a compelling argument for closing an area to fisheries exploitation

(Alonzo & Mangel 2004, Brannan *et al.* 2003, Barot *et al.*, 2004; Bertolo *et al.* 2004).

The tribe Epinephelini comprises a group of coastal, usually reef-associated fishes commonly known as groupers, which include more than 150 species distributed in 11 genera (Nelson 2006, Craig & Hastings 2007). These animals are widely distributed in all oceans, occurring in both tropical and subtropical areas, from subtidal depths down to more than 200 m. Their characteristic body shape is very easily recognizable by its large head and mouth (Heemstra & Randall 1993). Grouper species present a wide range of size classes and some of them can reach sizes greater than 2.5 meters (e.g., *Epinephelus itajara*). Generally these fishes are carnivorous, feeding swallowing large amounts of water by opening the mouth, which harbours inwardly-depressible sharp teeth to hold the food and swallow it whole (Heemstra & Randall, 1993; Sluka & Sullivan, 1996a). With rare exceptions, groupers tend to be solitary animals with high site fidelity and intimate association with habitat (Craig *et al.* 2011). However, the vast the majority of species, which are protogynous hermaphrodites, spawn in pairs (e.g., *Epinephelus akaara*), or in a large number of individuals forming spawning aggregations (such as the case of *Epinephelus striatus*) (Heemstra & Randall 1993). Once settlement habitat is chosen, individuals tend to spend most of their time within it (Farmer & Ault 2011). Because of their trophic level they have a high ecological value and are considered as key species in the reef community (Goeden 1982, Parrish 1987).

Groupers usually have small home ranges (Pastor *et al.* 2009) and can undergo small movements to surrounding areas possibly for feeding and/or reproduction (Sluka 2000). Besides the great habitat fidelity exhibited by some species (Lembo *et al.* 2002), recent studies suggest that groupers present homing movement, i.e. they are able to recognize and locate themselves within their area of use, often returning to their place of origin (Spedicato *et al.* 2003, Kaunda-Arara & Rose 2004a).

The Mediterranean Sea is the habitat of seven species of groupers; 5 native species and

2 immigrants from the Red Sea (Louisy 2006). Among the native species, the dusky grouper (*Epinephelus marginatus*) is the most emblematic, abundant and well studied. This species lives in rocky reefs throughout the entire Mediterranean basin (Harmelin-Vivien & Harmelin 1999). Juveniles are restricted to shallow reefs of coastal zones, while adults seek refuges at greater depths (Harmelin-Vivien & Harmelin 1999, Reñones *et al.* 1999, Hackradt *et al.* in prep.). Their distribution is closely related to the large-scale characteristics of the habitat (La Mesa *et al.* 2002) and may vary with habitat complexity and heterogeneity (Hackradt *et al.* Chapter 1). They normally do not move apart from reefs for too long (Gibran 2007). Their principal feeding tactic to feed on cephalopods, crustaceans and fish is the 'sit and wait' (Gibran, 2007). Supposedly, dusky grouper does not form spawning aggregations as other grouper species do (Hereu *et al.* 2006), however small and localized groups can be formed during the reproductive period (Zabala *et al.* 1997a). These groups make up hierarchical formations leaded by dominant males which are maintained during the entire reproductive season (Zabala *et al.* 1997b). It is known that temperature and meteorological conditions can influence on their reproductive behaviour (Hereu *et al.* 2006). Yet, few or no information about behaviour patterns of activity and habitat use are available outside the reproductive season (summer).

The goldblotch grouper, *Epinephelus costae,* is the second most abundant grouper living in Mediterranean rocky reefs; nevertheless, very little information is available about this species, since most work done to now focused on ex-situ larval development and breeding (Glamuzina *et al.* 2000, 2003). It is also typical from rocky reefs, although usually their abundance is lower than that of dusky groupers (Hackradt *et al.* in prep.). This species is mostly related to environments exposed to prevailing winds and swell (La Mesa *et al.* 2006), and recent studies have observed that they are directly affected by microhabitat availability (Hackradt *et al.* in prep. - Chapter II). Noteworthy, no data are available concerning activity patterns and behaviour for that species to date.

In this study we aimed to fulfil a gap of knowledge on the ecology of groupers by providing information on the activity patterns and depth-related behaviour for *E. marginatus* and *E. costae* in Mediterranean rocky reefs trough acoustic telemetry techniques. Also, we further investigate whether the displayed patterns of activity could be influenced by a set of environmental variables related mainly to structural habitat.

2. Materials & Methods

2.1 Study area

The study was conducted in the coast neighbouring the cape of Palos (37°38'N, 0°42'W) (Murcia, Southeast Spain) in SW Mediterranean Sea (Fig. 3.1).

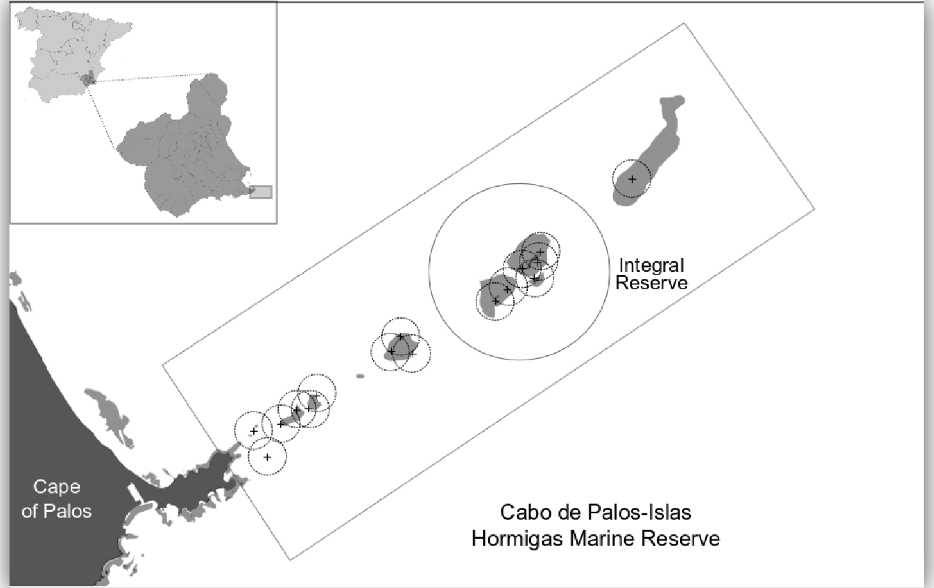


Figure 3.1: Map of Cabo de Palos - Islas Hormigas marine reserve and the position of the array and each VR2W receiver position. +: indicate VR2W position; dashed line indicate range of detection of each VR2W (~250 m).

The area is dominated by rocky reefs surrounded by sandy and detritic bottoms (Pérez-

Ruzafa *et al.* 1991, García-Charton & Pérez-Ruzafa 1998). At shallower areas rocky boulders of various sizes interspersed with extensive patches of *Posidonia oceanica* form a narrow belt following the coast, while at deepest portions (>16 m) detritic formations predominate, after which a series of steep rocky shoals and small islands are aligned seaward from the cape to the north-east. In this area, the Cabo de Palos – Islas Hormigas marine reserve was declared in 1995, encompassing a total surface of 1,934 ha, including a 270-ha no-take area around the Hormigas islets, where all uses are prohibited (except scientific research), while in the remaining MPA some artisanal fishing and diving activities are allowed (García-Charton *et al.* 2010).

2.2 Fish capture

Groupers were captured by using artisanal fishing traps in depths between 15 and 30 m. Field campaign to capture fishes started in October 2011 until mid-November 2011. Fish traps were installed manually by scuba diving, and placed near burrows and crevices where target species are commonly found. After installation of the traps, we put inside each trap bait composed of pieces of octopus (Octopus vulgaris) and squid (Loligo spp.). The traps were verified after a period ranging from 12 to 24 hours, depending on weather conditions. In order to increase catches, we did underwater fishing using line-and-hook and using squid as bait. The hooks used were previously devoid of barb to minimize the damage it could cause to the animals.

2.3 Transmitter implantation

After capture, the animals were taken to the boat and placed in a tank filled with seawater. Fish caught were measured at total length (TL in cm) and weighted (in g) and after that an external mark (dart tag) was inserted to allow further identification both underwater and in case of recapture. After tagging, a small incision of 10 to 20-mm in length was done between pectoral fins in order to insert the transmitter (model V9P or V13P, VEMCO Inc., depending on size of the animal) into the abdominal cavity. Surgical glue was used to close the incision. To prevent infections we applied an external layer of antiseptic and also a dressing layer in spray to avoid the direct contact of the wound with seawater or the bottom. Finally, animals were released in the same location where they were caught. Each marked individual was followed visually while descending to the bottom to ensure that they were in good conditions. Marked individuals were then monitored by scuba diving in all marking sites to ensure that all fishes exhibited a normal behaviour after 2 to 3 days after surgery intervention.

2.4 Telemetry

Only data from passive tracking were used in this study. An array of 16 receivers, model VR2W (Vemco, Inc.), arranged throughout the Cabo de Palos-Islas Hormigas marine reserve was used (Fig. 3.1). The first 14 receivers were installed in September 2011, one month before the beginning of the marking campaign. In January 2012, two additional receivers were installed to better cover two shadow zones inside the integral reserve. Data from VR2W receivers were downloaded every 3 months, in January and May 2012.

2.5 Data analysis

Data from VR2W, relocations and depth, were extracted from VUE (Vemco, Inc.) software and introduced into a data matrix. False detections and tag ID’s that did not correspond to our transmitters were removed from the matrix before analysis. We also removed animals that had less than 100 detections over the entire study period from the analysis (4 individuals of E. marginatus, #5128, 5130, 5131 and 5133). Then, with the raw data we constructed a matrix of relocation and depth by month, day and hour for each individual tagged. In order to compare relocation data with environmental variables we excluded detections made before December, due to the low number of individuals marked until this date..

Environmental data were obtained from the oceanographic buoy number 2610 from XTNET, a Spanish government monitoring network (Puertos del Estado, Ministerio de Fomento, http://www.puertos.es/), in which data was registered every hour. Wind and current data were combined into a single coarse component reflecting both the intensity and direction of predominant blow and flow regimes existing in the region (based on Milicich 1994 and Bergenius et al. 2005). Winds (in ms-1) and current (in ms-1) from NE-SW direction were categorized as positive if they comprised between 315º-134º (NW-SE, Northern sector) and negative if 135º-314º (SE-NW, Southern sector). Also, significant wave heights measure (in m), defined as the mean of the highest third of the wave height reading records, together with temperature in Celsius scale (ºC) and atmospheric pressure (Hpa), were also registered.

Temporal variation of activity and the influence of moon phases in such patterns were determined using the average number of relocations of each individual per hour, using multivariate analysis of variance PERMANOVA, which is adequate to analyse unbalanced field experimental designs as this (Anderson, 2001). The 2-way orthogonal design consisted of factors month (M, random, 5 levels, from January to May) and moon phase (MP, fixed, 4 levels: 1st quarter, new, 3rd quarter, and full moon) and their interaction. All analyses were done with 9999 permutations under a full model, using PRIMER v.6 program. On the other hand, a one-way ANOVA (using GAD package in R) was used to evaluate differences in depth occurrence among months. Prior to analyses, homogeneity of variances was checked using Cochran's test, and in the cases where variances were not homogeneous we performed the analyses anyway, since analysis of variance is quite robust to departures from their assumptions, especially when the design contains a large number of samples or treatments (Underwood 1997). When the temporal factor Month showed to be significant, multiple comparisons among months were run using the Student-Newman-Keuls (SNK) procedure in order to detect which months were significantly different to the others.

To evaluate the response of both species on the set of environmental variables considered, we used generalized additive modelling (GAM). GAM is known to be useful when the relationship between the variables is unknown and expected to be of a complex form, not easily fitted by standard linear or non-linear models (Hastie & Tibshirani 1990). The number of relocations and mean depth was introduced as a continuous smooth variable modelled non-parametrically using a smooth cubic line and default degrees of freedom using "gam" package in R. We applied to model Gaussian variance and identity link functions, both based on Hastie & Tibshirani (1990) and Venables & Ripley (2000). Model selection was done using Akaike information criteria (AIC) after exhaustive evaluation of all possible combinations of predictors using package "MuMIn". The relative importance of each added predictor to the selected model was evaluated by R2 statistic. In the case of a model with multiples predictors the statistics F and p were provided for each individual variable when the complete model was fitted. Additionally, we used Chi-square tests to evaluate the goodness-of-fit of adding a new variable to the model until the model selected by AIC was complete. Variables with lower residual deviance, and thus higher explanatory power, were added first, and subsequently those with the lower residual deviance. Results were graphically plotted and the proportion of effect of predictors over evaluated variables was indicated by positive and negative y-scale.

3. Results

An amount of 38 fishes, from which 30 individuals belonged to *E. marginatus* and 8 to *E. costae*, were marked. Sizes ranged from 40 to 90 cm for dusky groupers and 36 to 75 cm for goldblotch groupers. Locations of capture and number of individuals marked within each location are summarized in Table 3.1. A total of more than 747,000 valid detections were registered between October 2011 and May 2012.

Table 3.1: Fishes marked on Cabo de Palos marine reserve. Species size range and number of individuals marked in each zone (T: Testa; P1: Pile 1; P2: Pile 2; D: Dentro; Hg: Hormigón island; Ha: Hormiga island; M:Mosquito) and total.

Specie	**Size Range**	**Number of fish marked**							
		T	P1	P2	D	Hg	Ha	M	Total
E. marginatus	41,3 - 91,2	0	7	2	5	5	6	5	30
E. costae	36,7 - 74,5	1	1	1	3	1	0	0	8

PERMANOVA results indicate a significant effect of the interaction term M×MP for all variables tested (Table 3.2), evidencing that monthly variation in the patterns of activity and position in the water column was not independent of the lunar phases, so that lunar synchronization was not a general pattern. In the case of *E. marginatus*, higher average number of relocations has been observed during full and 3rd-quarter moons except for January and February, when greater activity was related to new and 1st quarter moons (Fig. 3.2a); on March no significant differences were found. For its part, in the case of *E. costae*, the average number of relocations was greater on 3rd quarters only in December and March. In January and April, higher means were recorded around new and 1st quarter moons, respectively. In February and March however, activity predominated on 1st quarter and 3rd quarter moons, in this order (Fig. 3.2b).

Table 3.2: Results from multivariate permutational analyses of variance (PERMANOVA) on data of detections and depth. Factors as M: Month; MP: Moon phase; M x MP: interactions between factors.

			E. marginatus		***E. costae***	
		df	**F**	**P**	**F**	**P**
Number of Relocations	**Month (M)**	4	33.075	0.0001	36.563	0.0001
	Moon phase (MP)	3	4.6122	0.0001	7.1018	0.0001
	MxMP	12	3.4643	0.0001	6.1911	0.0001
Mean depth	**Month (M)**	4	13.203	0.0001	86.404	0.0001
	Moon phase (MP)	3	2.5969	0.0001	5.8529	0.0001
	MxMP	12	3.0673	0.0001	5.5548	0.0001

Mean depth of detection of the grouper individuals showed significant differences across the studied period. The ANOVA evidenced a significant temporal variation in mean depth for the marked individuals of *E. marginatus* ($F_{(3,1240)}$ = 3767; $P<0.01$); this pattern consisted namely in that on October, fishes were concentrated in a shallower strata around 14 m, and during subsequent months until May, fishes occupied a deeper portion of the reef, around 18-20 m (Fig 3.3a). A similar pattern of spatial occupation was depicted for individuals of *E. costae*, so that from February to May goldblotch groupers were detected at deeper strata, although they showed lower temporal fluctuations in depth ($F_{(3,657)}$ = 3651; $P<0.01$): (Fig. 3.3a). Closer inspection of individual data, however, permits to detect that this pattern is due to a few individuals among all marked fishes, since only six dusky groupers (#5121, 5122, 5123, 5124, 5125 and 5136) out of 30 varied in depth strata (more than 5 m) along the sampling period (Fig. 3.3b, c, d), and in the case of *E. costae* only two individuals (#5146 and 5147) presented this depth pattern (Fig. 3.3e). Therefore, in general, fishes did not change very much their vertical position throughout the sampling period.

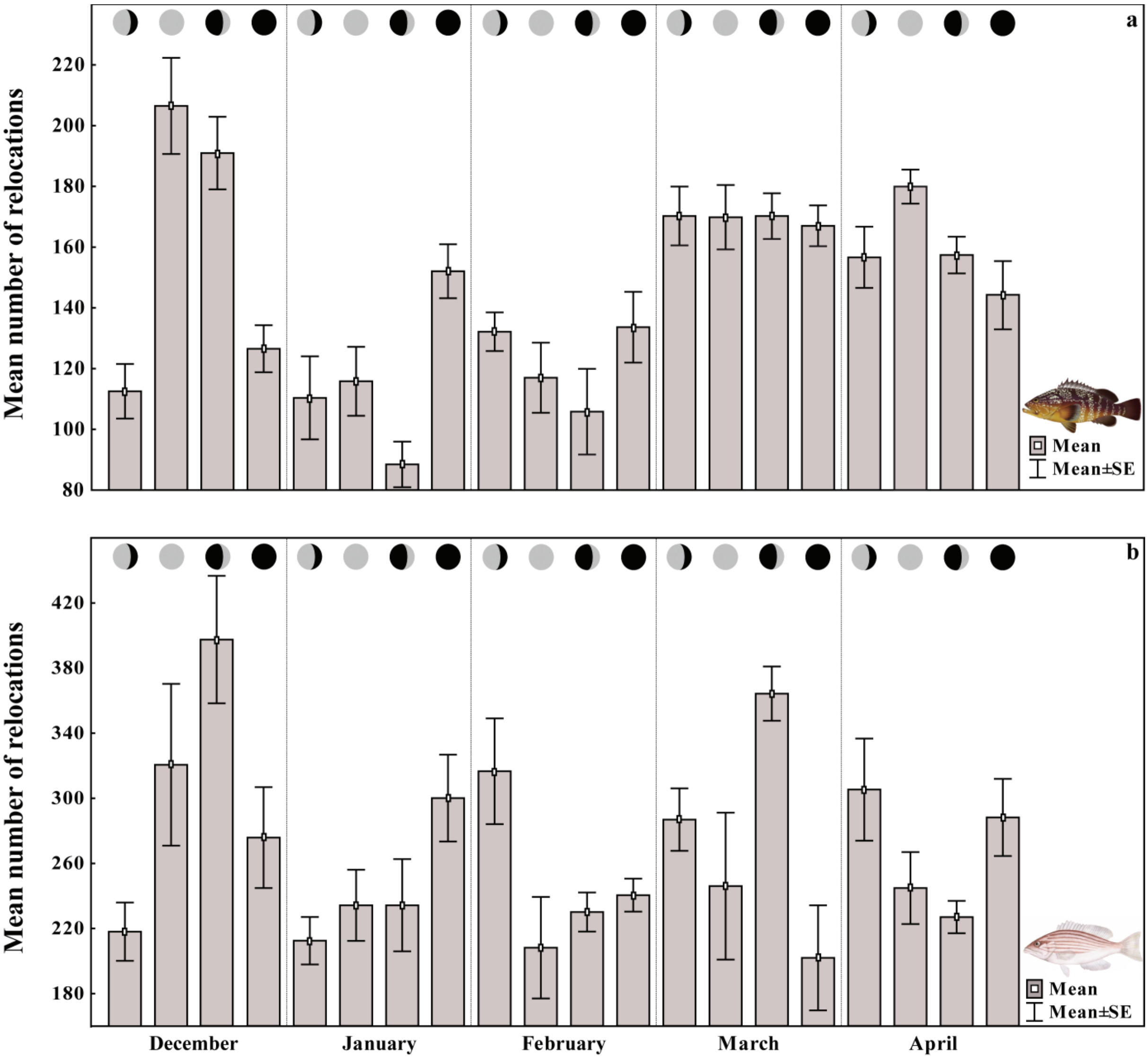


Figure 3.2: Mean number of relocations detected on VR2W array by moon phase in each sampling month. a) *E. marginatus*; b) *E. costae*

