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Ecology of Mediterranean reef fish early life history stages, population connectivity and implications for marine protected areas design

Ecología de los estadios iniciales del ciclo de vida de los peces mediterráneos de fondos rocosos, conectividad poblacional e implicaciones para el diseño de áreas marinas protegidas

Tesis Doctoral
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Doctoral Thesis

Ecology of Mediterranean reef fish early life history stages, population connectivity and implications for marine protected areas design

Submitted by MSc. Fabiana Cézar Félix Hackradt in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the University of Murcia

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“*A mí Carlos por estar ahí.*

Siempre.”

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Resumen

Resumen

Los ecosistemas costeros se cuentan entre los más productivos del planeta y los hábitats que albergan juegan un papel preponderante en las pesquerías costeras al proporcionar recursos esenciales (alimento, espacio, refugio) a las poblaciones que las sustentan. Existe un consenso generalizado acerca del papel de la sobrepesca como la perturbación antropogénica más importante causante de extinciones locales. Aunque los océanos hayan perdido gran parte de su biomasa de peces y megafauna debido a la explotación extractiva, otras causas pueden asimismo explicar la pérdida de especies marinas. Otras fuentes de erosión de la biodiversidad marina son la contaminación, la invasión por especies exóticas y los cambios catastróficos inducidos por el cambio climático. Así, los ecosistemas costeros son particularmente vulnerables a las acciones humanas que causan la erosión de la biodiversidad marina y se está perdiendo a tasas alarmantes.

Las AMP han sido propuestas como una herramienta central para la protección de la biodiversidad marina y la conservación de los recursos marinos. En esencia, un AMP es una porción de superficie marina y sus características geomorfológicas, biológicas y culturales, que se cierra total o parcialmente a las actividades humanas y, en especial, a la pesca. La planificación espacial de las actividades humanas a través del establecimiento de redes de AMPs se plantea como una aplicación concreta de la “gestión basada en el ecosistema”. En el Mediterráneo, el número de AMPs ha crecido desde el establecimiento del parque nacional de Port-Cros en 1963. No obstante, aunque actualmente existen 94 AMPs sin embargo en conjunto protegen menos del 1% del *mare nostrum*.

El éxito de las áreas marinas protegidas (AMPs) para conservar los recursos pesqueros y proteger la biodiversidad marina radica en gran medida en dar respuesta a las actuales lagunas de conocimiento sobre los procesos biológicos y ecológicos que sostienen su funcionamiento. Un vínculo crucial para ello es la comprensión de las primeras fases del ciclo de vida de los peces, incluyendo la dinámica espacial y temporal de la instalación y reclutamiento de especies de peces

en los hábitats costeros.

Con base a lo expuesto nos propusimos a contestar la siguiente pregunta: ¿Son las AMPs capaces de exportar larvas hacia áreas adyacentes no protegidas y, con ello, repoblar las áreas explotadas? Para contestar esta pregunta es necesario abordar otras innumerables que resultan esenciales. Entre ellas están: 1) ¿Cuándo, cómo y dónde se instalan las larvas de peces costeros?, 2) ¿Cuál es la variabilidad temporal y espacial del asentamiento larvario?; 3) ¿Cuál es el éxito del reclutamiento?; 4) ¿Cuál es el efecto del reclutamiento sobre el poblamiento adulto?; 5) ¿Cuál es el efecto de las AMPs sobre los estadios iniciales del ciclo de vida de las especies de peces?; 6) ¿Cuáles son las escalas de dispersión larvaria?

Estas y otra preguntas dirigieron la conducción y ejecución de esta tesis doctoral y culminaron en los siguientes objetivos, agrupados en cinco capítulos:

Capítulo 1

- ✓ Describir la temporalidad del aporte larvario a las zonas costeras adyacentes al área del cabo de Palos;
- ✓ Estudiar la influencia de las variables ambientales en el aporte y distribución de las larvas de peces costeros.

Capítulo 2

- ✓ Evaluar la correlación temporal entre el aporte larvario y el asentamiento;
- ✓ Estudiar la variabilidad inter-anual del asentamiento y reclutamiento;
- ✓ Estimar la mortalidad entre años sucesivos para las distintas especies;
- ✓ Verificar los posibles efectos de la mortalidad sobre la dinámica de las poblaciones involucradas.

Capítulo 3

- ✓ Evaluar las escalas de variación espacio-temporal del reclutamiento de peces de arrecifes rocosos costeros;
- ✓ Estudiar la importancia del hábitat en dicha variación espacial;

Capítulo 4

- ✓ Investigar el efecto de la protección pesquera en una red de reservas marinas sobre diferentes estadios de vida de los peces;
- ✓ Evaluar si dichos efectos varían dependiendo del nivel de explotación o de la movilidad de las especies;

Capítulo 5

- ✓ Estimar la variabilidad genética poblacional de dos especies bentónicas de gran interés pesquero, *Mullus surmuletus* y *M. barbatus;*
- ✓ Evaluar el origen poblacional de las larvas recolectadas dentro de la reservas marinas estudiadas;
- ✓ Determinar las escalas de conectividad para ambas especies.

En el primer Capítulo se describe la temporalidad del aporte larvario a la región costera del cabo de Palos y la influencia de las variables ambientales oceanográficas en dicho proceso. A través del empleo de trampas de luz se evaluó mensualmente la llegada de post-larvas de peces en los alrededores de isla Grosa y el cabo de Palos durante dos noches sucessivas en días de luna de cuarto mengante o creciente. Se observa que las larvas que llegan a la costa son producidas en eventos reproductivos predecibles que son determinados según la história de vida de cada espécie de pez, y dispersadas por mecanismos oceanográficos. En regiones templadas como en el mar Mediterrnáneo, hay dos estaciones reproductivas muy bien definidas – verano e invierno – sin embargo, la gran mayoría de las especies se reproducen durante el periodo de mayores temperaturas. Como es esperable, se registró un gran número de larvas de peces en el ambiente costero durante los meses de primavera y verano, en sincronía con el período de máxima productividad del fitoplancton. Las principales fuerzas que determinaron la distribución de larvas a las áreas costeras fueran los vientos y corrientes. No obstante, a escalas locales, en la cercanía de los arrecifes rocosos, las larvas son capaces de orientarse y escoger activamente el hábitat preferencial para instalarse, como se evidencia por la gran variabilidad del asentamiento larvario a pequeña escala. También se han encontrado fuertes indicios de que es la selección del hábitat y no el aporte larvario diferencial el responsable de la distribución larvaria, como queda puesto de manifiesto por la observación de que en las mismas condiciones oceanográficas, las larvas de peces se distribuyan de forma desigual en localidades que no distaban mas de 10 km entre si.

La variabilidad temporal e inter anual del asentamiento se aborda en Capítulo II. La temporalidad del periodo de asentamiento para cada especie fue muy conservativa, habiendo una alta correlación temporal ente los picos de asentamiento en años sucesivos. Sin embargo, debido a variaciones en el éxito repdroductivo y en el proceso dispersivo, se produce una alta variabilidad en la intensidad del asentamiento, que según nuestros datos llegó a ser 2000 veces mayor en abudancia wn 2011 teniendo en cuenta un mimso mes de referencia. Además del aporte variable de juveniles del plancton, eventos pós-asentamiento como la mortalidad pueden causar grandes alteracciones en la demografía poblacional. Las pérdidas en el ambiente bentónico alcanzaran el 80% de la densidad inicial de assentados, superando el 90% en algunas especies, y dependiendo si se alcanza un valor tope de abundancia, que es variable de especie para especie. Dependiente dr si se alcanza la un valor tope de abundancia, que es variable de especies para espcies, la mortalidad sufre efectos denso-dependientes. Esta alta mortalidad justo trás el asentamiento larvario contribuyó al desacoplamiento entre la abundancia de asentados y el aporte larvario. Po el contrario, para la gran mayoría de los juveniles de las especies estudiadas, se observó que la abundancia de reclutas reflejaba las abundancias establecidas en el asentamiento larvario, indicando que la regulación de la clase anual (cohorte) tiene lugar durante los primeros momentos del ciclo de vida de los peces. Esto significa que la demografía de las poblaciones locales se establece probablemente en las primeras fases de la vida, y tendrá un importante efecto en el poblamiento adulto. Por otro lado, una parte significativa de las relaciones asentados~reclutas se dió en especies bentónicas que poseen fuertes relaciones con el hábitat (Capítulo III), indicando que éste puede haber jugado un papel importante en el control de la mortalidad reduciéndola gracias a la disponibilidad de refugios.

El Capítulo III se centra en la variabilidad espacio-temporal del reclutamiento y en el papel del hábitat en determinar la distribución de los reclutas. Se registraron mayores tasas de reclutamiento en la zona costera con respecto al ambiente insular, en cuya zona se encuentran los hábitats requeridos por la mayoría de las especies de peces para asentarse, conocidos como áreas guarderías o de crecimiento. La gran variabilidad en el reclutamiento es atribuída principalmente a las diferentes preferencias de hábitat de cada especie, y su variabilidad a pequeña escala se corresponde con la composición en mosaico de los fondos consteros locales, confirmando los resultados del Capítulo I. Normalmente los peces no se asientan en los mismos hábitats que los adultos con el fin de evitar la competición a través de la partición espacial del recurso. Se ha observado que muchas especies realizan cambios ontogenéticos en el hábitat a medida que los reclutas crecen. Al optimizar sus habilidades de forragear y aumentar su capacidad para defenderse de los depredadores y competidores, los reclutas se desplazan a otros áreas, normalmente de mayor profundidad y complejidad, lo que les permite explorar nuevos hábitats y recursos y finalmente incorporarse a la población adulta local.

El efecto de las reservas marinas en los diferentes estadios del ciclo de vida de los peces fue investigado en una red de reservas marinas en la costa de Andalucía, en el Parque Natural de Cabo de Gata-Níjar, y constituye el tema del Capítulo IV. El efecto de la protección solamente fue perceptible en el poblamiento adulto, a través del aumento de la abundancia y biomasa. Este efecto fue todavía mas intenso en los estadios poblacionales adultos de especies económicamente importantes y con gran movilidad. Tambien se ha podido demostrar que la falta de vigilancia en una reserva marina impide que sus objetivos se cumplan en su totalidad y que sus efectos potenciales beneficiosos sean perceptibles. Aunque los efectos de la protección puedan ser percibidos en términos de aumento de la abundancia, y que se hayan observado grandientes de abundancia através de los bordes de las reservas marinas estudiadas, estos no indican directamente un aumento en el tamaño médio de los individuos. Muy probablemente la constante retirada de grandes inviduos através de la pesca ilegal submarina que ocurre en las áreas de aceso restringido en el Parque Natural de Cabo de Gata, impida la aparición de los efectos de la protección sobre la biomasa de las poblaciones de peces. Por ese motivo no ha sido posible constatar la existencia de gradientes de biomasa através de los bordes de las reservas marinas aunque su efecto en la abundancia era claro. Por otro lado, por primera vez, se ha registrado un efecto negativo de la protección sobre los estadios larvarios de los peces, pero solamente para especies económicamente importantes, sean pelágicas o demersales. Para explicarlo, se plantean hipóstesis no mutuamente excluyentes. Por un lado, las características geográficas locales podrían permitir la retención de las larvas producidas en las reservas o zonas costeras vecinas. Por otro lado, debido a las diferencias del hábitat dentro y fuera de las áreas no protegidas se produce una selección por parte de las larvas de las áreas externas a la reserva. Sin embargo, el reclutamiento parece no sufir el efecto de las AMP, no obstante es esperable que las AMP alteren las tasas demográficas y la forma en que las poblaciones son reguladas. Através del aumento de las tasas de mortalidad, de la calidad y complejidad de hábitat, el incremento del número de depredadores, entre otros, las AMPs pueden ejercer gran influencia en la dinámica poblacional. No obstante, todavía se necesitan de muchos otros estudios para comprobar la veracidad de tales premisas teóricas.

En el Capítulo V, através de técnicnas de genética molecular se estudió el patrón de conectividad genético en dos especies bentónicas de elevado interés comercial, el salmonete de roca, *Mullus surmuletus,* y el salmonete de fango, *Mullus barbatus.* A pesar de ser dos especies simpátricas y con características de historias de vida muy similares, como por ejemplo, con la misma duración del estadio larvario pelágico y el mismo período reproductivo y de asentamiento larvario, se han encontrado diferentes patrones de conectividad genética. Mientras las poblaciones muestreadas de *M. barbatus* muestran una completa homogeneidad genética, las poblaciones de *M. surmuletus* están estructuradas genéticamente. La repartición del hábitat evidenciada en los individuos adultos de ambas especies, sumada a las características geológicas de la estructura de la plataforma continental y, sobre todo, las condiciones oceanográficas locales, pueden haber sido los responsables por los patrones divergentes observados. Aunque nuestra intención inicial era poder identificar el origen poblacional de las larvas capturadas dentro de las reservas marinas estudiadas y asi poder concluir sobre su potencial de exportación larvaria, no se ha podido relacionar ningunas de las poblaciones adultas encontradas con las post-larvas. A pesar de la imposibilidad de evaluar la exportación larvaria, sí se ha podido verificar un efecto positivo de las reservas marinas en la conservación de la diversidad genética para ambas especies tanto dentro de la reserva como en las zonas periféricas como es el caso de la bahía de Cartagena. Hay disponible nuevas aproximaciones genéticas, además de los tests de asignación, para la evaluación de la exportación larvaria, como el análisis parental y microquimica de otolitos, sin embargo deben de ser empleadas junto a modelos oceanográficos con el fin de comprehender los patrones de conectividad junto las fases larvarias de los poblamientos marinos.

En suma se concluye que el hábitat es una factor esencial en el asentamiento y reclutamiento larval, determinando la distribución de los recién asentados y regulando la población a través del control de la mortalidad. La constatación de que las AMPs ofrecen hábitats de mejores calidad y que dicho hábitat difiere en complejidad de los hábitats externos a la reserva, indican un potencial efecto modelador de la reserva marina sobre los patrones demográficos de las poblaciones. El reconocimiento de dichos efectos es esencial para la comprensión de su repercusión en el poblamiento adulto. Adicionalmente observamos que las escalas de conectividad varian enoermemente entre as espécies y que características de historia de vida similares no garantizan

escalas de dispersión semejantes como fue observado en las dos especies de salmonetes. El estudio con técnicas de mejor refinamiento espacial puden indicar hasta que distancias se esblace el flujo génico lo que permite la ubicación de reservas marinas orientadas a favorecer determinadas especies, aumentando la capacidad de estas poblaciones a resistirem a los efectos deleterios de la pesca. Nuestra aportación más efectiva es la recomendación vehemente de la inserción de hábitats esenciales a las especies de pez de interés en los límites de las reservas marinas con el fin de garantizar todo el ciclo de vida bajo protección.

General Introduction

General Introduction

1.1 ELHS Concept

Early life-history stages (ELHS) concept refers to the events occurring during the first phase of a species life cycle until the complete development into an “adult-like shape form”. In fished it can be divided into 3 main phases (Miller and Kendall, 2009). The **egg or embryonic stage** begins at fertilization and ends at hatching. For live-bearing fishes this phase is spent within the female, and for oviparous species the development takes place in nests, attached to some substrate, or is spawned and experiences its complete development in the plankton. Following, **larval stage** emerges at hatching, and lasts at the transformation into the juvenile form. During their larval stage, fishes develop morphologically to adapt themselves to a different source of food other than yolk-sac. By the end, when all fin rays have been formed and scales begin to cover the body, metamorphosis is complete and **juvenile stage** begins. The metamorphosis is normally accompanied by a transition from planktonic life to a schooling pelagic or demersal existence (being the latter more common in reef fishes). Juvenile stage ends with the sexual maturity, when they began the adult life (Miller and Kendall, 2009).

1.2 Problem statement

Since Johan Hjort, who was the first to realize that fluctuation on fish stocks could be due to variations in survival of fish early life stages as a consequence of yolk exhaustion or variability in the distribution of larvae by oceanographic factors (Hjort, 1926), the importance of ELHS was recognised as a crucial step to understand fish population dynamics; this idea dominated fisheries science research until the 90's (Grorud-Colvert and Spounagle, 2009). Although much has been done, field ecological work is very time consuming, and often large time series are necessary to describe important variations of stock abundances. Moreover, detailed information regarding stock size, spawning date, larval duration, etc. were only available for economic important species. By this mean, basic knowledge about a number of species inhabiting reef formation, mainly rocky reefs, was lacking. Considering that every population relies on the supply of young fish to compound the adult stock, understanding of such important phases is determinant in marine ecology studies.

1.3 The larval phase

Most marine fish display bipartite life cycles with pelagic, potentially dispersive larvae, and a sedentary phase as juvenile and adult (Cowen, 2001). Larvae can move from their natal areas by complex hydrodynamic process (Milicichi, 1994), and in some species, by active swimming (Leis *et al*., 1996). This process, known as **larval dispersal**, is directly related to species distribution and abundance patterns, and ultimately, with their persistence and evolution (Mora and Sale, 2002). Larvae can persist days to weeks in the water column (Macpherson and Raventós, 2006) and show a variable swimming capacity, which can reach 30 cm s^{-1} (Fisher *et al*., 2000). Larval dispersal, which is characterized by a dynamic horizontal connection, is vital to guarantee the mutual exchange of gene pools within local subpopulations (Pérez-Ruzafa *et al*., 2006). The connection distances can be largely variable between species and also be influenced by spatial-temporal patterns of oceanographic conditions as coastal currents (González-Wangüemert *et al*., 2004, 2007; Pérez-Ruzafa *et al*., 2006; Leis, 2007). In some cases, dispersal spatial scales result to be more restricted than expected (Cowen *et al*., 2006; Almany *et al*., 2007). Therefore, acquisition of knowledge about patterns of dispersal of marine species and the factors determining them are mandatory for a better comprehension of marine population dynamics (source-sink, metapopulations, etc.), and ultimately for a correct application of management measures (Mora and Sale, 2002; Grorud-Colvert and Sponaugle, 2009).

1.4. Settlement process

When larvae reach a determined stage of development, known as **late stage or post-larval stage**, it will be ready to settle, leaving the pelagic environment in direction to the benthos (Sale, 1991). This transition is known as **settlement,** and it is followed by morphological adaptations to an exogenous food intake and a new way of life (Leis and Carson-Ewart, 2001). Settlement can be influenced by three general factors: physical transport, larval behaviour and availability of settlement habitat (Schmitt and Holbrook, 1998). Oceanographic features can influence directly on the delivery of propagules to coastal shores (Milicich *et al.,* 1992, 1994; Sponaugle and Cowen, 1996; D'Alessandro *et al.,* 2007). Competent larvae (post-larvae) can perceive the reef environment at distances of ~ 1km (Leis *et al.,* 1996), using odour, sound and visual stimulus as cues for orientation to their preferred habitat (Kingsford *et al*., 2002; Leis and Carson-Ewart, 2003; Leis *et al*., 2003; Leis and Lockett, 2005; Raventós and Macpherson, 2005; Lecchini *et al*., 2007; Dixson *et al*., 2008). Finally, variation in the availability of suitable settlement (or nursery) habitats is known to influence the intensity of colonization (Schmitt and Holbrook, 1996).

Once settled, post-larvae will be exposed to the complex benthic environment with numerous habitats, competitors and predators (Ohman *et al*., 1998). During this phase, mortality rate is very high (Jones, 1991), mainly due to strong predation (Planes *et al*., 2000; Doherty *et al*., 2004), and it appears to be higher during and just after settlement (Caley, 1998). Although some works have seen a strong selection for larger larvae and faster growing individuals (Vigliola *et al.,* 2007; McCormick, 2009), the effect of such bottleneck on population structure is poorly understood (Planes and Romans, 2004).

Survival probability is conditioned by several factors such as fish condition (Macpherson and Raventós, 2005; Raventós and Planes, 2008), substrate type (Jones, 1988), habitat structure (Almany, 2004; Johnson, 2007; Juanes, 2007), abundance of predators (Holbrook and Schmitt, 2003) or presence of conspecifics (Almany, 2003). Mortality rates can be density-dependent through compensatory mechanisms such as increased levels of competition and predation at high fish densities (Dingsor *et al,*. 2007) or be density-independent, as a consequence of environmental factors (North *et al.,* 2005). The effect of mortality on subsequent juveniles densities will be directly reflected on adult population densities (Caley *et al.,* 1996), thus highlighting the importance of measuring mortality and factors which may influence in its rates.

1.5 Recruitment phase

Following settlement there is a **recruitment** phase, which can be defined as the addition of post-larval individuals to resident population which had survived enough to be counted by an observer (Keough and Downes, 1982). This practical definition is due to the fact that the settlement event occurs mainly at night, and recruitment is measured instead as a proxy of settlement intensity (Schmitt and Holbrook, 1999). As a consequence, recruitment can be extremely variable in space and time at diverse scales due to the effect of different ecological and physical processes (Sale *et al.,* 2005b). Temporal variation in recruitment is related to species life-history patterns which involve synchronization of reproductive periods with temperature, lunar phases and photoperiod (Robertson *et al.,* 1990, 1992; Danilowicz, 1995; Tsikliras *et al.,* 2010). Stochastic variation of larval supply (Grorud-Colvert and Spounagle 2009), active settlement choice (Jenkins, 2005), differential mortality (Carr and Hixon, 1995; Caley, 1998) or even post-settlement movements (Jones, 1991) have been suggested as main causes of spatial variability in recruitment.

Traditional fishery science has used recruitment indices to predict the abundance of the adult population (Axenrot and Hannson, 2003) since patterns in abundance of young-of-year individuals tend to be reflected on subsequent adult population (Hare and Able, 2007). Therefore, variations in recruitment can have important implications for management (Fogarty, 1993) particularly for species of high economic interest such as reef apex predators.

1.6 ELHS, population connectivity and fishery management

An important advance of fisheries management was the adoption of the ecosystem approach (Jennings, 2004) with the usage of Marine Protected Areas (MPAs) as spatial management tools for conserving exploited fish populations (Planes *et al.,* 2000). By protecting the spawning stock biomass, MPAs can promote effects on ELHS such as the increase in fecundity and production of eggs and larvae, recruitment protection, habitat restoration and changes in density-dependence relationships (García-Charton *et al.*, 2008). However, at present gaps in knowledge restrain the correct use of MPAs as conservation and coastal management tools, as much of the effects described previously have not been assessed yet (Planes *et al.,* 2000; Sale *et al.,* 2005a). Additionally, in order to warrantee the protection of the entire fish life cycle, it is necessary to link both adult spawning stock and recruits.

Population connectivity can be achieved by adult movements and migrations or, more commonly, by larval dispersal. The quantitative knowledge of population connectivity trough larval dispersal can enlighten several topics of ecological interest such as population dynamics (Caley *et al.,* 1996), local extinction processes and recolonization (Strathmann *et al.*, 2002), self-recruitment (Almany *et al.,* 2007), scales of adaptation (Warner, 1997), marine reserves design (Palumbi, 2004), invasion by alien species (Neubert and Caswell, 2000) and species response to climate change (Harley *et al.*, 2006; Brierley and Kingsford, 2009; Rijnsdorp *et al.,* 2009; Hoegh-Guldberg and Bruno, 2010; Doney *et al.,* 2012). Studies on larval dispersal are a requisite for the characterization of population connectivity, by coupling oceanographic data to spatial models of larval supply or recruitment. The conjunction of population connectivity information associated to ELHS constrains, such as the availability of nurseries habitat, can assist on the implementation of marine reserves network (García-Charton *et al*., 2008).

Objectives

Chapter I main objective - Evaluate the temporal dynamics of larval supply.

- ✓ Describe the composition of post-larval assemblage reaching coastal zones next to Cape of Palos;
- ✓ Describe the timing of arrival of species composing post-larval pool;
- ✓ Study the influence of environmental variables on fish post-larvae distribution;

Chapter II main objective – Evaluate the relationship between abundance of larvae, settlers and recruits of reef fish species.

- ✓ Evaluate the temporal correlation between larval supply and settlement;
- ✓ Study the inter-annual variability of settlement and recruitment;
- ✓ Estimate mortality between sucessive years and species;
- ✓ Evaluate the effects of mortality in demography;

Chapter III main objective – Evaluate spatio-temporal variation in recruitment and the effect of fish-habitat relationship in such variation.

- ✓ Evaluate the spatial and temporal scales variation of recruitment;
- ✓ Investigate the importance of habitat in spatial variation of recruits;
- ✓ Describe post-settlement spatial distribution between successive life stages;

Chapter IV main objective – Test the effect of protection over distinct fish life stages.

- ✓ Investigate the effect of protection over larval, juvenile and adult abundances;
- ✓ Evaluate if such effect can be influenced by species economic value or mobility category;
- ✓ Evaluate the existence of gradients of abundance and biomass across reserve

boundaries;

Chapter V main objective – Evaluate population connectivity of two sympatric species.

- ✓ Estimate the genetic avariability of *Mullus surmuletus* and *M. barbatus*;
- ✓ Evaluate the origin of larvae colected inside studied marine reserves;
- ✓ Determine the scales of connectivity for both species.

Thesis structure

Thus, this thesis is structured in 5 successive and complementary chapters which culminate in several recommendations to managers to account for ELHS in marine protected areas science (Fig. 1).

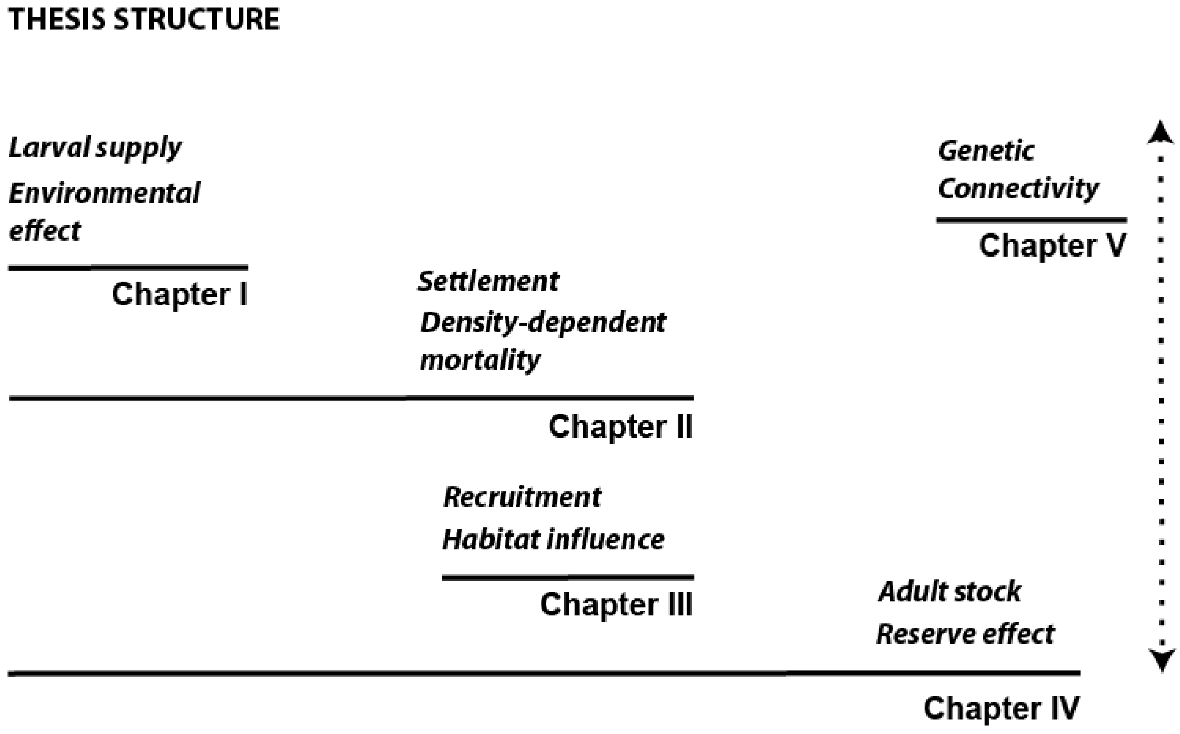

Figure 1 Scheme showing how thesis is composed

Firstly, in Chapter I we investigated the effects of environmental variables on determining temporal patterns and intensity of larval supply reaching coastal areas in an MPA (Cabo de Palos – Islas Hormigas marine reserve). Then, once evaluated the composition of fish assemblage in the plankton, in Chapter II we identify what proportion of this larval diversity was effectively installed on benthic habitat and how mortality affected demographic structure of recruit assemblage; for doing so, we tested for temporal correlations between larval supply and settlement intensity, and also the temporal synchrony between successive settlement years among species, and we investigated the density-dependence effects on mortality rates. In Chapter III, we investigated the habitat use by juvenile fishes and the likely existence of ontogenetic movements in the studied species, so that we determined the importance of habitat structure for defining such spatial and temporal variability in recruitment. In Chapter IV, as we already have determined the optimal recruitment period of the fish the species commonly observed and the habitat in which they occurred, we tested the effect of protection on different ELHS in Cabo de Gata natural Park during a period of high settlement, where we found the ideal location with homogeneous habitats and replicated marine reserves, and we also tested whether such effects changed according to species mobility or economic importance and we evaluated the effectiveness of MPA to export individuals by analysing gradients of abundance and biomass across the no-take zones. And finally, in order to evaluate the scales of larval dispersal in population connectivity, in Chapter V we used genetic tools to investigate the structure of adult populations and the assignment of post-larvae to distinct population sources, using the species *Mullus surmuletus* and *Mullus barbatus* as models, attending to their high economic importance in Mediterranean fisheries, so that these results can be applied to MPA design.