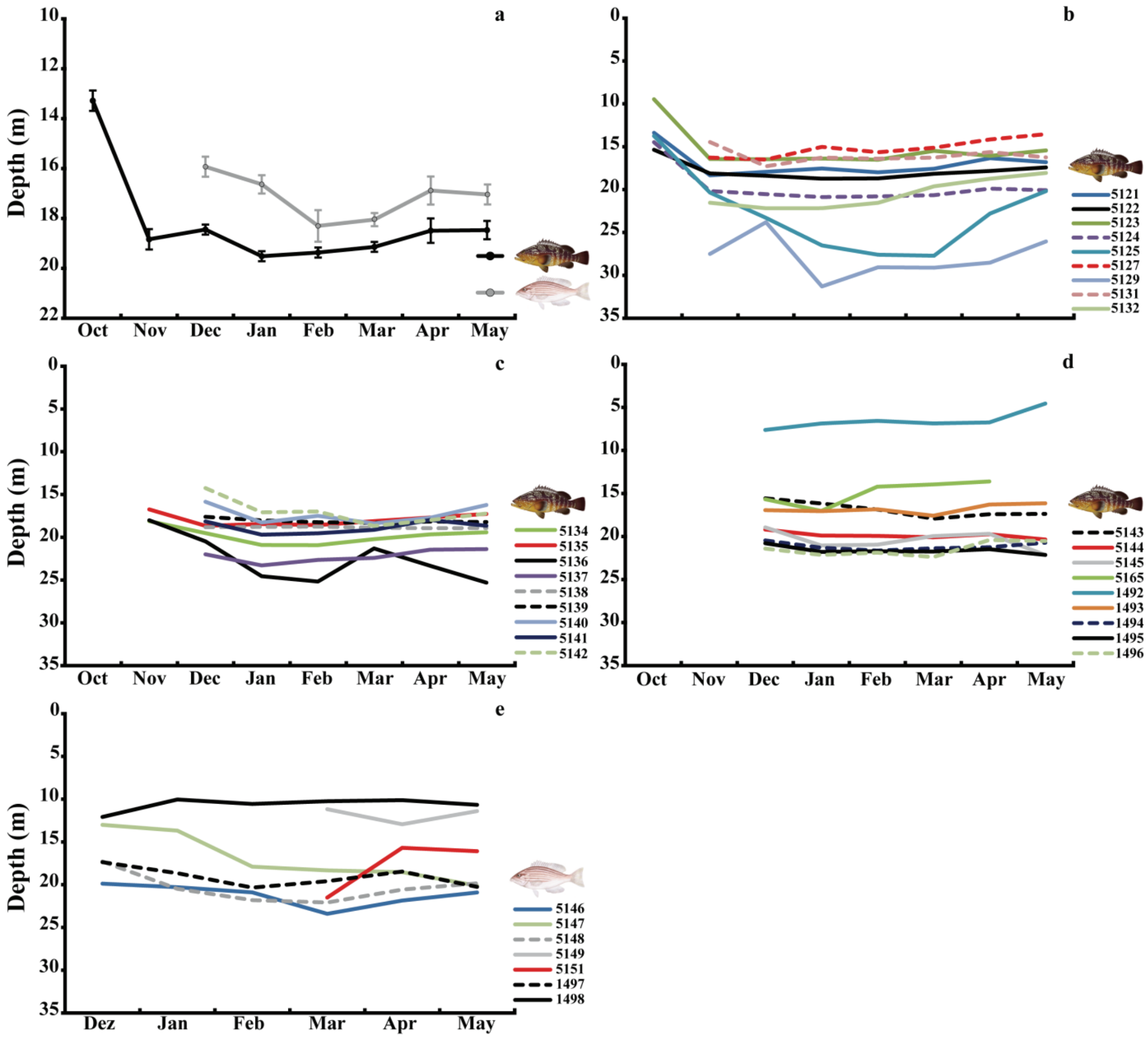


Figure 3.3: Vertical distribution of groupers on Cabo de Palos - Islas Hormigas marine reserve. a) mean depth of all groupers (*E. marginatus* and *E. costae*); b, c, d) depth of nine individuals of *E. marginatus*; e) depth of eight individuals of *E. costae,* on Cabo de Palos marine reserve.

Regarding diel pattern of activity both species can be considered as diurnal (Fig. 3.4 a). For *E. marginatus* higher mean number of detections occurred after 08:00 h lasting until 17:00 h, when a sharp reduction in the number of detections was registered. Peak activity of *E. costae* was in twilight hours; the first was around 07:00 h, folowed by a reduction in detections along the day, and a second small peak after sunset, at 20:00 h (Fig. 3.4a). In the case *E. marginatus,* 15 individuals displayed a clearly diurnal pattern of activity, other 10 specimens did not differ among day and night, and only one fish showed greater probability of detection at night than day (Fig. 3.4 b, c, d). For *E. costae,* 3 fishes showed the diurnal pattern, other 3 did not show any pattern, and one was nocturnal (Fig. 3.4e).

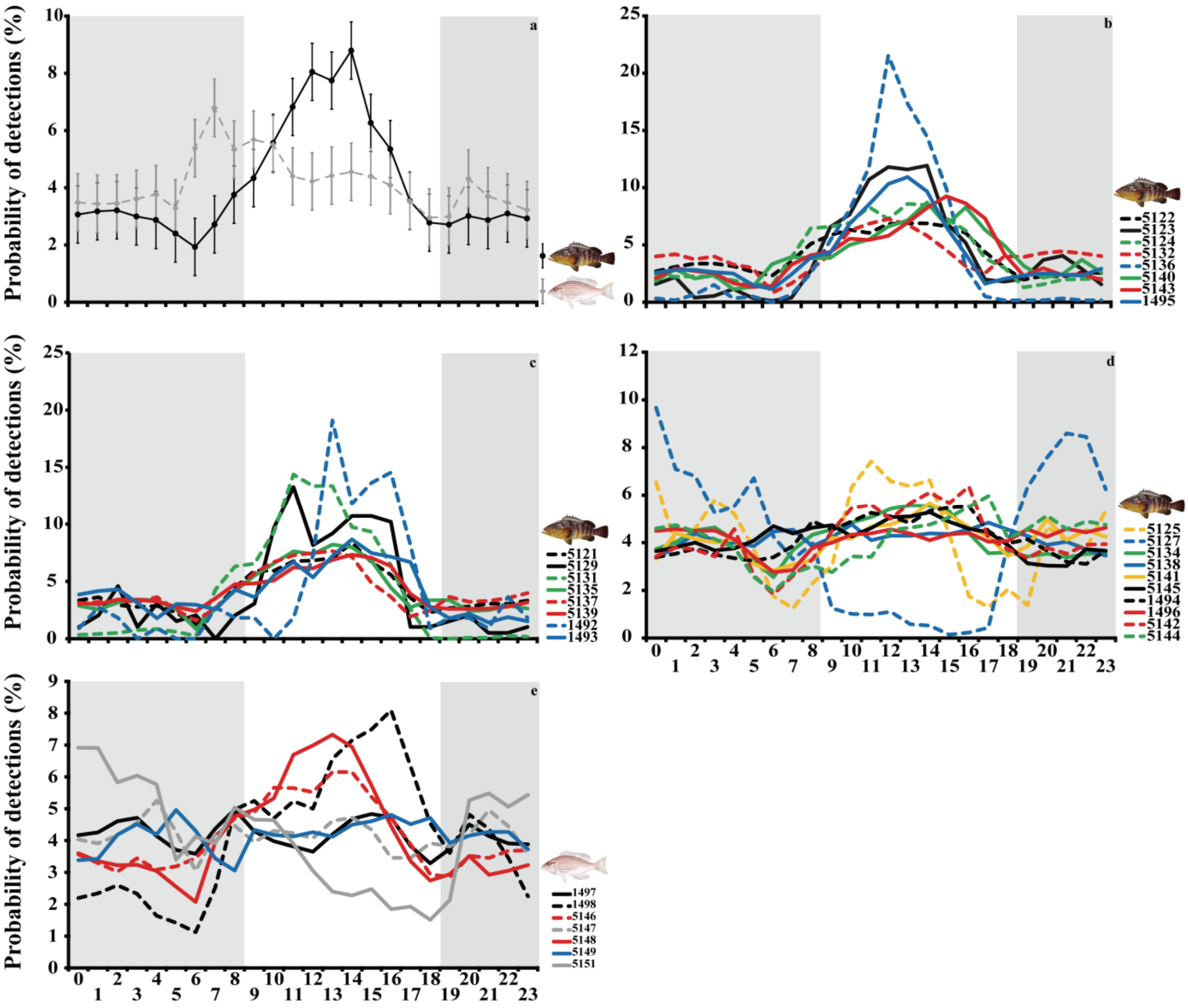


Figure 3.4: Daily probability of detections of each marked individual. a: mean probability for both species, *E. marginatus* and *E. costae*. b, c, d: *E. marginatus*; e: *E: costae*.

A great influence of environmental variables on behavioural patterns of both species was found. In general, low explanation power was obtained for the selected models; notwithstanding, the pattern of activity of *E. marginatus* (expressed as the mean number of relocations) exhibited the higher values for the coefficient of determination (Table 3.3). The best model obtained to explain the mean number of detections for dusky groupers *E. marginatus* included all environmental variables, although the addition of current intensity as the last environmental predictor did not improved fitting as depicted by Chi-square test (Table

3.3); hence, more detections occurred with higher atmospheric pressure (Fig. 3.5a) and temperatures (Fig. 3.5i), and significantly lower number of detections coincide with strong winds and bigger waves (Fig. 3.5c, e). In the case of mean depth of dusky groupers, the atmospheric pressure was the most important predictor followed by the intensity of currents (Table 3.3), so that fishes were recorded in deeper sites in periods of low atmospheric pressure and weaker current intensity (Fig. 3.6). Similarly, all environmental predictors were selected for the best model to explain the activity pattern of *E. costae*, but a significant reduction in deviance occurred only when wave height and atmospheric pressure were included in the model (Table 3.3): the mean number of relocations in goldblotch grouper was higher with high atmospheric pressure, and was lower with increasing wave height (Fig. 3.5d). On the other hand, depth variations of this species were influenced by the same environmental variables explaining *E. costae* activity patterns, plus temperature (Table 3.3), so that higher atmospheric pressure and lower wave height caused the displacement of individuals to shallower sites, but, somehow surprisingly, *E. costae* individuals searched for greater depths when seawater temperature increased (Fig. 3.6).

Table 3.3: Results of GAM fitted models between number of relocations and mean depth locations from groupers (*E. marginatus* and *E. costae*) with environmental variables (AP: atmospheric pressure; WH: wave height; Wi: wind; WT: water temperature; CU: current). Df – Residual Degrees of freedom, R.Dev – Residual Deviance, Red. Dev – Reduction in deviance by the inclusion of a new predictor regarding the null model, P(Chi) – significance by Chi square test between models of increasing predictor variable, R^2 – variance explained by each variable by the model, F – Fisher's statistic, *P* - significance at α=0.05, * - P<0.05, ** - P<0.01, *** - P<0.001, wi – Akaike weights of model selected by AIC model selection. *Statistics values of each variable predictor contained in the full model selected.

Variable	Model	Df	R.Dev	Red.Dev	P(Chi)	R^2	F^*	P^*	wi
Number of relocations: ***E. marginatus***	Null	1587	5949567						
	AP	1583	4370994	1578573		0.27	17.80	***	
	AP+WH	1583	4442148	1507419	<0.001	0.25	13.75	***	
	AP+WH+Wi	1583	5048163	901404	<0.001	0.15	40.93	***	
	AP+WH+Wi+WT	1583	4328567	750030	<0.001	0.27	38.92	***	
	AP+WH+Wi+WT+Cu	1583	5571917	377650	ns	0.06	16.36	***	0.99
Number of relocations: ***E. costae***	Null	623	519346						
	WH	619	469282	50064		0.10	20.79	***	
	WH+AP	619	468790	50556	<0.001	0.10	12.69	***	
	WH+AP+Cu	619	505323	14023	<0.001	0.03	2.06	ns	
	WH+AP+Cu+Wi	619	489169	30177	ns	0.06	6.75	**	0.57
	WH+AP+Cu+Wi+WT	619	475693	20340	ns	0.08	3.87	ns	0.43
Depth: ***E. marginatus***	Null	1579	7954						
	AP	1575	6560	1394		0.18	43.98	***	
	AP+Cu	1575	7150	804	<0.001	0.10	21.67	***	
	AP+Cu+WH	1575	7145	810	ns	0.10	5.40	**	
	AP+Cu+WH+WT	1575	7238	805	<0.001	0.16	18.60	***	
	AP+Cu+WH+WT+Wi	1575	7420	534	ns	0.07	22.41	***	0.99
Depth: ***E. costae***	Null	600	5571						
	AP	596	4921	650		0.12	10.19	***	
	AP+WH	596	4810	761	ns	0.14	5.14	**	
	AP+WH+WT	596	4942	645	<0.01	0.18	6.24	**	
	AP+WH+WT+Wi	596	5022	548	ns	0.10	0.98	ns	
	AP+WH+WT+Wi+Cu	596	5383	187	ns	0.03	0.67	ns	0.97

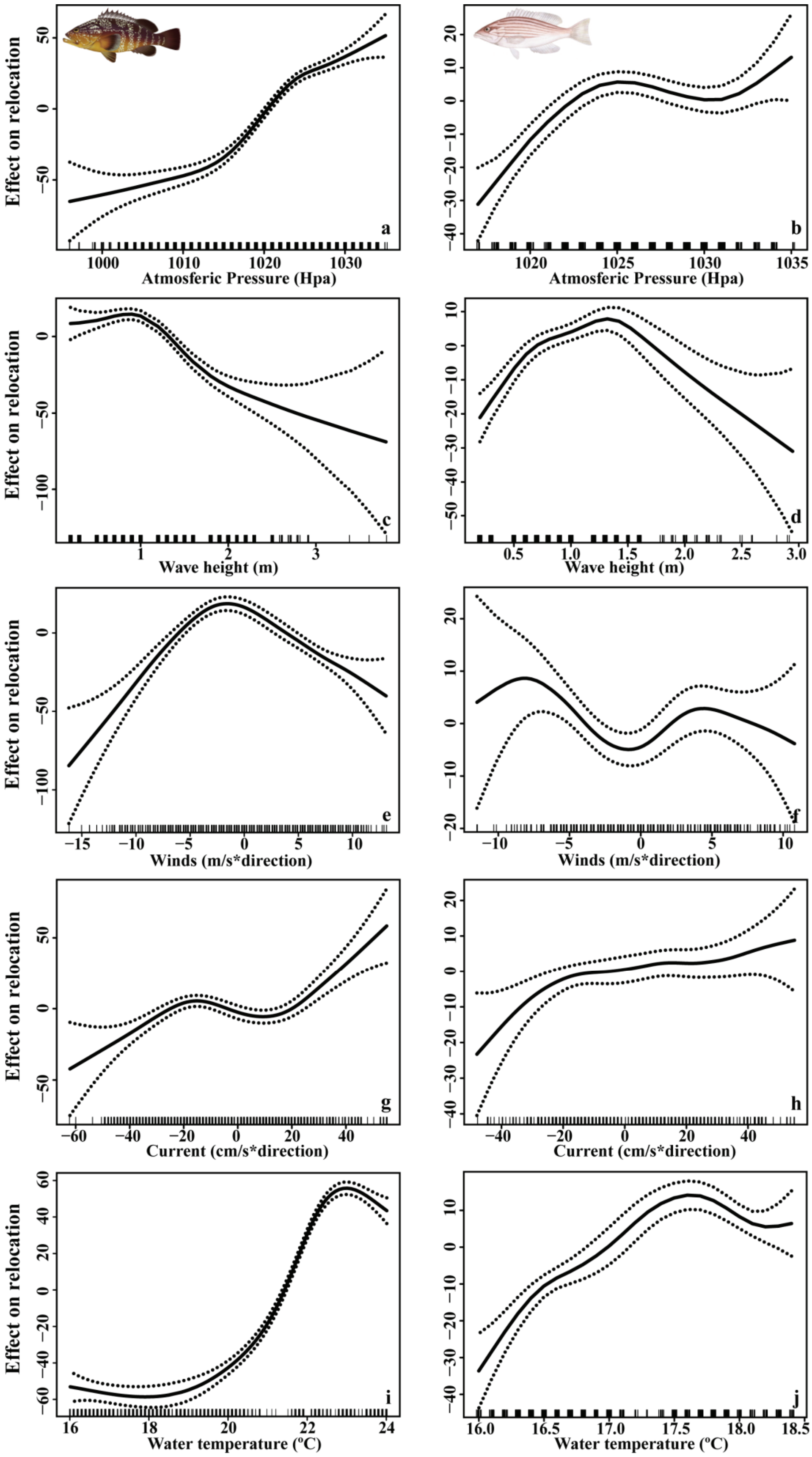


Figure 3.5: Generalized additive model (GAM) fitted as a smooth line of the number of relocations with the environmental variables. (*E marginatus*: a, c, e, g, i: *E. costae*: b, d, f, h, j).

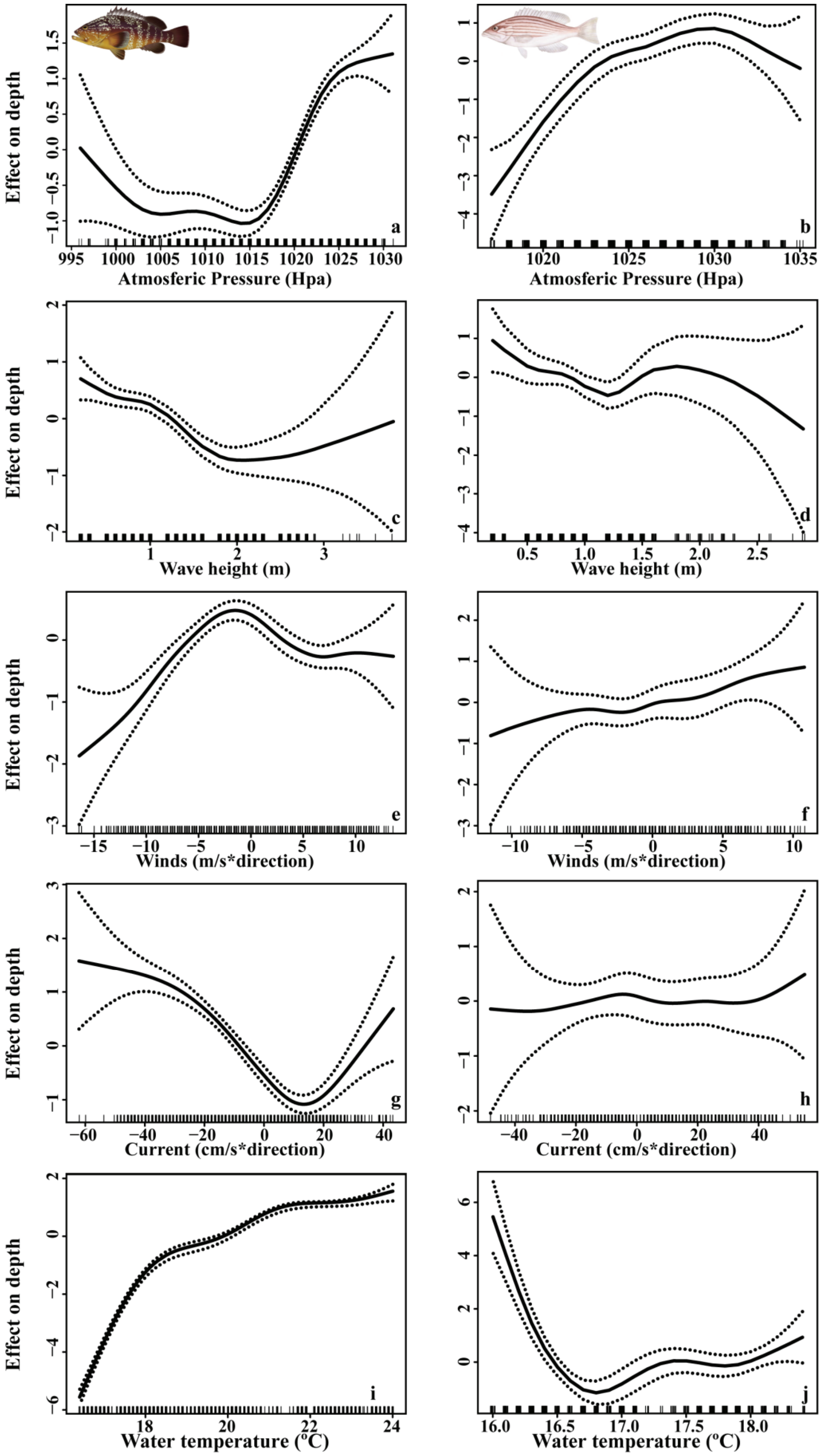


Figure 3.6: Generalized additive model (GAM) fitted as a smooth line of the depth of groupers with the environmental variables. (*E marginatus*: a, c, e, g, i: *E. costae*: b, d, f, h, j).