Chapter I

Félix-Hackradt FC, Hackradt CW, Treviño-Otón J, Segovia-Viadero M, Pérez-Ruzafa A, García-Charton JA. Environmental determinants of temporal variation in fish post-larval assemblage in coastal areas of South-western Mediterranean Sea.

Submitted to: Estuarine, Coastal and Shelf Science

Environmental determinants of temporal variation in fish post-larval assemblage in coastal areas of South-western Mediterranean Sea

Abstract

Supply-side ecology investigates the importance of larval production on local population dynamics. Late-stage larvae is often used instead of larvae as a more reliable estimator of the relationship between stock size and larval abundance. We present the first temporal assessment of larval supply in the Mediterranean Sea using light traps and the effects of environmental variables on larval abundances. A seasonal effect on larval supply was found in which greater abundances occurred on warmer months and was strongly influenced by local winds and currents. Lower diversity and post-larval abundances was found in an area protected from fishing. Although not tested, diversity of habitat types in coastal areas appears to be determinant on post-larval distribution. In order to ensure the accomplishment of the conservation objectives of marine reserves, early life history patterns must be taken into consideration during the design and establishment of marine protected areas.

Key-words: larval supply; light traps; currents; winds; seasonality; Mediterranean Sea

1. Introduction

Significant efforts have been made since the ‘80s to understand spatial and temporal patterns of marine fish larval supply, especially in the coral reef environments (Doherty and Williams, 1988; Milicich and Doherty, 1994; Valles *et al*., 2001; D'Alessandro *et al*., 2007). Since larval incoming is the first step of a chain of successive events which culminate on the adult population, the comprehension of early life history of marine fishes is determinant for population dynamics (Grorud-Colvert and Spounagle, 2009). However, due to the difficulties to sample competent larvae, since they avoid commonly used town nets as a result of their high swimming capability (Thorrold and Williams, 1996), the magnitude of larval supply was usually estimated by the settlement success at a given habitat (Schmitt and Holbrook, 1999; Almany *et al*., 2007; Hamilton *et al*., 2008). Nevertheless, a number of forces acting on settlers, such as intra- and inter-specific interactions, habitat requirements, environmental disturbances, etc., contribute to the uncoupling between larval availability and settlers (Spounagle and Cowen, 1996; Shima, 2001). Additionally, past attempts to relate spawning output and larval supply provided no strong evidence of linkage between events, indicating that both processes have very different dynamics (Dixon *et al*., 1999; Milicich and Doherty, 1994).

Larval dispersal was considered a passive process for a long time, yet recent studies indicate that late transitional larvae have well developed sensory abilities (Kingsford *et al*., 2022; Jones *et al*., 2005; Lecchini *et al*., 2005) and high swimming capacities, reaching up to 20 cm s^{-1} (Belwood and Fisher, 2001; Armsworth, 2001), suggesting that they are far from being just passive particles. Indeed, larvae can use reef signatures as chemical cues (Atema *et al*., 2002, 2012; Wright *et al*., 2005), noise (Leis *et al*., 2003; Leis and Lockett, 2005; Simpson *et al*., 2005; Radford *et al*., 2011) and also visual *stimuli* (Sabatés *et al*., 2003; Lecchini *et al*., 2005) to orient themselves towards the preferred habitat. For some species this behaviour is decisive to decouple any link between transport process and variation in supply (Wilson and Meekan, 2001). But the extent to which larvae may perceive such signals from the reef is still uncertain, and until this limit on which they could actively orient by themselves directly to the reef, hydrodynamic forces may be of huge importance to take larvae to the shore.

Many researchers have addressed the possible influence of environmental variables on fish during their larval pelagic phase. Physical factors such as winds, currents, temperature, rainfall, river discharge, among others, have all been addressed as possible *stimuli* for dispersal and larval survival (Spounagle and Cowen, 1996; Wilson and Meekan, 2001; Wilson, 2003; Carassou *et al*., 2008). Other oceanographic events such as fronts, eddies, and topographic features had also been identified as important influences to larval supply (Cowen and Castro, 1994; Sabatés and Masó, 1990; Vidal-Peñas *et al*., 2001; Bakun, 2006). However, the hydrodynamic processes which may have large impact on transitional larvae are those occurring on the vicinities of the reef (Milicich, 1994), as high settlement variability – i.e patchiness, could be found at reduced spatial scales.

Light traps have been used worldwide as a reliable method to measure larval supply at multiple spatial scales (e.g., Milicich and Doherty, 1994; Spounagle and Cowen, 1996; Grorud-Colvert and Spounagle, 2009; Carassou *et al*., 2008), but it is noteworthy that no previous works have used this approach in the Mediterranean Sea. Most Mediterranean supply-side studies have focused on the description of the distribution of ichthyoplankton assemblages inside coastal lagoons (Perez-Ruzafa *et al*., 2004) and marine coastal habitats (Sabatés, 1990; Townsend and Rashidi, 1991; Sabatés *et al*., 2003), and the evaluation of the influence of environmental variables on such distribution (Vidal-Peñas *et al*., 2001; Catalán *et al*., 2006; Sabatés *et al*., 2007; López-Sanz *et al*., 2011), in some cases related to the effect of protection measures (Sabatés *et al*., 2003; Crec'hriou *et al*., 2010; López-Sanz *et al*., 2011). On the other hand, other studies on Mediterranean fish early life history stages have been dedicated to understand settlement variability at a variety of spatio-temporal scales (García-Rubies and Macpherson, 1995; Harmelin-Vivien *et al*., 1995; Vigliola *et al*., 1998; Macpherson and Zika, 1999; Félix-Hackradt *et al*., *in prep.* Chapter II), and exploring the environmental influences on settlement patterns (e.g., Macpherson and Raventós, 2005; Raventós and Macpherson, 2005). Though, there is a lack of information regarding the links between the pelagic and the benthic phases at the Mediterranean Sea.

Our objective here is to present the first temporal assessment of larval supply in the Mediterranean Sea using light traps as sampling method. Additionally, we investigate the possible effect of several environmental variables (winds, currents, wave height, atmospheric pressure and moonlight) on larval abundances. Identifying the predictors which may drive larval supply is the initial stage to build predictive models. Considering that this work was done in the context of a marine reserve, the capacity to anticipate the temporal and spatial patterns of marine fish settlement is of paramount importance for the proper management of reef resources.

2. Material and Methods

2.1 Study site

The study was conducted on the coast neighbouring the cape of Palos (37°38’N, 0°42’W), including Grosa island (Murcia, Southeast Spain) in SW Mediterranean Sea (Fig. 1.1). The studied area is dominated by rocky reefs surrounded by sandy and detritic bottoms (Pérez-Ruzafa *et al.*, 1991; García-Charton and Pérez-Ruzafa, 1998). At shallower areas, rocky boulders of various sizes interspersed with *Posidonia oceanica* meadows 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 seawards 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).

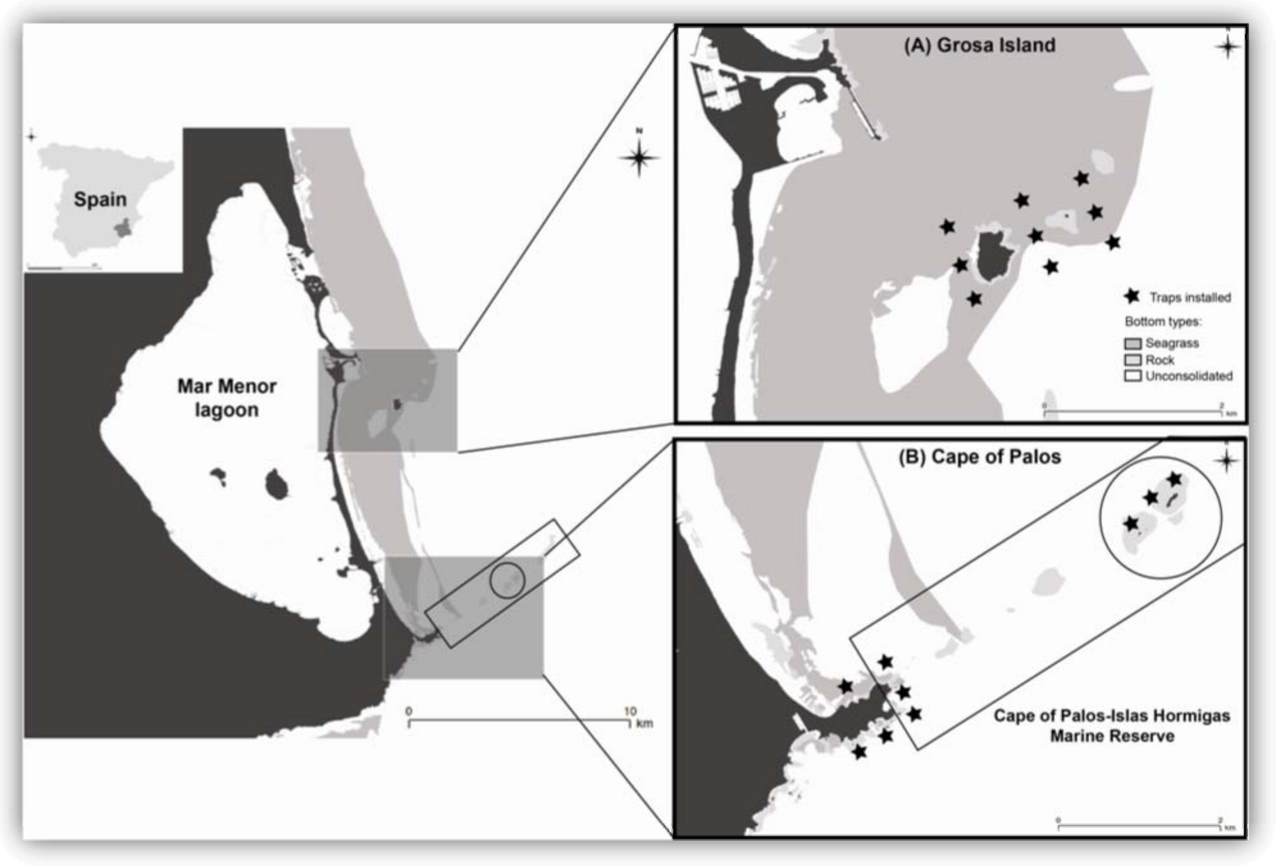

Figure 1.1 Map of Murcia coast, Southeast Spain, showing the location of Grosa Island, Cape of Palos and spatial delimitation of Cabo de Palos – Islas Hormigas Marine Reserve.

2.2 Sampling design and data collection

During one year interval, between July 2010 and June 2011, the abundance of post-larvae was surveyed on a monthly basis in two locations situated 10 km apart: Cape of Palos and Grosa Island (Fig. 1.1). These locations were selected to represent the range of habitats existing in the region, and thus represented a spatial replication factor for exploring variability in larval supply to coastal areas. Post-larvae is defined here as a synonym of late-stage or competent larvae, which is the transitional form from the pelagic to benthic realm (Leis and McCormick, 2002; McCormick *et al*., 2002). At each location, 3 sites separated by hundreds of meters were randomly chosen. In each site within each combination of months and localities, 3 light traps were installed at sunset and retrieved at sunrise during two consecutive nights. In Cape of Palos, 2 additional nights were surveyed in June 2010, so that sampling effort would result in 450 replicates, however due to losses, bad functioning or uncommon events such as jellyfish blooms, only 436 were used in the dataset.

Light traps (CARE®, Ecocean, Montpellier, France) were deployed on surface using a buoy moored at 10-40m depth, depending on how close sampling site was to the coast, maintaining a minimum distance of 300 m apart from each other to ensure independence. According to many authors (e.g., Milicich, 1994; Spounagle and Cowen, 1996; Wilson *et al*., 2001) the higher larval abundances occur on days around the new moon, although the timing of response is species-specific in most cases. To control as much as possible the influence of lunar phase on larval settlement patterns, sampling was restricted to 1st and 3rd quarter moons, when illuminated portion of moon is quite similar, and logistically it allows working in more than one locality per month. Due to oceanographic or logistic constrains we were not able to sample at Grosa Island on June 2010 and at Cape of Palos on January 2011.

Samples were recovered each morning, labelled and preserved in alcohol 70% until arriving to the laboratory. Each trap sample was then sorted using a light microscope to the lowest identification level using the appropriate bibliography (e.g. LoBianco, 1931; Arias and Drake, 1990; Ré, 1999; Fahay, 2007; Victor, 2012). Some individuals belonging to species from the genus *Atherina, Arnoglossus, Symphodus, Diplodus* and *Tripterygion* were not identified at the species level due to the identification difficulties they posed. For the same reason, all blennids and mugilids individuals and some unidentified clupeids were grouped at the family level.

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), as follows. Winds (in ms^{-1}) were divided into 2 components: alongshore, from NE-SW direction, and on-offshore winds, mainly SE-NW direction, while currents (measured in cms^{-1}) were only categorized as alongshore. Alongshore winds were categorized as positive if they comprised between 1º-90º (N-E, Northern sector) and negative if 181º-270º (S-W, Southern sector). In the case of on-offshore winds, positive values were attributed to blowing winds from 91º-180º (E-S, offshore winds) and negative ones to 271º-0º (W-N, onshore winds). For each day, wind intensity was calculated by summing velocities of winds belonging to each component (N-E, E-S, S-W, W-N) and dividing by the number of hours in which such wind was active, resulting in a daily mean velocity for each component. Alongshore and cross-shore components was then averaged to give a unique mean intensity. Each signal was then assigned to the intensity of blowing winds and flow currents and represented graphically. Also, significant wave heights measure, in meters (m), and defined as the mean of the highest third of the wave height reading records, together with temperature in Celsius scale (ºC) and illuminated portion of the moon (%), were also registered. Although sampling was restricted to quarter's moons phase, weather conditions did not allow us to fulfil these requirements every month and thus a small part of samples (mainly on winter) was acquired under different moon phases. Hence, to evaluate the possible influence of such variation on larval supply, we recorded the illuminated (or uncovered) portion of the moon (%), as a measure of moonlight intensity. All variables were averaged by day of sampling, resulting on 50 days registered. Although temperature, salinity, rainfall and river discharges are important physical factors which may affect larval distribution (Wilson and Meekan, 2000; Lemberget *et al.*, 2009), we did not consider them into analyses because temperature varies seasonally, and thus this component will be intrinsically evaluated when performing the temporal (monthly) analysis. For their part, salinity, river discharges and rainfall are directly related each other, so that the first variable is inversely related to the others. In addition, the studied area has the lowest pluviosity amongst those measured in Spain, and the nearest river influence is 70 km to the North. For these reasons, we do not expect that these variables could be relevant to local patterns of larval supply, and hence were not further considered.

2.3 Data analysis

To evaluate the possible spatial differences among months, sources of variation in the intensity of larval supply were tested by means of multivariate permutational analysis of variance (PERMANOVA), which is suitable to handle unbalanced sampling designs such as this. The effects of fixed factor Month (M) (orthogonal, 13 levels) and random spatial factors Location (L) (orthogonal, 2 levels) and Site (S) (3 levels, nested in the MxL interaction) on the value of community descriptors (richness and log-transformed total abundance) and log-transformed abundance of the commonest species were tested based on Bray-Curtis dissimilarities under 9,999 permutations using PRIMER v. 6.

Due to the fact that locations are not separated by more than 10 km from each other and environmental data collected from nearby monitoring stations (own unpublished data) indicate similar oceanographic conditions between locations, we used environmental variables data recorded from the same oceanographic buoy. Thus, we expect that, if occurring, environmental differences among samples were caused by the interval time between successive sampling rather than by spatial differences. To investigate the existence or not of divergent oceanographic and meteorological conditions between locations, and by this mean corroborate our hypothesis, a principal component analysis (PCA) was done with all samples (50 nights) from both locations and standardized environmental variables. Analysis were done using “vegan” package for R. Due to lack of support for considering localities as distinct (see Results section), either in relation to environmental conditions and also regarding to post-larval assemblage, all further analysis were done considering both locations together.

In order to evaluate the influence of the environmental variables considered on community descriptors and species abundances, data from trap captures from different locations and sites were averaged by night (CPUE) and tested using generalized additive models (GAM; Hastie and Tibshirani, 1990). GAMs were used due to expectancy of non-linear response of species abundance and predictor variables, as they performed better fitting over generalized linear models (GLM) (Dixon *et al*., 1999). Models were constructed using a smooth cubic line and default degrees of freedom using “gam” package in R. As data was averaged by night we used a Gaussian error distribution which is more appropriate when dealing with continuous measures than others (i.e. Poisson, binomial). 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 R^2 statistic, as well as the complete model selected. 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

3.1 Fish assemblage description

Light-traps sampled 5103 post-larval individuals during the year period, which belong to 51 *taxa* from 27 families. The most abundant *taxa* were Blenniidae (26% of total abundance), *Sardina pilchardus* (15%), *Mullus barbatus* (11%), *Pagellus bogaraveo* (10%), *Trachurus trachurus* (9%) and *Diplodus annularis* (7%), all together accounting for 78% of total abundance. Among the most representative families sampled we found Sparidae with 10 *taxa*, followed by Carangidae and Serranidae, with 5 and 4 species each, respectively (Table 1.1). Almost 60% of species captured were represented by less than 10 individuals, and 15 *taxa* were represented by only 1 specimen.

Table 1.1 Total abundance, mean number of individuals (± standard error) per location (Cape of Palos and Grosa Island) and size range (in mm) of post-larval species captured on light traps during sampling period.

Family	**Species**	**Cape of Palos**		**Grosa Island**		**Total**	**Size range (mm)**
		Mean	**SE**	**Mean**	**SE**		
Apogonidae	*Apogon imberbis*	1.3	0.13	3.0	0.28	10	17-28
Argentinidae	*Argentina sphyraena*			1.0		1	30
Atherinidae	*Atherina boyeri*	1.2	0.12	1.8	0.21	14	48-93
	Atherina spp.	5.0	0.00			5	30-43
Blennidae	Blenniidae	73.5	36.37	57.7	40.54	1312	9-32
Bothidae	*Arnoglossus laterna*	1.0				1	21
	Arnoglossus spp.			1.0		1	30
	Bothus podas			1.0		1	35
Carangidae	*Caranx chrysos*			1.0		2	20-27
	Caranx ronchus			1.0		1	23
	Seriola dumerilii	1.0	0.00			1	150
	Trachinotus ovatus	17.0	5.14	6.7	2.03	71	7-40
	Trachurus trachurus	17.6	6.35	39.9	9.62	460	5-58
Clupeidae	*Sardina pilchardus*	12.0	3.74	92.2	35.97	798	13-96
	Clupeidae	12.3	2.88	8.5	1.62	71	11-81
Congridae	*Conger conger*	1.0	0.00	3.0	0.00	5	80-120
Dactylopteridae	*Dactylopterus volitans*	2.0	0.00	1.0	0.00	3	38-42
Engraulidae	*Engraulis encrasicolus*	4.0	0.55	1.0	0.00	9	22-29
Gadidade	*Gaidropsaurus mediterraneus*	13.0	2.39	7.6	2.04	90	12-42
Gymnamodytidae	*Gymnammodytes semisquamatus*	1.5	0.14	3.7	0.86	14	40-70
Labridae	*Symphodus* spp.			5.0	0.55	10	6-11
	Thalassoma pavo	1.0	0.00	2.0	0.28	5	14-18
Mugilidae	Mugilidae	3.5	1.22	4.7	1.08	40	8-30
Mullidae	*Mullus barbatus*	151.5	63.61	6.0	0.00	612	23-62
Pomacentridae	*Chromis chromis*	17.0	4.85	35.7	10.08	175	6-14
Pomatomidae	*Pomatomus saltatrix*	1.0	0.00	2.5	0.42	6	26-42
Sciaenidae	*Sciaena umbra*	1.0	0.00			1	9
Scombridae	*Auxis rochei*	3.0	0.00			3	17-25
	Sarda sarda			1.0	0.00	1	28
Scorpaenidae	*Scorpaena porcus*	2.3	0.35	2.5	0.57	17	7-11
	Scorpaena scrofa	1.0	0.00	1.0	0.00	3	10-12
Serranidae	*Mycteroperca rubra*	1.0	0.00			1	13
	Serranus cabrilla			1.0	0.00	1	12
	Serranus hepatus	2.0	0.00	1.0	0.00	3	13-14
	Serranus scriba	2.0	0.00			2	13-14
Soleidae	*Monochirus hispidus*			1.0	0.00	1	82
	Solea senegalensis			1.0	0.00	1	11
Sparidae	*Boops boops*	2.0	0.23	2.0	0.00	8	8-58
	Diplodus annularis	65.2	16.02	21.0	2.22	368	7-37
	Diplodus puntazzo	13.0	0.00	29.0	0.00	42	6-15
	Diplodus sargus			1.0	0.00	1	10
	Diplodus spp.			2.0	0.00	2	7-8
	Oblada melanura	101.7	25.37	12.5	2.63	330	6-11
	Pagellus bogaraveo	21.6	7.77	101.2	47.99	513	20-38

Family	Species	Cape of Palos		Grosa Island		Total	Size range (mm)
		Mean	**SE**	**Mean**	**SE**		
Sparidae	*Pagrus pagrus*	5.0	0.28	3.0	0.55	16	8-26
	Sarpa salpa	7.3	1.47	2.4	0.38	35	11-38
	Spondyliosoma cantharus	5.5	1.25		0.00	11	10-19
Syngnathidae	*Hippocampus hippocampus*			1.0	0.00	1	6
Trachinidae	*Echiichthys vipera*	1.0	0.00	1.0	0.00	2	11-12
Tripterygiidae	*Tripterygion* spp.			1.5	0.14	3	12-14
Uranoscopidae	*Uranoscopus scaber*			1.0	0.00	1	18
	No Identified	1.0	0.00	3.4	0.69	18	

There were more *taxa* captured in Grosa Island (46 species) than in Cape of Palos (39 species). Among them, 15 *taxa* occurred exclusively in Grosa Island *(Argentina sphyraena, Arnoglossus* spp.*, Bothus podas, Caranx chrysos, Caranx ronchus, Diplodus sargus, Diplodus* spp.*, Hippocampus hippocampus, Monochirus hispidus, Sarda sarda, Serranus cabrilla, Solea senegalensis,, Symphodus* spp.*, Tripterygion* spp. and *Uranoscopus scaber*, and another 8 only in Cape of Palos (*Arnoglossus laterna*, *Atherina* spp., *Auxis rochei, Mycteroperca rubra, Serranus scriba, Sciaena umbra, Seriola dumerili* and *Spondyliosoma cantharus*) (Table 1.1). Differences in species composition among studied locations relied on the occasional occurrence of rare species. In both localities a group of 6 species dominated the catches, as they represented more than 85% of total abundance. This group was formed by almost the same species in both localities, although in different order of contribution to abundance. In Cape of Palos they were, in rank order, Blennidae (27%), *Mullus barbatus* (23%)*, Diplodus annularis* (12%)*, Oblada melanura* (12%)*, Trachurus trachurus* (7%) and *Pagellus bogaraveo* (4%), while in Grosa Island, *Sardina pilchardus* (30%), Blennidae group (24%), *P. bogaraveo* (16%)*, T. trachurus* (12%)*, Chromis chromis* (4%) and *D. annularis* (2%) were the most abundant *taxa*.

3.2 Spatio-temporal patterns of larval supply

Multivariate species abundance and total number of larvae, as well as the abundance of a set of species were significantly affected by the interaction between month and location factors (MxL)

(Table 1.2, Fig. 1.2), which means that these parameters varied differently in time depending on the location; the observed differences, however, were mostly due to the exclusive occurrence of some species in each location, and the different abundances of the shared species measured in both locations, and not to the temporal occurrence of abundance peaks of these species. For its part, post-larval fish species richness was significantly affected by main factors Month and Location, so that the variation in the number of species was concordant in time throughout the year for the two locations, while its average values were significantly higher in Grosa Island than in Cape of Palos (Table 1.2, Fig. 1.2). The abundance of Blennidae, *G. mediterraneus, S. salpa* and *S. cantharus* varied monthly but not between locations, while other species, such as *A. imberbis, A. boyeri, G. semisquamatus* and *S. porcus,* did not show any influence of the factors tested (Table 1.2). In addition, all community descriptors and most of the species evaluated were highly variable at fine spatial scale as evidenced by the significant effect on the spatial factor site [S(MxL)] (Table 1.2). Due to the strong seasonal trends observed of total and species abundance in both locations (either if the influence of this spatial factor is significant or not), focus will be made on the temporal variation of post-larvae occurrence.

Total post-larval abundance and species richness were positively related, so that significant higher values of species richness and total abundance were registered during summer period, namely June-August. Interestingly, a significantly high number of individuals were also captured during November, February and March. About 55% of total species sampled settled during summer months, between June-August; another 25% did it in autumn (September-November), and the remaining 9 and 11% occurred on winter and spring season, respectively. Fig. 1.3 illustrates the temporal variation in larval incoming of all species caught by light traps ordered by their date of appearance. The most abundant group, the blennids, were caught in all monthly samples likely due to the multispecific composition of this group. Although the presence of multiple species impede us to estimate settlement period more accurately, we can state that the greatest peak occurred during summer (June-August), thereafter abundance drops significantly and recover back again by the end

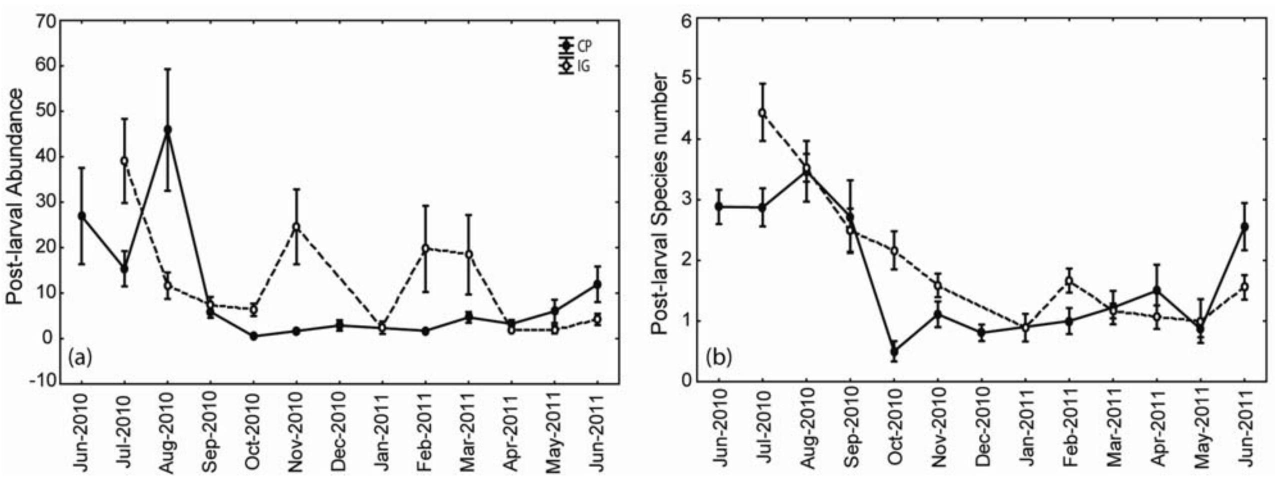

Figure 1.2 Variation of post-larvae abundance (a) and number of species (b) between Cape of Palos (filled circle) and Grosa Island (open circles) localities among months studied.

of May. Other abundant species, the Atlantic horse mackerel *T. trachurus,* showed the larger settlement period observed which last 5 months starting on May. Red mullet *M. barbatus* postlarvae were first registered in June, however the great peak of abundance was during August. Postlarvae of the damselfish *C. chromis* and the pompano *T. ovatus* started their catches in July extending it until September. The scorpaenids *S. porcus* and *S. scrofa* were both represented in low numbers during July and August respectively. At the end of summer we registered the first arrival of sardine postlarvae (*S. pilchardus*) which was much less intense than the second one in January. In autumn, the first species reaching the sampling sites were mugilids in September, followed by sparids. Sparidae was the most well represented family among. A clear lag in arrival period among species was found; red porgies *Pagrus pagrus* and black seabreams *S. cantharus* were caught around May-June, while *D. annularis* and *O. melanura* arrived between June and August. Other *Diplodus* species presented a temporal segregation, so that *D. puntazzo* arrived in October-November and *D. sargus* in May-June. Post-larvae of *S. salpa* and *P. bogaraveo* presented an expanded and overlapped arrival period, although peaks did not coincide, occurring in December and February for the first and November, for the second; for its part, *B. boops* were recorded from April-May. Finally, species such as *G. semisquamatus* and *G. mediterraneus* were typically from winter, ending in March and May, respectively.