4. Discussion

The number of detections of an individual reflects the presence of such individual within the detection range of the array of receivers. However the absence of detection of any individual does not necessarily mean that individual is not in the site surveyed. Instead, the animal may just be outside the detection range, inside crevices, holes or in a shadow site (i.e., behind a rock) in which detection is not possible (Lindholm *et al.* 2009). As groupers are considered animals with diurnal habits with crepuscular peak of feeding activity and small home ranges, we believe that reduction in detection, considering the biology and life history of both species, indicates a direct reduction on the activity patterns of the species rather than the disappearance of the individuals from the study site. Our results suggest a strong temporal fluctuation of activity patterns of the studied individuals. During cold months (December until March) lower mean number of detections was registered for both species. For temperate fish, in general, a reduction in the metabolic rhythms is expected during cold months (Egli & Babcock 2004).

No clear synchrony with lunar cycles was observed in the activity patterns of both species. It is noteworthy, however, that this study is analysing data obtained from a period which is outside the summer reproduction period of groupers (Hereu *et al.* 2006, Reñones *et al.* 2010). For tropical groupers, there is a marked synchrony of reproduction activity with lunar cycles (Domier & Colin 1997). However, the synchronism would be related to strong tidal currents of spring tides which could optimize the egg transport to coastal zone (Johanes, 1978). In the Mediterranean, Hereu *et al.* (2006) observed that there is not such relation between moon phases and reproduction activity of *E. marginatus*, possibly due to the fact that tidal range is too small to have an effect on larval dispersal. Therefore, our results corroborate that this asynchrony between monthly activity patterns and moon phase is maintained during winter months.

We also found a distinct depth occupation depending on the temperature of seawater. Little empirical information is available for this issue; direct observations described higher abundances during summer compared to the other months (Harmelin & Harmelin-Vivien 1999, Reñones *et al.* 1999, LaMesa *et al.* 2006). Hereu *et al.* (2006) recorded that during the reproductive period on summer, dusky groupers responded to temperature by positioning themselves above the thermocline and even stopping their courtship behaviour. This might be an indication that low temperatures may affect behaviour responses of these species. At Cape of Palos, during winter groupers went down to greater depths and remained more time inside their refuges, reducing the number of detections.

Groupers are recognized as diurnal predators (Bshary *et al.* 2006), exhibiting higher feeding activity during twilight periods (Parrish, 1987; Sluka & Sullivan, 1996, Gibran 2007). Nonetheless, our data suggest a peak of intensity of their activity during intermediate hours of the day, where luminosity is at maximum. Because in temperate environments the daylight period varies greatly with seasonality, and together with the fact that groupers remain on visual cues to find prey, it is expected to find an enlargement followed by an narrowing on diel activity pattern across seasons. To evaluate this possibility, a whole year period must be covered to detect and characterize such seasonal variability.

Hereu *et al.* (2006) found some relationships of the behaviour of *E. marginatus* with environmental variables. Our data reinforce and complement this work by showing that not only activity patterns but also vertical positioning (depth) of both species was affected by environmental variables. Environmental conditions such as low atmospheric pressure, high wave heights and strong winds indicate the presence of storms in the studied area. Within these conditions, low detections could be translated into a reduction of activity, the search for deeper areas and seeking for refuge inside coves, crevices, etc. Another important result of this study is the additional influence of currents on grouper behaviour. Cape of Palos is located on an area where a change of orientation of the coast from E-W to N-S occurs. Though, this location is often subjected to strong currents. In our study, a weak but significant relationship of activity pattern and depth of groupers with currents was observed. Indeed, field observations verified this relationship between grouper positioning and the intensity of currents by observing that both species usually position themselves in the water column facing the prevailing current, holding there for large periods of time (CWH, personal observation).

The research done up to date focused on describing the behaviour of *E. marginatus* in aspects such as reproduction, feeding, diel activity, etc., and concentrated only on warmer months, and thus left uncovered a great part of temporal variation associated to cold months. For its congener, the gaps of knowledge are even larger. This is the first contribution to add new information about the behaviour of *E. costae* and to provide relevant complementary information about behaviour patterns of *E. marginatus*. The better understanding of behaviour patterns of such reef apex predator species is mandatory for conservation and management purposes, even more if we consider their ecological, commercial and cultural importance for the Mediterranean littoral.

Chapter IV

Home range and movement pattern of two sympatric groupers (Serranidae: Epinephelinae) and implications for their conservation.

Espacio vital y patrones de movilidad de dos especies simpátricas de meros (Serranidae: Epinephelinae) e implicaciones para su conservación.

Home range and movement pattern of two sympatric groupers (Serranidae: Epinephelinae) and implications for their conservation.

ABSTRACT

Worldwide Marine protected areas have been recognized as effective management tools in recovering population numbers of exploited species, mainly those sedentary species such as groupers. However, for the understanding the effects underlying spillover on MPAs or for assist MPA design and implementation some information about species mobility and area of use must be assessed. By this mean, we marked 152 fishes externally and 38 with acoustic transmitters belonging to the two most abundant groupers in Mediterranean rocky reefs, *E. marginatus* and *E. costae,* and monitored them for 9 months. Both species presented similar home range (HR) sizes, being total area occupied by *E. costae* slightly larger than *E. marginatus*. HR increased with size in *E. costae* individuals while no relationship was found to *E. marginatus*, which might be related to differences in behaviour, being *E. marginatus* much more site attached than *E. costae*. No differences on HR size among sexes were found for both species. On patchy habitats like Cabo de Palos-Islas Hormigas Marine Reserve, great overlaps among HR size among individuals and between species were found. Higher densities within a limited space can cause unwanted collisions between individuals HR, producing higher movement rates. These movements were corroborated by both marking techniques, and spillover effects were detected to both species. As spillover is based on density-dependent movements, the enhance rate of such events foresee a potential increase on fishery benefits with increasing densities. Understanding mobility patterns in groupers could provide useful information for comprehend how species interact with their habitat and sympatric species, and ultimately for helping to design effective spatial measures to protect marine biodiversity.

Key words: *Epinephelus marginatus*, *Epinephelus costae*, acoustic tracking, spill-in, spillover, marine protected areas.

1. Introduction

Marine protected areas (MPAs) are recognized as an essential tool for the spatial management of marine and coastal environments (Hilborn *et al.*, 2004, Gaylord *et al.* 2005, García-Charton *et al.* 2008). The beneficial effects of MPAs to protect fish stocks and increase fishery in adjacent areas through spillover and larval export, among others, has been often empirically demonstrated (Russ & Alcala 1996, Roberts *et al.* 2001, Goñi *et al.* 2008, Harmelin-Vivien *et al.* 2008). The effects of the protection provided by MPAs are directly related to the life history of the species (Chapman & Kramer 1999, Claudet *et al.* 2010). For migratory species, such as tuna and mackerel, MPAs may have little or no effect as these species spend much of their time in unprotected areas (Grüss *et al.* 2011). Even in the case of very mobile species, such as many carangid species, effect of protection can apply to only part of the time spent within the boundaries of the protected area (Wetherbee *et al.* 2004, Kerwath *et al.* 2009). For their part, species with low mobility rate, normally site-attached with small home range, spend much of their time within the boundaries of the MPA and ultimately are positively affected by protection (Kramer & Chapman 1999, Grüss *et al.* 2011).

Some studies have related the fish life history to protection efficiency (Claudet *et al.* 2010, Grüss *et al.* 2011), and more recently a number of studies have attempted to relate the size of the home range and movement patterns of the species to the design of MPAs and MPA networks (Claudet *et al.* 2010, Langebrake *et al.* 2012). In theory, the home range is the basis for spillover across MPA boundaries to occur via a density-dependent diffusion process (Rodwel *et al.* 2003, Kellner *et al.* 2008, Pérez-Ruzafa *et al.* 2008, Kerwath *et al.* 2009).

With increasing density inside an MPA, an increase in the number of fish encounters could force some individuals to migrate out of the boundaries of a protected area, causing spillover. However, as a result of the intrinsic patterns of movement, an inverse direction towards inside the protected area, known as spill-in (Russ & Alcala 1996, Russ & Alcala

2004), could occur, a process that has received little attention in the literature. Though, understanding the spatial scales of movement and home range size of target species is fundamental to help in the design and implementation of MPAs, and therefore for effective protection to occur.

Groupers are an emblematic group of reef apex predator fish species that generally respond very well to protection (Russ & Alcala 1996, Sluka *et al.* 2001, McClanahan *et al.* 2007) due to their site-attached behaviour. Nevertheless, response of groupers to protection can delay some decades for population to recover historical levels (Russ & Alcala 2004, McClanahan *et al.* 2007). In the Mediterranean, studies on grouper mobility patterns and home range have been done solely on dusky grouper (*Epinephelus marginatus*), which is the most economically important and abundant grouper species in rocky reefs in the region. These studies focused on evaluating home-range size (Lembo *et al.* 2002, Pastor *et al.* 2009) and site fidelity (Lembo *et al.* 1999, Spedicato *et al.* 2002) as well as their homing behaviour (Lembo *et al.* 2002). In general these works marked a small number of fishes (between 6 to13) or they were done for a very short period of sampling (1 month), limiting conclusions about mobility patterns of groupers. Despite of their findings a lot of questions about movement patterns of the dusky grouper remains open. For example, none of them evaluated the *E. marginatus* behaviour in relation to other species, or the effect of MPA or habitat size in mobility, or even the influence of fish densities on movement patterns. Additionally, the complete absence of previous studies in other economic important Epinephelinae species such as goldblotch grouper (*Epinephelus costae*), reinforce the need of more studies on mobility.

Thus, the aims of this work are: a) determine the home range and movement patterns for *E. marginatus* and *E. costae*; b) compare patterns of both species and c) evaluate the responses of both standards front the effects of a marine reserve.

2. Materials & Methods

2.1 Study area

This study was conducted on rocky reefs in the Cabo de Palos – Islas Hormigas (hereafter CP) marine reserve (Fig. 4.1). The CP marine reserve (37º38' N, 0º42' W), which was established in 1995, is rectangular in shape and occupies 1898 ha. It is divided into a no-take zone (NTZ) with 270 ha around the Hormigas islands where all activity is prohibited (except scientific research). The remaining area is a buffer zone (BZ), in which some local artisanal fisheries and recreational diving are allowed. The bottom in this area is formed in the shallower areas by rocky boulders of various sizes interspersed with extensive patches of *Posidonia oceanica* forming a narrow belt following the coast, while at deepest portions (>16 m) detritic formations predominate. After that, a series of steep rocky shoals and small islands are aligned seawards from the cape to the north-east, where extensive algal communities cover the infra-littoral zone, while the circalittoral zone is dominated by coralligenous habitats dominated by the gorgonians *Paramuricea clavata* and *Eunicella singularis* (Calvín *et al.* 1999, García-Charton *et al.* 2010).

2.2 Fish capture

Marking campaigns were conducted in October and November 2011 in seven sites throughout the CP marine reserve. We utilized two methodologies to capture groupers. Firstly, we installed manually traps made of wicker at depths between 15 and 30 m by scuba diving. We looked for places near burrows and crevices where target species were commonly found. After installation, a bait composed of pieces of octopus (*Octopus vulgaris*) and squid (*Loligo* sp.) was put inside each trap, and then verified after a period ranging from 12 to 24 hours, depending on weather conditions. In addition, we did underwater fishing using line-and-hook and squids as bait. The hooks used were previously devoid of barb to minimize the damage it could cause to the animals.

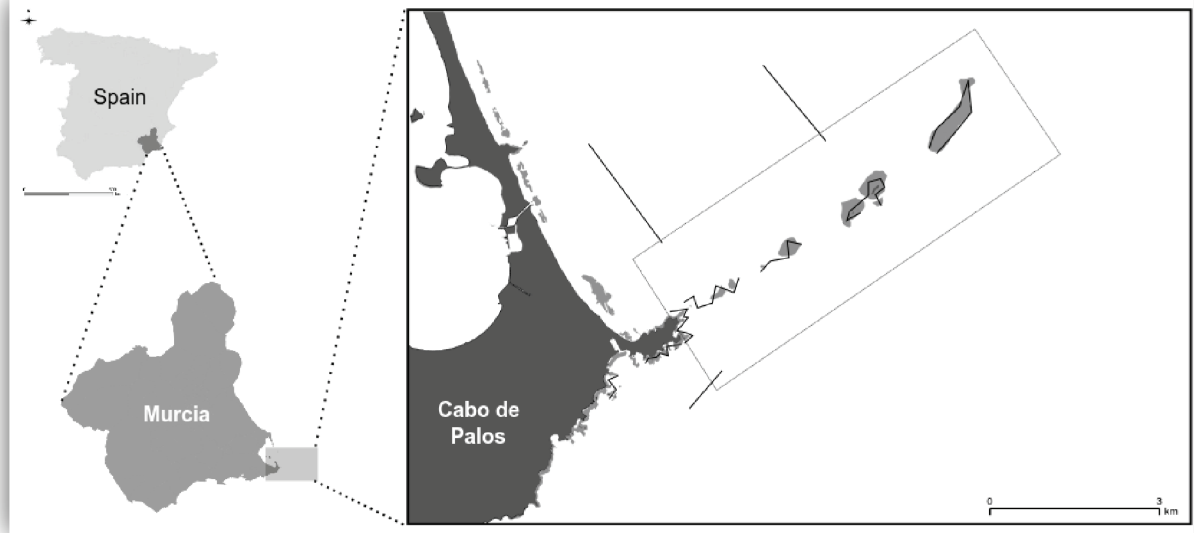


Figure 4.1: Map of Cabo de Palos. Lines indicates transects performed by VR100 active tracking within the limits of MPA (delimitaed by the retangle) and outside it.

All fishes captured were taken to the boat and placed in a tank filled with seawater. Once in the tank, the fish was 'vented' with a hypodermic needle (18 gauge, 1.5 inch) inserted into the swim bladder, using a perpendicular lateral approach to the base of the pectoral fin. Venting was deemed complete once the excess air from the swim bladder had been fully expelled, as seen by the slowing of air bubble production from the needle hub when held under water. This approach permitted the fish to return more easily to the bottom, thus reducing barotrauma problems after venting, fish was weighted using a net and digital dynamometer (in kg) and measured at total length (TL in cm).

2.3 Fish tagging procedure

We inserted an external dart-tag (Floy Tag Inc.) in the base of the dorsal fin, between the 3th and 4th rays. Each tag has a number code that permits individual identification in case of recapture, and harbour a distinct colour for each sampling site. That colour allows identifying fish movements from the site where they were marked to different sites, in the case of these movements to occur. We applied a broad-spectrum antiseptic on the tag insertion to prevent infections. After tagging, we did a small surgery incision of 10 to 20-mm long between pectoral fins in order to insert the acoustic coded transmitter into the abdominal cavity. Fishes smaller than 50 cm (TL) received a V9P-2H transmitter (9×47mm 20-40 sec interval delay and 105 day tag life), while in individuals greater than 50 cm (TL) V13P-1L transmitters (13×45mm, 40-80 sec interval delay and 399 day tag life, VEMCO Inc.) was inserted. Surgical glue was used to close the incision. An external layer of antiseptic was applied to protect it from inflammation and a dressing layer in spray was applied on top to prevent direct contact of the wound with seawater or the bottom. Animals were kept into the tank before releasing into the water to ensure it was in good conditions. When animals displayed a normal behaviour they were immediately released in the same location as they were caught. A diver followed each animal while descending to the bottom to ensure its good condition. Marked individuals were then monitored by scuba diving in all marking sites and all fishes exhibited a normal behaviour after 2 to 3 days after surgery.

2.4 Fish tracking

Monitoring campaigns started on December 2011 and lasted until August 2012. Monitoring was done using a directional hydrophone (VH110) coupled to a receiver tracking system VR100 (VEMCO, Amirix division Inc.). Monthly surveys were conducted to recover information of fish relocations. Each campaign was conducted during 24 h coinciding with the full moon phase. We performed transects of 10 min (Fig. 4.1) from a boat around each reef marking site. If no fish were detected, another site was visited. Otherwise, if there was a positive sign, then we positioned the boat to acquire the stronger signal as possible from the transmitter and followed the mark as long as it was detectable. When a pinger was lost we continued the transect until the 10 minutes was completed. When a new pinger was detected by the mobile receiver, we proceed to complete the tracking with this new fish. Tracking was done until the animal was lost to achieve a maximum of 45 minutes in a single location.

2.5 Underwater visual sampling

Tagged fish were monitored through underwater visual censuses carried out in each marking site. Transects of 50×5-m were conducted to count and estimate the sizes of all individuals of the two grouper species. Animals were carefully inspected to search for tag marks and colour of tag recorded in the spreadsheet. When colour identification was impossible on a given individual due to fouling or bad visibility, the corresponding re-sight was recorded, but this individual was excluded from movement analyses.

2.6 Data analysis

Tracking data downloaded by using VR100 PC Host Software (VEMCO). After selection and removal of false detections, wrong detections and the ones without GPS position, data were analysed with adehabitatHR package of R software. Minimum convex polygons (MCP) were used to estimate the maximum area covered by each fish (Kimley & Nelson, 1984) and Kernel utilization distribution (KUD) with least square cross-validation estimation was used to increase resolution of area usage by fish (Seaman & Powel 1996). KUD home ranges were calculated for both 95% (vital domain) and 50% (core zones) and displayed for each fish in a geo-referenced position system using Qgis v.1.8 program. We estimated the percentage of home range overlap by calculating the common area of both KUD and CA estimates shared by two fishes occurring in the same place using vector spatial tools in Qgis. Percentage of overlap was then investigated regarding the sex and fish size for both species. Additionally, adehabitatLT package was used to explore trajectories of individual fish of both species and estimate mean daily moved distance of each fish during the whole period of survey.

Tagging data were used to evaluate movement patterns from inside to outside the NTZ (spillover) and in the opposite direction (spill-in) across NTZ boundaries. Although the number of marked individuals was relatively low, we had more than 40% on recapture rates. For this reason we used large movement variables, such as Immigration and Emigration rates, to assess spillover and spill-in rates. Immigration rate (I) was defined as Ni/Tr, where Ni is the number of individuals entering the integral reserve and Tr the total number of recaptured individuals. Similarly, Emigration rate (E) was estimated as No/Tr, where No is the number of individuals leaving integral reserve. Also, tagging procedure was used to assess the maximum distance travelled (D) by all marked individuals of each species, and was defined as the linear distance between the farthest two sighting points.

Dispersal potential as function of body size was assessed by linear regression with maximum distance travelled (D) by each marked fish as dependent variable. This analysis is useful to understand in which size fish has the greatest potential to disperse.

3. Results

3.1 Home range & movement pattern

A total of 152 fishes were marked with external tag, from which 124 belonged to *E. marginatus* and the remaining 28 to *E. costae*. From these, 38 *E. marginatus* and 13 *E. costae* were acoustically tagged and followed manually until August 2012. After the whole monitoring period an amount of 1350 relocations were detected for both species. No fish individuals were lost.

Both species displayed a similar home range size, with *E. marginatus* mean home range slightly greater than *E. costae,* though this difference is not statistically significant (MCP: t=0.21, p > 0.05; KUD: t= -0.77, p > 0.05 ; CA: t=-0.53,p > 0.05). Mean home range size for *E. marginatus* was 3.51 ha (35.100 m^2) for 95% kernel utilization distribution (KUD) and 0.97 ha (9.700 m^2) for 50% core area (CA). For their part, home range of *E. costae* individuals defined by KUD was 2.87 ha (28.700 m^2) and 0.84 ha (8.400 m^2) in CA. Also, home range differed regarding sex of individuals, so that male goldblotch groupers showed a greater home range area than females, while the opposite pattern was found for dusky groupers. Female goldblotch groupers showed a home range of 2.81 ha (KUD) and 0.78 ha (CA) and males 4.30 ha (KUD) and 1.35 (CA). For female dusky groupers the home range was 3.62 ha (KUD) and 1.01 ha (CA), and males the estimated home range was 1.69 ha (KUD) and 0.58 ha (CA) (Table 4.1).