Table 1.2 PERMANOVA results on the effects of fixed factor Month, and random spatial factors Locality and Sector on total post-larvae abundance, species richness, multivariate abundance and the abundance of each species.

Variables	**M**		**L**		**M×L**		**S(M×L)**	
	F	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Abundance	1.632	ns	7.232	**	3.361	***	2.141	***
Species number	3.308	*	6.774	*	1.957	ns	2.001	***
All species	2.754	**	3.624	**	4.421	***	1.952	***
A. imberbis	1.121	ns	0.054	ns	1.693	ns	0.757	ns
A. boyeri	2.072	ns	0.697	ns	0.708	ns	1.352	ns
Blenniidae	20.168	***	0.043	ns	1.016	ns	2.862	***
C. chromis	1.074	ns	2.968	ns	14.557	***	1.489	*
D. annularis	1.795	ns	1.266	ns	2.103	ns	2.931	***
D. puntazzo	0.560	ns	0.141	ns	4.313	**	2.667	***
G. mediterraneus	13.405	**	1.290	ns	1.376	ns	0.793	ns
G. semisquamatus	2.232	ns	2.324	ns	1.269	ns	1.144	ns
Mugilidae	2.269	ns	0.652	ns	1.282	ns	1.767	**
M. barbatus	1.682	ns	19.230	***	9.271	***	0.899	ns
O. melanura	3.676	ns	0.103	ns	0.937	ns	2.938	***
P. bogaraveo	1.769	ns	9.914	**	13.200	***	1.023	ns
P. pagrus	3.376	ns	0.156	ns	0.424	ns	3.016	***
S. pilchardus	1.178	ns	18.900	***	6.829	***	1.153	ns
S. salpa	10.231	**	0.299	ns	0.403	ns	1.147	ns
S. porcus	2.591	ns	2.424	ns	2.049	ns	0.640	ns
S. cantharus	14.869	*	0.862	ns	0.814	ns	0.269	ns
Symphodus spp.	0.753	ns	2.296	ns	1.037	ns	1.908	*
T. ovatus	0.817	ns	10.042	**	28.966	***	0.574	ns
T. trachurus	1.430	ns	2.010	ns	5.186	***	2.586	***

Many other species have occurred at shorter time intervals (Fig. 1.3); the species *S. hepatus*, *P. saltatrix*, *Trypterigion* spp., *S. dummerilli, S. sarda, S. cabrilla, Diplodus* spp. and *S. umbra* were registered only during summer months, while *D. volitans, T. pavo, A. rochei, S. scriba, S. sphyraena, S. ronchus, H. hippocampu*s and *B. podas* were in autumn. For their part, *M. hispidus* post-larvae were caught in winter and *E. encrasicolus, Arnoglosus* spp., *A. laterna, C. conger* and

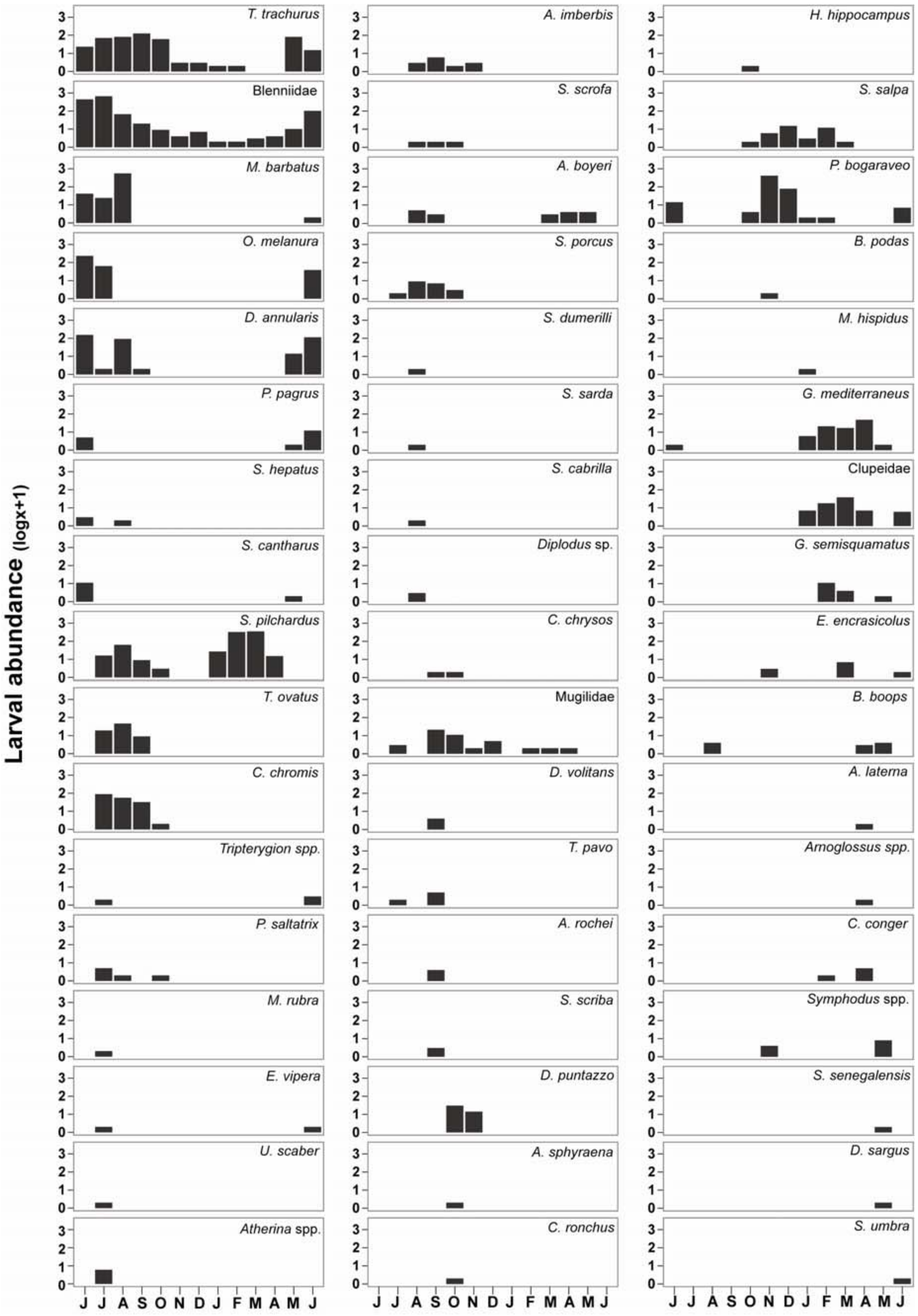

Figure 1.3 Monthly variation of abundance of post-larval species sampled. Species were sorted according to first and most abundant month of occurrence. Letters refers to months surveyed, starting on June/10 and ending on June/11.

S. senegalensis during spring (Fig. 1.3).

The size range of the post-larvae captured was highly variable amongst abundant species and reflected their different life history patterns (Table 1.2). Species such as *C. chromis, D. puntazzo, D. annularis, O. melanura, P. pagrus*, and *S. porcus* were captured at very small initial size (<10mm) and at few size classes intervals while *G. mediterraneus, M. barbatus, P. bogaraveo, A. imberbis, A. boyeri, S. pilchardus* and *G. semisquamatus* were initially greater (between 12 to 48 mm) and were represented by distinct size class range depending on the species. On the other hand, Blennidae, Mugilidae and *T. trachurus* were sampled at a very long period of time and thus were represented by many size classes (Table 1.2).

3.3 Environmental characteristics

Mean water temperature started to increase around April and peaked in July, reaching 26ºC, and by then decreased gradually until February, when the lowest value (13ºC) was recorded (Fig. 1.4a). Despite the fact that during winter and early spring the higher frequency of storms events, as indicated by higher variation of atmospheric pressure and mean wave height (Fig. 1.4b,c), made difficult to always install the light-traps in the preferred moon phases (1st and 3rd quarters), we managed to perform the sampling campaigns within 40-60% of full moon (Fig 4d). Strong northern currents occurred mostly during late summer and early autumn, being replaced by lighter southern component during the majority of the sampling period (Fig 4e). Alongshore winds were usually from the North, and blew weakly during summer months, being stronger and more variable from December to May (Fig 4f). In general, cross-shore winds were of lesser intensity than alongshore ones, and were predominantly from offshore direction almost all year round (Fig 4g).

Even though each location was sampled each month in different nights, both locations were sampled under the same environmental conditions, as evidenced by ordination analysis, in which the first principal component (PC) denotes a clear gradient between cross-shore and alongshore winds, while the second PC illustrates the effect of current and waves. The first two PCs accounted for 50% of total variance (Fig. 1.5). As a consequence, hereafter we evaluate the environmental influence on post-larval assemblage along time averaging the replicates by night, regardless of the location.

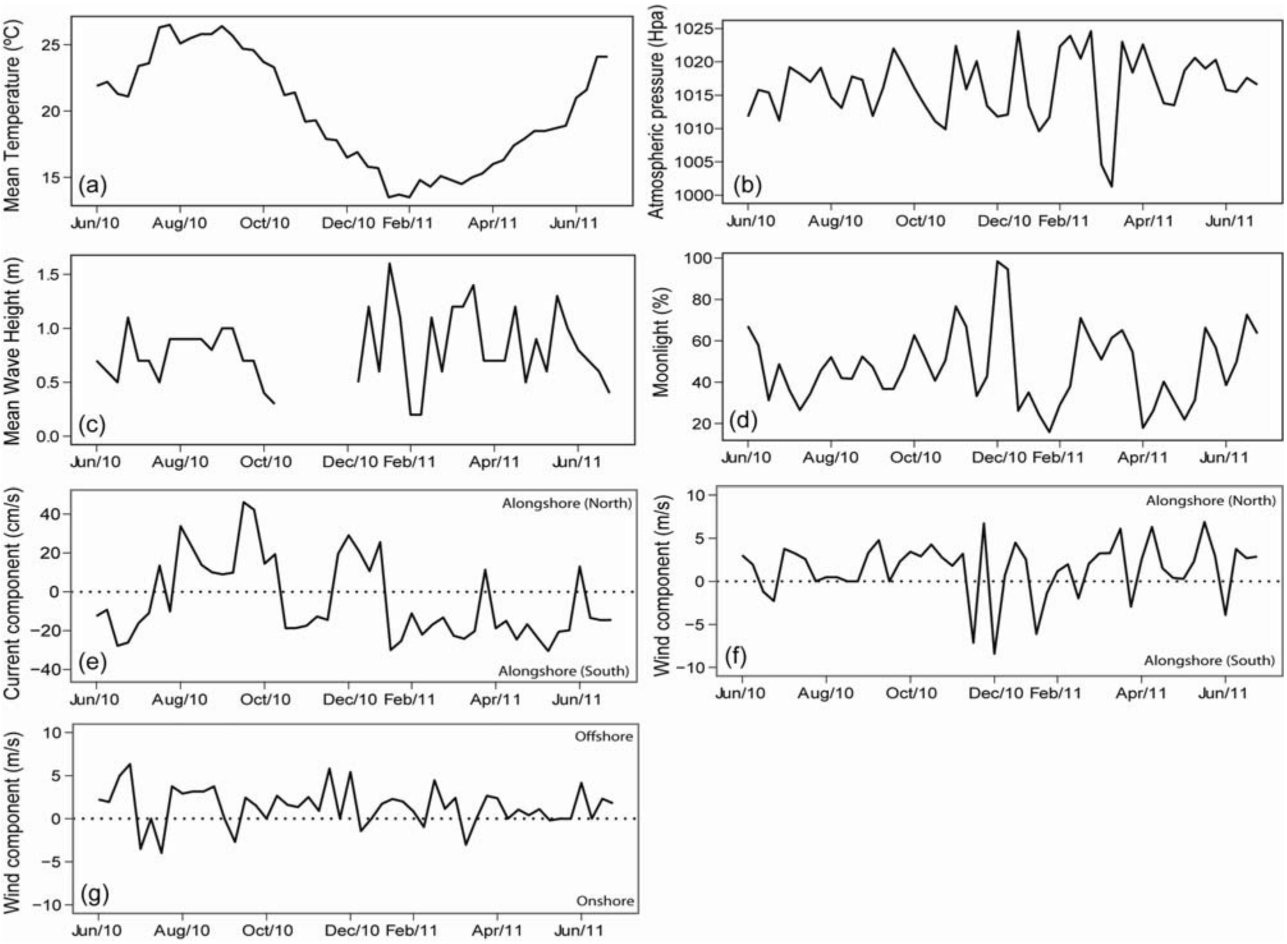

Figure 1.4 Environmental variables obtained during the sample period. (a) Mean Temperature, (b) Atmospheric pressure, (c) Mean Significant Wave height, (d) Moonlight, (e) Current component (f) Alongshore and (g) Cross-shore winds component.

3.4 Relationships between environmental variables and larval supply

The relationship with environmental variables of total abundance, species richness and the abundance of the most abundant species is shown in Table 1.3. In most cases, models retained more than one predictor variable, being Cross-shore winds and current the most important influential variables. The proportion of variance explained by the models was very variable (R^2 values ranging 0.03-0.71), although in general it was rather high (R^2 > 0.25, averaging 0.37; Table 1.3) indicating a relatively strong relationship between the studied variables and post-larval arrival.

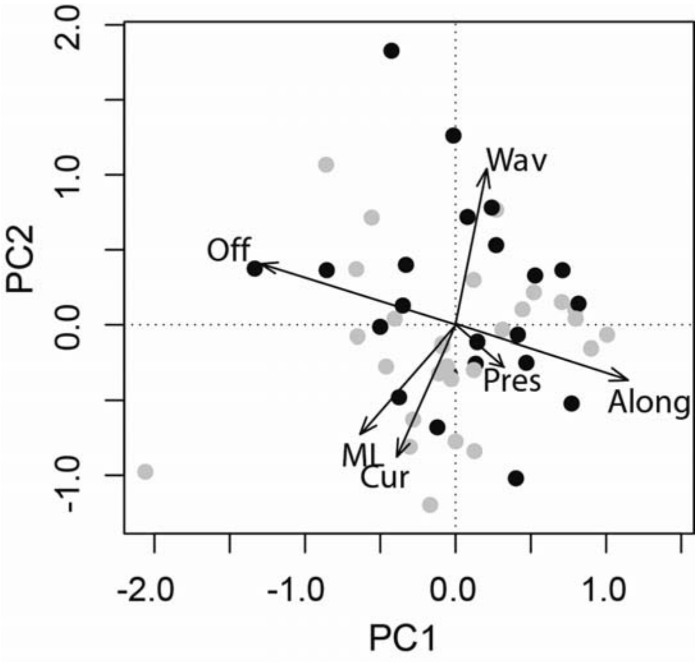

Figure 1.5 Principal components analysis (PCA) performed on environmental variables by nights sampled in Cape of Palos (grey) and Grosa Island (black) locations.

Total abundance and species richness were related to low (or absent) alongshore winds, and strong offshore (+) and onshore (-) winds, and to intermediate levels of moonlight (30-50% of full moon); also, higher number of species was further affected by intermediate levels of Atmospheric pressure (Table 1.3, Fig. 1.6, Appendix I).

For the majority of species, current intensity was the principal predictor related to an increase in post-larval abundance, followed in importance by cross-shore winds (Table 1.3, Appendix I). Strong NE currents were positively related to higher abundances of *A. imberbis, T. ovatus,* Mugilids and *T. trachurus*, while *S. porcus* was more abundant with weak NE current. Abundance of *D. annularis* and *O. melanura* were beneficed by strong offshore winds, while the opposite occurred for Blennids, *C. chromis* and *T. ovatus*, for which onshore winds were more

Table 1.3 Results of GAM fitted models between post-larval abundance, richness, diversity and abundance of most common species per sampling night with environmental variables. Res Df – Residual Degrees of freedom, Res Dev – Residual Deviance, Reduct. 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 complexity, R^2 – variance explained by each variable by the model, F – Fisher's statistic, P - significance at $\alpha=0.05$, * - $P<0.05$, ** - $0.05<P<0.001$, *** - $P<0.001$, wi – Akaike weights. [1]Statistics values of each variable predictor contained in the full model selected.

Variable	Term	Res. Df	Res. Dev	Reduct. Dev	P(Chi)	R^2	F [1]	p[1]	wi
Abundance	Null	49	11935.72						
	Off	45	8641.20	3294.52		0.28	1.65	0.194	
	Off+Along	41	6863.90	5071.82	***	0.42	7.32	0.001	0.35
	Off+Along+ML	37	5924.50	6011.22	ns	0.50	1.69	0.186	0.20
Richness	Null	49	62.55						
	Off	45	53.27	9.28		0.15	4.61	0.008	
	Off+ML	41	38.73	23.82	***	0.38	3.83	0.019	
	Off+ML+Along	37	24.70	37.84	***	0.61	10.41	0.000	
	Off+ML+Along+Pres	33	19.48	43.07	ns	0.69	2.80	0.055	0.33
A. imberbis	Null	49	0.24						
	Cur	45	0.14	0.11		0.44	6.32	0.001	0.39
A. boyeri	Null	49	0.22						
	Wav	45	0.17	0.05		0.21	2.58	0.065	0.22
Blenniidae	Null	49	2283.10						
	Off	45	1180.66	1102.44		0.48	3.70	0.020	
	Off+Pres	41	1025.13	1257.97	ns	0.55	3.06	0.040	
	Off+Pres+Along	37	746.73	1536.37	**	0.67	21.66	0.000	0.25
C. chromis	Null	49	60.36						
	Off	45	39.15	21.22		0.35	7.70	0.000	0.33
D. annularis	Null	49	274.95						
	Off	45	187.49	87.46		0.32	2.92	0.045	
	Off+Along	41	146.30	128.64	***	0.47	7.31	0.000	0.41
D. puntazzo	Null	49	9.08						
	Wav	45	6.53	2.55		0.28	9.34	0.000	
	Wav+Along	41	4.99	4.09	***	0.45	4.42	0.009	0.44
G. semisquamatus	Null	49	9.08						
	Pres	45	6.53	2.55		0.28	8.07	0.000	
	Pres+Wav	41	4.99	4.09	***	0.45	2.34	0.088	0.14
Mugilidae	Null	49	2.56						
	Cur	45	2.19	0.37		0.14	3.95	0.014	
	Cur+Pres	41	1.61	0.95	***	0.37	3.30	0.030	0.43
O. melanura	Null	49	609.34						
	Off	45	288.52	320.82		0.53	6.29	0.000	
	Off+Along	41	175.97	433.37	***	0.71	28.90	0.001	0.38
P. bogaraveo	Null	49	1120.05						
	Along	45	829.60	290.45		0.26	6.61	0.001	
	Along+Wav	41	626.76	493.29	***	0.44	1.07	0.371	0.56

Variable	Term	Res. Df	Res. Dev	Reduct. Dev	P(Chi)	R^2	F^1	p^1	wi
P. pagrus	Null	49	0.63						
	Along	45	0.50	0.13		0.21	3.42	0.025	0.50
S. pilchardus	Null	49	2204.47						
	Pres	45	1593.94	610.53		0.28	5.46	0.003	0.60
S. porcus	Null	49	2204.47						
	Cur	45	2137.20	67.27		0.03	4.31	0.010	
	Cur+Along	41	2061.30	143.17	***	0.06	2.59	0.066	0.25
S. salpa	Null	49	1.52						
	Pres	45	0.93	52.33		0.38	7.52	0.000	0.23
T. ovatus	Null	49	13.78						
	Cur	45	10.47	3.31		0.24	3.04	0.041	
	Cur+Off	41	9.09	4.69	ns	0.34	2.56	0.070	
	Cur+Off+Wav	37	7.68	6.10	ns	0.44	2.13	0.113	0.23
T. trachurus	Null	49	213.35						
	Cur	45	162.27	51.08		0.24	4.09	0.012	0.36

relevant. On the other hand, the only significant effect driving *A. boyeri* and *D. puntazzo* larvae was wave height, being the first associated to intermediate waves (>1m) while the second to the absence of them. The sparids *P. pagrus* and *P. bogaraveo* were mainly affected by alongshore winds, being the first related to medium intensity South-western winds and the second to both strong NE and SW winds. For its part, atmospheric pressure was the poorest predictor and was more commonly found as an additional variable in the model selected rather than the main predictor. The exceptions were *G. semisquamatus* and *S. pilchardus*, the first occurring in both high and low pressures periods and the latter predominantly in lowest ones, characteristic from winter months (Fig. 1.4, Appendix I). Finally, moonlight did not appear in any model selected for post-larval species, contrary to what observed in the models accounting for the variations in total abundance and species richness (Table 1.3).

4. Discussion

Light-traps have been first developed and applied in freshwater and riverine habitats and further expanded to marine environments (Ruck, 1975), so that they have been used worldwide, e.g. Great

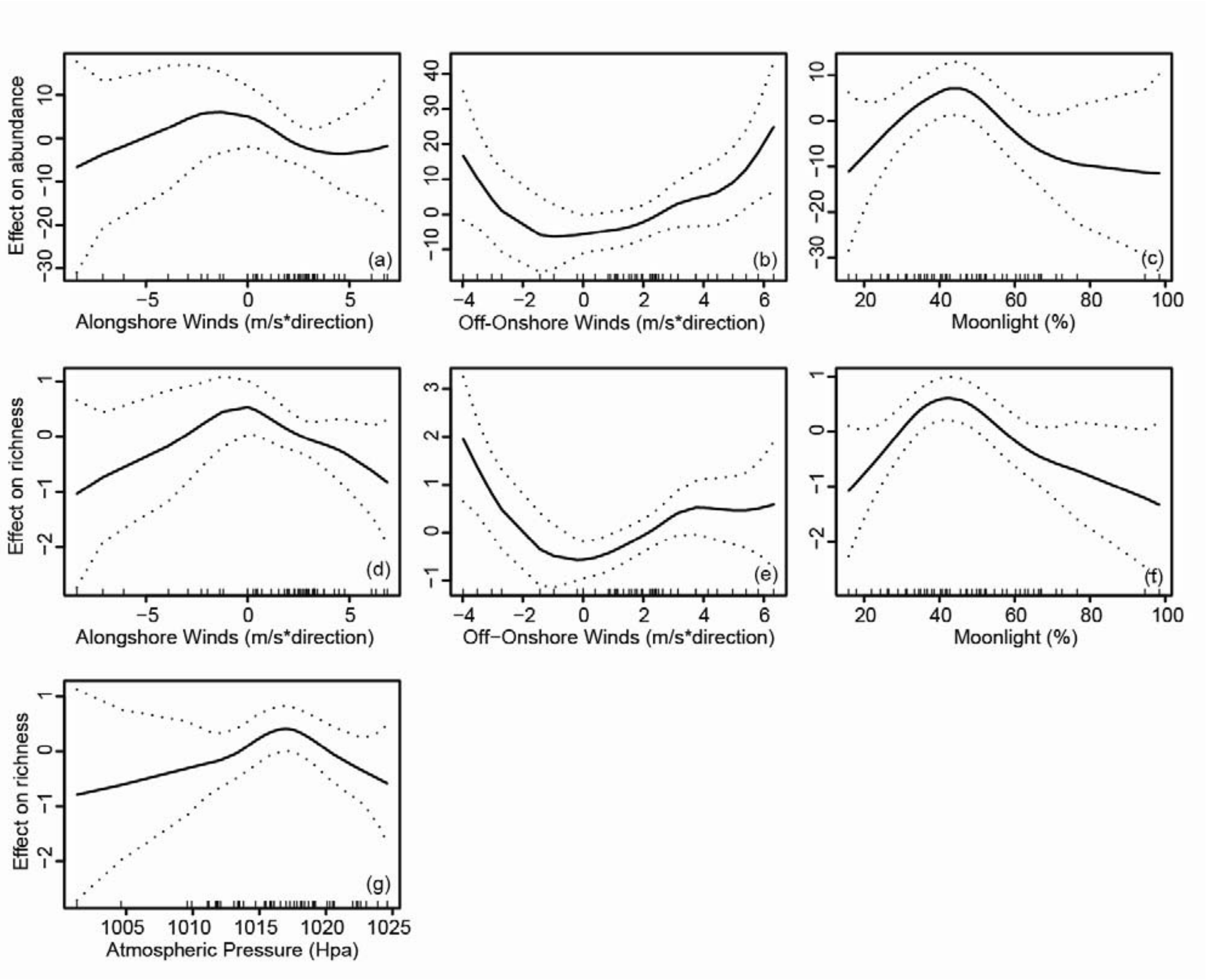

Figure 1.6 Output from GAM analysis illustrating relationships of post-larval abundance (a-c) and richness (d-g) with environmental variables. Dashed lines are standard errors. Positive winds are Offshore and North-Alongshore winds, and negative are Onshore and South-Alongshore winds. Positive currents are North-Alongshore and negative, South-Alongshore. Both wind and currents are a combination of direction (+ and – values) and intensity (velocity).

Barrier Reef (Doherty, 1987; Milicich and Doherty, 1994; Thorrold, 1992; Milicich, 1994; Fisher and Bellwood, 2002), Gulf of California (Brogan, 1994), Caribbean (Spongaugle and Cowen, 1996; Hendriks *et al.*, 2001; Wilson, 2001; Jones, 2006; Lemberget *et al.*, 2009), NW Atlantic coast (Hernandez and Lindquist, 1999), New Zealand (Hickford and Schiel, 1999), tropical NW Australia (Meekan *et al.*, 2001), coast of California (Steele *et al.*, 2002), North Sea (Øresland, 2007), New Caledonia (Carassou and Ponton, 2007, 2009), and East China Sea (Nakamura *et al.*, 2009). The present work aimed at fulfilling an important gap of knowledge on fish early life history stages in the Mediterranean Sea, as we present the first results of species composition and temporal variability in Mediterranean fish post-larval supply using light-traps.

Although light-traps are not efficient enough to measure post-larval densities, they appear to be useful for providing measures of relative larval supply (Meekan *et al*., 2000). Also, this sampling device is selective because not all species and families present phototactic response, as it is the case of labrids (Spounagle *et al*., 2005; D'Alessandro *et al*., 2007; Grorud-Colvert and Spounagle, 2009). In our study, however, light-traps were able to sample as much as species as visual census technique in the same area (García-Charton and Pérez-Ruzafa, 1998; García-Charton *et al*., 2001, 2004). Nevertheless, such comparison must be made with caution, as light-traps attract species from different types of habitats, while visual census focuses on reef-associated species.