Table 4.1: Home range (in hectare) for *E. costae* and *E. marginatus* on Cabo de Palos marine reserve calculated by MCP (minimum convex polygon, 100%), KUD (kernel utilization distribution, 95%), CA (core area from KUD 50%). SR: fish size range. F: female, M: male (number of individuals).

		MCP	**KUD**	**CA**	**SR**
E. costae	All (13)	8.59 ±3.13	2.87 ±0.60	0.84 ±0.20	36-75
	F (4)	8.71±3.7	2.23±0.60	0.62±0.19	
	M (4)	8.32±6.69	4.30±1.23	1.35±0.40	
E. marginatus	All (38)	7.03 ±4.06	3.51 ±0.45	0.97 ±0.12	45-93
	F (13)	7.89±4.69	3.72±0.51	1.01±0.14	
	M (2)	1.48±0.88	2.20±0.43	0.70±0.15	

No significant relationship was found between home-range area and individual sizes for *E. marginatus* (MCP: R^2=0.03, *P*>0.05; KUD: R^2=0.003, *P*>0.05; CA: R^2=0.002, *P*>0.05) (Fig. 4.2a). For its part, in the case of *E. costae*, although the area estimated by MCP did not show a significant relationship with size (R^2=0.076, *P*>0.05), those estimated by KUD (R^2=0.352, *P*<0.05) and CA (R^2=0.328, *P*<0.05) showed a positive relationship with fish size (Fig. 4.2b).

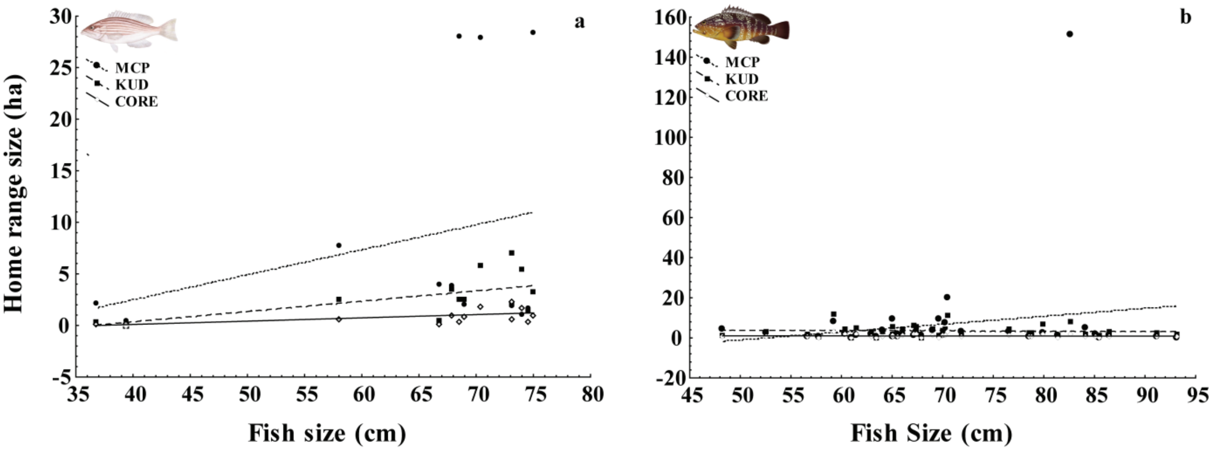


Figure 4.2: Linear regression between the three methods for estimating home range size (ha) and fish size (cm). a: *Epinephelus marginatus*; b: *Epinephelus costae*.

Higher vital areas (MCP) were found in *E. costae* individuals when compared to *E. marginatus* ones (Fig. 4.3, Table 4.1). The only exception to this rule was individual #5128 of *E. marginatus*, which showed the largest vital area of all marked individuals (Figure 4.3b). Comparatively, a greater overlap between individual vital areas within each species and between species was observed. Similarly, higher spatial overlaps were seen when KUD and core areas were plotted for each individual (Fig. 4.4). Individuals of *E. costae* showed higher percent overlap than *E. marginatus*, both regarding vital (*E. costae* = 58.6% ± 6.25*, E. marginatus* = 41.1% ± 5.28) and core areas (*E.costae* = 33.2% ± 5.18*, E. marginatus* = 28.5% ± 5.24). Marked fish used similar zones within each studied sites, however a more segregated distribution was found for fishes from the NTZ (Fig. 4.4f). Additionally, no relationship between percentage of KUD and CA overlap as a function of fish body size was found for both species [*E. costae* 95 (β=0.287, R^2=0.07, P>0.05); 50 (β=-0.242, R^2=0.008, P>0.05); *E. marginatus* 95 (β=0.-0.031, R^2=0.0002, P>0.05); 50 (β=0.190, R^2=0.002, P>0.05)].

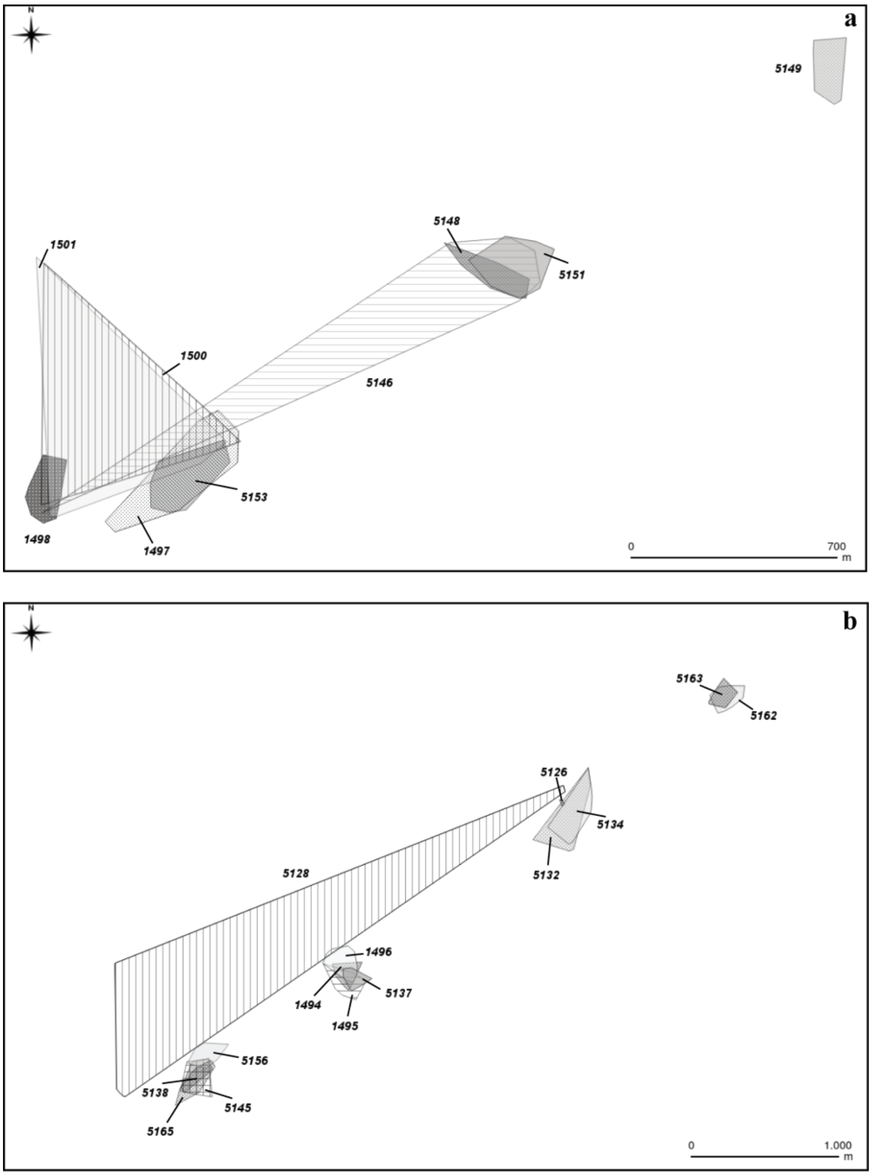


Figure 4.3: Minimum convex polygon (MCP) for both species studied; a: *E: costae*, b: *E. marginatus*.

Figure 4.5 displays the home range overlap (KUD and CA) by sexes for both species; only sites in which males were detected were represented. Although more males were recorded in the surveyed sites, we included only individuals with more than 5 relocations, resulting in 3 males of *E. costae* and 1 of *E. marginatus*. The greatest overlap between males and females territories was found for *E. costae* as compared to *E. marginatus* (Fig. 4.5a). For *E. marginatus* however, significant differences were found in home range overlap between sexes, with higher mean overlaps found among females (t=15.73; *P*<0.01) (Fig. 4.5b).

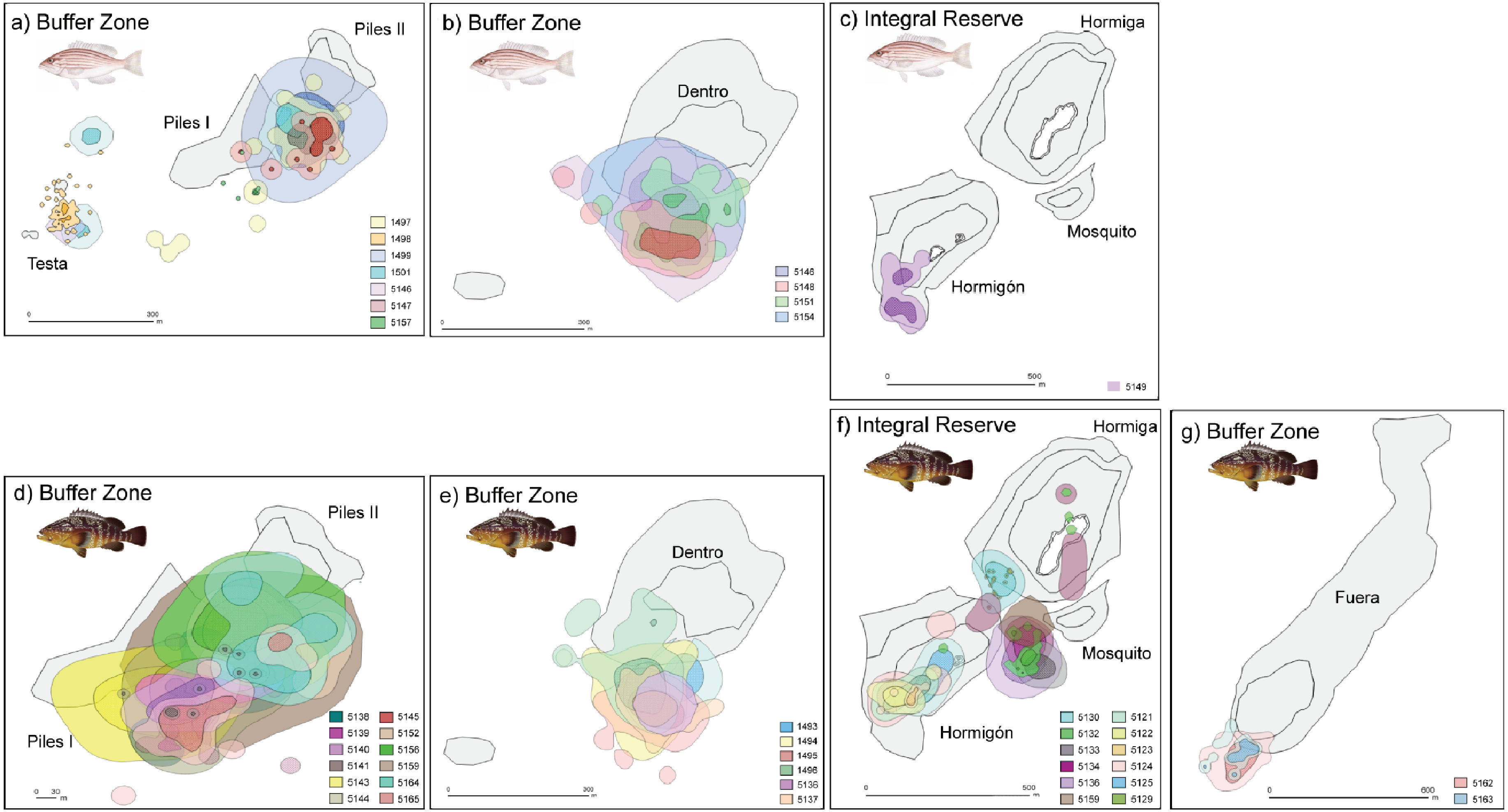


Figure 4.4: Home range (HR) of groupers in Cabo de Palos. Kernel Utilization Distribution (KUD 95%, coloured area), and core area (KUD 50%, dashed area) based on LSVC (Least square cross-validation) in each rocky shoal of the marine reserve. *E. costae* HR on a) "Piles" zone; b) on "Dentro" zone and c) inside integral reserve. *E. marginatus* HR on d) "Piles" zone; e) "Dentro" zone; f) integral reserve and g) "Fuera" zone.

Illustrative main movement patterns of selected individuals for both species are shown in Fig. 4.6. In general, both species presented similar movement patterns. It is noteworthy, however, that individuals of *E. costae* which undergoes large excursions normally return to their place of origin (Fig. 4.6a), while *E. marginatus* individuals stayed in the place of destination after the long displacement (Fig. 4.6b). Although not shown, only one individual of *E. marginatus* (#5128) returned to its natal site, in contrast to other 4 fishes that did not returned, establishing in the new place.

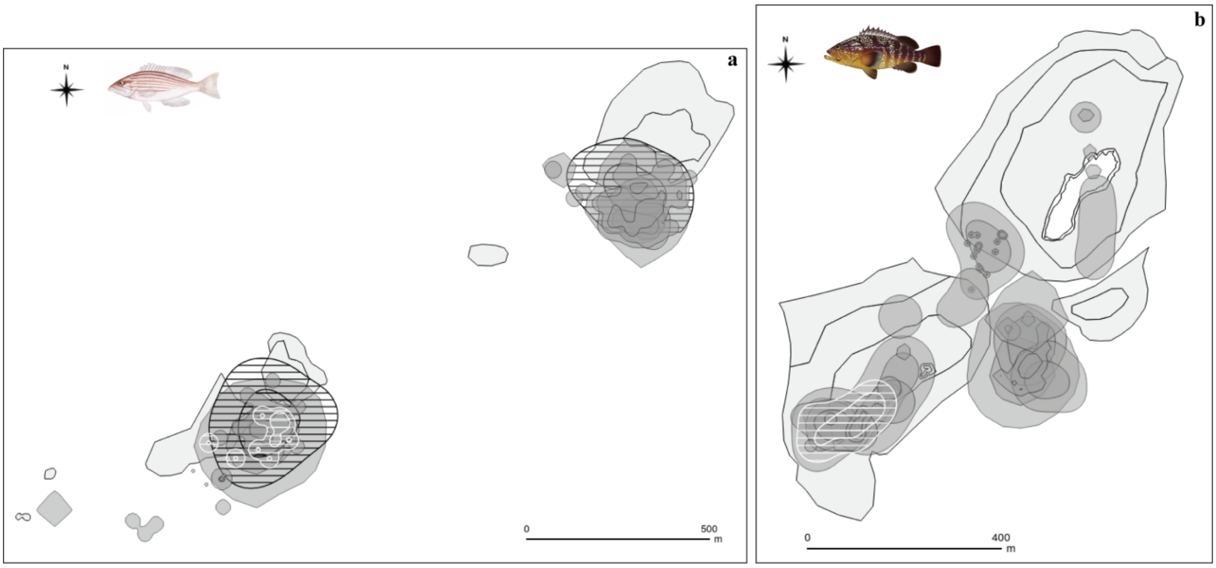


Figure 4.5: Home range (KUD) and core area by sex of both grouper species; a) *E. costae* and b) *E. marrginatus* on Cabo de Palos. Males were dashed lines (both white and black) and females in grey.

Daily mean movement was tested as a response to fish size by each species. *E. marginatus* moved around 100-m per day; no correlation with fish body size was found (R^2= 0.01; P>0.05) (Fig. 4.7a). On the other hand, *E. costae*, which showed lower mean daily movement than dusky grouper, presented a positive relationship between individual size and movement pattern (R^2=0.37, P<0.05), in which smaller fishes exhibited more restricted movements than big ones (Fig. 4.7b).

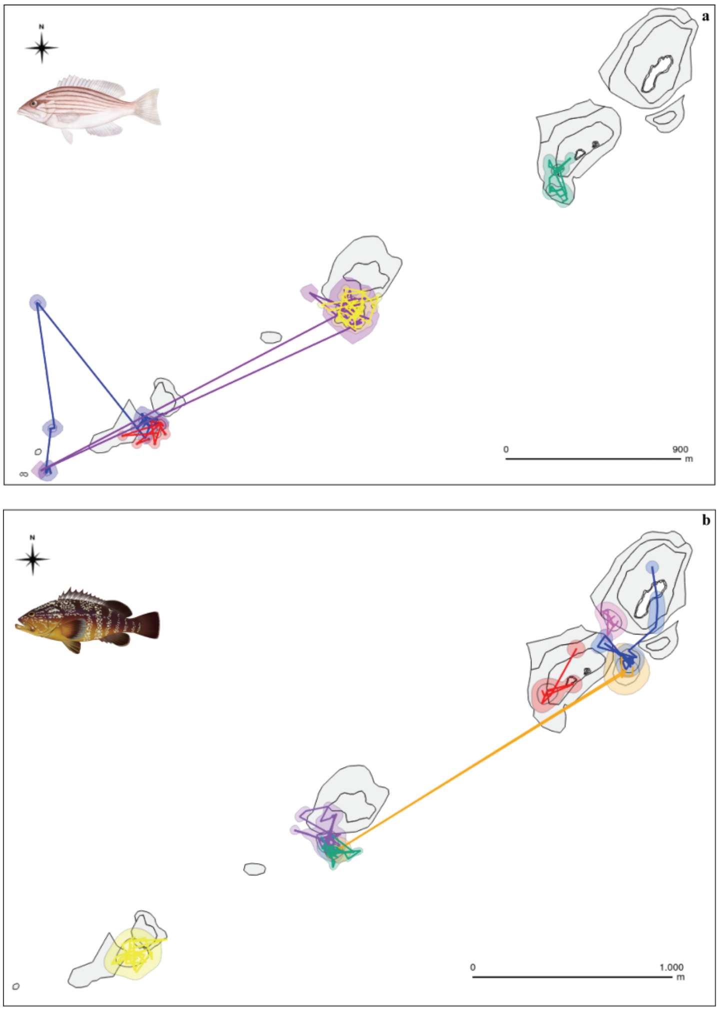


Figure 4.6: Trajectories displayed by some individuals of each species a) *E. costae,* b) *E. marginatus* plotted in relation to KUD home range.

3.2 Movement on relation to MPA

Based on mark-and-recapture data we estimated the emigration and immigration rates from and to BZ and NTZ. We assume that spill-in and spillover are the immigration and emigration rates for the case of the NTZ, respectively. In the case of *E. marginatus*, the emigration rate from both zones was basically the same (Fig. 4.8a), but the immigration rate was slightly higher in relation to the BZ than in NTZ. This indicates that an equal rate of individuals is moving in opposite directions inside (spill-in) and outside (spillover) the NTZ. On the other hand for *E. costae*, we only identified a directional (spillover) movement from NTZ to BZ (Fig. 4.8b).

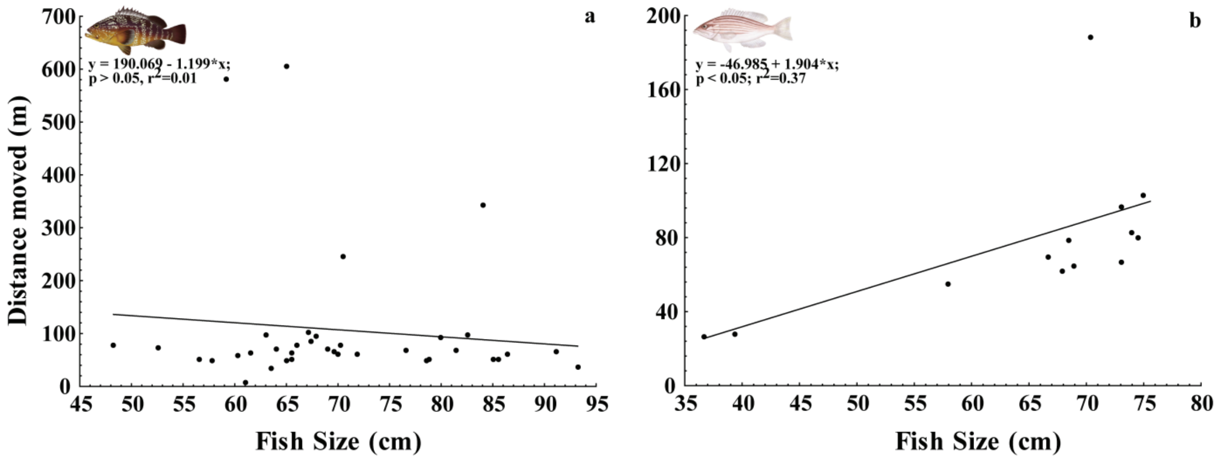


Figure 4.7: Linear regression between mean daily distance moved by each fish with fish size, a) *E. marginatus*, b) *E. costae.*

For both species a significant relationship between distance travelled and body size was detected; *E. marginatus* showed a negative relationship (R^2=0.24, P<0.05) demonstrating that small sized fish undergone larger displacements (Fig. 4.9a) while the opposite pattern was found for *E. costae* (R^2=0.41, P<0.05), in which larger fishes travelled longer distances (Fig. 4.9b).