In this work, a spatio-temporal heterogeneity appeared in postlarval assemblage structure, as detected by a significant effect of the MxL interactions in PERMANOVA applied to multivariate post-larval abundance and the abundance of a series of species, as well as a fine-scale spatial variability as showed by the detection of significant effects of the factor S(MxL). These spatial differences can be attributed to the exclusive occurrence of particular species in each location, mainly represented by the higher occurrence of rare species at Grosa Island, as well as single very abundant catches in one location of common species shared by both locations, as it is the case of *S. pilchardus*. The exclusive occurrence in Grosa Island of species such as *B. podas*, *S. senegalensis*, *U. scaber, H. hippocampus*, which mainly settle in seagrass beds and/or sandy habitats (Cabral and Costa, 1999; Guidetti, 2000; Procaccini *et al.,* 2003; Morato *et al.,* 2007), and in Cape of Palos of *M. rubra, S. scriba, S. umbra*, which typically settle in rocky areas (Moreno *et al.,* 1994; Biagi *et al.,* 1998; Louisy *et al*., 2007), indicate that habitat could have influenced on such spatial differences. In effect, although both studied locations are predominantly rocky, habitat is patchy at several spatial scales, as it is typical in Mediterranean nearshore areas (García-Charton and Pérez-Ruzafa, 2001; García-Charton *et al*., 2004), forming mosaics of different compositions of rocky boulders, *Posidonia* meadows and sandy bottoms within the rocky matrix. Cape of Palos is predominantly rocky with *Posidonia* patches restricted to south and northern sites surrounding the cape, while steeper, deeper and more complex environment is found at insular habitat, Hormiga Island, where the no-take zone of Cabo de Palos-Islas Hormigas Marine Reserve is located. On the other hand, as Grosa Island is closer to the coast, a gentler slope combined with shallower depths and large and dense *Posidonia* beds interspersed in a sandy matrix is the dominant habitat feature (Ros *et al*., 1984; Felix-Hackradt *et al*., unpublished data). In addition, the proximity of Grosa Island to the principal communication channel to Mar Menor coastal lagoon (<2.5km) may have exerted some influence on species composition found at the island with contributions such as *S. senegalensis*, *S. pilchardus* (Franco *et al*., 2012) and *H. hippocampus*. Besides, the implication of differential composition on catches in different habitats may support that late stage larvae are able to identify the preferred habitat and swim actively to it, and therefore contributing to patchiness observed on settlement distribution. However, it was not the intention of this work to test for habitat influence on larval spatial distribution and thus no conclusion could be made about it. Therefore, a comparative study of larval supply and settlement intensity in controlled habitats at multiple spatial scales must be made to evaluate if spatial differences are caused by active choice or merely by chance.

Temporal variation in larval supply followed a clear seasonal pattern in which higher abundances and species number occurred during the warmer months, namely June to September. About 80% of species sampled were caught during summer and autumn months (June-November) reflecting the spawning period of adult stocks (Tsikliras *et al*., 2010). This seasonal synchronization has been reported overall at Mediterranean Sea and was closely related to the abundance of preys for larvae (e.g. phyto- and zooplankton peaks); moreover, the duration of the period of larval supply varied greatly among species, however most species settlement periods lasted 2-4 months, coinciding with the duration of the spawning period of adult populations (Tsikliras *et al*., 2010).

Three different settlement strategies were observed among the species collected: species with short larval phase that settle immediately after metamorphosis (e.g., *C. chromis, S. porcus, P.*

pagrus, and *O. melanura*), which has the advantage to reduce mortality at pelagic phase; those with extended larval period and better swimming capability by virtue of their bigger size (*P. bogaraveo*, *M. barbatus*), which is adequate to enhance the probability of finding a suitable habitat; and finally those with protracted spawning periods and almost all-year occurrence (*T. trachurus, S. pilchardus*, *A. boyeri*), a strategy that allows fishes to increase offspring survival opportunities. Other species, such as sparids, may avoid competition by segregating offsprings at different spatial or temporal scales, so that species having the same habitat requirements may display temporal lags, or occupy different habitats when settlement coincides on time (García-Rubies and Macpherson, 1995; Tsikliras *et al*., 2010). For example, red porgies *Pagrus pagrus* and black seabreams *S. cantharus* were caught around May-June and occupy different habitats: the first prefers sandy habitats while the other occurs at *Posidonia* meadows (Guidetti and Bussoti, 1997; Labropolou *et al*., 1999); for their part, *D. annularis* and *O. melanura* arrived between June and August but one occur at seagrass and the other at rocky habitats, respectively (García-Rubies and Macpherson, 1995; Guidetti and Bussoti, 1997). Other *Diplodus* species all settle in shallow gravel dominated beaches, and thus presented a temporal segregation, with *D. puntazzo* arriving in October-November and *D. sargus* in May-June.

Temporal fluctuations are thus highly predictable as they reflect species life history traits. Yet, the magnitude or intensity of larval pulses are stochastic and may be associated to local or mesoscale oceanographic features (D'Alessandro *et al*., 2007). A number of studies demonstrated that larvae can obtain information of the surroundings to help them on the navigation to preferable habitat (Simpson *et al*., 2005; Leis, 2006; Gagliano *et al*., 2008). However the capability of larvae to perceive a habitat cue depends on the type of signal and the distance to the given signal source (Staaterman *et al*., 2012). Until reaching this frontier, larvae could take advantage of driving forces such as winds and currents to be delivered nearby required areas.

Many studies have tried to predict larval supply patterns by correlating them with local environmental variables, with contrasting results. In some scenarios, wind was identified as the most influential force acting on larval supply intensity, notwithstanding, greater spatial variability of such effect indicated that the interaction with topographic features must be also considered (Milicich, 1994; Carassou *et al*., 2008). Bergenius *et al*., (2005) suggest that not only winds but also solar radiation and rainfall have important effects on larval delivery, in a positive and negative way, respectively. On the other hand, D'Alessandro *et al*., (2007) showed that temporal variation is largely cyclic and related to lunar and tidal cycles, in which winds had little effect on larval flow to nearshore areas, but they are more related to the passage of oceanographic transient features such as eddies. Finally, others were unable to find any correlation with environmental variables except with temperature (Wilson and Meekan, 2001). In this work, we found that alongshore currents and cross-shore winds were the most important variables predicting larval supply in the studied locations. Currents appears to be more important to the transport of pelagic species such as *T. trachurus, T. ovatus* and Mugilids, while winds drive the dispersion of benthic ones as Blenniidae and *C. chromis* (onshore)*, D. annularis* (offshore), and *P. pagrus* (alongshore). Although in a minor extent, levels of lunar illumination had a negative effect on total larval abundance and richness, corroborating the findings of D'Alessandro *et al*. (2007). The little effect of moonlight on this study could be explained by the fact that tidal variation in Mediterranean Sea is minimal, only few centimetres, or virtually absent (McElderry, 1963). Under these circumstances, other hydrological variables such as winds, currents and waves, increase in importance for explaining the dispersion of larval species.

The coast of Murcia region (Spain) is characterized by the encounter of two important coastal currents, the Liguro-Provenzal originated in Gulf of Lyon flowing Southwards, and the Modified Atlantic Water, flowing Northwards until Murcia coast, where it diverges Southwards to compose the Algerian Current (Millot, 1999). Additionally, the continental shelf differs abruptly through North and South of Cape of Palos. Northwards, the continental shelf extends 25 km offshore, while southwards it becomes narrower (< 6 km). Thus, the width of the continental shelf may interfere with the effects of current and wind forces on larval supply, as highlighted by Milicich (1994) and Carassou *et al*. (2008).

Overall, GAM models selected have intermediate to high explanatory power; for those cases where the models account for a low-to-intermediate part of variation, larval response to environmental constrains is more complex, suggesting that other underlying mechanisms must be acting on larval supply process. The particular topographic characteristics of the region under study may contribute to the creation of mesoscale features such as local eddies, as suggested by Vidal-Peñas *et al*. (2001) to explain the divergent ichthyoplankton assemblage from North and South of Cape of Palos. Such transition oceanic features have been related with mass events of larval arrival (Cowen *et al*., 2003; Spounagle *et al*., 2005; D'Alessandro *et al*., 2007). But, due the absence of oceanographic studies in the region, the hypothesis is left without empirical support. Besides, the lack of relationship of intensity of larval supply and the environmental variables explored here may be due to the fact that variability in larval supply is mainly driven by seasonality while environmental variables did not show any temporal pattern. Additionally, the active orientation of larvae to settlement habitat (Jenkins, 2005; Staaterman *et al*., 2012) may confound effects of environment variables. Only recently researchers have focused on understanding how larvae orientate during their pelagic phase. Signals as gravitational field, relative position of the sun, polarization of the light (Kingsford *et al*., 2002) among others, are potential cues likely to account, at least in part, for larval supply. Therefore, more effort must be done to elucidate which mechanisms larvae must use to orient themselves during the period spanning from hatching to settling to the reef, in order to evaluate at what distances from reef a larvae can actively select a suitable habitat to settle.

5. Conclusions

In summary, this work provides the first temporal assessment of post-larval assemblage in the Mediterranean Sea. Larval supply was temporally synchronized with temperature in which greater intensity occurred on warmer months and was strongly influenced by local winds and currents.

Additionally, supply intensity varied at both large and fine spatial scales. Unexpectedly, lower diversity and post-larval abundances was found in an area protected from fishing. Although not tested here, the higher diversity of habitat types in coastal environments appears to be determinant on post-larval spatial distribution. Our findings highlight the importance that spatial and temporal variability of fish early life stages may have on adult population dynamic. In order to ensure the accomplishment of the conservation objectives of marine reserves, early life history patterns must be taken into consideration during the design and selection of spatial management units for the establishment of marine protected areas.

Chapter II

Félix-Hackradt FC, Hackradt CW, Treviño-Otón J, Pérez-Ruzafa A, García-Charton JA. Temporal patterns of settlement, recruitment and post-settlement losses in a rocky reef fish assemblage in the South-western Mediterranean Sea

Manuscript

Temporal patterns of settlement, recruitment and post-settlement losses in a rocky reef fish assemblage in the South-western Mediterranean Sea

Abstract

The transition from the pelagic to benthic realm is a critical step during the initial phase of most marine fishes. Post-settlement events can alter significantly the density distribution of settlers, which will be reflected on adult population structure. Understanding the temporal and inter-annual variability of settlement and the effects of subsequent mortality on defining recruit densities was investigated across 2 years in the vicinities of Capo de Palos-Islas Hormigas Marine Reserve by visual census technique and light trap sampling. Settlement had two well-defined periods: summer an winter, being the first more important in terms of species richness and abundance. Although temporal synchronization was observed between larval supply and settlement, densities of settlers could not be predicted from post-larval abundances. High inter-annual variability with differences in abundance of 2000-fold between years, but timing of settlement was consistent between years for most species. High mortality (~80% in average) and general decoupling of post-larval stage and settlement indicates that early mortality is most responsible for such patterns. Nevertheless, especially for benthic species such as *Diplodus* and *Symphodus,* with strong recruit-habitat relationships, recruits abundances reflect those at settlement, indicating an effect of habitat in mediating mortality. These findings evidence that post-settlement losses occurring just after settlement are more important in shaping population demography than later ones.

Key-words: mortality; habitat-recruit relationship; larval supply; temporal synchronization

1. Introduction

Most marine populations have a pelagic stage which is potentially dispersive and thus contribute to their demographically open state (Jones *et al.*, 2009). This implies that juvenile replenishment is likely to be greatly dependent on larval supply (Caley *et al.*, 1996). The paradigm prevailing until recent dates wants that the greater proportion of new settlers comes from elsewhere (Caley *et al.*, 1996), and thus local production will not be reflected in local larval densities (Victor, 1986); nevertheless, a number of current works have shown that self-recruitment is more common than previously thought (Jones *et al.*, 2009; Swearer *et al.*, 2002; Spounagle *et al.*, 2002; Almany *et al.*, 2007). Notwithstanding, a number of studies have successfully described the linkage between larval input and settlement rates (Victor 1986; Milicichi *et al.*, 1992; Watson and Munro, 2004; Jenkins *et al.*, 2005), while for others these relationships were less clear (Pineda *et al.*, 2010; Pan *et al.*, 2011; Zimmer *et al.*, 2012). A variety of factors such as methodological bias, physical environment, species interactions (competition, predation), larval behaviour and habitat availability, were identified as potential modifiers of the correlation between larval supply and settlement (Pineda *et al.*, 2010), while density-dependence settlement, growth and mortality were advocated as the principal factors controlling recruitment success (Juanes, 2007).

Recruitment of reef fishes can be defined as the settlement of pelagic larvae or juveniles to the bottom where fish is in straight contact with substrate and had survived enough to be counted by an observer (Keough and Downes, 1982). Settlement and recruitment processes are known to vary greatly on multiple spatial and temporal scales (Caley *et al.*, 1996; Vigliola *et al.*, 1998; Wilson *et al.*, 2008). Temporal variation in recruitment is related to species life history patterns which involve synchronization of reproductive periods with temperature, lunar phases and photoperiod (Robertson *et al.*, 1990, 1992; Danilowicz, 1995; Tsikliras *et al.*, 2010). Fluctuations in settlement and recruitment can have major effect on local population dynamics (Ammann, 2004) and therefore being determinants on adult population size (Macpherson *et al.*, 1997).

Differing from coral reef environment where much of the early life history science has been developed, in temperate zones, especially in the Mediterranean context, most studies about recruitment of fishes have been focused on highly economically valuable species such as cods (Lloret and Lleonart, 2002; Morales-Nin and Moranta, 2004), sardines and anchovies (García *et al.*, 2003; Palomera *et al.*, 2007), sparids (Harmelin-Vivien *et al.*, 1995; Macpherson *et al.*, 1997, 1998; Vigilola *et al.*, 1998), and to a lesser extent, non-commercial species such as labrids (Raventós and Macpherson, 2005; Raventós, 2009) and blennids (Macpherson and Zika, 1999); on the other hand, available information about recruitment patterns at the community level is still scarce (García-Rubies and Macpherson, 1995).

The settlement (i.e., the transition from pelagic to benthic environment) is a period in the fish life-history cycle that generally occurs in the moment of the metamorphosis from the post-larval to the juvenile form, which is accompanied by shifts in the diet as an adaptation to the new environment (Kaufman *et al.*, 1992). This transition, rather than the larval phase, is widely recognised as the critical period for marine species as postulated by the “Hjort hypothesis” (Chambers *et al.*, 2001; Almany and Webster, 2006). Studies on coral reef environments suggest that post-settlement losses can reach 99% (Jones, 1991) and are higher during and immediately after settlement (Hixon, 1991; Caley, 1998). Almany and Webster (2006) showed that predation can alter the density of settlers within a short period of time, reducing their abundance by 50% 2 days after settlement. However, some evidences point that the phase of fish development in which mortality will be critical is species-specific (Dahlberg, 1979; Houde, 1989). Furthermore, species differ markedly in their early post-settlement mortality rates (Macpherson *et al.*, 1997). Hence, in order to understand the possible effects that mortality could have on community composition, a multi-species approach should be applied. Due to the spatio-temporal multi-scale nature of the recruitment process, post-settlement losses largely fluctuate between recruitment seasons; however, few works have evaluated the temporal variability of mortality.

Here we evaluate the inter-annual, multi-species variability in recruitment of Mediterranean rocky reef fish species. We specifically addressed the following questions: Is the larval supply temporally correlated to settlement? Is the temporal synchrony of both processes maintained between years and among species? Does post-settlement mortality change among species and years? Ultimately, we intend to explore to what extent the mortality of settlers is responsible for a decoupling between settler and recruit abundances.

2. Material and Methods

2.1 Study site

The study was conducted on the coast neighbouring the cape of Palos (37°38'N, 0°42'W), including Grosa island (Murcia, Southeast Spain) in SW Mediterranean Sea (Fig. 2.1). The studied area is dominated by rocky reefs surrounded by sandy and detritic bottoms (Pérez-Ruzafa *et al.*, 1991; García-Charton and Pérez-Ruzafa, 1998). At shallower areas, rocky boulders of various sizes interspersed with *Posidonia oceanica* meadows 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 seawards 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 Sampling design and data collection

During two consecutive years, between August 2009 and June 2011, the abundance of fish recruits was surveyed in a monthly basis in 4 locations: two in the shallow reefs surrounding the cape of

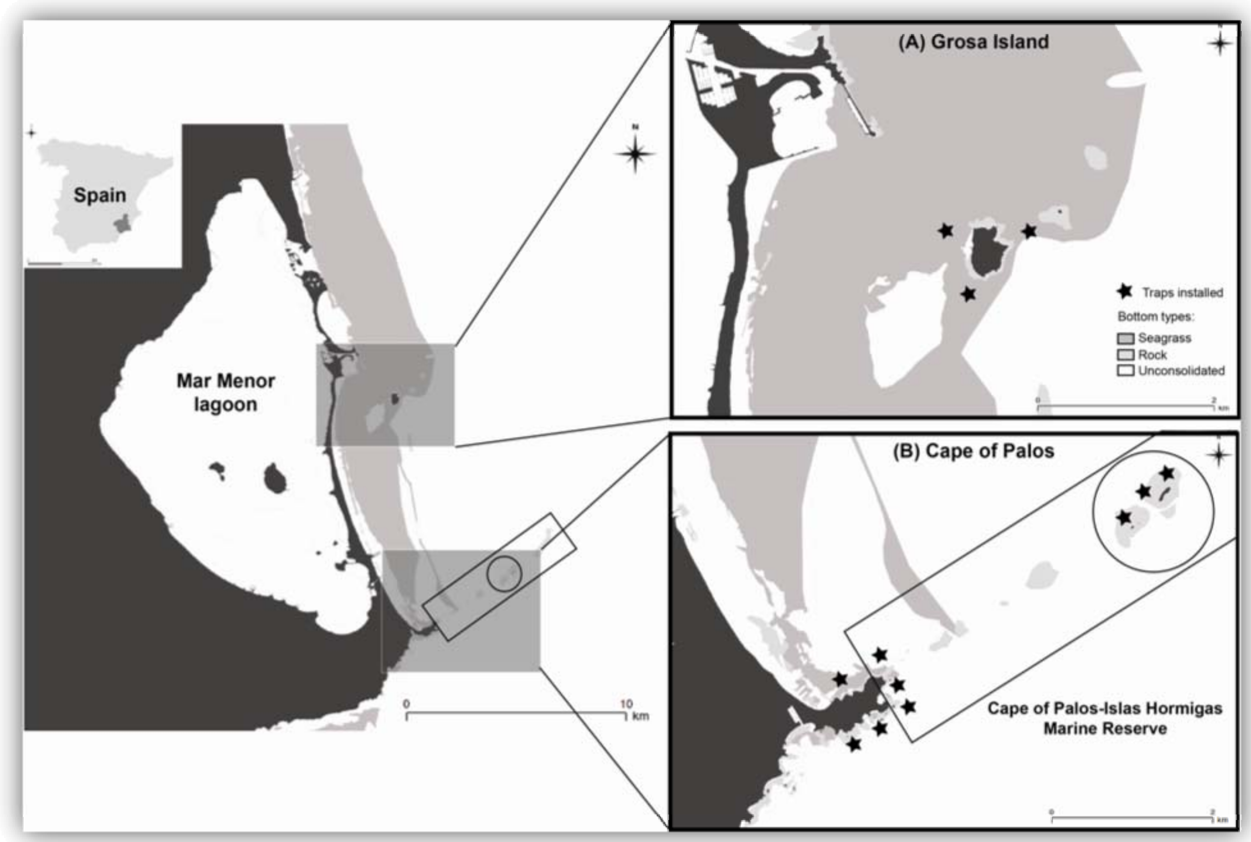

Figure 2.1 Map of Murcia coast, Southeast Spain, showing the location of Grosa Island, Cape of Palos and spatial delimitation of Cabo de Palos – Islas Hormigas Marine Reserve.

Palos, one around the Hormiga Island, and one at Grosa island (Fig. 2.1). These locations were selected to represent the range of habitats existing in the region. At each location, 3 sites separated by hundreds of meters were randomly chosen. In each site within each combination of months and localities, young fishes were counted visually by SCUBA diving on ten 10×2-m replicate transects set out haphazardly. A depth range of 0-8 m was covered, in such a way that the whole range was evenly explored in each site each month. Recruits of all species were recorded except those from families having cryptic habits (blenniids, gobiids, etc.). Each observation was assigned to one of eleven abundance classes, whose limits coincide with the terms of a base-1.6 geometric series, constructed considering the most frequent figures of observed juveniles abundance (Harmelin-Vivien *et al.*, 1985; García-Charton *et al.*, 2000), namely 1, 2, 3, 4, 5-7, 8-10, 11-30, 31-50, 51-70, 71-100, >100. Geometric means of each fish abundance class were used for the calculation of abundances. The size of each observed fish was visually estimated to the nearest cm. To minimize bias in size estimation a pilot study was done in order to calibrate all 3 observers (FCFH, CWH and JTO) and accuracy of data was compared among observers 3 times, resulting in no significant differences. All censuses were done between 10:00 and 15:00 h GMT, when water conditions (turbidity and swell) were optimal (Harmelin-Vivien *et al.*, 1985). During the first year all localities were visited within 2 weeks interval, however during the second year, surveys at Cape of Palos were done with 15 days interval from Grosa Island sampling to follow post-larval sampling schedule (see below). Owing to logistic limitations it was infeasible to sample in all locations on August/2010.

During the second year period (from June 2010 to June 2011), post-larval abundances were estimated using light-traps (CARE®, Ecocean, Montpellier, France). Lighting devices were deployed on surface using a buoy moored at 10-40m depth, depending on how close sampling site was to the coast, maintaining a minimum distance of 300 m apart from each other to ensure independence. According to many authors (e.g., Milicich, 1994; Spounagle and Cowen, 1996; Wilson, 2001) the higher post-larval abundances occur on days around the new moon, although the timing of response is species-specific in most cases. To control as much as possible the influence of lunar phase on post-larval settlement patterns, sampling was restricted to 1st and 3rd quarter moons, when the illuminated portion of moon is quite similar, and logistically it allows working in more than one locality per month. Due to oceanographic or logistic constrains we were not able to sample at Grosa Island on June 2010 and at Cape of Palos on January 2011. Samples were retrieved each morning and later identified using appropriated bibliography (e.g. LoBianco, 1931; Arias and Drake, 1990; Fahay, 2007; Victor, 2012). Some individuals belonging to species from the genus *Pagellus, Atherina* and *Mullus* were not identified at the species level and mugilids individuals were grouped at the family level due to the identification difficulties they posed.

2.3 Data analysis

Only fish comprising the 0+ cohort were used in the analysis, and they were divided into two size classes, settlers and recruits. Due to the fact that not all species settle at the same size, settlers were defined as those individuals within the first two size classes as indicated in Table 2.1, as a proxy estimate of settlement. Though recruits were those individuals belonging to the remaining size classes until the upper limit of the size range (Table 2.1). Recruits are characteristically distinct form settlers due to its colour and body similar to adult, while settlers are unpigmented and do not display many of the adult features. The size criteria of defining 0+ class for each species was based upon the existent literature focusing as possible on regional studies, and also FishBase data (Froese and Pauly, 2011) when such regional studies were lacking, and finally field experience.

To investigate the existence of a temporal synchrony between post-larval supply and settlement intensity, and also between the settlement period of each species of the two consecutive years, we used cross-correlation techniques (Raventós and Macpherson, 2005; Taylor *et al.*, 2009). For both time series comparisons (post-larvae – settlers and settlers – recruits), we grouped the total amount of individuals from all four locations to give a better representation of monthly settlement intensity or larval abundance in the region. Abundance of settlers and post-larvae were ln(x+1) transformed prior to analysis. Only species present in samples of consecutive years in numbers higher than 30 individuals were considered. The correlation coefficient was then compared with a distribution generated by 10,000 permutations for each species and significance was tested by *t*-test using R statistical software. If any of the cross-correlations is significantly different from 0 it will indicate a correlation between the two time series.

Temporal variability of settlement seasons was evaluated for each species by plotting size class abundances of 0+ cohort for each month. We considered the initial of settlement period the month in which the first size class (i.e. 1 cm) was observed, and the end of settlement was considered the month in which settlers' class(es) size(s) were not seen any more.

The relative importance of settlement versus post-settlement processes was investigated by computing the change in recruit abundance for each location by subtracting the number of recruits counted in one month from the number of recruits of the next census (1 month later) (based on Levin, 1994). The positive or negative sign of change indicates which process (post-larval settlement or post-settlement losses) is predominant in producing the observed changes: a mean positive change indicates that gains from post-larval settlement were, on average, relatively greater than post-settlement losses; likewise, a mean negative change suggests that post-settlement losses were larger than gains from settlement (Levin, 1994). In establishing this relation, we assume that recruits are relatively site-attached, so that loss of recruits is assumed to be due to mortality rather than migration.

Additionally, to evaluate the effects of post-settlement losses on recruit abundance, mortality rates and their variation across sampling years were estimated for those species for which recruits were recorded every sampling month during both years. Recruit abundance from all localities were pooled and then ln(x+1) transformed prior regression analysis with time (in months). The slope of linear regression gave the instantaneous mortality rate (M) (Adams and Howard, 1996; Macpherson *et al.*, 1997). Also we expressed the proportional loss in abundance for each species by subtracting the total abundance at the end of the settlement period (N_t) from the initial abundance at the peak of the settlement season (N_0) and gave it as a percentage. Additionally, we further evaluated whether mortality was density-dependent for each species and among years. For doing that we compared the coefficient of variation at the beginning (CV_{N0}) and at the end of settlement season (CV_{Nt}): a reduction in the coefficient of variation between these 2 stages would provide confirmation that mortality is density-dependent (Macpherson *et al.*, 1997).

Finally, the coupling between early life stages was assessed by fitting the ln-transformed abundances of post-larvae and settlers, and those of settlers and recruits, using linear regression analysis. We hypothesised that the absence of relationship would indicate decoupling during the pelagic transition to the demersal habitat, or yet in the demersal environment. Since one of the major causes for decoupling is higher mortality rates, we expect to infer in which life-phase mortality is more important for defining population structure.

3. Results

3.1 Temporal variability of post-larval supply and settlement

Between June 2010 and June 2011 light-traps sampled 5,103 post-larval individuals belonging to 51 *taxa* from 27 families. The most abundant *taxa* were Blenniidae (26% of total abundance), *Sardina pilchardus* (15%), *Mullus* spp. (11%), *Pagellus bogaraveo* (10%), *Trachurus trachurus* (9%) and *Diplodus annularis* (7%), all together accounting for 78% of total abundance. Among the most representative families sampled we found Sparidae with 10 *taxa*, followed by Carangidae and Serranidae, with 5 and 4 species each, respectively (Table 2.1). Almost 60% of species captured were represented by less than 10 individuals, and 15 *taxa* were represented by only 1 specimen. Higher species richness and total abundance were registered during summer, namely June-August. Interestingly, a notably high number of individuals were also captured during November, February and March (Fig. 2.2). About 55% of total species sampled settled in summer, between June-August; another 25% did it in autumn (September-November), and the remaining 9 and 11% occurred on winter and spring season, respectively.

A total of 103,343 fish individuals belonging to the 0+ cohort were visually censused from August 2009 to June 2011, in which 30,558 were settlers and 69,785 were recruits (Table 2.1). Abundance of young fishes in the second year was 2.4-fold higher than the first one and, similarly, 28% more species were registered in the second year compared to the first one (Table 2.1). About 6.3 times more settlers were registered in the second year while only 1.75 times more recruits were detected in the same period. A relative change in species composition of the annual rank abundance was observed for settlers' species. In the first year most important species, in numbers, were *Thalassoma pavo* (29%), *Chromis chromis* (21%), *Sarpa salpa* (19%) and *Diplodus vulgaris* (9%), while in the following year *Oblada melanura* (25%), *S. salpa* (20%), *C. chromis* (11%) and *Atherina* spp. (10%). For their part, recruits most abundant species composition was more conservative among years, although species varied much in their relative importance (Table 2.1). Recruits of Mugilidae (28%), *O. melanura* (14%), *S. salpa* (10%) and *C. chromis* (9%) were the more representative in the first sampling period, and *S. salpa* (24%), *T. pavo* (18%), *C. chromis* (16%) and Mugillidae (13%) in the second one.

Temporal variability of post-larval supply, settlement and recruitment were strongly seasonal and temporally lagged for the three life stages (Fig. 2.2). The inspection of Fig. 2.2 allows recognising two reproductive seasons according to the incoming of post-larval fish to the sampling areas: the first and more important during summer (June to August) and a second one during winter (December to March). Abundance peaks for post-larvae, settlers and recruits were 1-2 months lagged in relation to the previous life phase and occurred on June, July and September, and for the second peak February, April and May, respectively (Fig. 2.2).

Although for the great majority of fish species analysed we found high correlation coefficients between post-larval and settler abundances, few of them were statistically significant (Table 2.2), indicating that settlement for those species could not be predicted by the intensity of post-larval supply, except for *D. sargus, S. salpa, Atherina* spp. and *T. pavo*. Most species displayed temporal lags which varied in interval and sign among species (Table 2.2); the predominance of negative signs amongst species means that settler time-series are $t+n$-lagged (being n the lag number in Table 2.2) months in relation to post-larval time-series, while a positive sign indicates that settlers were observed before that the post-larvae were captured in the light-traps. Regarding cross-correlations between settlement years, few species displayed temporal lags representing a temporal synchrony between years in their settlement period, thus indicating the great variability in settler abundance between years (Table 2.2).