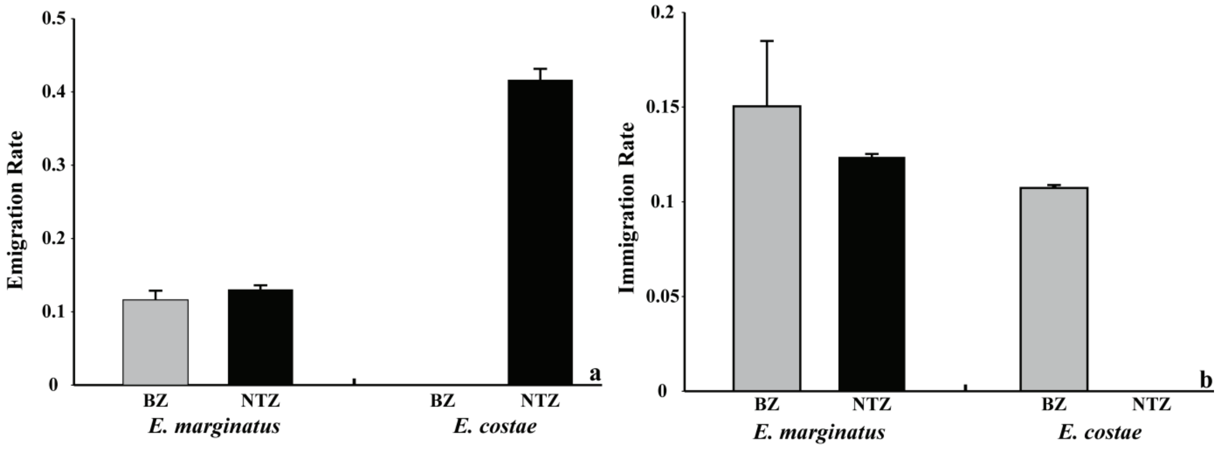
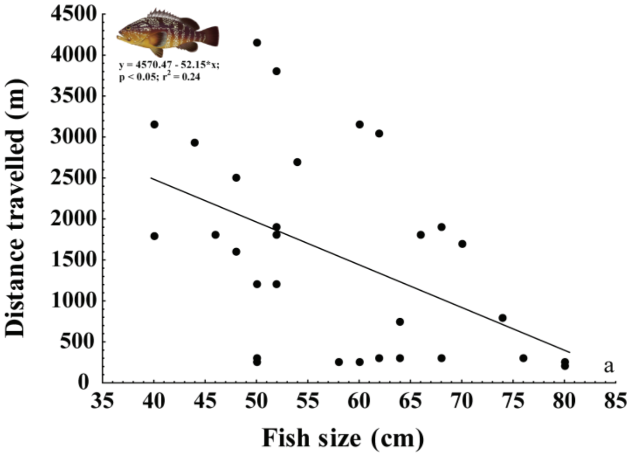


Figure 4.8: Number of individuals changed between reserve protections level (BZ: buffer zone; NTZ: no take zone). a) emigration rate; b) immigration rate.

4. Discussion

This study is the first one describing the movement pattern and home range estimation for *Epinephelus costae*, a poorly studied grouper species, which was categorized as data-deficient in the recent IUCN red list publication, though any additional contributions are welcome to enhance knowledge on biology and ecology of this species and support management and conservation measures (Craig *et al* 2011).



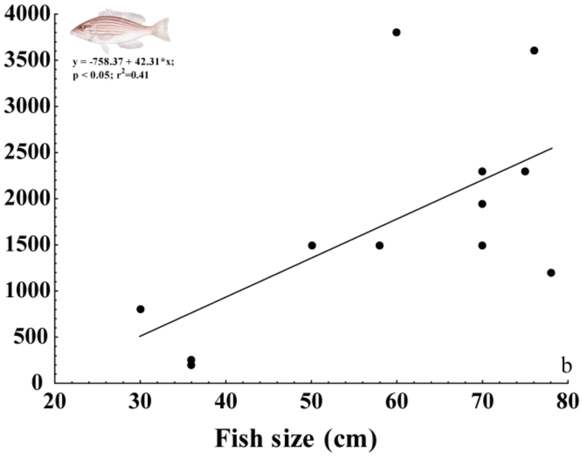


Figure 4.9: Linear regression between distance travelled (m) and fish size (cm). a) *Epinephelus marginatus*; b) *Epinephelus costae*

Comparatively, both grouper species studied here showed very similar home range sizes as synonym of vital area (KUD $_{95\%}$ ~ 3 and ~ 3.5 ha, for *E. costae* and *E. marginatus*, respectively). The lack of previous information about *E. costae* hinders its comparisons with other studies; however, such comparisons could be made for *E. marginatus* instead. Other studies done on the dusky grouper in the Mediterranean described distinct home range values; in Ustica (Sicily, Italy), dusky groupers showed mean home ranges estimates of ~ 5 ha (Lembo *et al.*, 2002), i.e. similar to ours, while in the Cerbère-Banyuls marine natural reserve (France), a mean area of ~ 13 ha was observed for the same species (Pastor *et al.*, 2009). Several studies have focused on understanding the amplitude of groupers vital domain in coral reef environments of different localities. As example, relatively low mean estimated areas of 0.08 ha was found for *E. guttatus* in the Caribean Sea (Shapiro *et al* 1994) while approximately 4 ha was characterized for *Plectropomus areolatus* in Pohnpei, Micronesia (Hutchinson & Rhodes 2010) and an even bigger area (~18 ha) was recorded for *E. striatus* in the Bahamas (Bolden 2001). Divergent patterns exhibited by species that may share common life history characteristics such as similar diet, behaviour, morphology, such as *E. striatus* and *E. marginatus*, can be caused by an environmental influence rather than be determined by biological imprinting. This hypothesis is supported by the findings of Meyer (2008) in a study comparing two populations of *Cephalopholis argus*, one introduced in Hawaii and a natural population in Moorea Island (French Polynesia), showing that both populations diverged in home range and use of spatial resources according to location.

Additionally, it was postulated that home-range size increases linearly as a response to fish body size (Kramer and Chapman 1999). Although several works support the existence of such relationship, e.g. for *Lutjanus decussatus* (Nanami & Yamada 2008), *Achoerodus gouldii* (Bryars *et al* 2012), *E. costae* (this study), others did not find any correlation, as is the case of *E. morio* (Farmer &Ault 2011) and *E. marginatus* (this study), or even found a negative relationship between size of home range and individual size, such as for *E. tauvina* (Kaunda-Arara & Rose 2004a). These results indicate that the response might be species-specific.

Estimates of home range provided by MCP and kernel methods provide different images of fish spatial activity. MCP methods have been criticized because they could overestimate vital areas used by fish (Worton 1989) by incorporating all relocation points in space, which ultimately may not be commonly used by an individual fish; nevertheless, these methods give us a spatial dimension of how far fishes can move and how variable are the movement patterns among individuals. Conversely, by analysing density distribution, kernel estimates tells much more about the area in which species spend the majority of its time (Worton 1989), and give insights of social structure component that MCP does not. In our data, no difference was found between species home range for all three estimates methods; however, there is a tendency of *E. costae* to show larger total areas (MCP) meanwhile *E. marginatus* presented higher KUD and core areas. Similarly, no difference was found between area used and percent overlapped area between both sexes for the two studied species.

On fragmented landscapes, spatial distribution of resources can affect spatial use and behaviour (Atwood & Weeks 2003). Due to the patchy nature of Cabo de Palos rocky reefs, it is expected to found greater overlaps between individuals owing to limited space (Farmer & Ault, 2011). Additionally it is known that for *E. marginatus* an ontogenetic shift on diet occurs (Harmelin & Harmelin-Vivien 1999), which can contribute to the flexibility in sharing common territories, given that feeding niche is not overlaid. Underwater observations confirm that individual fish can withstand one neighbour within very short distances, reacting agonistically only when its small "comfort" zone is disturbed. Furthermore, both grouper species occurred in the same reef zones, thus causing overlapping between their respective territories. However, as both species make differential use of reef resources (Hackradt CW unpublished data, Chapter II) interactions are avoided, hence allowing the use of a common spatial area.

Despite the high site-fidelity showed by dusky groupers in many studies (e.g. Lembo *et al* 1999, Lembo *et al* 2002, Pastor *et al* 2009), and the strong territorial behaviour exhibited by groupers (Schenkel 1966, Sale 1991), both species studied here demonstrated their ability to make incursions outside their home-range area, travelling between rocky shoals that are separated by hundreds to thousands of meters, with deep (in some cases >80 m), detritic bottoms between them. In coral reef habitats, large displacements are usually related to reproductive period (e.g. spawning aggregations) that is concentrated far from the home reef (Bolden 2000, Pina-Amargós & González-Sansón 2009). In the case of Mediterranean groupers, and especially of *E. marginatus*, no evidences have been found to now of large displacements for spawning aggregation purposes. Some studies highlighted an extended reproductive period, lasting about 2-3 months (Marino *et al* 2001; Reñones *et al* 2010), and suggested that during summer months small scale movements occur which might be related to a reproductive gathering (Marino *et al* 2001). For its part, *E. costae* displayed a similar pattern to that found for *E. marginatus*, and for both species no seasonal differences in movement patterns were detected. This pattern, however, is not widespread for *E. marginatus*, since individuals of dusky groupers never left their vital domain inside the integral zone in Cerbère-Banyuls marine natural reserve (Pastor *et al.*, 2009). Our result thus indicate that the high movement rates found at Cabo de Palos may be driven by some force acting on Cabo de Palos groupers populations which does not occur at Banyuls, and we believe that might be the result of different population densities within reserves. In fact, density-dependent movement is the basis for spillover theory which relies on movement in a directional way from areas of high fish density to areas with lower densities (Abesamis & Russ 2005, Pérez-Ruzafa *et al*. 2008). As a consequence of the biomass build-up inside the no-take zones, an increase in fish home range overlap occurs, and consequently a likely greater number of fish contacts would cause a density-dependent diffusion process (Lisazo *et al.* 2000, Grüss *et al.* 2011). Spillover of groupers populations have been detected for a number of Mediterranean MPAs by analysing biomass gradients across reserve boundaries, including the Cabo de Palos – Islas Hormigas marine reserve (Hackradt *et al.*, *in prep*); in the abovementioned work it was demonstrated that spillover was only detectable in locations harbouring high fish abundances, as was the case of Cabrera and Cabo de Palos – Islas Hormigas MPAs, and was not detected at Cerbère-Banyuls reserve, where relatively low densities were found. Our results from both tagging and tracking procedures corroborate the biomass exportation as we were able to detect movement of groupers individuals from both inside no-take zone (NTZ) to buffer zone (BZ) and from BZ to outside MPA. We used immigration and emigration rates as proxy of measurements of spill-in and spillover effects, taking into consideration only movements observed regarding the NTZ. We observed similar immigration and emigration rates for *E. marginatus* regarding the NTZ, while positive emigration of *E. costae* to outside NTZ was recorded. According to

Russ & Alcala (2004), spill-in movements are produced as a behaviour response to disturbance on fished areas, but it can also be driven by habitat features such as habitat quality (Grüss *et al.* 2011). Whatever the cause of spill-in movement, it will be maintained as a function of the available resources (space, food, shelter), and as soon as carrying capacity (*K*) is reached only spillover effects would be detectable. Thus, at intermediate levels of abundance, spill-in and spillover movements can co-occur and their net result will depend on differences on abundance from inside to outside NTZ. Hence, in Cabo de Palos – Islas Hormigas marine reserve, high quality habitat inside the NTZ may contribute for the spill-in movements to occur. Moreover, the higher grouper densities found within the MPA as compared to outside make spillover effects detectable for both species. However, in the case of *E. marginatus* population the net balance of spillover and spill-in rates was equal to zero, indicating that Cabo de Palos population did not reached their potential *K*. The growing phase in which *E. marginatus* population is going trough was described by García-Charton *et al* (2010), indicating a potential increase of MPA benefits to fished areas in the following years.

Fish mobility generally increases with body size (Grüss *et al* 2011), as found for *E. costae* in this work. Conversely, for long-lived, slow-growing, late-maturing and territorial species such as *E. marginatus* adults are extremely site-attached and thus smaller individuals tend to move more as they do not yet fully developed territoriality and site attach behaviour displayed by larger ones (Kramer & Chapman 1999, Kaunda-arara & Rose 2004b). These opposite patterns detected in both species have important implications for determining their population structures, since smaller *E. marginatus* and larger *E. costae* were the ones potentially dispersive, contributing to adjacent fished population.

MPAs are nowadays the most effective management tool for conserving grouper populations at sustainable levels worldwide. A great number of species is at present under some level of threat, mainly due to overfishing and habitat destruction (Craig *et al* 2011, Sadovy de Michelson *et al.* 2012). The comprehension of mobility patterns in groupers could provide useful information for the understanding of how species interact with their habitat and sympatric species, and ultimately for helping to design effective spatial measures to protect marine biodiversity.

Chapter V

Response of rocky reef top predators (Serranidae: Epinephelinae) in and around marine protected areas in the Western Mediterranean Sea.

La respuesta de depredadores apicales en arrecifes rocosos (Serranidae: Epinephelinae) dentro y cerca de aéreas marinas protegidas en la cuenca oeste del Mediterráneo.

Response of rocky reef top predators (Serranidae: Epinephelinae) in and around marine protected areas in the Western Mediterranean Sea.

ABSTRACT

Groupers species are extremely vulnerable to overfishing and many species are threatened worldwide. In recent decades, Mediterranean groupers experienced a dramatic decline of their populations. Marine protected areas (MPAs) can protect populations inside their boundaries and provide individuals to adjacent fishing areas through the process of spillover and larval export. This study aims at evaluating the effectiveness of six marine reserves in Western Mediterranean Sea to protect the populations of three species of grouper (*Epinephelus marginatus*, *E. costae* and *Mycteroperca rubra*) and understanding in which circumstances MPAs are able to export biomass to neighbouring areas. All the studied MPAs, except one where no grouper was observed, were able to maintain high abundance, biomass and mean weight of groupers. Size classes were more evenly distributed inside than outside MPAs. In two reserves, biomass gradients could be detected through the boundaries of the reserve as an indication of spillover. In some cases, habitat structure appeared to exert a great influence on grouper abundance, biomass and mean individual weight, influencing the gradient shape. Because groupers are generally sedentary animals with small home range, we suggest that biomass gradients could only occur where groupers attain sufficient abundance inside MPA limits, indicating a strongly density-dependent process.

Key words: Groupers, Marine Protected Areas, Spillover, Biomass, Population recovery

1. Introduction

Marine biodiversity is seriously threatened by an array of anthropogenic actions, like habitat destruction (Lotze *et al.* 2006), land-based sediment load (Syvitski *et al.* 2005) and pollution (Johnston & Roberts 2009, McKinley & Johnston 2010), invasion by alien species (Gollasch 2006, Occhipinti-Ambrogi & Galil 2010), and catastrophic shifts induced by global warming (Harley *et al.* 2006, Philippart *et al.* 2011, Doney *et al.* 2012). Overfishing, however, is likely to be the main human activity causing marine biodiversity erosion, as well as a facilitating factor for the synergistic effects of all other sources of perturbation (Jackson *et al.* 2001, Jackson 2008). Worm *et al.* (2009) stated that although increasing efforts to restore marine ecosystems and rebuild fisheries are under way, most fish stocks worldwide still require rebuilding. Lower exploitation rates are needed to reverse the collapse of vulnerable species, which are likely to cause upheavals in the global ecosystem through the loss of particular functions played by key species.

The instauration and maintenance of marine reserves or marine protected areas (MPAs), is one of the most effective tools to break the loss of marine diversity and also stop habitat loss (Pauly *et al.* 2005, Fenberg *et al.* 2012). Its application is based on a strategy shift in coastal management towards the spatial planning of human activities and the implementation of an ecosystem-based approach to fisheries management (Pauly *et al.* 2002, Agardy 2005, Gilliland & Laffoley 2008).

Recent studies, based on meta-analyses and reviews, show that MPAs can reverse most deleterious effects of fisheries on the marine environment (Claudet *et al.* 2008, 2010, García-Charton *et al.* 2008, Higgins *et al.* 2008, Pérez-Ruzafa *et al.* 2008, Planes *et al.* 2008, Lester *et al.* 2009, Fenberg *et al.* 2012) provided that they are properly managed (Samoilys *et al.* 2007, Guidetti *et al.* 2008). The notable effects of marine reserves are an increase in abundance and an enlargement of the average size of individuals of the target species inside the boundaries of the protected area, so that greater abundance and size theoretically imply an increase in reproductive potential (Claudet *et al.* 2008, 2010, García-Charton *et al.* 2008, Lester *et al.* 2009, Fenberg *et al.* 2012). The eggs and larvae from restored spawning stocks inside MPA could then be exported by currents to adjacent fishing areas (Gell and Roberts 2003, Alcala *et al.* 2005, Crec'hriou *et al.* 2010, but see Pelc *et al.* 2010). On the other hand, because of increased density inside the MPA, adults and juveniles fishes from target species may emigrate from inside the protected locations to outside, where the density is lower ("spillover", Rowley 1994). An indirect method to estimate the magnitude and importance of such export of larvae, juveniles and adults fishes from MPA to neighbouring areas is to look for the likely existence of gradients of biomass of target species across MPA limits, under the rationale that, if spillover occurs, there would be more fishes near than far away from the MPA (Rakitin & Kramer 1996, Chapman & Kramer 1999, Pérez-Ruzafa *et al.* 2008). This research strategy has been used in several studies in the Mediterranean (e.g. Guidetti 2006, Harmelin-Vivien *et al.* 2008, Goñi *et al.* 2008, Stobart *et al.* 2009) and worldwide (e.g., MacClanahan & Mangi 2000, Russ *et al.* 2003, Ashworth & Ormond 2005, Francini-Filho & Mora 2008, Amargós *et al.* 2010). It has been hypothesized that the shape of biomass gradient for a given fish population responds to the distribution of the fishing pressure outside the reserve, and to the flux of individuals over the reserve boundary, which in turn would depend on the extent to which the system's carrying capacity is reached by the population (Pérez-Ruzafa *et al.* 2008). The instantaneous population growth rate of a species would affect the speed of recovery of the population after cessation of fishing activity and the ability of the species to maintain abundances close to the carrying capacity inside the integral reserve, and, even more importantly, it likely affects the population size in the fished area and therefore determines the fishing mortality that the population can support without collapsing (Pérez-Ruzafa *et al.* 2008). For its part, flux of adults through MPA limits will depend on movement patterns, home range and spatial use of the species concerned (Pérez-Ruzafa *et al.* 2008). Therefore, the efficiency of a marine reserve to enhance fish abundance and biomass within their limits and to spillover to adjacent areas can vary depending on the biological characteristics of each species (Kramer & Chapman 1999, Claudet *et al.* 2010).

On the other hand, habitat structure is one of the factors explaining the small-scale spatial variability of fish assemblage (García-Charton & Pérez-Ruzafa 2001), and spatial variations in habitat structure is likely to affect the strength and even the occurrence of biomass increase within MPA boundaries and spillover (Chapman & Kramer 1999, Kramer & Chapman 1999, García-Charton *et al.* 2004, Harmelin-Vivien *et al.* 2008), by influencing resource availability (food or refuge against predators or fishing), and ultimately affecting population growth and mobility.