Table 2.1 Post-larval, settlers and recruits abundance over the sampling period (09-10 and 10-11), the range size and the size criteria (in cm) used to define settlers and recruit stages. Y1= 09-10 and Y2=10-11. * All size classes not available in the range

Species	**Larvae**	**Settlers**		**Recruits**		**Size range (cm)**	**Size settlers (cm)**
	Y2	**Y1**	**Y2**	**Y1**	**Y2**		
Atherina spp.	19	186	2724	1997	366	1-5	≤2
Apogon imberbis	10	5	25	1	100	3-4	≤3
Argentina sphyraena	1						
Arnoglossus laterna	1						
Arnoglossus spp.	1						
Auxis rochei	3						
Belone belone			15		9	10-20*	≤14
Blenniidae	1312						
Boops boops	8	0	779	1996	1586	1-7	≤2
Bothus podas	1		2		2	4-10*	≤4
Caranx chrysos	2						
Caranx ronchus	1						
Chromis chromis	175	890	2929	2239	7125	1-4	≤2
Conger conger	5						
Coris julis		88	302	658	1589	1-5	≤2
Dactylopterus volitans	3						
Diplodus annularis	368	3	517	49	538	1-5	≤2
Diplodus cervinus		0	343	1	40	1-5	≤2
Dentex dentex		1	4	34	17	6-16	≤7
Dicentrachus labrax			5		16	7-14	<8
Diplodus puntazzo	42	10	93	20	355	1-7	≤2
Diplodus sargus	1	30	347	556	346	1-7	≤2
Diplodus spp.	2						
Diplodus vulgaris		373	1510	584	296	1-7	≤2
Echiichthys vipera	2						
Engraulis encrasicolus	9						
Epinephelus costae		0		2	0	8-10	
Epinephelus marginatus		2	2	2	6	5-12	≤6
Gaidropsaurus mediterraneus	90						
Gymnammodytes semisquamatus	14		102		246	3-6	≤4
Hippocampus hippocampus	1						
Labrus merula		3	4	16	10	4-11	≤5
Litognathus mormyrus			14		86	2-8	≤3
Labrus viridis		2		3	1	6-11	≤7
Monochirus hispidus	1						
Mycteroperca rubra	1				1	12	
Mullus spp.	612	0	56	23	11	4-10	≤5
Mugilidae	40	192	1035	7126	5911	1-10	≤2
Oblada melanura	330	7	6625	3603	2021	1-6	≤2
Pagellus bogaraveo	513		32			1-6	≤2

Species	**Larvae**	**Settlers**		**Recruits**		**Size range (cm)**	**Size settlers (cm)**
	Y2	**Y1**	**Y2**	**Y1**	**Y2**		
Pagrus pagrus	16						≤2
Pomatochisthus spp.			592		121	1-4	≤2
Pomatomus saltatrix	6						
Sarda sarda	1						
Sardina pilchardus	798						
Sardina spp.	71						
Sarpa salpa	35	822	5332	2456	10514	1-6	≤2
Sciaena umbra	1				4	12	
Scorpaena porcus	17						
Scorpaena scrofa	3						
Seriola dumerilii	1						
Serranus cabrilla	1				5	6-8	
Serranus hepatus	3						
Serranus scriba	2	0	8	199	126	2-8	≤3
Solea senegalensis	1						
Sparus aurata					4	8-10	
Spicara maena		139	3	55	25	6-8	≤6
Spondyliosoma cantharus	11		8		2	3-10	≤4
Sphyraena viridensis		0	228	15	57	3-12	≤4
Symphodus spp.	10						
Symphodus cinereus		0	12	2	8	3-4	
Symphodus mediterraneus		17	175	143	195	3-6	≤4
Symphodus ocellatus		42	324	597	3191	1-4	≤2
Symphodus roissali		25	91	275	386	2-5	≤3
Symphodus rostratus		0	8	19	11	3-6	≤4
Symphodus tinca		4	53	715	811	1-7	≤2
Thalassoma pavo	5	1251	1894	1238	8136	1-5	≤2
Trachinotus ovatus	71	124	149	665	221	3-10	≤4
Trachurus trachurus	460				1	8	
Tripterygion spp.	3						
Uranoscopus scaber	1						
Total abundance	5085	4216	26342	25289	44496		
Total richness	50	30	36	30	42		

Temporal patterns of settlement intensity for each species are shown in Appendix II, and some of the most common patterns exhibited by species are illustrated in Fig. 2.3. The vast majority of species settled during the warmer months, between June and September (e.g. *S. roissali*, *C. chromis, C. julis, D. annularis,* and all *Symphodus* species). Nevertheless, an important number of species reached the coast during winter and early-spring months, such as *S. salpa, D. vulgaris, D.*

puntazzo, D. sargus, D. cervinus, among others. Settlers of species such as *T. ovatus*, *Atherina* spp. and *S. viridensis* were present during short periods of time. For other species (*B. belone, B. podas, E. marginatus, M. rubra, E. costae, D. labrax, S. aurata, S. umbra* and *T. trachurus*) the accurate determination of their settlement season was not possible due to the limited number of specimens observed in the visual counts. A notable coherence between years was observed in the settlement period of species. On the other hand, high variability between years in abundance was observed for most species, so that in general the first year presented relatively low rates of settlement compared to the second year, displaying, for example, differences of as much as 2000-fold more recruits (all species considered together) in September 2010 compared to the same month on 2009 (Fig. 2.3-2.4, Appendix II).

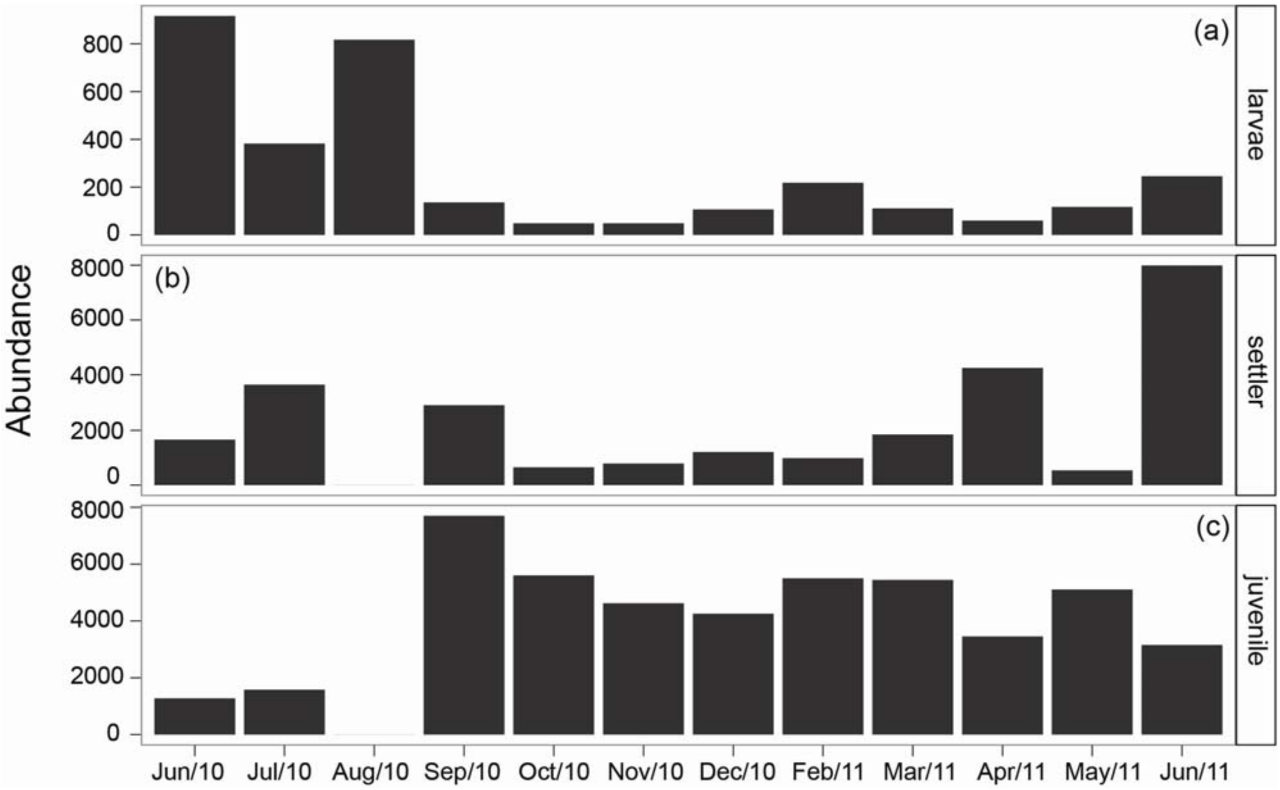

Figure 2.2 Temporal variability of a) larval, b) settler and c) recruit abundances over sampling locations during the second year (10-11 settlement season).

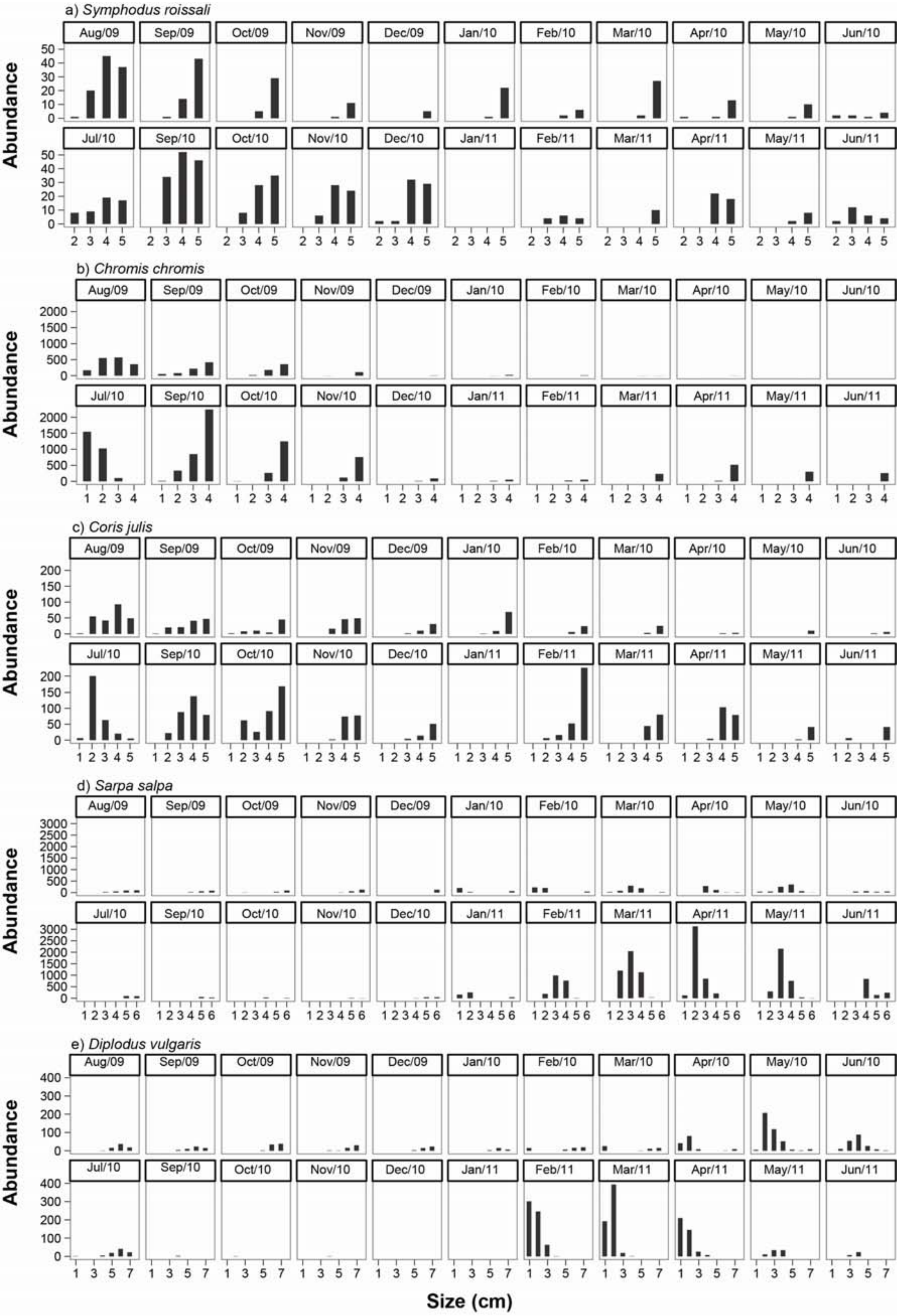

Figure 2.3 Intensity and temporal synchrony of settlement period of some abundant species as example of summer settlers a) *S. roissali*, b) *C. chromis* and c) *C. julis*, and winter-spring settlers, d) *S. salpa* and e) *D. vulgaris*. See appendix II for all species.

Table 2.2 Cross correlation coefficients between larvae and settlers abundances sampled on year 10-11 and among settlers abundance in consecutive years (09-10, 10-11). Only species with higher settlement intensity were included. Correlation lag time in months shown in brackets. Asterisk denotes significance at *P*=0.05.

Species	**Larvae-Settlers**	**Years**
	r coefficient (lag)	**r coefficient (lag)**
A. imberbis	0.84 (-1)	0.99
Atherina spp.	0.57 (1) *	0.71
B. boops	0.57 (5)	
C. chromis	0.65	0.87
C. julis		0.89
D. annularis	0.43 (-1)	0.37 (1)
D. puntazzo	0.63 (-3)	0.66
D. sargus	0.71 (-1) *	0.65
D. vulgaris		0.81 (1)
G. semisquamatus	0.88	
Mullus spp.	0.79 (2)	
Mugilidae	0.58	0.81
O. melanura	0.94	0.93
Pagellus spp.	0.66 (6)	
S. ocellatus		0.7
S. scriba	0.67 (2)	
S. salpa	0.60 (-2) *	0.65
S. maena		0.76 (-5)
S. cantharus	0.19 (-1)	
T. ovatus	0.55 (-1)	0.99
T. pavo	0.61 *	0.76

3.2 Settlement vs. post-settlement processes

Important changes in the number of recruits between successive months were observed along the entire sampling period, these changes being of much more magnitude during the second year of sampling (Fig. 2.4). Variations in settlement intensity were more important than post-settlement losses during the summer and early-autumn months and later on winter; during the rest of the studied period, post-settlement losses became more relevant in determining population structure (Fig. 2.4). The net effect of cumulative losses on recruit abundance trough time was assessed by regression techniques, separately for each year (Table 2.3). Mortality rates ranged from 0.09 month^{-1} for *D. sargus* during the first sampling year to 0.67 month^{-1} for *C. chromis* in the same year. No

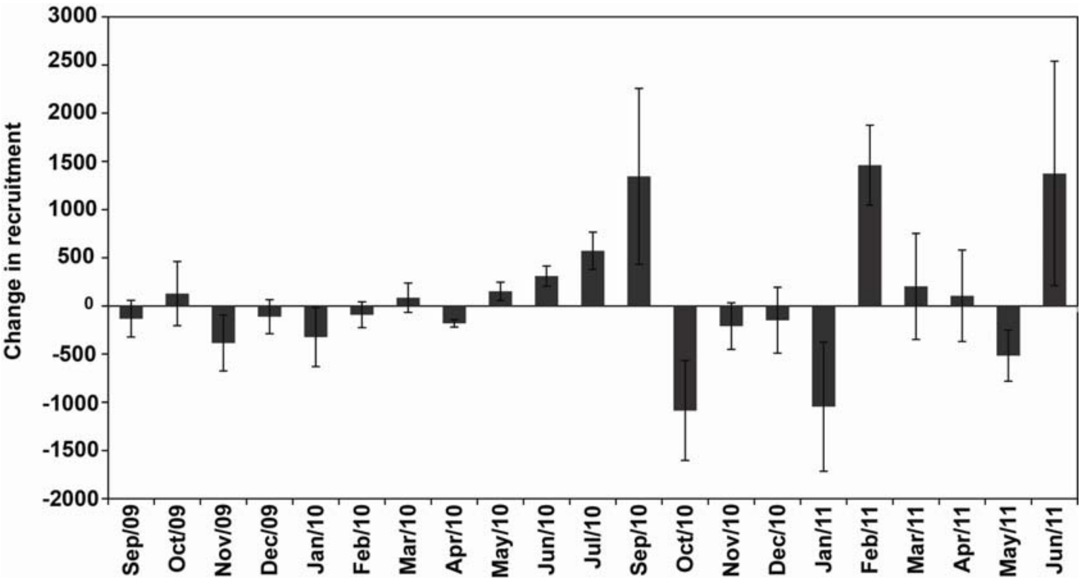

Figure 2.4 Mean relative importance of settlement vs post-settlement losses across studied localities for each month. Bars are standard errors.

significant differences were found in mortality rates between years of sampling (*t* = -1.4132, df=11, *p*>0.05). Density-dependent mortality was found for 4 species, namely *D. annularis*, Mugilidae, *S. mediterraneus* and *S. ocellatus* and was consistent over successive years. For its part, *Symphodus tinca* also showed density-dependence, but only in the second year. In general, recruitment losses were high, ranging 46.3-99.3%, although they varied for each species among years (Table 2.3).

Positive and significant relationships were found between abundance of distinct life stages only for *C. chromis* during the transition from pelagic to benthic habitat (Table 2.4); *Mullus* spp. was only marginally significant, while for the remaining species the relation was not significant. On the other hand, recruits belonging to the species *Atherina* spp., *D. annularis, D. puntazzo, D. sargus, D. vulgaris, Pagellus* spp., *S. mediterraneus, S. roissali, S. ocellatus* and *T. pavo* were directly related to abundances of settlers (Table 2.4, Fig. 2.5).

4. Discussion

Temporal patterns of post-.larval supply, settlement and recruitment were strongly seasonal, and defined two clear reproductive seasons: summer and winter, being the summer peak greater in abundance for all life-history stages than in winter. Taking into account that the majority of Mediterranean species are late-spring/early-summer spawners (Tsikliras *et al.*, 2010) and planktonic larval duration (PLD) estimated for 42 species is on average 25 days (Raventós and Macpherson, 2001), settlement period will be concentrated throughout summer and early autumn. For their part, Mediterranean ichthyoplankton surveys made to now (e.g. Sabatés *et al.*, 2007) have detected the highest species diversity and abundances in summer, while during winter the species composition varied substantially, and is composed by a few *taxa*, corroborating our findings. However, when we evaluated synchronization of post-larval supply and settlers abundance for each species, few

Table 2.3 Mortality rates (in month^{-1}) and associated statistics, initial and final abundance of recruits, proportional recruitment losses and difference of coefficient of variation (CV_d = CV_{N0} - CV_{Nt}) for most abundant species which had occurred in all sampling months. Significance: * - $P<0.05$, ** - $0.001<P<0.01$, *** - $P<0.001$. Positive values of Cv_d indicate density-dependent mortality.

Species	**Year**	**M**	**SE**	**R^2**	**P value**	**N_0**	**N_t**	**Losses %**	**CV_d**
C. chromis	1	-0.674	0.119	0.76	***	1643	25	98.5	-179.22
	2	-0.269	0.110	0.33	*	2676	168	93.7	-0.40
C. julis	1	-0.334	0.043	0.85	***	241	8	96.7	-71.31
	2	-0.266	0.093	0.44	*	294	4	98.6	-17.75
D. annularis	1	-0.206	0.036	0.78	***	114	1	99.1	51.16
	2	-0.307	0.107	0.51	*	141	12	91.5	0.86
D. sargus	1	-0.092	0.053	0.17	ns	147	63	57.1	-46.28
	2	-0.126	0.162	-0.05	ns	131	53	59.5	-31.62
Mugilidae	1	-0.168	0.049	0.52	**	1224	411	66.4	83.58
	2	-0.199	0.123	0.15	ns	2315	167	92.8	15.83
O. melanura	1	-0.387	0.093	0.65	**	322	8	97.5	-35.40
	2	-0.208	0.071	0.43	*	700	131	81.3	-122.45
S. mediterraneus	1	-0.187	0.068	0.40	*	32	2	93.8	52.61
	2	-0.152	0.048	0.56	*	103	23	77.7	9.66
S. ocellatus	1	-0.320	0.071	0.66	**	333	7	97.9	12.00
	2	-0.102	0.100	0.00	ns	180	78	56.7	7.33
S. roissali	1	-0.168	0.067	0.35	*	103	53	48.5	-43.82
	2	-0.231	0.117	0.23	ns	132	8	93.9	-14.92
S. scriba	1	-0.263	0.057	0.64	**	33	4	87.9	-164.52
	2	-0.220	0.074	0.53	*	63	8	87.3	-57.33
S. tinca	1	-0.142	0.037	0.58	**	82	44	46.3	-14.48
	2	-0.206	0.072	0.45	*	161	11	93.2	126.01
T. pavo	1	-0.345	0.063	0.74	***	948	9	99.1	-79.60
	2	-0.288	0.197	0.11	ns	2158	16	99.3	-102.37

Table 2.4 Relationship between larval, settlers and recruits abundances during the second year of sampling period. Significance: * - *P*<0.05, ** - 0.001<*P*<0.01, *** - *P*<0.001.

Model	**R^2**	**P value**	**intercept**	**slope**
Larvae ~ Settlers				
C. chromis	0.42	*	3.770	0.747
D. annularis	0.13	ns	0.697	0.585
D. puntazzo	0.20	ns	0.545	0.855
Mugilidae	-0.10	ns	1.892	-0.229
Mullus spp.	0.26	ns	-0.381	0.350
O. melanura	-0.09	ns	5.941	-0.071
Pagellus spp.	-0.02	ns	-0.114	0.309
S. salpa	-0.07	ns	4.700	0.466
T. ovatus	-0.10	ns	1.446	-0.121
Settlers ~ Recruits				
A. hepsetus	0.64	**	0.407	0.681
B. boops	-0.07	ns	2.023	0.175
C. chromis	-0.06	ns	6.979	-0.158
C. julis	0.10	ns	5.138	-0.173
D. annularis	0.63	**	0.596	0.672
D. cervinus	-0.04	ns	0.369	0.133
D. puntazzo	0.66	***	0.988	1.045
D. sargus	0.57	**	1.023	0.753
D. vulgaris	0.66	***	-0.319	0.652
Mugilidae	0.03	ns	1.989	-0.218
Mullus spp.	-0.10	ns	6.091	-0.002
O. melanura	0.05	ns	7.722	-0.505
Pagellus spp.	0.45	*	0.424	1.119
Pomatochistus spp.	0.05	ns	0.401	0.284
S. mediterraneus	0.96	***	0.160	0.980
S. ocellatus	0.40	*	3.907	0.487
S. roissali	0.54	**	1.908	0.743
S. salpa	-0.02	ns	4.343	0.282
S. tinca	0.17	ns	3.417	0.442
S. viridensis	-0.02	ns	0.727	0.174
T. ovatus	0.04	ns	0.986	0.425
T. pavo	0.53	**	0.533	1.028

significant correlations were found, indicating that in most cases settlement intensity cannot be predicted from post-larval supply data. Only in the case of *D. sargus* and *S. salpa*, where post-larval and settler abundances were positive correlated, that settlers lagged 1 and 2 months in advance larvae occurrence, respectively (mean PLD 27.6 and 31.7, respectively; Macpherson and Raventós, 2006). An opposite pattern was found for *Atherina* spp. in which settlers were registered 1 month before the capture of larvae, and no lag at all was observed for *T. pavo* individuals. These results might have happen due to the time interval between samplings on distinct locations. As 15 days were left between one visit to another (Cape of Palos and Grosa Island) larvae may be captured in one site and settlers recorded 15 days later but within the same month of larvae recording, as happened to *T. pavo,* or 15 days earlier but in the previous month, as the case of *Atherina* spp. Despite these seasonal trends, greater fluctuations in abundance between consecutive years were found for all settlers species analysed which resulted in lack of significant correlations between species abundance among years. Yet, correlation coefficients between yearly series were quite high and few species showed temporal lags indicating that settlement timing is conservative for each species between years, meaning that settlement occur in the same months across years. The lack of significance of between-years correlations were likely due to the huge differences in abundance (as much as 2000-fold more recruits) in one year compared to the same month on previous year. Similarly, Doherty (1987) also found no consistency in recruitment magnitude of *Pomacentrus wardi* from one year to the next nor in the timing of recruitment episodes (other than seasonal limits), but only at the scale of the reef. As the spatial scale of our study was larger, the predictability of replenishment probably increased as well due to the cancellation of the variability recorded at smaller scale. Although temporal variations were detected at every spatial scale, recruitment was relatively consistent and predictive at larger spatial scales, indicating that the major variations in replenishment occur over finer scales.

Numerous studies worldwide provided information about fish recruitment, mortality, and the effect of these processes on adult population size (e.g., Jones, 1984; Holm, 1990; Hixon, 1991; Holbrook and Schmitt, 2003); however, few studies focusing on the dynamics of Mediterranean fish early life stages estimated mortality as well (e.g., Macpherson *et al.*, 1997; Macpherson and Raventós, 2005). The importance of recruitment in determining local population size cannot be interpreted without the knowledge of subsequent mortality patterns (Caley *et al.*, 1996).

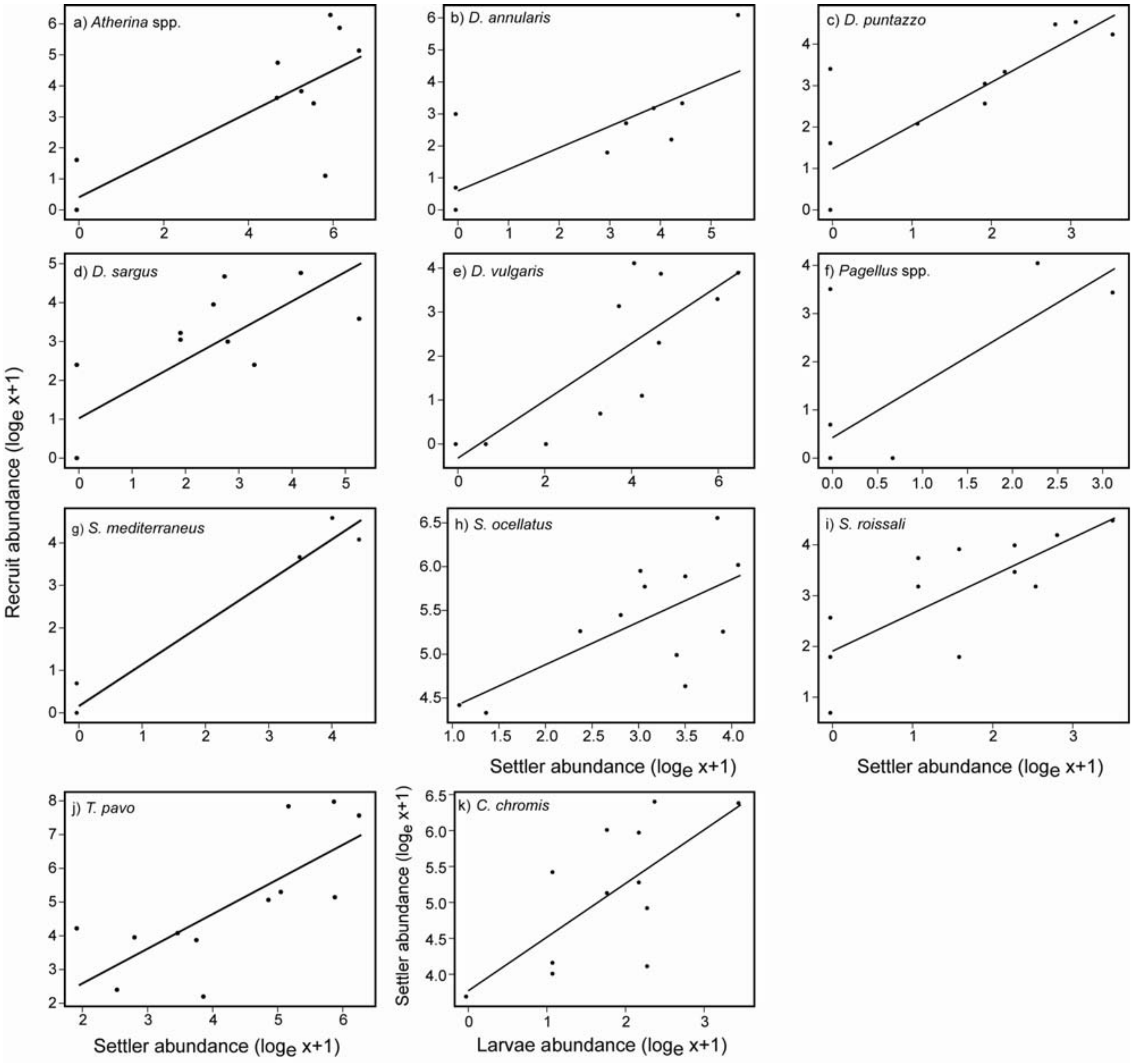

Figure 2.5 Significant regression relationships between settler and recruit abundance for a) *Atherina* spp., b) *D. annularis*, c) *D. puntazzo,* d) *D. sargus,* e) *D. vulgaris,* f) *Pagellus* spp. g) *S. mediterraneus*, h) *S. ocellatus*, i) *S. roissali,* j) *T. pavo*, and among larvae and settler abundance, k) *C. chromis*, during the second year of sampling period.