Among the most common species favoured by the reduction in fishing pressure are top predators (Shears & Babcok 2002, McClanahan *et al.* 2007, Stevenson *et al.* 2007, DeMartini *et al.* 2008, Sandin *et al.* 2008, Stallings 2009), and especially groupers (Sluka *et al.* 1997, Chiappone *et al.* 2000, Unsworth *et al.* 2007). Groupers (Epinephelinae: Perciformes) are emblematic species around the world, as they are of great importance for both recreational and artisanal fisheries (Harper *et al.* 2000, Coll *et al.* 2004, Morales-Nin *et al.* 200, Sadovy de Mitcheson *et al.* 2012). Most species of Epinephelinae are endangered, and about half the species are under some level of threat (Morris *et al.* 2000, Aguilar-Perera 2006, Sadovy de Mitcheson *et al.* 2012). The high susceptibility of grouper species to overfishing and habitat loss is likely due to their biology and life style, which promote a synergetic effect with anthropogenic activities (Eklund & Shufle 2001). High site fidelity, high longevity, late maturity, formation of spawning aggregations, slow growth rate and low resilience (5 to 14 years to minimum population doubling time) are some of the characteristics that determine a high to very high level of vulnerability of these species (Cornish & Harmelin-Vivien 2004, De

Almeida Rodrigues Filho *et al.* 2009; Sadovy de Mitcheson *et al.* 2012). Six autochtonous grouper species live in the Mediterranean Sea (five from the genus *Epinephelus* and one *Mycteroperca*), three of them (*Epinephelus marginatus*, *E. costae* and *Mycteroperca rubra*) being common in Western Mediterranean coastal waters. All of them are exploited by commercial and/or recreational fisheries (Coll *et al.* 2004, Lloret *et al.* 2008). During the last decades a notable decline of their populations was observed worldwide, and particularly in the Mediterranean Sea (Chauvet 1991, Gracia 1996, Sadovy de Mitcheson *et al.* 2012).

The aim of this study is to evaluate the efficiency of protection measures to promote the recovery of populations of three groupers species both within and around MPAs in Western Mediterranean Sea. The hypotheses to be tested are whether, and to what extent, there are higher grouper abundances inside than outside marine reserves, and whether biomass gradients can be found across the boundaries of the studied MPAs, suggesting spillover to neighbouring areas. Moreover, the present study aims at exploring the possible interference of the spatial distribution of structural habitat on grouper abundance within MPAs, and the occurrence of spillover towards adjacent areas.

2. Materials and methods

2.1. Study Area

The work was conducted from July to October of 2003 and 2004 (depending on the MPA) on six MPAs spread over the Western Mediterranean Sea (Fig. 5.1): the natural marine reserve of Cerbère-Banyuls and the Carry-le-Rouet marine park (hereafter referred to as Banyuls and Carry, respectively) in France, and the National park of Cabrera and the marine reserves of Medes islands, Tabarca island and Cabo de Palos – Hormigas islands (hereafter Cabo de Palos) in Spain. All MPAs (except one, Carry) were designed under the recommendations of the IUCN, with no-take / no-use zones (called integral reserves - IR) surrounded by buffer zones (BZ), where some uses are permitted (usually recreational diving and some kind of artisanal fishing). Carry is formed exclusively by a no-take zone, although it belongs to a larger conservation unit in the region. Three MPAs are located on archipelagos (Medes, Tabarca and Cabrera), two are exclusively coastal (Banyuls and Carry), and Cabo de Palos includes both coastal and island sites. Common criteria used to select the MPAs involved in this study are that they were established for more than 10 years, and presented a high level of enforcement. All marine reserves involved in this study are similar regarding the composition and constitution of the seabed, presenting *Posidonia oceanica* meadows and rocky bottoms.

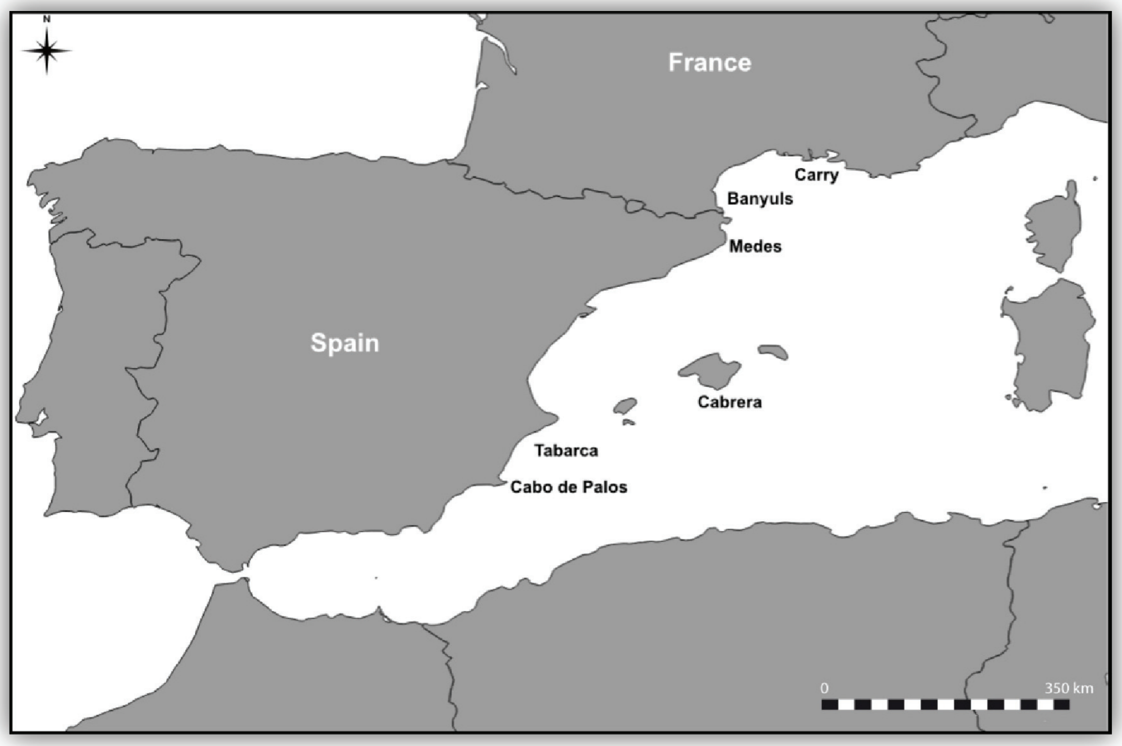


Figure 5.1: Location of the Mediterranean marine protected areas (MPAs) studied. Carry-le-Rouet, Banyuls, Medes, Cabrera, Tabarca, Cabo de Palos.

2.2. Sampling design and data acquisition

Seven to nine sectors, separated by 1000's of metres, were positioned at increasing distances from the core of each MPA. In each sector, three zones were haphazardly located at a scale of

100s of metres. Finally, six transects (replicates) separated by 10s of metres were sampled in each zone. In three MPAs (Banyuls, Carry and Cabo de Palos), gradients in fish parameters were studied in two opposite directions. In such cases, three sectors were located inside the MPA (one inside the IR, and two within BZ) and six in fished areas: three in one direction and three in the opposite direction. In the three MPAs located on islands (Cabrera, Medes and Tabarca) only one direction (northward) could be studied due to the absence of suitable rocky habitats southward. In Cabrera, 3 sectors were located within IR, 3 sectors inside BZ, and 3 outside the MPA. For Medes, three sectors were located within e IR, one in BZ, and three outside the MPA. In Tabarca, the sampling was performed considering one sector inside IR, three sectors in BZ, and the rest outside the reserve. In this MPA, the sampling was done on two different bottom types: rocky bottoms and *Posidonia oceanica* meadows [see Table 1 in Harmelin-Vivien *et al.* (2008) for further information].

Fish abundance of three grouper species, the dusky grouper *Epinephelus marginatus*, the goldblotch grouper *E. costae* and the mottled grouper *Mycteroperca rubra,* was assessed by visual census in 25×5-m transect belts located at 6-12 m depth and parallel to the coast. As *Posidonia oceanica* beds covered large areas around Tabarca island, seagrass beds were surveyed in this habitat in 50×5 m transects, as fishes were more dispersed. In each transect, fishes were identified and their abundance recorded on a polyester sheet clipped on a PVC board, and the size of each individual was recorded within 2-cm size classes, so that fish weight could be estimated from length-weight relationship found on the literature, using the ECOCEN software (Bayle-Sempere *et al.* 2002). Within each transect a series of descriptors of structural habitat were also registered, namely the number of rocky boulders (classified as small, medium and big) and the type of substrate on percentage of cover (by rock, sand, *Posidonia* and pebbles) [for further details on habitat data acquisition, see García-Charton *et al.* (2004) and Harmelin-Vivien *et al.* (2008)].

2.3. Data analysis

The effect of protection in each MPA was evaluated separately, because differences in sampling design precluded making a unique analysis with all MPAs. Thus, distance-based permutational multivariate analysis of variance (PERMANOVA, Anderson 2001) based on Bray-Curtis dissimilarities (Bray & Curtis 1957) on log-transformed data was run in univariate mode, using abundance, biomass and individual mean weight of the three target species of groupers as response variables. In general, the field experimental design for each MPA consisted of three factors: Location (factor L, fixed, comparing two or three levels of protection in each MPA, depending on the number of sectors included in the IR and BZ as compared to the unprotected –UP– locations), Sector (factor S, 3 levels, random, nested in L) and Zone (factor Z, 3 levels, random, nested in S).). For Medes and Tabarca, one sector was excluded from the analyses to get a balanced design (BZ in Medes, and IR in Tabarca). In the case of MPAs where 6 sectors were surveyed outside the protected location against 3 sectors within it, an asymmetrical design was applied, for which the Location term was partitioned into two portions: the one degree-of-freedom contrast Protected (P) *vs*. UPs locations, and the variability between UPs. The overall mean squares of the terms S(L) and Z(S(L)) were similarly partitioned into S(P *vs* UPs) and S(UPs), and Z(S(P *vs* UPs)) and Z(S(UPs)), respectively. In order to minimize the effect of habitat variability on data and exploring only the effect on species protection, we used the environmental data as co-variables. For all analysis 9999 permutations were applied under a full model, using PRIMER v.6 package.

Trends and significance of gradients of groupers biomass across MPAs boundaries were calculated using linear correlation of dependent variables with the distance from MPA limits at the scale of zones. Negative correlations would indicate that biomass decreases from the core of MPA to distant fished zones. The border of IR was defined as zero, so negative distances indicate zones inside IR, and positive distances zones outside the IR. To explore the actual shape of grouper biomass gradients across MPA borders, generalized additive modelling (GAM) were applied using gam v.1.06.2 package. GAM is known to be useful when the actual relationship between the variables is unknown and expected to be of a complex form, not easily fitted by standard linear or non-linear models (Hastie & Tibshirani 1990). Distance to the integral reserve boundary was introduced as a continuous smooth variable modelled non-parametrically using a loess smoother (lo(Distance)). We applied to model Gaussian variance and identity link functions, both based on Hastie & Tibshirani (1990) and Venables & Ripley (2000). The gradients were tested for both sides of MPA (South and North), or as a unique gradient, depending on the study case.

The likely influence of habitat structure on gradient shape was further assessed by performing multiple linear regression analyses to measure the strength of the relationship between the whole set of habitat variables (including their quadratic and cubic terms) and each species' population parameter (abundance, biomass and individual mean weight). Prior to analyses, the extreme and influential cases were detected and removed by carrying out analysis of residuals (McCullagh & Nelder 1989; García-Charton & Pérez-Ruzafa 1998). Then, the residuals of these analyses were used as dependent variable in linear correlations and GAMs with the distance from MPA limits (Harmelin-Vivien *et al.* 2008). If habitat quality is equal both inside and outside the MPA, or does not influence fish biomass, we hypothesize that the extraction of habitat influence would not affect the shape of biomass gradient as depicted by GAMs (Fig. 5.2a and b). If habitat quality is better inside than outside the MPA (i.e. it promotes higher fish biomass within the MPA because structural habitat provides either enhanced food or/and refuge resources as compared to surrounding areas), the shape of biomass gradient across MPA limits would be smoothed once extracted the influence of habitat from raw data (Fig. 5.2c).

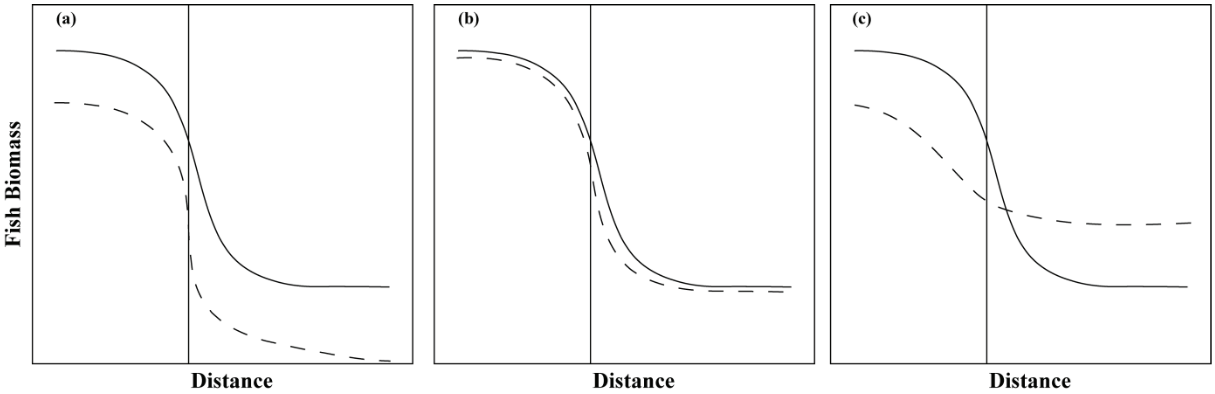


Figure 5.2: Hypotheses for the shape of gradient of fish biomass across MPA boundaries with raw data (solid line) and residual data after extracting habitat influence (dotted line) where habitat quality (a) is equally good or (b) has no influence both inside and outside the MPA, and (c) where habitat quality is better within the MPA. The vertical line indicates the limit of the MPA.

3. Results

3.1. Effects of protection

The dusky grouper (*Epinephelus marginatus*) was recorded in all MPAs, except Carry. In Banyuls and Medes, dusky groupers were censused only in IR, and in Tabarca – *Posidonia* this species was censused both in IR and BZ, but not outside the MPA. Thus, the effect of protection levels and nested spatial factors will not be further explored by statistical methods for these three MPAs (Fig. 5.3). The two other species, goldblotch grouper (*E. costae*) and mottled grouper (*Mycteroperca rubra*), were censused only in Cabrera, Tabarca – rocky and Cabo de Palos MPAs. Cabrera and Cabo de Palos MPAs presented the highest grouper abundance amongst all reserves, while the lowest abundance values were recorded in the northernmost MPAs (Banyuls and Medes). As no grouper was recorded in Carry during this survey, no further reference will be done to this MPA.

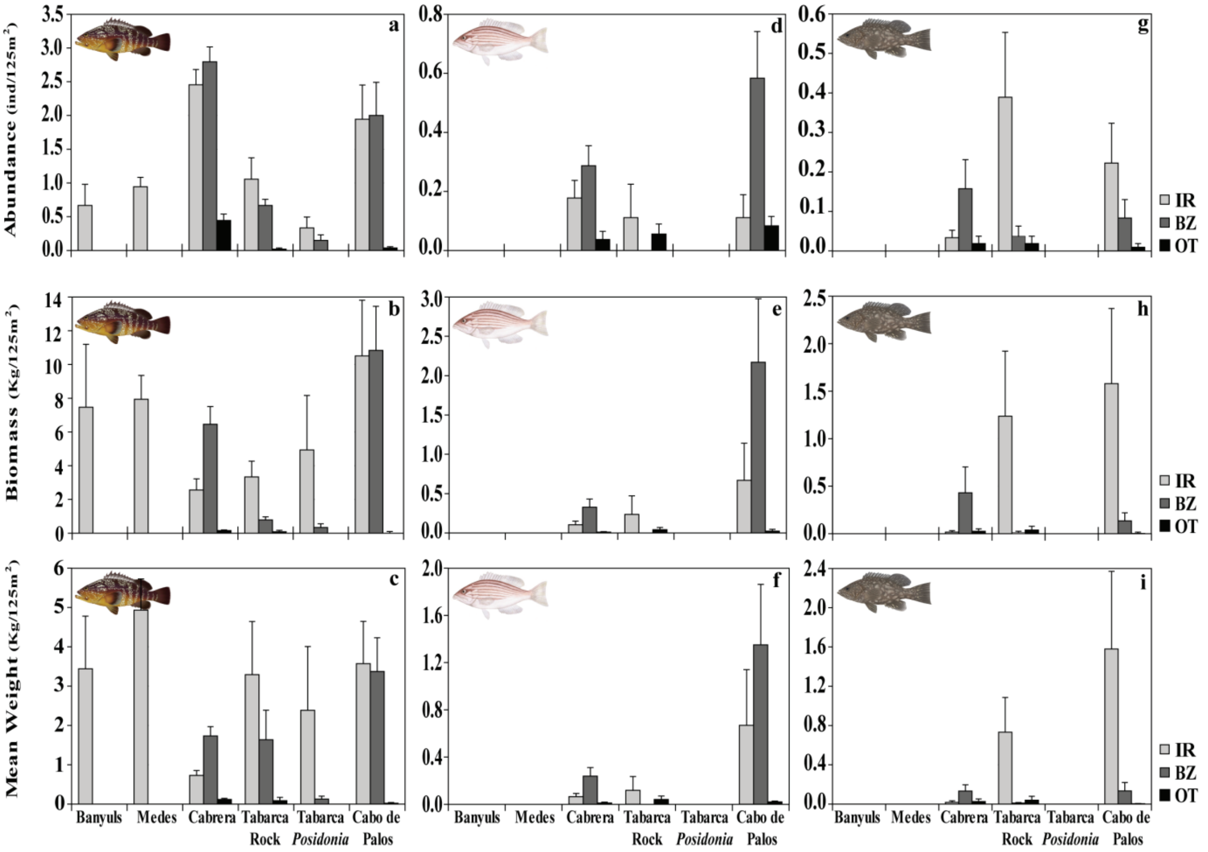


Figure 5.3: Abundance, biomass and mean weight of the tree species of groupers, *E. marginatus*, *E. costae* and *M. rubra* on the tree levels of protection (Integral reserve IR, Buffer zone, BZ and unpotected, OT) on all MPA studied.

The abundance, biomass and mean weight of dusky grouper were significantly higher within the protected areas than in the unprotected ones in all analyzed MPAs, as showed by the statistically significant effect of fixed factor Location (Table 5.1, Fig. 5.3). In Cabrera and Cabo de Palos, the values of the analysed parameters in IR and BZ were very similar (Fig. 5.3), and presented much higher values than in unprotected areas (Table 5.1 and Fig. 5.3). In the case of Tabarca (both in rocky bottoms and *Posidonia* meadows) the IR, although not included in the analyses, showed much higher values of all analysed parameters compared to both BZ and UP areas (Fig. 5.3). A significant medium-scale (among sectors separated by 1000's of meters) variability was evidenced in Cabo de Palos, mostly in the unprotected locations, and a fine-scale (among zones separated by 100's of meters within sectors) variability was detected for all dependent variables in Tabarca – both rocky and *Posidonia*, and Cabo de Palos (Table 5.1).

Table 5.1: Permutational multivariate analysis of variance (PERMANOVA) results for abundance, biomass and mean weight of *E. marginatus* found in the locations studied on Western Mediterranean Sea where groupers are present both inside and outside the MPA.