Annual mortality rates were considerably high for most species (in average ~80%), except for *D. sargus* which presented moderate recruit losses (~50%), although this parameter varied between species and sampling years. In general, early post-settlement mortality may vary greatly among species and can alter patterns of abundance established at settlement (Caley *et al.*, 1996).

Usually mortality estimates rely on density series acquired at short time intervals, normally expressed in days (Victor, 1986; Levin, 1994; Macpherson *et al.*, 1997), thus differing from the methodology implemented here, as we measured recruitment on a monthly basis. Notwithstanding, we found similar percentage losses as those reported in other studies both in the Mediterranean (Macpherson *et al.*, 1997; Planes *et al.*, 1998) and elsewhere (Levin, 1994; Schmitt and Holbrook, 1999; Valles *et al.*, 2008). Additionally, consistently lower correlation coefficients between all species abundances and times were found during the second year. Considering the 2000-fold difference in settler abundance from one year to the other, we attribute the lower correlation of mortality rates in the second year to an increase in the fine-scale variation in fish abundances. Despite of lower correlation, for most species percentage losses were constant between years. In fact, only 3 out of 12 species analysed (all *Symphodus* species – *S. ocellatus, S. roissali* and *S. tinca*) showed more than 40% of difference between post-settlement losses from one year to another. If mortality were density-dependent, a higher mortality rate would be the result of the increase in settlement intensity. This might be the case of *S. ocellatus*, which had proportionally less settlers in the second year compared to the previous one, as well as lower mortality, and higher proportional survivorship. Conversely, both *S. tinca* and *S. roissali* presented higher mortalities rates in the second year. Mortality in *S. tinca* was density-dependent but only for the second year, indicating that fish abundance must exceed some threshold level so that mortality could be distinguished from density independence (Osenberg *et al.*, 2002). Such level was then probably not attained in the first year due to low settlement intensity. Yet, density-independent regulation must be the case for *S. roissali,* in which variation in abundance is directly reflected on subsequent life stages. Density-dependent mortality was also found for *S. mediterraneus*, Mugilidae and *D. Annularis*

Significant coupling between early life-history stages were found for a number of species. Only settlement intensity of *C. chromis* was predictable from post-larval supply. However, recruit patterns reflected the abundance of settlers for a greater number of species (*Atherina* spp., *D. annularis, D. puntazzo, D. sargus, D. vulgaris, Pagellus* spp., *S. mediterraneus, S. ocellatus, S. roissali* and *T. pavo*). Larval-juvenile coupling is supported by several studies (Victor, 1986;

Grorud-Colvert and Sponaugle, 2009; Taylor *et al.*, 2009) and unverified by others (see review in Pineda *et al.*, 2010) mainly due to microhabitat selection or differential habitat-specific post-settlement mortality of juveniles (Schmitt and Hollbrook, 2000; Juanes, 2007; Johnson, 2007). According to Levin (1994), the shorter the lag between measurements of settlement and recruitment, the stronger the relationship between them. For other part, increasing lag between measurements will lead to stronger relationship between surviving juveniles and subsequent adult densities (Caley *et al.*, 1996). So, the lack of relationships between settlement and post-larval stages for most species tested in this study could be related to the timing of measurements, as samples were taken monthly; therefore, higher mortality rates just after settlement could have contributed to weaken the observed relationships.

Noteworthy, those species in which significant relationship between recruits and settlers were found were mostly benthic species, with strong recruit-habitat relationships, such as *Symphodus* and *Diplodus* species (Félix-Hackradt *et al.*, *in prep.* Chapter III). Habitat complexity is though the major factor mediating the mortality during recruitment phase; at low levels of habitat complexity mortality is strong and recruitment has little effect on population size. At higher complexity levels limitation by recruitment is substantial; although density-dependence mortality will ultimately regulate population size, population dynamics can reflect variations in recruitment even in the presence of density-dependent mortality (Johnson, 2007). Although was outside the scope of this work to test survival for habitat-mediated relationships, we have evidences that support the importance of benthic habitat complexity in controlling mortality rates in 0+ year-class.

5. Conclusions

Recruitment was strongly seasonal, with two well-defined settlement periods: summer and winter months, the first period being the more important in terms of species richness and abundances. High temporal synchronization between intensity of post-larval supply and abundance of settlers was depicted by cross-correlations techniques; however, settlement intensity could not be predicted from post-larval abundances. Moreover, timing of settlement was consistent between years for most species. Additionally, a huge inter-annual variability in recruitment was observed, with a difference in abundance as big as 2000-fold between years. Post-settlement losses were high for all species (~80% in average), but density-dependent mortality was found for few species. Decoupling of post-larval supply and settlement was the general rule for all species tested except for *C. chromis*. These results could be related to the temporal allocation of sampling, as samples were taken at monthly intervals, but higher mortality rates just after settlement could have contributed to weaken the observed relationships. At contrary, for many species recruit abundances reflected those of settlers. Most species for which such relation was significant were benthic species with strong recruit-habitat relationships, such as *Symphodus* and *Diplodus* species. Although not tested, these findings would make clear the importance of habitat-mediated mortality for controlling mortality rates and thus shaping population structure.

Chapter III

Félix-Hackradt FC, Hackradt CW, Treviño-Otón J, Pérez-Ruzafa A, García-Charton JA. Habitat use and ontogenetic shifts of fish life stages at rocky reefs in South-western Mediterranean Sea.

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Habitat use and ontogenetic shifts of fish life stages at rocky reefs in South-western Mediterranean Sea

Abstract

The present study aimed at exploring the habitat use by fish life stages and ontogenetic shifts in a South-western Mediterranean rocky reef. During one year interval, the abundance of young-of-the-year (YOY) and juveniles and their relation with rocky reef habitat structure (complexity, heterogeneity, total cover and depth) were visually surveyed on a monthly basis in four localities. Fish assemblage responded significantly to spatial and temporal variation of structural microhabitat, this response being species-specific. Abundance of fish life stages was directly related to temperature variation, with higher abundances occurring on warmer months. Also, habitat differences occurred between YOY and juveniles of a series of species from shallow steeper rocky habitats to deeper complex rocky bottoms with high total cover. Spatial differences on YOY and juvenile abundance across localities could be attributed to fine-scale habitat preference, from purely rocky, more complex sites, with greater slopes and depths, to sites harbouring more heterogeneous habitats. By characterising the microhabitat association of fish life stages (both YOY and juveniles), and identifying for what species this influence may interfere with spatial and temporal patterns, the present study can be used as a basis for pluriannual monitoring of fish recruitment success in the area in order to better understand their population dynamics.

Key-words: recruitment; spatial scale; microhabitat; young-of-the-year; juvenile

1. Introduction

Recruitment of reef fishes can be defined as the settlement of pelagic larvae or juveniles to the bottom where fish is in straight contact with substrate and had survived enough to be counted by an observer (Keough and Downes, 1982; Hixon and Webster, 2002). The recruitment process is highly variable in space and time due to the effect of different ecological and physical processes (Sale *et al.*, 2005b). Temporal variation in recruitment is related to species life history patterns which involve synchronization of reproductive periods with temperature, lunar phases and photoperiod (Robertson, *et al*. 1990, 1992; Danilowicz, 1995; Tsikliras, *et al*., 2010). Additionally, pelagic larval duration greatly varies among fish species, ranging from days to months (Macpherson and Raventós, 2006), thus influencing the capability of species to disperse and finally causing differential larval supply. During the pre-settlement period, larvae are subject to high mortality (Carr and Hixon, 1995; Caley, 1998), leading to high variability in recruitment at multiple scales. The degree to which variability could be attributed to either stochastic (e.g. oceanographic variation on larval supply) or deterministic process (e.g. non-random habitat utilisation by recruits) (Ault and Johnson, 1998) is still uncertain.

Once settled, juvenile reef fish face several threats to their survival, for which characteristics of reef structural habitat will be decisive. A number of studies have focused on describing the underlying mechanisms that determine microhabitat use by fish; many features such as reef complexity (Armswort, 2002), substrate type (Jones 1988), habitat structure (Almany, 2004), predation (Holbrook and Schmitt, 2003), presence of co- and hetero-specifics (Almany, 2003), may influence fish habitat preference and can be constraining factors limiting recruitment success. Furthermore, refuges may be important for controlling density-dependence processes (Anderson *et al.*, 2001). Ultimately, habitat features can affect directly mortality and other demographic rates (Beukers and Jones, 1997; Almany and Webster, 2006). If habitat attributes are significantly related to the presence and abundance of recruits, then spatial variation of favourable habitat characteristics may have huge effects on fish community structure (Johnson *et al*., 2007)

For Mediterranean coastal reef species, most essential nursery habitats are located on nearshore zones (García-Rubies and Macpherson, 1995; Harmelin-Vivien *et al*., 1995; Macpherson, 1998; Planes *et al*., 2000). During the early life stages fish often undergo a series of ontogenetic changes which are related to optimizing their feeding ability and increase their capability of defence against predators and competitors (Bailey, 1994). Due to these morphological changes fish normally shift from their nursery habitat (Eggleston, 1995) to a more appropriate one in order to fulfil their new requirements, but such behavioural response may vary according to species (Macpherson, 1998).

Beyond the natural inherent spatial variability of recruitment, other factors could positively or negatively affect recruitment rate or survival. Protection from fishing has been hypothesized to affect recruitment success and variability affecting it negatively by enhancing predation and reducing recruit survival, however the extent of this effect might depend on whether settlement occurs away from the adult habitat or within it (Planes *et al.,* 2000). Therefore, the evaluation of spatial recruitment variability inside a Marine Protected Area is the first step to understand the underlying mechanisms which may affect settlement and recruitment, and ultimately population dynamics.

In this context the present study aimed to evaluate a) the importance of habitat structure on defining recruitment variability, b) the influence of spatial and temporal variation on determining such patterns and c) the presence of ontogenetic habitat shifts within fish life stages in a Mediterranean coastal rocky reef area.

2. Material and Methods

2.1 Study site

The study was conducted on the coast neighbouring the cape of Palos (37°38’N, 0°42’W) (Murcia, Southeast Spain) in SW Mediterranean Sea (Fig. 3.1). The studied area is dominated by rocky reefs surrounded by sandy and detritic bottoms (Pérez-Ruzafa *et al.*, 1991, García-Charton and 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 Sampling design and data collection

During one year interval between August 2009 and July 2010, the abundance of young-of-the-year (YOY) and juvenile fish and their relation with habitat was surveyed on a monthly basis in four localities situated thousands of meters apart: Cape (CA), Coves (CO), Hormiga island (IH) and Grosa island (IG). These localities were selected to represent the range of habitats existing in the region, and thus represented just a spatial replication factor for exploring recruitment variability. Both CA and IH sampling localities were located within the Cabo de Palos – Islas Hormigas Marine Reserve, while remaining localities were located outside the marine reserve limits, with CO situated on the coastal zone of the cape of Palos and IG 10 km northwards of the cape of Palos (Fig. 3.1). At each locality, 3 sites separated by hundreds of meters were randomly chosen each month.

In each site within each combination of months and localities, young fishes were counted visually by SCUBA diving on ten 10x2-m replicate transects set out haphazardly and marked with a plastic measuring tape deployed by the diver while advancing slowly at a constant speed. A depth range of 0-8 m was covered, in such a way that the whole range was evenly explored in each site

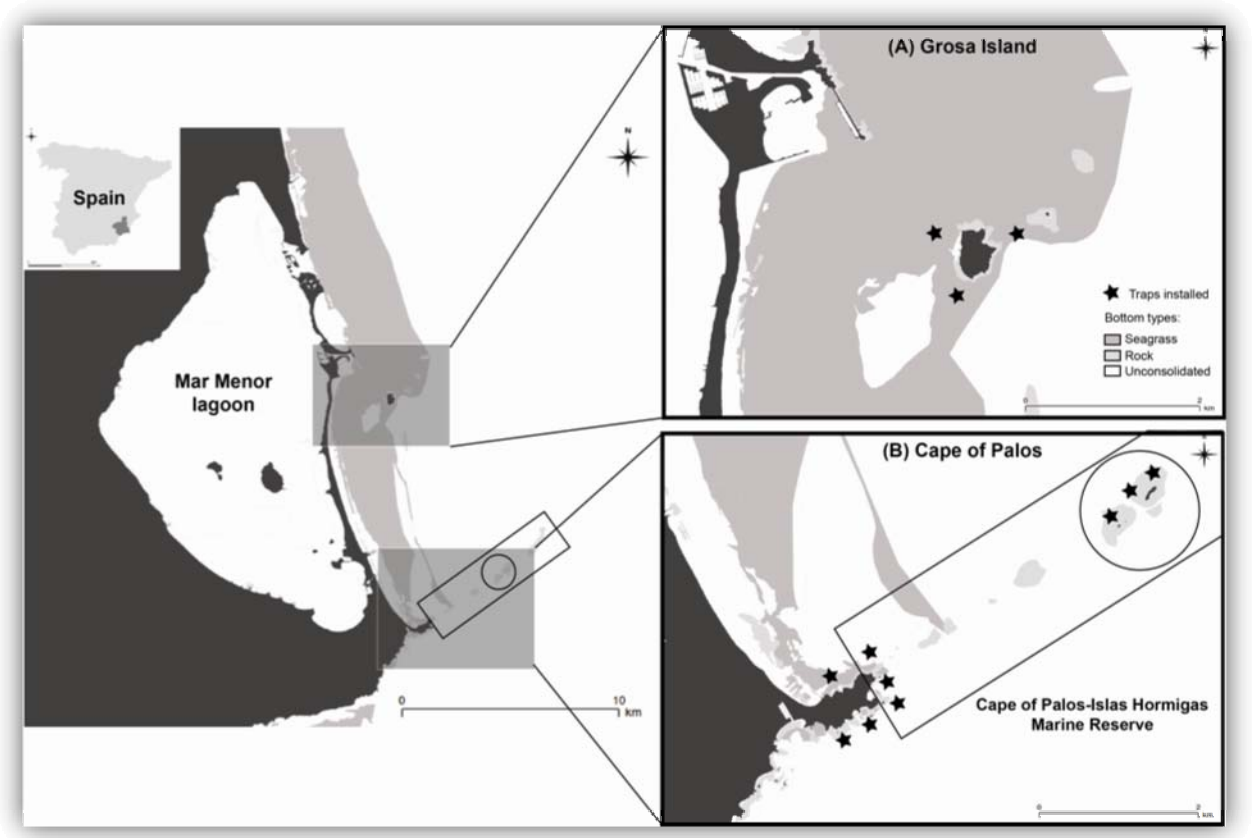

Figure 3.1 Map of Murcia coast, Southeast Spain, showing the location of Grosa Island, Cape of Palos and spatial delimitation of Cabo de Palos – Islas Hormigas Marine Reserve.

each month. Juveniles of all species were recorded except those from families having cryptic habits (blenniids, gobiids, etc.). Each observation was assigned to one of eleven abundance classes, whose limits coincide with the terms of a base-1.6 geometric series, constructed considering the most frequent figures of observed juveniles abundance (Harmelin-Vivien *et al*., 1985; García-Charton *et al*., 2000), namely 1, 2, 3, 4, 5-7, 8-10, 11-30, 31-50, 51-70, 71-100, >100. Geometric means of each fish abundance class were used for the calculation of abundances. The size of each observed fish was visually estimated to the nearest cm. To minimize bias in size estimation a pilot study was done in order to calibrate all 3 observers (FCFH, CWH and JTO) and accuracy of data was compared among observers 3 times, resulting in no significant differences. All censuses were done between 10:00 and 15:00 h GMT, when water conditions (turbidity and swell) were optimal (Harmelin-Vivien *et al*., 1985). Furthermore, all localities were visited within 2 weeks interval, minimizing timing recruiting differences.

After counting fish individuals, the same observer covered the transect length in the opposite direction to measure a series of habitat characteristics, considering 3 categories: (a) “heterogeneity”, defined as cover of the entire transect by different substrate types embedded in the rocky matrix, either sand, gravel or/and *Posidonia* meadows; (b) “complexity” of the rocky substrate, including rugosity, slope and number of rocky boulders classified by size; and (c) “total cover”, which comprised both algal cover and the number of sea urchins (mainly *Arbaxia lixula, Paracentrodus lividus*) and sponges (mainly *Ircinia* spp., *Chondrosia reniformis, Phorbas tenacior, Haliclona mediterranea*) observed on the rocky substrate. Urchins and sponges were selected due to their great abundance on the region in comparison to others invertebrates like ascidians and anemones, and their potential to be used as refuge by juvenile fish (José A. García-Charton, *pers. observ.*). Slope (in degrees) and rugosity (visual scale of increasing complexity from 1 to 4, adapted from Gratwicke and Speight, 2005) were estimated visually, twice, using a 1-m^2 random quadrat per transect. Also substrate cover (visual estimation of substrate %) and the number of rocky boulders within each transect was recorded classifying them by the size of their major length as follows: small (<50 cm), medium (50-100 cm) and large (>1 m). Both were measured 5 times at 2-m interval along transect length. Algal percent cover, distinguishing amongst encrusting, filamentous, bubble, articulated, tubular / foliose and arborescent / branched algae (based on Littler and Littler, 1984 and Steneck and Dethier, 1994), was estimated by means of 6 photoquadrats of 625 cm^2 haphazardly placed on the rocky substrate along the transect. The number of encrusting and massive sponge colonies, and the number of sea urchins occurring on the rocky reef were counted on two 1-m^2 random quadrats per transect. Depth (in m, also at 2-m interval along transect length) and temperature (in ºC) were recorded at each transect with the diving computer; the temperature measures were compared with those recorded by an offshore buoy located at Cabo de Palos-Islas Hormigas Marine Reserve, and, provided that no significant differences were detected, we kept the local measures.

2.3 Data analysis

For further analyses we separated all counted fishes into 2 life stages: (a) young-of-the-year (YOY) – comprising all fish from a minimum size of 10 mm that belong to 0+ cohort, and (b) juveniles – all individuals belonging to the remaining age classes but not yet sexually mature. The size criteria of inclusion of each fish to one or the other life stage are displayed on Table 3.1 and were based upon existent literature for each species, focusing as possible on regional studies, and also FishBase data (Froese and Pauly, 2011) when such regional studies were lacking, and finally field experience. Some *taxa* were classified at genus or family level, due to the impossibility of accurate visual identification of YOY at the species level: *Pagellus* spp., *Atherina* spp. and Mugilidae.

YOY and juvenile fish assemblages were specified for each transect by their species richness, abundance (total, and excluding pelagic as well as rare species), Shannon-Wiener's *H'* (log base 2) diversity, and species composition. For each species we estimated its relative abundance and frequency of occurrence. We defined as rare species those whose frequency of occurrence did not exceed 1% and their individual abundance represented less than 0.1% of the total. For analytical purposes we defined as pelagic the species *Atherina* spp., Mugilidae, *T. ovatus*, *B. boops, S. maena, Pagellus* spp.; although *S. viridensis* and *O. melanura* are considered pelagic species when adult, as young they are much more related to bottom, so that they were included in the group of demersal species.

The effects of fixed factor Month (M) and random spatial factors Locality (L) and Site (S) (nested in the MxL interaction) on the value of community descriptors (computed both for all species and excluding pelagic and rare species) and the abundance (log-transformed) of the commonest species were tested with ANOVA (using GAD package in R). Prior to analyses, homogeneity of variances was checked using Cochran's test, and additional control on heterocedasticity was done by using graphical methods (residual plots, variance vs means plots) to check the distribution of observations and variances, and groups with large variances were checked for errors and outliers (McGuinness, 2002). 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 is balanced and contains a large number of samples or treatments (Underwood, 1997). In these cases, tests have to be taken cautiously when the significance of the effect is $0.01 < P < 0.05$. 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.

For further analyses, and due to the large amount of habitat variables, we reduced the dimensionality of environmental data by compressing them into principal components (PCs) separately for each category of habitat characteristics – heterogeneity, complexity and total cover, and only first principal components (PC1's) of each analysis were retained, as they described clear environmental gradients (see results).

Multiple linear regression models were conducted to test for fish-habitat relationships and to explore possible habitat shifts between YOY and juveniles. For each life stage, log-transformed species abundance and community descriptors were exhaustively regressed to all possible combinations of habitat variables (PC1's and depth) and selected using information theoretical approach. Rare species were excluded from the analysis. Linear models were tested using the identity link function and Gaussian distribution. Models were compared using the bias-corrected Akaike's information criterion (AICc), and ranked by Akaike weights and AICc differences (ΔAICc) (using AICcmodavg package, R sofware). ΔAICc is the difference between the AICc of each model and the AICc of the best model, and Akaike weights is the probability of model to be the best one (Burnham and Anderson 2004).

To evaluate the relationship between the species matrix and the environmental variables from a multivariate perspective, a redundancy analysis (RDA) was performed. This analysis is only valid if data respond linearly to the environmental gradient, which seems to be the case for our data set, as the axis length (in standard deviation units) of detrended correspondence analysis performed on YOY and juvenile data is always shorter than 3 (Lepš and Šmilauer, 2003). Log-abundance data were averaged by sites within localities and months and used to perform calculations and to test the relative contribution of each variable on the ordination; a Monte Carlo permutation test was used after forward selection of variables with a 1% level of significance to test the null hypothesis of no relationship between environmental characteristics and species abundance. All calculations were done using CANOCO v. 4.5. For this multivariate approach we excluded pelagic and rare species from statistical procedures as they may produce noise on visualization of spatial and temporal patterns of recruitment of rocky reef species.

3. Results

3.1 YOY and juveniles fish assemblage

In the 1,440 replicate transects performed over the sampling year we recorded 63,832 fish recruits, from which 60% (37,668 individuals) were classified as young-of-the-year (YOY) and the remaining as juveniles. Species richness was higher among YOY stage, in which 36 *taxa* were registered against 28 juvenile *taxa* (Table 3.1). A group of 7 *taxa* accounted for more than 80% of YOY total counts; they were, in descending order, Mugilidae (21.8%), *Chromis chromis* (15.4%), *Oblada melanura* (13.0%), *Sarpa salpa* (9.6%), *Atherina* spp. (9.2%), *Thalassoma pavo* (6.7%) and *Boops boops* (5.4%). Among juvenile stage *C. chromis* (42.5%) was by far the dominant species, followed by *Atherina* spp. (11.3%). *S. salpa* (9.6%), *O. melanura* (9.0%) and *C. julis* (7.0%). Families Sparidae and Labridae included the higher number of species in both life stages, representing more than 60% of total YOY and juvenile species richness (Table 3.1). Additionally, some *taxa* as *Sphyraena viridensis*, *Spicara maena*, *Pagellus* spp., *Trachinotus ovatus* and *Mullus surmuletus* were common at YOY stage, but were not registered as juveniles.

Table 3.1 Total species abundance of YOY and juveniles in the studied localities. Standard length (SL, in cm) used to define both life stages according to the cited bibliography is also indicated. CO – Coves, IG – Grosa Island, CA – Cape, IH- Hormiga Island.

Species	Abbreviation	YOY					Juveniles				
		CO	IG	CA	IH	SL (cm)	CO	IG	CA	IH	SL (cm)
Apogonidae											
Apogon imberbis	Apoimb	1	2	4	0	<4[1]	1	0	0	0	<5[1]
Atherinidae											
Atherina spp.	Athsp	508	2350	608	0	<5[2]	74	1327	1557	0	<7[2]
Carangidae											
Trachinotus ovatus	Traova	301	30	359	133	<10[3]	0	0	0	0	<20
Centracanthidae											
Spicara maena	Spimae	178	16	0	0	<8[4]	0	0	0	0	<10
Labridae											
Coris julis	Corjul	237	160	386	265	<5[5]	426	468	340	603	<9[6]
Labrus merula	Labmer	5	2	4	8	<11[5]	3	0	0	0	<15[7]
Labrus viridis	Labvir	2	1	2	0	<11[5]	3	0	0	0	<15[7]
Symphodus cinereus	Symcin	1	1	0	0	<4[7]	0	0	0	0	<5
Symphodus mediterraneus	Symmed	0	5	2	163	<6[7]	3	3	6	52	<9[7]
Symphodus ocellatus	Symoce	180	125	124	397	<4[9]	247	832	144	1128	<5[7]
Symphodus roissali	Symroi	121	63	108	70	<5[10]	130	55	108	109	<6[5]
Symphodus rostratus	Symros	7	0	13	0	<6[7]	30	7	14	0	<9[7]
Symphodus tinca	Symtin	345	198	130	110	<7[11]	54	68	66	37	<12[12]
Thalassoma pavo	Thapav	164	654	332	1378	<5[13]	135	157	168	988	<12[13]
Mugilidae											
Mugilidae	Mugsp	1355	2034	796	4040	<10[14]	36	63	6	12	<20[14]
Mullidae											
Mullus surmuletus	Mulsur	92	25	76	0	<10[15]	359	145	101	333	<15[15]
Pomacentridae											
Chromis chromis	Chrchr	901	2239	1585	1080	<4[16]	1635	1562	1740	6180	<7[16]
Sciaenidae											
Sciaena umbra	Sciumb	0	1	0	0	<10[17]	0	1	0	0	<25[17]
Serranidae											
Epinephelus costae	Myccos	0	2	2	0	<12[7]	0	2	0	0	<30[7]
Epinephelus marginata	Mycmar	1	0	2	2	<12[7]	0	0	0	8	<43[7]
Mycteroperca rubra	Mycrub					<12[7]	0	0	1	0	<32[7]
Serranus cabrilla	Sercab	0	0	1	0	<8[18]	0	2	5	0	<15[19]
Serranus scriba	Serscr	74	39	43	48	<8[20]	40	26	16	40	<17[20]
Sparidae											
Boops boops	Booboo	704	114	337	866	<7[21]	54	15	12	505	<10[22]
Dentex dentex	Denden	21	11	14	0	<15[23]	1	1	1	0	<20[24]
Diplodus annularis	Dipann	132	40	22	0	<5[25]	104	32	12	0	<10[25]
Diplodus cervinus	Dipcer	0	0	1	0	<7[26]	0	0	0	0	<27[26]
Diplodus puntazzo	Dippun	19	25	5	0	<7[27]	13	9	16	0	<16[27]

Species	Abbreviation	YOY					Juveniles				
		CO	IG	CA	IH	SL (cm)	CO	IG	CA	IH	SL (cm)
Diplodus sargus	Dipsar	141	423	130	0	<7[28]	179	344	130	0	<20[29]
Diplodus vulgaris	Dipvul	417	305	509	1	<7[28]	175	196	191	5	<17[30]
Litognathus mormyrus	Litmor	4	0	0	0	<7[31]	3	1	0	0	<12[7]
Oblada melanura	Oblmel	571	1540	999	1784	<6[32]	0	0	0	0	<12[32]
Pagellus spp.	Pagsp	117	1	44	0	<6[25]	0	0	0	0	<15[25]
Sarpa salpa	Sarsal	652	705	1551	707	<6[33]	586	859	1037	27	<18[33]
Sparus aurata	Spaaur	4	0	0	0	<10[7]	0	0	0	0	<30[7]
Spondyliosoma cantharus	Spocan	3	10	0	0	<10[34]	0	0	0	0	<17[34]
Sphyraenidae											
Sphyraena viridensis	Sphvir	22	0	26	0	<12[35]	0	0	0	0	<26[35]
Total		7280	11121	8215	11052		4291	6175	5671	10027	

[1]Klein (2007); [2]Pombo *et al.* (2005); [3]Mourad (1999); [4]Dulcic *et al.* (2000); [5]Gordoa *et al.* (2000); [6]Alonso-Fernandez *et al.* (2011); [7]Fishbase; [8]Raposeiro-Azevedo (2009); [9]Raventós and Macpherson (2005a); [10]Raventós (2009); [11]Pallaoro and Jardas (2003); [12]Ghorbel *et al.* (2002); [13]Guidetti *et al.* (2002); [14]Hotos *et al.* (2000); [15]Reñones *et al.* (2004); [16]Dulcic and Kraljevic (1995); [17]Grau *et al.* (2009); [18]Tserpes and Tsimenides (2001); [19]García-Diaz *et al.* 1997; [20]Tuset el al (2005); [21]Monteiro *et al.* (2006); [22]El-Agamy *et al.* (2004); [23]Morales-Nin and Moranta (1997); [24]Loir *et al.* 2001; [25]Pajuelo and Lorenzo (2001); [26]Pajuelo *et al.* (2003); [27]Pajuelo *et al.* (2008); [28]Gordoa & Moli (1997); [29]Mouine *et al.* (2007); [30]Gonçalves *et al.* (2002); [31]Lorenzo *et al.* (2002); [32]Pallaoro *et al.* (1998); [33]Criscoli *et al.* (2006); [34]Pajuelo and Lorenzo (1998) and [35]Allam *et al.* (2004)

3.2 Spatial and temporal dynamics of fish recruits

Fish recruitment was clearly a seasonal phenomenon in the studied area, in which mean water temperature varied from 12ºC in February to 28ºC during August. In the case of YOY, higher abundances, richness and diversity were found during warmer months (mainly during June-September) (Fig. 3.2). A temporal lag between maximum values in abundance, species richness and *H*' diversity of YOY and juveniles was observed, so that in the case of juveniles these parameters showed higher values by the end of autumn, from September-December (Fig. 3.2).