	Marine Reserve		**Abundance**			**Biomass**			**Mean Weight**		
	Source	**df**	**MS**	**PF**	**P**	**MS**	**PF**	**P**	**MS**	**PF**	**P**
Cabrera	**L**	2	9960.3	14.43	**0.006**	29525	22.05	**0.001**	26915	23.99	**0.001**
	S(L)	6	660.0.	1.92	0.120	1278.3	1.13	0.374	1070.8	1.01	0.454
	Z(S(L))	18	344.9	1.53	0.073	1137	1.18	0.277	1065.5	1.16	0.310
	Res	128	224.6			965.7			922.0		
Tabarca Rock	**L**	1	3174.3	92.15	**0.001**	21930	271.77	**0.0001**	22613	275.57	**0.0001**
	S(L)	4	28.8	0.10	0.981	67.4	0.035	0.998	68.6	0.003	0.998
	Z(S(L))	12	290	2.83	**0.002**	2032.2	2.97	**0.002**	2190.7	3.15	**0.0005**
	Res	83	102.3			682.2			695.8		
Cabo de Palos	**L**	2	2436.2	4.09	0.059	11668	5.03	**0.037**	11267	5.11	**0.030**
	P*vs*UPs	1	4816	6.83	**0.044**	22996	8.79	**0.020**	22204	8.97	**0.025**
	UPs	1	0.002	0.0005	0.984	3.4	0.008	0.984	3.4	0.008	0.984
	S(L)	6	623.6	3.35	**0.009**	2419.7	2.71	**0.027**	2298.1	2.66	**0.031**
	S(P*vs*UPs)	4	905.9	3.44	**0.031**	3327.9	2.67	0.067	3141.9	2.61	0.069
	S(UPs)	4	59.9	4.25	**0.023**	450.5	3.95	**0.017**	450.5	3.95	**0.021**
	Z(S(L))	18	186.7	1.45	0.108	895.8	1.69	**0.042**	868.4	1.69	**0.046**
	Z(S(P*vs*UPs))	12	270.5	2.20	**0.010**	1282.3	2.50	**0.006**	1241.3	2.49	**0.005**
	Z(S(UPs))	12	14	0.60	0.859	113.59	0.67	0.807	113.6	0.67	0.802
	Res	128	129.1			531.2			515.2		

No significant effect of the factor Location was found in any MPA analysed for goldblotch groupers and mottled groupers (Tables 5.2 and 5.3), despite these species were more abundant inside the protected area of Cabrera and Cabo de Palos (Fig. 5.3). This result was likely due to the high spatial variability at several scales, including among replicates. At Cabo de Palos a significant spatial variability among sectors within the protected location in abundance, biomass and mean weight of *E. costae* was evident (Table 5.2). Significant variability among zones within sectors was also found in all MPAs analyzed for *E. costae*, and in Tabarca – rocky and Cabo de Palos for *M. rubra* (Table 5.3). This pattern was attributable to the unprotected sites in Cabo de Palos for both species.

Table 5.2: Permutational multivariate analysis of variance (PERMANOVA) results for abundance, biomass and mean weight of *E. costae* found in the locations studied on western Mediterranean Sea where groupers are present both inside and outside the MPA.

Marine Reserve			**Abundance**			**Biomass**			**Mean Weight**		
	Source	**df**	**MS**	**PF**	**P**	**MS**	**PF**	**P**	**MS**	**PF**	**P**
Cabrera	**L**	2	428.8	2.85	0.129	3034.1	3.52	0.082	3015.3	3.61	0.083
	S(L)	6	144.3	0.92	0.507	826.9	0.73	0.640	800.4	0.71	0.652
	Z(S(L))	18	157.6	2.12	**0.010**	1140.9	2.24	**0.005**	1133.4	2.26	**0.005**
	Res	135	74.4			509.0			501.6		
Tabarca Rock	**L**	1	13.5	0.24	0.750	143.5	0.34	0.693	175	0.36	0.681
	S(L)	4	69.6	1.38	0.29	476.9	1.16	0.398	542.1	1.14	0.406
	Z(S(L))	12	52.5	2.18	**0.019**	428.7	2.59	**0.006**	495.9	2.68	**0.006**
	Res	83	24.1			165.8			185.3		
Cabo de Palos	**L**	2	170	0.26	0.798	1443.5	0.35	0.733	1444	0.36	0.732
	P*vs*UPs	1	40.3	0.05	0.869	363.31	0.07	0.832	352.8	0.07	0.826
	UPs	1	150.9	4.82	0.089	1257.4	4.89	0.084	1262.7	4.90	0.084
	S(L)	6	680.7	6.09	**0.0001**	4343.7	5.86	**0.0001**	4260.5	5.85	**0.0001**
	S(P*vs*UPs)	4	1041.5	7.87	**0.0001**	6643.4	7.54	**0.0001**	6516.9	7.53	**0.0001**
	S(UPs)	4	31	0.60	0.684	256.1	0.75	0.585	256.4	0.76	0.577
	Z(S(L))	18	112.4	1.80	**0.030**	744.2	1.69	**0.044**	731.8	1.68	**0.048**
	Z(S(P*vs*UPs))	12	136	2.16	**0.015**	904	2.03	**0.026**	888.1	2.02	**0.025**
	Z(S(UPs))	12	51.4	1.03	0.431	342.5	0.94	0.525	338.3	0.94	0.522
	Res	128	62.5			439.8			435.5		

The size class distribution of the dusky grouper was more evenly distributed in Banyuls and Medes, with the presence of large individuals, than in the other MPAs, where individuals larger than 60 cm were scarce (Fig. 5.4). In all MPAs, larger individuals, if censused, were only seen within MPAs limits, while small dusky groupers were abundant outside MPA in Cabrera and Cabo de Palos (Fig. 5.4). Small specimens (< 40 cm) of *E. costae* were also more frequently seen outside MPAs, while large individuals (> 60 cm) of *M. rubra* occurred exclusively within MPA limits (Fig. 5.4).

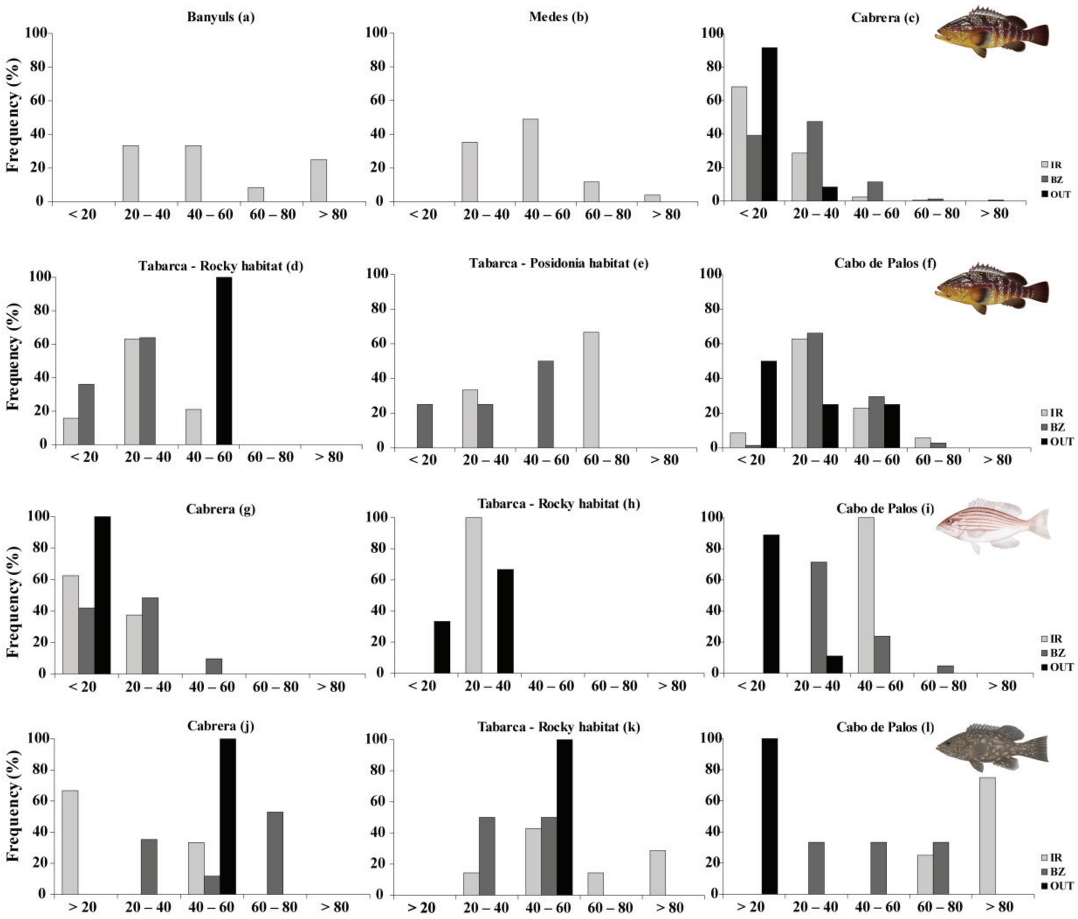


Figure 5.4: Distribution of frequencies of groupers size classes in each MPA studied (a-f, *E. marginatus*; g-i, *E. costae*; j-l, *M. rubra*).

Table 5.3: Permutational multivariate analysis of variance (PERMANOVA) results for abundance, biomass and mean weight of *M. rubra* found in the locations studied on Western Mediterranean Sea where groupers are present both inside and outside the MPA.

Marine Reserve			Abundance			Biomass			Mean Weight		
	Source	**df**	**MS**	**PF**	**P**	**MS**	**PF**	**P**	**MS**	**PF**	**P**
Cabrera	**L**	2	1.2	0.11	0.897	14.3	0.13	0.909	14.2	0.13	0.903
	S(L)	6	17.9	1.31	0.290	153.9	1.40	0.251	153.8	1.39	0.259
	Z(S(L))	18	13.6	0.83	0.685	110.0	0.83	0.685	109.9	0.82	0.683
	Res	135	16.3			131.8			131.8		
Tabarca Rock	**L**	1	18.6	0.46	0.576	168.8	0.43	0.596	166	0.42	0.602
	S(L)	4	54.2	0.67	0.624	497.5	0.77	0.580	507	0.76	0.574
	Z(S(L))	12	83.9	2.17	**0.016**	674.9	2.27	**0.014**	689.4	2.30	**0.014**
	Res	83	38.6			297.1			299.2		
Cabo de Palos	**L**	2	44.2	1.35	0.325	441.9	1.32	0.330	441.9	1.32	0.341
	P*vs*UPs	1	87.3	2.06	0.218	873.1	2.03	0.208	873.1	2.03	0.214
	UPs	1	1.3	0.38	0.597	11.2	0.38	0.598	11.2	0.38	0.598
	S(L)	6	32.7	0.88	0.570	334.8	0.92	0.550	334.8	0.92	0.553
	S(P*vs*UPs)	4	46.6	0.91	0.544	481.0	0.95	0.521	481.3	0.95	0.535
	S(UPs)	4	3.6	0.57	0.744	29.6	0.57	0.736	29.6	0.57	0.746
	Z(S(L))	18	37.3	1.35	0.164	366.4	1.40	0.131	366.4	1.40	0.135
	Z(S(P*vs*UPs))	12	52.8	2.00	**0.031**	522.1	2.11	**0.021**	522.1	2.11	**0.017**
	Z(S(UPs))	12	6.3	1.15	0.327	52.3	1.15	0.331	52.3	1.15	0.324
	Res	128	27.8			261.9			261.9		

3.2. Influence of habitat

Habitat structure appeared to exert a great influence on the dusky grouper abundance, biomass and mean individual weight in Tabarca (both in rocky and *Posidonia* bottoms) and Cabo de Palos (Table 5.4). In Banyuls the habitat structure had a positive influence on the dusky grouper abundance, and in Cabrera, Tabarca – rocky and Cabo de Palos on the *E. costae* abundance. The Mean weight was positively influenced by habitat only in Tabarca rocky and Cabo de Palos. The influence of habitat was positive on biomass of *E. costae* in Tabarca-rocky and Cabo de Palos and the biomass and mean weight of *M. rubra* in Cabo de Palos. In Banyuls, Medes and Cabrera, apart from the positive relationship with abundance of *E. marginatus* in Banyuls and with abundance of *E. costae* in Cabrera, no significant influence appears (Table 5.4). In the cases where it resulted to be significant, the influence of habitat on grouper populations' parameters attained in average around 33% of total variability (range 14-53%) (Table 5.4).

Table 5.4: Summary of results of multiple linear regressions (adjusted R^2 and significance level) of mean abundance (Ab), biomass (Biom) and mean individual weight (W) of the three species of groupers studied against linear, quadratic and cubic terms of all habitat characteristics measured in the transects for each MPA (ns: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$)

		Banyuls	Medes	Cabrera	Tabarca – rocky	Tabarca – *Posidonia*	Cabo de Palos
E. marginatus	**Ab**	0.210 (*)	0.117 (ns)	0.062 (ns)	0.296 (**)	0.525 (***)	0.331 (***)
	Biom	0.176 (ns)	0.048 (ns)	0.086 (ns)	0.350 (***)	0.473 (***)	0.359 (***)
	W	0.083 (ns)	0.066 (ns)	0.112 (ns)	0.323 (**)	0.451 (***)	0.374 (***)
E. costae	**Ab**			0.139 (*)	0.257 (*)		0.253 (**)
	Biom			0.060 (ns)	0.173 (ns)		0.191 (ns)
	W			0.084 (ns)	0.300 (**)		0.211 (*)
M. rubra	**Ab**			0.049 (ns)	0.397 (***)		0.210 (*)
	Biom			0.048 (ns)	0.366 (***)		0.159 (ns)
	W			0.044 (ns)	0.378 (***)		0.159 (ns)

3.3. Gradients of biomass export

Where groupers have been censused both inside and outside the MPA limits (Cabrera, Tabarca – rocky, Cabo de Palos – North and Cabo de Palos – South), raw values of grouper biomass decreased with increasing distance from the boundary of MPAs, except for *E. costae* in Tabarca – rocky, for which no significant relationship with distance appeared (Table 5.5). Most negative relationships, 72% (8 out of 11) were significant. When residuals of biomass data obtained from multiple linear regressions were used as dependent variables instead of raw data, the number of significant negative correlations with distance decreased. The non-significant negative relationship of *E. costae* raw biomass southwards of Cabo de Palos even became a significant positive relationship. These shifts when using residuals instead of raw data as dependent variables suggested a high habitat effect which could mask the real effect of protection (Table 5.5).

Table 5.5: Results of linear correlation performed on raw data and residuals after extracting habitat effects on the biomass of three species of groupers

	E. marginatus				***E. costae***				***M. rubra***			
	Raw		**Res**		**Raw**		**Res**		**Raw**		**Res**	
	r	**P**	**r**	**P**	**r**	**P**	**r**	**P**	**r**	**P**	**r**	**P**
Cabrera	-0.483	**0.000**	-0.450	**0.000**	-0.140	**0.027**	-0.109	0.084	-0.056	0.374	-0.074	0.239
Tabarca–rocky	-0.413	**0.000**	-0.158	0.077	0.012	0.890	-0.071	0.429	-0.076	0.397	-0.021	0.814
C. Palos North	-0.516	**0.000**	-0.181	0.061	-0.216	**0.025**	0.047	0.627	-0.276	**0.004**	-0.057	0.556
C. Palos South	-0.573	**0.000**	-0.085	0.380	-0.156	0.107	0.226	**0.019**	-0.316	**0.001**	-0.121	0.212

Results of general additive models (GAM) on fish raw biomass as a function of distance to reserve boundaries yielded significant non-linear relationships in 8 out of 12 studied crossings between MPAs and species, these significant models explaining 16-58% of total data variability (Table 5.6). When using residual biomass resulting from multiple linear regressions with habitat variables, the number of significant non-.linear relationships with distance dropped to 5, and the range of percentage variability explained by the models decreased to 10-39% (Table 5.6). Graphical representation of GAMs evidenced two major patterns: the biomass of grouper species decreased rapidly between the integral reserve and the buffer zone (Tabarca – rocky), or at the edge of the MPA, in the fishing area (Cabrera and Cabo de Palos). No change in the pattern could be observed for raw data and using the residuals for Cabrera, which was expected when habitat structure does not influence grouper abundances either inside or outside the MPA. However, in Tabarca and Cabo de Palos, residuals' slopes were less pronounced and curves' shapes smoother, following what is hypothesized when habitat quality is better inside than outside the MPA (Fig.5. 2 and 5-7). The actual shape of the observed biomass gradients suggested in these cases that there was no export, or it occurred at a very short distance (< 1000 m).

Table 5.6: Analysis of deviance for the generalised additive models (GAMs) fitted with raw data and residuals of multiple linear regressions with habitat variables on biomass of three grouper species

		Term	Res df	dev	F	P	R^2
E. marginatus							
Cabrera	**Raw**	lo (distance)	246	162.91	3.73	0.005	0.28
	Res	lo (distance)	246	132.62	3.80	0.004	0.25
Tabarca – rocky	**Raw**	lo (distance)	120	90.77	6.87	<0.001	0.32
	Res	lo (distance)	120	11.91	1.48	0.211	0.07
C Palos North	**Raw**	lo (distance)	102	197.78	13.64	<0.001	0.54
	Res	lo (distance)	102	23.37	2.37	0.05	0.12
C Palos South	**Raw**	lo (distance)	102	189.35	12.00	<0.001	0.51
	Res	lo (distance)	102	18.02	3.25	0.02	0.10
E. costae							
Cabrera	**Raw**	lo (distance)	246	9.45	1.20	0.31	0.04
	Res	lo (distance)	246	6.30	1.11	0.35	0.03
Tabarca – rocky	**Raw**	lo (distance)	120	2.50	2.42	0.053	0.07
	Res	lo (distance)	120	0.91	1.15	0.33	0.03
C Palos North	**Raw**	lo (distance)	102	98.34	29.12	<0.001	0.58
	Res	lo (distance)	102	43.27	14.68	<0.001	0.39
C Palos South	**Raw**	lo (distance)	102	74.61	20.61	<0.001	0.40
	Res	lo (distance)	102	37.89	10.97	<0.001	0.29
M. rubra							
Cabrera	**Raw**	lo (distance)	246	2.89	1.55	0.18	0.03
	Res	lo (distance)	246	2.71	1.35	0.25	0.03
Tabarca – rocky	**Raw**	lo (distance)	120	5.37	2.09	0.08	0.07
	Res	lo (distance)	120	1.10	0.70	0.59	0.02
C Palos North	**Raw**	lo (distance)	102	13.32	2.37	0.05	0.16
	Res	lo (distance)	102	4.11	1.47	0.21	0.06
C Palos South	**Raw**	lo (distance)	102	13.32	2.37	0.05	0.16
	Res	lo (distance)	102	4.11	1.47	0.21	0.06

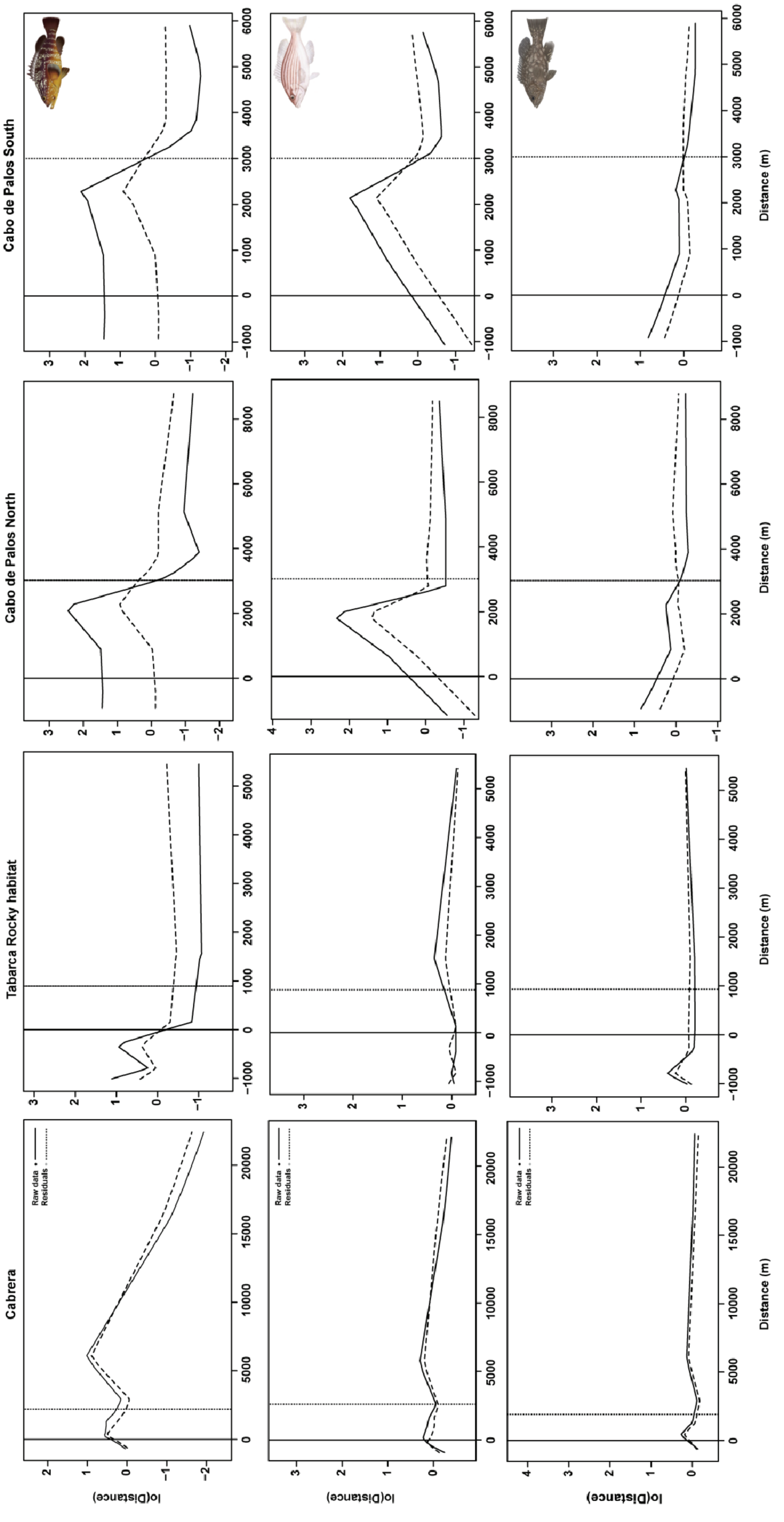


Figure 5.5: General additive model fitted for raw data and residuals of biomass ($\mathrm{Log}_{[x+1]}$ transformed) of *E. marginatus*, *E.costae* and *M. rubra* as a function of the smooth variable distance (m) from the boundaries of integral reserve. The y-axis is scaled so zero corresponds to the mean in log scale. The vertical line indicates the limit of integral reserve (IR) and dashed vertical line indicates the limit of the buffer zone.