Analyses of variance showed that YOY and juvenile abundance, species richness and diversity differed across months and localities, as evidenced by the significant effect of the MxL interaction (Table 3.2, Fig 3.2a). In the case of YOY assemblage, the removal of pelagic and rare species caused the loss of significance at the interaction MxL for species richness and *H*' diversity index, giving preponderance to the effect of main factors Month and Locality (Table 3.2), so that both YOY diversity and richness were consistently higher from July to December, as compared to all remaining months, and spatial variability at the scale of locality (separated by thousands of meters) was significant (Fig. 3.2). In addition, significant fine spatial scale variability among sites within each combination of month and locality was detected for all community descriptors of YOY and juvenile

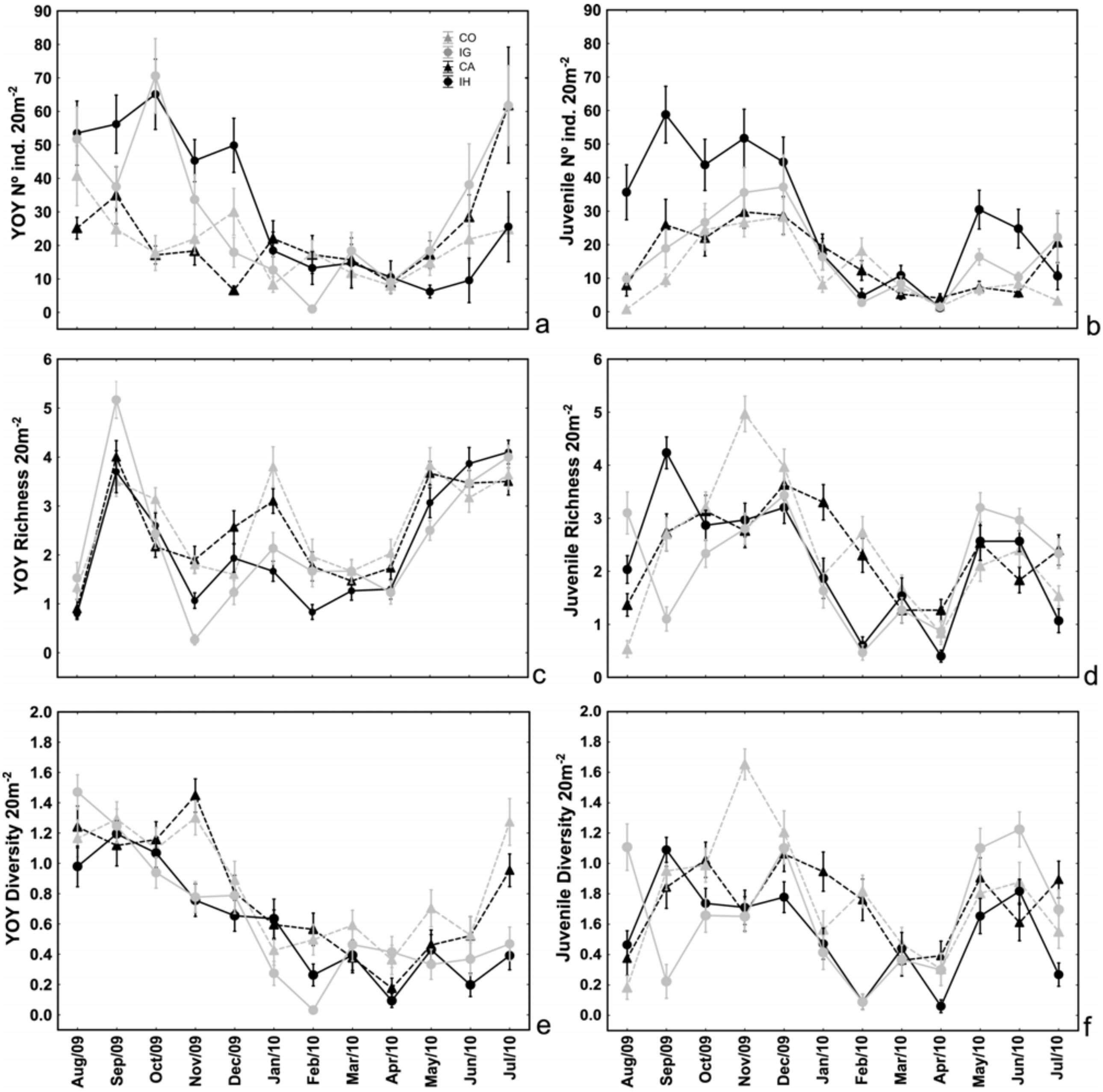

Figure 3.2 Monthly variation in abundance (a-b), species richness (c-d) and *H*' diversity (in bits/ind; e-f) of YOY and juveniles at each sampling locality. Localities are: Hormiga Island (black circle), Grosa Island (grey circle), Cape (black triangle) and Coves (grey triangle). Vertical bars are standard errors of the mean.

stages (Table 3.2). A total of 11 YOY species among those analysed showed a significant effect of the MxL interaction (Table 3.2), which means that temporal variation in abundance was not consistent across localities, or, more likely, that their occurrence was not spatially homogeneous but still showed a clear temporal pattern. Hence, abundance of most of these species consistently peaked in warmer months, mainly during July to September (for example, *C. chromis, C. julis, S. mediterraneus, S. ocellatus,* and *S. viridensis*). Other species such as *O. melanura, T. pavo* and Mugilidae were more abundant in October-November, while *Pagellus* spp. peaked in June. On the other hand, some species showed only the significant effect of one of the main factors. This is the case of *T. ovatus a*nd *S. scriba*, whose abundances were higher during August-September in all localities, while the abundance of *D. puntazzo* and *D. dentex* was spatially heterogeneous among localities, but no temporal effect was detectable. Another group of 5 species (*Diplodus vulgaris, Sarpa salpa, Mullus surmuletus, Symphodus roissali* and *S. tinca*) responded significantly to both main factors, so that sparids, *M. surmuletus* and labrids peaked on May, June and July-August, respectively, with marked differences in abundance among localities. Finally, almost all species showed significant variability at fine spatial scale (among sites separated by hundreds of meters) (Table 3.2).

Regarding the juvenile stages, nine out of the 18 analysed species presented a significant effect of the MxL interaction, while for other eight species a significant effect of either one or the other, or both main factors was evidenced (Table 3.2). In general, analogously to YOY stages, a non-random temporal occurrence was also observed for almost all juvenile species. Peak in juvenile abundance of *C. chromis, C. julis, D. sargus, S. roissali, S. mediterraneus, O. melanura, S. salpa* and *S. ocellatus* occurred during September-December months and on June-July for *D. vulgaris*. Variation in juvenile abundance of *S. scriba* was only temporally significant, occurring mainly during September-December. For their part, *B. boops, D. annularis* and *D. puntazzo* were affected only by spatial factor Locality, in which occurrence was related to specific localities (Table 3.2). All species except *D. sargus* showed a significant fine spatial scale variability [i.e., significant effect of

S(MxL)] (Table 3.2).

Table 3.2 Results of ANOVA on community descriptors (total abundance, species richness and diversity) and the abundance of commonest species and of young-of-year (YOY) and juvenile size stages for fixed factor month (M) and random spatial factors locality (L) and site (S). ***: P<0.001; **: P<0.01; *: P<0.05; ns: not significant; ^{1} pelagic and rare species excluded.

Variables	**YOY**								**Juveniles**							
	M		**L**		**ML**		**S(ML)**		**M**		**L**		**ML**		**S(ML)**	
	F	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Abundance	6.74	***	1.01	ns	3.39	***	1.61	***	6.75	***	5.20	**	3.29	***	1.96	***
Abundance^1	11.50	***	0.49	ns	1.71	*	1.73	***	5.45	***	6.84	***	3.87	***	2.07	***
Richness	16.45	***	4.74	**	1.90	**	2.07	***	4.57	***	2.52	ns	5.03	***	1.82	***
Richness^1	19.46	***	5.48	**	1.37	ns	2.02	***	4.11	**	2.85	*	5.23	***	1.79	***
Diversity (H')	15.47	***	8.61	***	1.56	*	1.89	***	2.83	*	6.33	**	4.18	***	1.68	***
Diversity (H')^1	13.14	***	10.68	***	1.37	ns	1.61	***	2.67	*	6.57	***	4.36	***	1.63	***
Atherina spp.	2.70	**	6.16	**	1.02	ns	2.17	***	2.59	*	11.98	***	2.57	***	1.51	**
B. boops	1.95	ns	1.83	ns	1.35	ns	2.49	***	1.39	ns	3.62	*	1.16	ns	1.69	***
C. chromis	15.13	***	5.57	**	2.91	***	0.70	ns	3.57	**	29.09	***	3.02	***	1.58	**
C. julis	4.07	***	3.44	*	3.89	***	1.07	ns	4.07	**	2.57	ns	2.13	**	1.54	**
D. annularis	2.45	*	11.15	***	1.73	*	1.84	***	1.13	ns	15.97	***	1.11	ns	1.93	***
D. dentex	1.22	ns	4.52	**	0.97	ns	1.13	ns								
D. puntazzo	1.79	ns	3.49	*	0.94	ns	1.37	*	1.62	ns	3.25	*	0.79	ns	1.28	*
D. sargus	1.82	ns	32.63	***	2.38	**	1.58	***	1.59	ns	53.80	***	5.37	***	1.11	ns
D. vulgaris	2.94	**	22.80	***	1.16	ns	1.91	***	1.94	ns	24.21	***	1.91	**	1.57	**
M. sumuletus	5.69	***	3.54	*	1.05	ns	1.59	***								
Mugilidae	2.32	*	8.21	***	2.44	***	1.10	ns	1.20	ns	1.65	ns	0.77	ns	1.83	***
O. melanura	2.88	**	3.47	*	1.81	*	1.81	***	2.40	*	3.81	*	1.13	ns	2.37	***
Pagellus spp.	2.31	*	2.44	ns	2.44	***	2.05	***								
S. maena	1.10	ns	0.84	ns	0.97	ns	3.30	***								
S. mediterraneus	1.03	***	95.45	***	4.48	***	1.05	ns	1.59	ns	24.44	***	1.86	*	1.29	*
S. ocellatus	3.13	**	5.91	**	4.16	***	1.19	ns	3.69	**	5.59	**	1.31	ns	1.73	***
S. roissali	10.21	***	3.76	*	0.98	ns	1.74	***	3.13	**	6.13	**	2.00	**	1.37	*
S. rostratus	1.58	ns	7.15	***	1.47	ns	0.87	ns	2.37	*	8.35	***	1.19	ns	1.30	*
S. salpa	4.61	**	12.51	***	0.72	ns	1.39	*	4.07	**	14.06	***	1.31	ns	1.52	**
S. scriba	11.52	***	2.23	ns	0.76	ns	1.83	***	2.53	**	2.32	ns	1.04	ns	1.47	**
S. tinca	3.05	**	4.66	**	1.33	ns	1.77	***	2.12	*	1.60	ns	1.68	*	1.30	*
S. viridensis	2.97	***	3.09	*	1.57	*	1.22	ns								
T. ovatus	5.52	***	1.75	ns	1.08	ns	1.82	***								
T. pavo	15.83	***	38.91	***	1.76	*	1.77	***	1.93	ns	46.94	***	3.00	***	2.19	***

3.3 Habitat description

Compression of environmental data by PCA, separately for heterogeneity, complexity and total cover, resulted in 3 first principal components (PCs) which explained 48, 38 and 19% of total variance, respectively (Table 3.3). The first PC obtained on habitat heterogeneity variables distinguished habitat types from purely rocky to more heterogeneous habitats – i.e. with a notable proportion of sand, *Posidonia* meadows and/or gravel embedded in the rocky matrix. For their part, the first PC obtained from habitat complexity variables evidenced an increasing gradient of habitat complexity, from transects dominated by small blocks to higher rugosity, slope and number of large rocky boulders. Finally, cover variables were compressed into a first PC which characterized a gradient from sites with dominance of filamentous algae and barren rocky bottoms to those rocky substrates covered by algal morphotypes with *thallii* of increasing elevation above the ground and increasing number of sea urchins and/or sponges (Table 3.3).

3.4 Fish - habitat relationships

Table 3.4 summarises the fish-habitat relationships for both of YOY and juvenile stages. The relative likelihood of a model being the best in the set of all possible models, as indicated by AIC weights, ranged 16.9 – 77.1 for YOY, and 13.9 – 68.7 for juveniles. Higher total abundance of YOY was found in shallower heterogeneous rocky habitats of low architectural complexity, while total abundance of juveniles was higher in deeper rocky bottoms with high complexity and total cover. Pure rocky substrates harboured a richer fish assemblage than heterogeneous ones (i.e. those having a considerable proportion of sand, gravel and/or *P. oceanica*) whatever the life stage. Lower value of total cover was a better habitat feature propitiating YOY diversity, while depth was the most important variable related to diversity of juveniles.

Depth within the studied range (0-8 m) was the most important characteristic in determining fish species abundance, followed by heterogeneity, cover and at last, complexity (Table 3.4,

Table 3.3 Loadings of environmental variables onto the first principal components (PC1) identified for each category of variables. PC1 explained 48, 38 and 19% of total variance for heterogeneity (het), complexity (com) and cover (cov), respectively.

Heterogeneity variables	**PC1 het**
Rock	-0.861
Sand	0.199
Posidonia	0.320
Gravel	0.342
Complexity variables	**PC1 com**
Small blocks	0.028
Medium blocks	0.199
Rugosity	0.298
Large blocks	0.334
Slope	0.871
Total cover variables	**PC1 cov**
Filamentous algae	-0.772
Barren	-0.046
Bubble algae	0.020
Urchins	0.028
Massive sponge	0.031
Encrusting algae	0.039
Encrusting sponge	0.048
Articulated algae	0.052
Arborescent algae	0.193
Tubular algae	0.596

Appendix II).

A number of YOY fish species were related to shallower areas where they have been evenly distributed on available habitats (e.g. *D. sargus, M. surmuletus*, *S. roissali* and *S. viridensis*), while the abundance of other YOY species were more related to deeper transects (e.g. *C. chromis*, *C. julis*, *D. annularis*, *D. dentex, S. scriba* or *S. cantharus*). On the other hand, juvenile individuals were generally more related to rocky habitats situated at deeper waters. Recruits (both YOY and juveniles) of some species such as *D. annularis*, *M. surmuletus*, *Pagellus* spp. and *S. salpa* were more associated to heterogeneous habitats, and others (e.g. *C. chromis*, *S. mediterraneus, S. roissali* and *T. ovatus*) appeared to be more related to purely rocky bottoms. Total cover of rocky substrates by algae and invertebrates was also an important habitat feature determining YOY abundance (Table 3.4, Appendix II).

Importantly, ontogenetic shifts in fish-habitat relationship from YOY to juvenile phases were observed for some species, as showed by the different models retained for both phases (Table 3.4, Appendix II); for instance, YOY of *D. vulgaris* and *D. puntazzo* were related to shallow and heterogeneous habitats, but juveniles of *D. vulgaris* were more related to less covered and deeper waters, while juveniles of *D. puntazzo* were more commonly found on purely rocky habitat. Similarly, *D. sargus* also was more related to rocky areas when juvenile, occupying less complex and less covered (by algae) shallow habitats as YOY. As another example, YOY of *S. salpa* occurred preferentially in heterogeneous substrates with high total cover in the rocky portion of the habitat, and occupied homogeneous rocky bottoms with lower total cover as juveniles. On the other hand, *C. chromis* and *O. melanura* juveniles maintained themselves in the same depth strata as YOY, but with distinct habitat structure: juveniles of *C. chromis* searched for more complex and covered deep rocky areas than YOY of the same species, although both preferred deeper bottoms; for its part, *O. melanura* juveniles preferred hard substrate with higher complexity than YOY, but both stages remained in the shallower sites. Nonetheless, some species, such as *D. annularis, C. julis, S. scriba* and all *Symphodus* species seemingly did not undergo ontogenetic movements, remaining in the same habitat as YOY and juvenile population (Table 3.4, Appendix II).

Axes 1 and 2 of RDA cumulatively account for 15% of total variance in YOY species data, and about 90.9% of YOY species abundance variance is explained by environmental variables. In the case of juveniles, the proportion of total variance explained by the first two axis of RDA was 21.8%, while environmental influence on species abundance accounted for 90.3% of total variance. The stepwise forward selection of habitat descriptors retained only habitat heterogeneity (grossly indicating substrate type) as having significant influence on the fish YOY assemblage structure, this variable accounting for 7% of total variation (i.e. the inertia explained by the first 4 axes). In the case of juveniles, the retained environmental variables were heterogeneity (8% of variance

Table 3.4 Summary of best model selection for community descriptors (abundance, richness and Shannon-Wiener's *H*' diversity) and fish species belonging to YOY and juvenile size stages with habitat categories (PC1s for complexity – com, heterogeneity – het, and total cover – cov, and depth). Symbols "+" and "-" indicate positive or negative slope relation, respectively and "*"indicates that pelagic and rare species were excluded from calculations. Models were selected using Akaike's information criterion (AIC); wi: AIC weights.

Variables	**Best model selection**			
	YOY	**wi**	**JUV**	**wi**
Abundance	-com-het-depth	0.369	+com-het+cov+depth	0.375
Abundance*	-het+depth	0.298	+com-het+cov+depth	0.340
Richness	-het-cov	0.204	-het+depth	0.325
Richness*	-cov-het+depth	0.233	-het+depth	0.332
Diversity	-cov	0.206	+depth	0.294
Diversity*	-com-cov-depth	0.406	+depth	0.747
Atherina spp.	+com-cov-depth	0.301	-cov-depth	0.270
B. boops	+depth	0.330	-het	0.284
C. chromis	-het+depth	0.523	+com-het+cov+depth	0.611
C. julis	+depth	0.330	-com+depth	0.264
D. annularis	+het+depth	0.423	-com+het+depth	0.467
D. dentex	-cov+depth	0.277	-	
D. puntazzo	+het-depth	0.391	-het	0.183
D. sargus	-com-cov-depth	0.533	-com-het-cov	0.403
D. vulgaris	+het-depth	0.444	-cov+depth	0.364
M. surmuletus	-com+het+cov-depth	0.328	-	
Mugilidae	-het-depth	0.479	-com-depth	0.239
O. melanura	-depth	0.188	+com-het-depth	0.198
Pagellus spp.	+cov+het-depth	0.393	-	
S. maena	+het+cov	0.188	-	
S. mediterraneus	+cov-het+depth	0.555	+cov-het+depth	0.619
S. ocelatus	+cov+depth	0.308	+depth	0.326
S. roissali	-com-het-depth	0.441	+com	0.139
S. rostratus	-cov+depth	0.319	-cov+het+depth	0.687
S. salpa	+cov+het-depth	0.323	-cov-het-depth	0.342
S. scriba	-cov+depth	0.354	+cov+depth	0.287
S. viridensis	-depth	0.264	-	
S. tinca	-com	0.242	+depth	0.232
T. ovatus	-cov-het-depth	0.465	-	
T. pavo	+com-het+cov+depth	0.771	+com-het+cov-depth	0.480

explained), cover (3%) and depth (3%). Thus, the first axes of both analyses distinguishes between shallower, heterogeneous bottoms (i.e. including patches of *Posidonia*, sand and gravel embedded in the rocky matrix) in its positive part, from more homogeneous (purely rocky) bottoms in its negative part, which are also more complex, steeper and deeper (Fig. 3.3). It appears to be a spatial variation of habitat structure at the scale of localities situated thousands of meters apart, since the more homogeneous, deeper and complex rocky bottoms are more frequent in all sites surveyed in Hormiga island (IH), and in most sites in Grosa island (IG) as compared to the other two localities (CA and CO). Sites surveyed in IH were more associated with YOY as well as juveniles of *T. pavo, S. mediterraneus*, *O. melanura*, *Serranus scriba* and *Chromis chromis*, while heterogeneous, shallower sites were characterized by the presence and abundance of YOY and juveniles of species such as *Diplodus* spp., *Sarpa salpa*, *Mullus surmuletus* and *Symphodus* spp. (Fig. 3.3).

4. Discussion

In the present study we assessed the spatial and temporal patterns of recruitment of 36 fish *taxa* in the SW Mediterranean Sea along one year in relation with their structural habitat. Compared to studies carried out at the same locality on the adult fish assemblage (García-Charton and Pérez-Ruzafa, 1998; García-Charton *et al*., 2001, 2004) the early-life stages sampled in this study represented 70% of total richness of visually censused adult fish species. Also, among the few published works which focused on fish recruitment in the Mediterranean region, the majority concentrated on one or a few species (Planes *et al*., 1998; Macpherson and Zika, 1999; Raventós and Macpherson, 2005b; Macpherson and Raventós, 2005; Carreras-Carbonell *et al*., 2007) or at genus or family level (Harmelin-Vivien *et al*., 1995; Vigliola *et al*., 1998; Macpherson, 1998).

YOY and juveniles fish abundance distribution among species followed those showed by adult population (García-Charton and Pérez-Ruzafa, 1998; García-Charton *et al*., 2001, 2004) with the dominance of *C. chromis*, *B. boops*, *T. pavo*, *Atherina* spp. and *C. julis* at both life stages. Similarly, early-life stages and adult species number was distributed among the same main families - Labridae, Sparidae and Serranidae, comprising 75% of total richness.

Recruitment appears to be a markedly seasonal phenomenon in the Mediterranean,

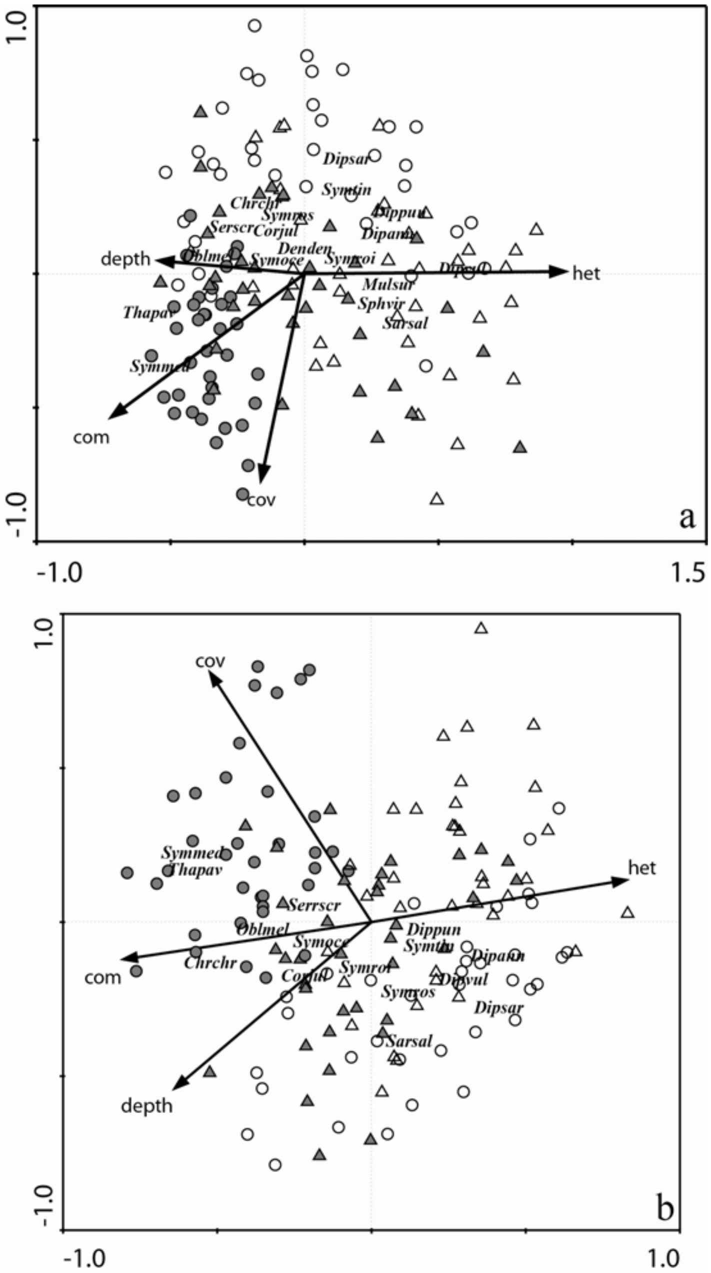

Figure 3.3 Triplot of RDA performed on a) YOY and b) juvenile species abundance at studied localities throughout the sampling period with respect to compressed environmental variables (vectors). Localities are: Hormiga Island (grey circle), Grosa Island (white circle), Cape (grey triangle) and Coves (white triangle). Environmental variables are: heterogeneity (het), complexity (com), total cover (cov) and depth. See Table 3.1 for species abbreviations.

corroborating previous observations (e.g., García-Rubies and Macpherson, 1995). Temporal variation in recruitment is a reflection of fish life history characteristics which include events such as mating, spawning and larval dispersion, which varies from one species to the other (Robertson *et* *al*., 1993). Recruitment peaks are expected to occur within warmer months in temperate reefs, due to the intrinsic relation of reproduction events with temperature, but timing among species may be delayed by short-time intervals. For example, it is known that for most *Diplodus* species temporal staggering of recruitment may be a mechanism for reducing interspecific competition as they all share the same habitat requirements for settlement (García-Rubies and Macpherson, 1995).

Also, we showed a marked spatial variability at the scale of localities (thousands of meters apart) as well as sites (separated by hundreds of meters), which are likely a reflection of habitat differences at these spatial scales. In this study we have characterized the relationship between fish recruit abundance and diversity and habitat features.

Importantly, rocky substrate conditioned a richer fish assemblage whatever the life stage, an observation which was also found by García-Rubies and Macpherson (1995) in NE Mediterranean littoral. It is known that habitat heterogeneity and complexity have an important influence on ecological patterns and processes, affecting species distribution, assemblage composition and diversity (García-Charton and Pérez-Ruzafa, 2001; Almany, 2004; Hewitt *et al*., 2005). Homogeneous substrates such as purely rocky bottoms usually harbour greater habitat complexity (rugosity, slope), which may provide additional refuge for a number of YOY and juvenile individuals In addition, YOY fish diversity was higher with decreasing cover by algae and benthic invertebrates, although some YOY species might take advantage of additional cover by photophylic algae recovering the first centimetres depth to keep protected against predators (see below), suggesting that the inherent complexity of rocky substrate could, by itself, provide necessary refuge for young fishes without any further structural addendum supplied by algal cover.