4. Discussion

In this work, we showed that grouper species are extremely favoured by protection measures in Western Mediterranean MPAs, especially the dusky grouper, and to a lesser extent, goldblotch and mottled groupers). Average abundance, biomass and individual weight of groupers are higher within the protected areas than immediately outside. In some MPAs (Banyuls, Medes, Tabarca – *Posidonia*), dusky groupers were observed only inside the MPA, as a proof of a high fishing effort just at the edge of the protected areas (Goñi *et al.* 2008, Stenzelmüller *et al.* 2008). Therefore, at present abundant grouper populations are found only within marine protected areas in the western Mediterranean, highlighting the heavy impact of fishing on the coastal fish populations and the importance of MPAs to maintain epinephelid populations. A noticeable recovery in grouper abundance as a response to fisheries closure has been already documented in the Mediterranean Sea (Harmelin *et al.* 1995, Reñones *et al.* 1999, Lenfant *et al.* 2003, García-Charton *et al.* 2004), corroborating that these species respond more or less rapidly to protection (Sluka *et al.* 1997, Chiappone *et al.* 2000, Russ & Alcala 2003, 2004, 2010, Claudet *et al.* 2010) even in small reserves (Unsworth *et al.* 2007). Thus, MPAs are very effective to counteract the rapid depletion of predatory fish observed worldwide (Myers & Worm 2003). Because high biomass of top predators can be considered the natural state of marine reef fish communities, as demonstrated by studying remote reefs (Pandolfi *et al.* 2003, Newman *et al.* 2006, De Martini *et al.* 2008, Jackson 2008, Sandin *et al.* 2008), MPAs would serve to recover pristine fish community structures (McClanahan *et al.* 2007). The key questions are how much protection time is required until local carrying capacity is attained (Russ & Alcala 2010), and which is the maximum value of apex predators biomass for unfished Mediterranean locations (McClanahan *et al.* 2007).

Regarding individual size of groupers, almost all size classes are represented within each MPA, with larger individuals generally restricted to inside MPA limits. This indicates that grouper populations protected from fisheries are well established and are constituted from both mature and juvenile individuals. The high abundance, large size and stable size class distribution can allow groupers' reproduction inside MPAs (Zabala *et al.* 1997, Hereu *et al.* 2006, Reñones *et al.* 2010). Due to high site fidelity and strong territorial behaviour displayed by mature *E. marginatus* (Pastor *et al.* 2009), younger individuals must swim larger distances in order to find food, shelter and constitute their own territory. During the search juveniles may establish themselves outside MPA as a density-dependent response to high competition for territory inside the reserve (Sánchez-Lizaso *et al.* 2000). Both mechanisms, egg and larvae exportation from the restored spawning biomass and density-dependent movement of juveniles can be reflected on an increased observation of younger *E. marginatus* outside the limits of marine reserves (Bodilis *et al.* 2003).

Geographical differences in grouper species composition, abundance and size distribution appeared in our study. Grouper species were generally more frequent and abundant in southernmost MPAs like Cabo de Palos, Tabarca and Cabrera, where the three species studied were found. Moreover, in the northernmost reserves, dusky groupers only were recorded (Banyuls and Medes) or even no grouper at all (Carry). This species showed also much lower abundances in northern MPAs than in southern ones. Additionally, the higher mean biomass and individual weight values recorded in Banyuls and Medes indicated that the dusky grouper populations were composed of larger and older individuals, which could be due to the older ages of these MPAs (dating from 1974 and 1983, respectively), compared to the southernmost MPAs (from 1986 for Tabarca to 1995 for Cabo de Palos). On the other hand environmental conditions could also shed light on spatial differences observed. Duration of the spawning activity and the spawning survivorship are limited by temperature thresholds which can largely influence in differential population demographic parameters observed (Hereu *et al.*, 2006, Reñones *et al.*, 2010). This pattern corroborates the southern affinity of grouper species, especially *E. costae* and *M. rubra*, and their usefulness as an early-warning signal of seawater warming in the Northern Mediterranean coasts (Francour *et al.* 1994, Harmelin & Robert 2001, Bodilis *et al.* 2003, Azurro 2008). Moreover, juveniles were virtually absent from the northern MPAs despite the recovery of the adult population and years of protection. Low recruitment levels could be limited by habitat availability or quality. Thus, latitudinal/oceanographic effects together with habitat could be limiting northern populations. Identifying life-stage that are critical to a species' population dynamics along its distribution range is essential to effectively design MPA in order to achieve conservation objectives. Based on the estimation of biomass gradients, significant evidences of groupers' spillover from within to outside MPA borders were detected in only two of the five marine reserves analyzed, Cabo de Palos and Cabrera, when considering the effect of habitat. In Cabo de Palos, an area with low artisanal fishing effort directed to grouper species (Goñi *et al.* 2008), the spatial scale of grouper spillover seemed to be of less than 1000 m, a value lower than that estimated by Harmelin-Vivien *et al.* (2008) for the whole visually-censused fish assemblage (3000 m). This observation corroborates the hypothesis that biomass gradients are sharper (and thus spillover lower) for low-mobility or high catchability species, provided that fishing pressure outside MPA is high compared to that exerted in BZ, which is clearly the case in Cabo de Palos (Goñi *et al.* 2008; Stenzenmüller *et al.* 2008; Esparza-Alaminos 2010). It is important to highlight that groupers are targeted by recreational fishing (mainly spearfishing) as heavily as by professional fishing (Morales-Nin *et al.* 2005; Lloret *et al.* 2008), and spearfishing is highly practiced around Cabo de Palos MPA (Carlos W. Hackradt, *personal observation*). In Cabrera, the closer unprotected sites are located at about 10 km from the MPA with unsuitable habitats for juvenile groupers in between. Therefore, grouper biomass export from Cabrera MPA is rather likely due to egg and larval dispersal, as showed by Crec'hriou *et al.* (2010). In Banyuls, Medes and Tabarca, no spillover of grouper outside the reserves could be detected. In Cabo de Palos and Cabrera, grouper abundances were the highest among the six MPAs studied, and there was no difference in grouper abundance and biomass between no-use areas (IR) and buffer zones (BZ), where artisanal fishing occurred, while in Tabarca higher abundances were recorded in IR than BZ. We hypothesised that the importance of biomass gradients (and thus the occurrence of fish spillover) is likely to be a function of fish density inside MPAs (Amargós *et al.* 2010). When groupers are abundant inside MPA, biomass gradient from inside to outside are to be evidenced, as a result of movement of individuals (usually small-sized ones). When there is a low number of individuals within an MPA, gradients could be detected from IR to BZ, but not from BZ to outside areas. Therefore, because the efficiency of an MPA for a target species will depend on its total size compared to the home range of this species (Kramer & Chapman 2000), the carrying capacity, zonation and size of the MPA should be taken into consideration when estimating its potential to spillover. Although several studies establish that Mediterranean groupers have high site fidelity and small home range (Lembo *et al.* 1999, 2002, Pastor *et al.* 2009), further studies about the mobility and home range of these species inside and outside Mediterranean MPAs are still needed, taking into account fish densities and sizes.

The influence of habitat structure on the shape of the curve depicting the relationship between the response variable and the geographical distance with MPA limits tells much about the relative importance of structural habitat to favour or, at contrary, to hinder spillover. In those MPAs where habitat structure appears to exert a great influence on grouper abundance, biomass and mean individual weight (Tabarca – rocky and Cabo de Palos), extracting the habitat effect smoothes the shape of biomass gradient, although it does not affect the estimated spillover distance. According to the theoretical model proposed here, this shift is likely due to the fact that habitat structure is of higher quality within than outside MPAs, which is a quite normal situation in the Mediterranean (García-Charton *et al.* 2004). In these situations, structural habitat, by providing additional food and refuge resources, would act as an "attractant" for groupers (García-Charton & Pérez-Ruzafa 2001), hence boosting the carrying capacity of protected sites and, consequently, reducing the strength of spillover to neighbouring, unprotected sites. However, Cabo de Palos – Islas Hormigas marine reserve can be considered a singularity within the Mediterranean context regarding the habitat structure. The offshore steep and complex rocky shoals, with significant water motion and currents, are not typical of most Mediterranean rocky reefs (Sala *et al.* 2012). The fact that, despite these particular features, some spillover can be detected, highlights that this process is possible even for highly sedentary fish species, once a minimum density has been attained. As demonstred by empirical models (Pérez-Ruzafa *et al*. 2008). Spillover is likely to be favoured by the existence of habitat continuity from inside to outside the MPA (Stelzenmüller *et al.* 2007, Goñi *et al.* 2008; Forcada *et al.* 2009). Spillover has not been observed when comparing continuous (*Posidonia oceanica* meadows) and discontinuous (rocky bottoms) habitats around Tabarca MPA, as pointed out by Forcada *et al.* (2008). The present study emphasizes the importance of considering explicitly habitat structure when evaluating biomass exportation patterns (Chapman & Kramer 1999, Garcia-Charton *et al.* 2004, Harmelin-Vivien *et al.* 2008).

These results provide novel insights into the debate about the consequences of establishing a buffer zone when designing an MPA. Some studies argue that BZ's can have detrimental effects on the protection of fish species (Claudet *et al.* 2008), and that only no-take marine reserves should be created, as partial protection is an ineffective conservation strategy (Denny & Babcock 2004). The present study found that the success of BZ's to protect grouper species will depend on the ecological as well as management conditions established in each particular MPA. Therefore, the ecological role of such a partially closed area to protect commercially important fish species would depend on a variety of aspects, such as the difference in habitat quality between both zones, the heterogeneity and spatial continuity of structural habitat between both areas of different protection levels and with unprotected ones, the intensity of fishing pressure allowed in the buffer zone compared to neighbouring unprotected areas, the relative size of both no-take and buffer zones, and the mobility and home range of the species involved (Claudet *et al.* 2008, 2010). On the other hand, it has been demonstrated that partial protection of coastal areas together with an adaptive co-management plan that involves fishers, scientists, and managers may benefit fishing communities and reduce overfishing (Guidetti & Claudet 2010).

In summary, the evidences presented here show that MPAs are an essential tool to protect overexploited populations and endangered species, such as Mediterranean groupers, provided that they are adequately enforced and managed (Di Franco *et al.* 2009; Guidetti *et al.* 2008, 2010). Moreover, MPAs are able to export fish biomass to neighbouring areas, even in the case of very sedentary species, if they are appropriately designed in terms of reserve location, size, zoning, and management. Further studies on grouper population mobility, connectivity, habitat preferences, and carrying capacity, are urgently needed in order to establish ecological criteria to optimize MPA design.

Conclusions

Conclusions

Linking biology and behavioural mobility is essential to really understand population ecology and to correctly apply management and conservation measures. The use of MPAs as a tool for conservation and management of marine resources can be totally developed only when basic data are available (Sale *et al.* 2005, García-Charton *et al.* 2008).

The study of habitat selection in fish and the spatial distribution remains a gap in MPA science (García-Charton *et al.* 2008). We know that many fish populations suffer density-dependent effects on their growth and habitat selection (Lindberg *et al.* 2006, Grüss *et al.* 2011), and important factors such as habitat structural complexity, predation risk and interspecific competition demonstrated to be affecting habitat selectivity in fish (Jordan *et al.* 1997, Munday *et al.* 2001, Schofield 2003). Here we showed that groupers species display a size-related distribution with depth, in which small-sized fishes occupy shallower habitats than larger individuals. Additionally, density-dependent habitat selection was observed for groupers. At higher abundances, fishes enlarge their niche breadth to compensate for higher competition intensity by occupying larger areas. Furthermore, mutual, usually negative correlation in abundance was observed between groupers and combers species, suggesting some degree of interspecific relationship. Large spatial niche overlaps between grouper species were only possible due to food niche partitioning. Conversely, as combers do not present the same spatial distribution, and apparently they are limited by depth, they can overlap feeding niches. Also, when groupers are in low densities, combers are much more abundant then when groupers populations are well structured, evidencing the importance of groupers for controlling meso-predator abundances through cascading top-down effect (Braum & Worm 2009).

We also investigated the activity patterns of two important groupers species in Mediterranean *Epinephelus marginatus* and *Epinephelus costae*, and we could observe that their activity is strongly influenced by thermal fluctuation. During cold months a reduction in metabolic rhythms is translated into a lower fish activity, as observed in other temperate fishes during winter (Egli & Babcock 2004). Despite groupers are recognized as diurnal predators (Bshary *et al.* 2006), exhibiting higher feeding activity during twilight periods (Parrish, 1987, Sluka & Sullivan, 1996, Gibran 2007), our data suggest a greater activity period during intermediated hours of the day, where luminous intensity is high. We suggest that, as in temperate environments the daylight period varies greatly along seasons, and together with the fact that groupers relie on visual cues to find preys, diel activity pattern would change throughout to seasons; however, a whole year period must be covered to confirm such seasonal synchronization. Nevertheless, these evidences must be analysed cautiously due to the fact that recent studies verified that the range of reception by acoustic devices could be affected by environmental noise (Simpfendorfer *et al.* 2008), fouling on receivers and environmental conditions (Heupel *et al.* 2006, Simpfendorfer *et al.* 2008). Thus, more data are necessary to conclude about patterns of activity on groupers (Payne *et al.* 2010).

Within the Cabo de Palos – Islas Hormigas marine reserve, the two groupers species showed very similar home range sizes (KUD95% ~ 3 and ~ 3.5ha, for *E. costae* and *E. marginatus* respectively). The inexistence of previous information about *E. costae* hinders its comparisons with other studies, however when *E. marginatus* results was compared with other works in the Mediterranean we observed a great variability of home range sizes depending on the place in which the study was done (Lembo *et al.* 2002, Pastor *et al.* 2009). Divergent patterns of spatial occupancy have been shown by species that share common life history characteristics such as similar diet, behaviour, morphology as for example *E. striatus* and *E. marginatus*, indicating that spatial use is probably determined by local resource availability and spatial distribution, as well as to specific environmental characteristics, rather than by biological imprinting, as was evidenced by Meyer (2008) when compared introduced and natural *Cephalopholis argus* populations in Hawaii. Although it was postulated that home range size increase linearly as response of fish body size (Kramer & Chapman 1999), we believe that this response might be species-specific as many species do present it, such as *E. costae*, and others don't, as *E. marginatus*.

On fragmented landscapes, spatial distribution of resources can affect spatial use and behaviour (Atwood & Weeks 2003). The high overlap between individual territories and among species observed in the present study could be due to the patchy nature of Cabo de Palos rocky reefs, leaving limited space for use (Farmer & Ault 2011). We suggest that niche partitioning, ontogenetic shifts and overabundance of resources allow the common use of the same area by fishes (Harmelin & Harmelin-Vivien 1999) as corroborated by differential habitat selection by species.

Despite the high site fidelity showed by groupers in many studies (Lembo *et al*. 1999, Lembo *et al*. 2002, Pastor *et al*. 2009), as well as their strongly territorial behaviour (Schenkel 1966, Sale 1991), both species demonstrated in this study their ability to make incursions outside their home range area, travelling between rocky shoals. Although large-scale movements may be related to the reproductive period (Bolden 2000, Marino *et al*. 2001, Pina-Amargós & González-Sansón 2009) we did not find any evidence of such relation as movements were done at different dates and not necessarily associated to a seasonal variation. Groupers from Cerbère-Banyuls marine natural reserve population never left their territory within the reserve limits, although it is coastal and therefore continuous (Pastor *et al.* 2009). By this mean we suggest that the patchy habitat at Cabo de Palos together with high densities of groupers may be sufficient to force some individuals to leave off and try different places; some of them have came back to the place of origin, but others don't.

In fact, density-dependent movement is the basis for spillover theory which relies on directional movements from areas of high fish density towards areas with lower densities

(Abesamis & Russ 2005). As a consequence of the biomass build-up inside the no-take zones, an increase in fish home range overlaps and consequently a greater number of fish contacts will cause a density-dependent diffusion (Lisazo *et al.* 2000, Grüss *et al.* 2011). Those large scale movements observed in some individuals illustrate that spillover does occur at Cabo de Palos but not in Banyuls as spillover is only detectable at high fish densities (Hackradt *et al.* in prep. - Chapter V). The spatial scale of grouper spillover seems to be of less than 1000 m, a value which is lower than which estimated by Harmelin-Vivien *et al.* (2008) in the same location for the whole visually-censused fish assemblage (3000 m). This observation corroborates that biomass gradients are expected to be sharper (and thus spillover would be lesser) for low-mobility species and for species presenting high catchability, provided that fishing pressure outside is high compared to that exerted in the buffer zone, which is clearly the case in Cabo de Palos (Harmelin-Vivien *et al.* 2008, Goñi *et al.* 2008, Stenzenmüller *et al.* 2008, Esparza-Alaminos 2010).

In this work, we showed that grouper species are extremely favoured by protection measures in Western Mediterranean MPAs. Average abundance, biomass and individual weight of groupers (especially dusky groupers, and in a lesser extent, goldblotch groupers and mottled groupers) are generally much higher within the studied protected areas than immediately outside. Therefore, at present remnant grouper populations with considerable abundances are found only within marine protected areas, while, with few exceptions, in unprotected sites its presence can be considered as relict. These results highlight the importance of MPAs to maintain grouper populations, as revealed by a number of studies which described a noticeable recovery in abundance of groupers as a response to fisheries closures in the Mediterranean Sea (Harmelin *et al.* 1995, Reñones *et al.* 1999, Lenfant *et al.* 2003, García-Charton *et al.* 2004), corroborating that these species respond more or less rapidly to protection (Sluka *et al.* 1997, Chiappone *et al.* 2000, Russ & Alcala 2003, 2004, 2010, Claudet *et al.* 2010) even in small reserves (Unsworth *et al.* 2007).

These results provide novel insights into the debate about the consequences of MPA designing and protection, and more specifically on the interest or not of establishing buffer zones around the no-take areas, where some level of artisanal fishing and other uses (such as recreational diving) are allowed. The ecological role of such a partially closed area to protect commercially important fish species would depend on a variety of aspects, such as the difference in habitat quality between both zones, the heterogeneity and spatial continuity of structural habitat between areas with different protection levels and with unprotected ones, the intensity of fishing pressure allowed inside reserve compared to neighbouring unprotected areas, the relative size of both no-take and buffer zones, and the mobility and home range of the species involved (Claudet *et al.* 2008, 2010).

In summary, the evidences presented here show that MPAs are an essential tool to protect overexploited populations and endangered species, such as Mediterranean groupers, provided that they are adequately enforced and managed (Di Franco *et al.* 2009, Guidetti *et al.* 2008, 2010).

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