Regression models of habitat features on young fish abundance at the species level further depicted important insights to explain fine (among sites) and medium (among localities) scale spatial variability in recruitment, and consequently their occurrence on the different localities. As highlighted above, for some species total cover by algae and invertebrates were important habitat features to favour their settlement and recruitment; for example, *Symphodus mediterraneus* and *S.*

ocellatus recruited at deeper sites with high algal cover; *S. rostratus* also recruited in zones however covered by turf algae, meanwhile *S. roissali* and *S. tinca* selected shallow areas with low complexity values (by the importance of small blocks). Also, *S. salpa* YOY used shallow heterogeneous coastal zones with high cover by algae and/or invertebrates. For its part, *T. pavo* recruited in zones similar to those used by most *Symphodus* species, but characterized by steeper slopes and hard substrate. The structural architecture provided by rocky steep slopes with algae cover is ideal for the development of urchins (namely *Arbacia lixula* and *Paracentrodus lividus*), which use crevices on steep rock formation as refuge from predators (Benedetti-Cecchi and Cinelli, 1995). Although a significant correlation was not detected, at multiple occasions we observed 10 to 20-mm *T. pavo* individuals hiding between urchins' spines, which could be interpreted as a strategy for predators' avoidance. Other studies on habitat preferences by juvenile fish showed the importance of the presence of macrophytes over shallow rocky bottoms for the recruitment of certain species, as it was the case for the labrids *Tautogolabrus adspersus* in the Gulf of Maine, USA (Levin, 1991), and *Achoerodus viridis* in New South Wales, Australia (Gillanders and Kingsford, 1998).

Some species (such as *Sarpa salpa, Pagellus* spp. and *Diplodus annularis*) displayed specific habitat association to heterogeneous habitats, mainly due to the extent of *Posidonia oceanica* meadows embedded in the rocky matrix, which is characteristic of Mediterranean coastal zones down to 30-m depth (Ruiz *et al*., 2010). In the case of *D. annularis* recruitment takes place at deeper (>5m) and heterogeneous sites (due to the presence of *P. oceanica* leaves), corroborating the findings of Harmelin-Vivien *et al*. (1995) concerning this species; the obligate relationship of *D. annularis* has been stressed before (García-Charton *et al*., 2004), and explain why this species is not found where the seagrass *Posidonia oceanica* is absent. Also abundant were the youngs of species belonging to the genus *Diplodus*; *D. vulgaris* and *D. puntazzo* recruited preferentially at shallow heterogeneous mixed bottoms composed by gravel, sand and small boulders, and mostly in gravel-dominated beaches, while *D. sargus* was more often associated to less complex rocky areas (predominance of small blocks), which is consistent to sparid recruitment patterns found by García-Rubies and Macpherson (1995) and Harmelin-Vivien *et al*. (1995). In contrast to these studies, however, in our study *M. surmuletus* preferred coastal shallow mixed patches of *Posidonia* surrounded by sandy areas with small to medium boulders with high cover, while García-Rubies and Macpherson (1995) observed that this species was strictly associated to seagrass beds.

Here we provide the first description for the Mediterranean of microhabitat used by YOY and juveniles of species such as *Serranus scriba, Serranus cabrilla, Sphyraena viridensis*, *Spicara maena*, *Trachinotus ovatus*, *Dentex dentex* and *Spondyliosoma cantharus*, Except for *Serranus cabrilla* (which was only registered as juvenile) and *S. scriba* (which both life stages were observed), all these species were only recorded as YOY. Moreover, *S. viridensis* and *T. ovatus*, which are pelagic species, were only registered during a very limited period of time (2 months: July-August and August-September, respectively), mainly in coastal zones. Depth and habitat heterogeneity were the main characteristics driving YOY distribution patterns for these species: YOY of *S. cantharus, D. dentex* and *S. scriba* were observed principally in deeper areas within the studied depth range (0-8 m), while shallower sites were preferred by YOY of *S. viridensis* and *T. ovatus*. The pompano *T. ovatus* YOY phase was more related to rocky substrates, while *S. maena* occurred most often at heterogeneous substrates, where *Posidonia* predominates.

A habitat difference from shallow rocky areas to deeper and more complex zones was observed between YOY and juvenile life stages of a number of species. In general YOY species were predominantly related to shallow rocky habitats, while juveniles occurred mainly at deeper rocky bottoms with high complexity and rocky cover with algae and conspicuous invertebrates. The relationship with depth is generally described as one of the main factors conditioning both juvenile and adult populations (García-Rubies and Macpherson, 1995; Harmelin-Vivien *et al*., 1995; García-Charton and Pérez-Ruzafa, 1998; Friedlander and Parrish, 1998; Macpherson, 1998; Claudet *et al*., 2011), this relationship being species-specific. Sparid species increase their ecological niche dimensions with an increase in size, going horizontally towards rougher waters and vertically to deeper zones (Harmelin-Vivien *et al*., 1995). This shift is probably due to their shoaling behaviour (Macpherson 1998): as individuals grow, shoals become fragmented, increasing the number of shoals that now are less dense and more evenly distributed. As a consequence, early life stages of sparids did not share the same habitat as adult population; the same appears to happen in the transition between YOY and juvenile stages. On the other hand, YOY and juveniles labrid species showed the same habitat requirements described earlier by García-Rubies and Macpherson (1995), which is the same as adults, so that the overlapped distribution between young stages and adult individuals of *Symphodus* species is strongly associated to the presence of high algae cover, as it is used for refuge by young fish and for building nests by adults. Likewise, *S. salpa* individuals take advantage of high cover and heterogeneous habitat when YOY, and move further to less covered rocky habitat while growing.

5. Conclusions

In this work we provide strong evidence that spatial differences on recruitment were mainly driven by species habitat requirements. The area studied is composed by a mosaic of habitats varying spatially at fine and medium-scale, and this spatial variation is responded by a corresponding variability in young fish abundance of many species, likely as a result of active selection by settlers on their preferred habitat. Moreover, ontogenetic differences on habitat use by distinct life stages were identified during the sampled year. Spatial partitioning of resources by co-specifics could help on maintain population structure. By characterising the microhabitat association of both YOY and juvenile stages, and identifying for what species this influence may interfere with spatial and temporal patterns, the present study can be used as a basis for pluriannual monitoring of fish recruitment in the area, in order to better characterise the actual influence of other factors, such as protection from fishing and habitat connectivity, on this crucial phase of fish life cycle, for an improved understanding of their population dynamics and ecology.

Chapter IV

Félix-Hackradt FC, Hackradt CW, Treviño-Otón J, Pérez-Ruzafa A, García-Charton JA. Effect of marine protected areas on distinct fish life-history stages: a case study in a marine reserve network in South-western Mediterranean Sea

Manuscript

Effect of marine protected areas on distinct fish life history stages: a case study in a marine reserve network in South-western Mediterranean Sea

Abstract

The role of Marine Protected Areas in recovering biomass of exploited populations is well recognized worldwide and their effect might be enhanced on species with high commercial importance and low mobility levels. Nevertheless little is known about their effects on initial life stages of fishes. We used a beyond-ACI approach to assess the effect of protection on different fish life stages – larvae, juveniles and adult – classified in economic and mobility categories, in a marine reserve network in South-western Spain. We found that only abundance and biomass of adult of commercial and demersal species were positively affected by protection. Gradients of abundance across reserve boundaries were observed but the same did not happen to biomass, indicating that higher abundances were composed by small sized fishes. On the other hand post-larvae of commercially important species (both pelagic and demersal) were negatively related to MPA. Active selection of settlement preferred habitats as well as larval retention favoured by the geomorphological configuration of the coast (i.e. predominance of embayments outside no-take areas) has been proposed to explain such findings. Recruits showed high spatial variability resulting in lack of response of fishing protection measures. Given the fact that MPA could change demographic rates by altering mortality rates, increasing habitat quality, enhance predator number etc, protection can exert huge influence on population dynamics. We highlight the need on including early life stages and overall suitable habitats for them on design of MPA networks due to the direct importance of these stages to successful fulfilment of MPA objectives.

Key-words: effect of protection; spillover; larvae retention; habitat selection; MPA network; enforcement

1. Introduction

Marine protected areas (MPAs) have been advocated as a major tool for recovery and conservation of marine resources (García-Charton *et al.,* 2008; Fenberg *et al.,* 2012). Their multiple benefits in protecting ecosystems and ecological processes while enhancing fisheries through density-dependence spillover and larval dispersal of target species (Roberts *et al.,* 2001) convert them as the most powerful tool for spatial management in the marine environment. Although growing evidences support the real benefits of MPA such as biomass or abundance increase (Russ *et al.,* 2004; McClanahan and Mangi, 2000; Claudet *et al.*, 2008, 2010), population structure restoration (Harmelin-Vivien *et al.,* 2007; Guidetti, 2006), spillover (Francini-Filho *et al.,* 2008; Harmelin-Vivien *et al.,* 2008), exportation of eggs and larvae (Cudney-Bueno *et al.,* 2009; Crec'hriou *et al.,* 2010; López-Sanz *et al.,* 2011) among others, little is known about the MPAs effect on settlement and recruitment events (Planes *et al*., 2000; Sale *et al.,* 2005a; García-Charton *et al.,* 2008).

Larval supply is the first component of a series of processes contributing to the sustainability of adult populations (Halpern and Warner, 2003). Patterns of larval supply may vary according to egg production (Meekan *et al.,* 1993), larval mortality (Jenkins *et al.,* 2005), environmental constrains (D'Alessandro *et al.,* 2007; Félix-Hackradt *et al., in prep.* Chapter I), etc. Moreover, it is expected that the intensity of fish larvae directly influence the magnitude of recruitment (Doherty and Fowler, 1994), which in turn can be further altered by post-settlement processes that may act to uncouple larval and juvenile dynamics. Recruitment losses, though, will be of main importance to future demography of adult population, introducing variability at multiple temporal and spatial scales (i.e., seasonal, interannual, site scale, etc.; Levin, 1994).

MPAs may have important effects on resident population structure (Planes *et al.,* 2000). Location of the MPA may determine whether nursery habitats will be protected and thus favour species settlement. In theory, mortality is supposed to be higher inside MPAs due to the increase of predator abundance; however, compensatory effects may offset net differences (Syms and Carr, 2001). In this context, it is paramount to address the ecological effects of MPAs on the first life-history stages of fish in order to properly design and implement this management tool. To achieve these goals, powerful sampling designs must be constructed in order to accurately tackle the questions of interest. The use of multiple control locations in an asymmetrical analysis of variance to test for differences between impact and control sites (beyond-BACI designs) allows sufficient power to detect changes owing to the impact source. This statistical approach, developed by Underwood (1992, 1993, 1994), controls for spurious effects of using pseudoreplicated data, and provide additional power to naturally restricted designs as those of environmental disturbances. The asymmetrical beyond-BACI designs can be modified and used to identify environmental impacts when only 'after data' are available, by comparing the disturbed location(s) to multiple controls – i.e. asymmetrical after control/impact design (ACI) (Chapman *et al.,* 1995; Roberts, 1996; Glasby, 1997).

Because of increased density inside the MPA, adults and juveniles fishes of target species may emigrate from inside the protected location to outside, where the density is lower (“spillover”, Rowley, 1994). In addition, it has been hypothesized that MPAs can act as a source of propagules due to increased density and fecundity (as a consequence of the recovery of larger size classes) of protected populations, thus replenishing unprotected areas by dispersal of eggs and larvae (Planes *et al.,* 2000). 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 and Kramer, 1996; Chapman and 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 and Mangi, 2000; Russ *et al.,* 2003; Ashworth and Ormond, 2005; Francini-Filho and Moura, 2008; Amargós *et al.,* 2010).

In this work we used the beyond-ACI approach to investigate the effect of protection on distinct stages of Mediterranean reef fish life cycle (post-larvae, juveniles and adults). We addressed the following questions: Is the intensity of larval supply affected by protection? What is the response of juvenile and adult abundance and richness? Is the success of protection effect dependent on species mobility and economic importance? In addition, we addressed the question of the existence of gradients of abundance of distinct fish life-history stages (post-larvae, recruits and adults) of species and species groups constructed attending to their pattern of spatial occupation and their economic importance, as an indirect way to detect and quantify the likely occurrence of spillover from no-take areas to neighbouring unprotected sites. The answer to these questions aimed to fulfil an important gap on the marine reserves science, by adding light to a complex period of fish life cycle and assist on the proper management of marine resources.

2. Material and Methods

2.1 Study site

The Cabo de Gata – Níjar Natural Park covers 38,000 ha of both marine and terrestrial areas in the province of Almería, SE Spain and extends one mile offshore along about 60 km of coastline (Fig. 4.1). It was created in 1987 and declared a Biosphere Reserve ten years later (Paruelo *et al.,* 2005). The marine area under regulation extends over 12,200 ha, from which 4,613.45 ha are within no-take zones, where all harvest and recreational activities are forbidden. There are 5 no-take zones (from South to North: Cabo de Gata, Loma Pelada, Polacra, Punta Javana and Media Naranja, Fig. 4.1). Local artisanal fisheries, sport fishing and diving are allowed within the park limits except inside the no-take zones where only scientific activities are authorized. The studied areas are dominated by rocky reefs surrounded by sandy and detritic bottoms interspersed with extensive patches of *Posidonia oceanica* and *Cymodocea nodosa* which forms a narrow belt following the coast (García-Raso *et al.,* 1992). *P. oceanica* beds are the commonest seagrass type at the studied locality, extending down to 30 m depth, while *C. nodosa* occurs at shallower areas over fine sand bottoms in smaller patches down to 10 m depth (Luque *et al.,* 2004).

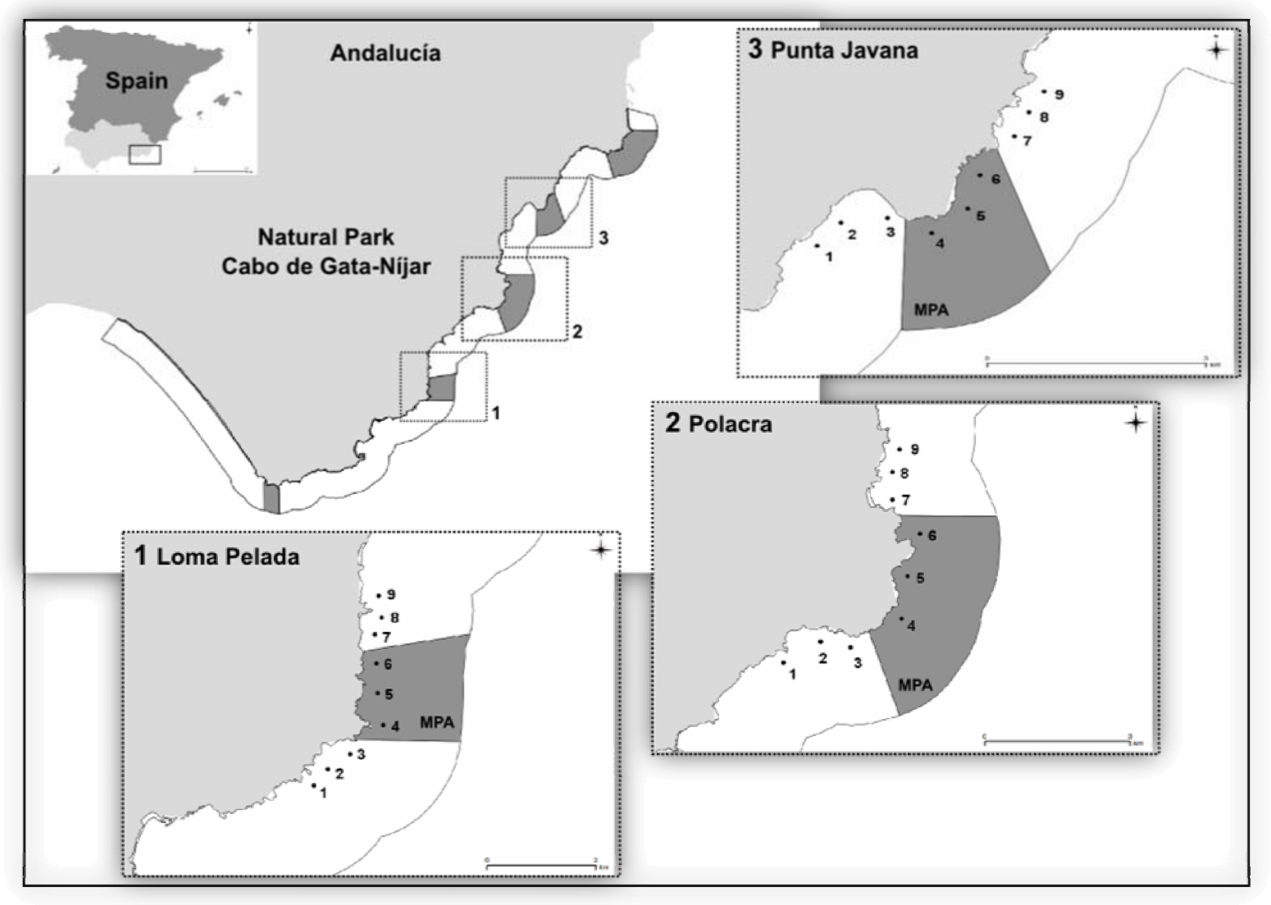

Figure 4.1 Map of Cabo de Gata-Níjar Natural Park, Southeast Spain, showing the spatial delimitation of the three marine reserves studied, Loma Pelada, Polacra and Punta Javana, and the location of light traps within each zone.

2.2 Sampling design

During the settlement peak months, namely between July and September 2011, the abundance of post-larvae, juvenile and adult fish was surveyed three random times in three locations situated thousands of meters apart: Loma Pelada (LP), La Polacra (PO) and Punta Javana (PJ). These locations were selected to represent a spatial replication of the “Reserve effect”. At each location, 3 zones were delimited: the no-take-zone (Protected) and 2 zones where fishing is permitted (Unprotected), located upstream (North) and downstream (South) the MPA boundaries (Fig. 4.1). In each zone, 3 sites separated by hundreds of meters were randomly chosen each time.

2.3 Collection of fish post-larvae

Light-traps (CARE®, Ecocean, Montpellier, France) were used to sample the post-larval pool reaching the coastal zone. Post-larvae is defined here as a synonym of late-stage or competent larvae, which is the transitional form from the pelagic to the benthic realm (Leis and McCormick, 2002; McCormick *et al.,* 2002). One light-trap per site was installed at sunset and retrieved at sunrise during two consecutive nights each campaign, resulting in 54 replicate light-trap samples per time period. Light-traps were deployed on surface using a buoy moored at 10-40 m depth, depending on how close sampling site was to the coast, maintaining a minimum distance of 300 m apart from each other to guarantee independence. Since the highest larval abundances occurs on days near the new moon (Milicich, 1994; Spounagle and Cowen, 1996; Wilson *et al.,* 2001), to optimize larval catches field campaigns were concentrated within the 3 days after and before the new moon period. Due to the loss of most light-traps at the beginning of the third campaign, the data issued from the last time period (September) were not included in the analysis.

Samples were recovered each morning, labelled and preserved in alcohol 70% until arriving to the laboratory. Each trap sample was then sorted using a light microscope to the lowest identification level using the appropriate bibliography (e.g., LoBianco, 1931; Arias and Drake, 1990; Ré, 1999; Fahay, 2007; Victor, 2012). Some individuals from the genus *Atherina, Bothus, Trachurus, Lepadogaster, Symphodus, Diplodus* and *Serranus* were not identified at the species level due to taxonomic difficulties. For the same reason, blennids and mugilids individuals were grouped at family level.

2.4 Juvenile and adult censuses

In each site within each combination of time, location and zone, we counted visually by SCUBA diving all non-cryptic recruits on five 10×2-m replicate transects set out haphazardly and marked with a plastic measuring tape deployed by the diver while advancing slowly at a constant speed. As recruit we considered all juvenile individuals from a minimum size of 10 mm to a size in which they are not yet sexually mature. A depth range of 0-5 m was covered, in such a way that the whole range was evenly explored in each site each time period. Each observation was assigned to one of eleven abundance classes, whose limits coincide with the terms of a base-1.6 geometric series, constructed considering the most frequent figures of observed abundance of juveniles (Harmelin-Vivien *et al.,* 1985; García-Charton *et al.,* 2000a), namely 1, 2, 3, 4, 5-7, 8-10, 11-30, 31-50, 51-70, 71-100, >100. Geometric means of each fish abundance class were used for the calculation of abundances. The size of each observed fish was visually estimated to the nearest cm.

For estimating the abundance of adults, three 50×5-m replicate transects were visually surveyed in each site and each time period; transects were located at a depth range of 10-15 m. Each observation was assigned to one of nine abundance classes following Harmelin (1987) and García-Charton *et al.* (2000a), namely 1, 2-5, 6-10, 11-30, 31-50, 51-100, 101-200, 201-500, >500. Analogously to the case of juvenile data, the geometric mean of each class was considered for further calculations. Individual sizes were estimated in classes of 2 cm; to minimize bias in size estimation, 4 well trained and intercalibrated observers were responsible for all censuses, which were done between 10:00 and 15:00 h GMT, when water conditions (turbidity and swell) were optimal (Harmelin-Vivien *et al.,* 1985).

After counting fish individuals, each observer covered the transect length in the opposite direction to measure habitat heterogeneity, defined as percentage cover of the entire transect by different substrate types embedded in the rocky matrix; this measure was made by visually estimating the percent cover by sand, gravel or/and *Posidonia* meadows at 2-m and 5-m intervals along the juveniles and adults transects, respectively. In addition, habitat complexity was estimated using a visual scale of increasing complexity from 1 to 4, adapted from Gratwicke and Speight (2005) for the recruit counts, and as the number of rocky boulders classified by size (small: <50 cm; medium: 50-100 cm; and large: >1 m) following García-Charton and Pérez Ruzafa (1998, 2001), in the case of adult transects.

2.5 Data analysis

Due to significant variation in the response of different fish species to protection (Claudet *et al.*, 2010), species were categorized into 4 groups with respect to their economic importance and spatial habitat occupation based on Harmelin (1987) and Claudet *et al.* (2010). Their relative importance to fisheries was defined as commercial or non commercial, and spatial categories were defined as pelagic, which includes categories 1 and 2 of Harmelin (1987), and demersal, including categories 3 to 6 (Table 4.1).

The effect of protection on fish abundance (log-transformed for coherence with the logarithmic scale of the abundance classes used when visually censusing fishes) of the different life stages (total, by species and by commercial and spatial categories) and on biomass of adult categories only was evaluated using an asymmetrical analysis of variance (ANOVA) based on Glasby (1997). A beyond-ACI design was constructed, starting from the original design which included the factors Location (L, random, 3 levels, including the 3 no-take zones – LP, PO, and PJ), and Zone (Z, 3 levels, random and nested in L). Due to the above mentioned loss of sampling devices, and also in order to homogenize the analysis for the three life stages surveyed, the time periods and the sites were ruled out as experimental factors, and thus all replicates were included within each zone, all times confounded. The asymmetrical analysis included the contrast between protected and unprotected zones (factor Protection P, fixed, 2 levels) and the interaction between this contrast and factor Location (factor L×P). Finally, there is a term comparing the two control zones that are nested within each location; only the control zones are compared in this way because the no-take zones are not replicated within each location. For a complete explanation of how to construct asymmetrical designs and the appropriate F-tests, see Glasby (1997). Prior to analyses, homogeneity of variance was checked using Cochran’s test; in the few cases where variances were not homogeneous we performed the analyses anyway, since analysis of variance is quite robust to departures from their assumptions (Underwood, 1997). In these cases, tests have to be taken cautiously when the significance of the effect is $0.01 < P < 0.05$. All analyses were done using "GAD" package in R software. Furthermore, *post-hoc* planned comparisons was used to evaluate the differences between Protection levels means (Control vs Reserve) when interaction term L×P was significant (see Glasby, 1997).

To visualize multivariate patterns among locations (protected or not) and zones within locations, distinguishing among post-larvae, juvenile and adult assemblages (total, and grouped by commercial interest and spatial position in the water column), non-metric multidimensional scaling (nMDS) ordinations were obtained from Bray-Curtis dissimilarity matrices calculated from log transformed data, using PRIMER v.6 package. Stress values were shown for each nMDS plot to indicate the goodness of representation, knowing that empirical evidence and simulation studies have shown that a stress value of <0.2 gives an ordination sufficient to be interpreted in ecological terms (Clarke and Warwick, 2001). Differences among Location and Protection groups were tested using a 2-way ANOSIM. In addition, we conducted a SIMPER analysis only for ANOSIM significant terms (Location and Protection) to search for the species which contributed, and in what proportion, to the differences encountered.

Additionally, in order to evaluate the existence of a gradient of abundance and biomass (in the case of adults) across MPA limits to both northwards and southwards directions, data from species categories in which a significant effect of protection was observed were tested using generalized additive modelling (GAM; Hastie and Tibshirani, 1990). GAMs were used due to expectancy of non-linear response of species abundance and predictor variables, as they performed better fitting over generalized linear models (Dixon *et al.,* 1999). Models were constructed using a smooth cubic line and default degrees of freedom using "gam" package in R. We used a Gaussian error distribution which is more appropriate when dealing with averaged measures than others (i.e. Gaussian, binomial).

Finally, linear regressions were used to test the mutual relationship between the intensity of post-larval incoming, recruitment and adult population abundance. For doing that, species which occurred in at least 2 consecutive life stages (larvae and juveniles or juveniles and adults) were included in the analyses. Selected species abundance, for both juvenile and adult life stage, were pooled among transects within each site to give an average density per 10 m^{-2}. Abundance data of each light-trap was used instead as they are not replicated at the finer spatial scale (within sites). Additionally, as data from light-traps were restricted to the first 2 months, only censuses from the two first time periods (July and August) were used in order to control the influence of distinct larval pools not measured on the subsequent time period (September).

3. Results

3.1 Fish assemblage

A total of 74 different *taxa* were recorded among the 3 life stages surveyed (Table 4.1). Light-traps captured 6,078 individuals belonging to 45 *taxa.* Blennidae (34% of total number of post-larval individuals captured), *Oblada melanura* (21%), *Boops boops* (18%) and *Chromis chromis* (10%) were the most representative *taxa* accounting for more than 80% of total post-larval catch. A total of 16,351 juvenile individuals of 34 species were identified in visual censuses, from which *C. chromis* (38% of total abundance), *Sarpa salpa* (11%) and *O. melanura* (7%) accounted for 56% of all observed fishes. Finally, 17,868 adult individuals from 46 species were censused, the species *S. salpa* (17%), *C. chromis* (16%), *B. boops* (10%) and *Diplodus vulgaris* (9%) accounting for 52% of total abundance. About 77% of all *taxa* were categorized as demersal species, and ~60% belonged to commercially important species. Twenty three species such as *Anguilla anguilla, Arnoglossus laterna, Trachinotus ovatus, Trachurus trachurus, Ariosoma balearicum, Dactilopterus volitans, Auxis rochei, Pagellus bogaraveo, Spondyliosoma cantharus, Syngnathus thyphle* among others were exclusively observed in the light-trap catches, while *Lichia amnia* was censused only as juveniles, and 12 species namely *Seriola dumerilli, Dasyatis pastinaca, Muraena helena, Parapristipoma octolineatum, Epinephelus costae, Symphodus melanocercus, Dicentrachus labrax,*

Table 4.1 Relative and total abundance values per life stage - larvae, juvenile and adult, and spatial and economic categories of species registered at Cabo de Gata Natural Park. D-demersal, P-pelagic, C-commercial, N-no-commercial.

Family	Species	Spatial	Econ.	Larvae	%	Juvenile	%	Adult	%
Anguilidae	*Anguilla anguilla*	D	C	1	0.02				
Apogonidae	*Apogon imberbis*	D	N			230	1.4	671	3.8
Atherinidae	*Atherina boyeri*	P	C	4	0.07				
	Atherina spp.	P	C	18	0.3	936	5.7		
Blennidae	Blenniidae	D	N	2078	34.2				
Bothidae	*Arnoglossus laterna*	D	N	3	0.05				
	Bothus spp.	D	N	3	0.05	1	0.01	1	0.01
Carangidae	*Lichia amnia*	P	C			9	0.06		
	Seriola dumerilli	P	C					20	0.1
	Trachinotus ovatus	P	C	183	3.0				
	Trachurus mediterraneus	P	C	5	0.08				
	Trachurus spp.	P	C	14	0.2				
	Trachurus trachurus	P	C	36	0.6				
Centracanthidae	*Spicara maena*	P	N					143	0.8
	Spicara smaris	P	N					98	0.5
Clinidae	*Clinitrachus argentatus*	D	N	36	0.6				
Clupeidae	*Sardina pilchardus*	P	C	149	2.4				
Congridae	*Ariosoma balearicum*	D	N	41	0.7				
Dactylopteridae	*Dactylopterus volitans*	D	N	4	0.07				
Dasyatidae	*Dasyatis pastinaca*	D	C					1	0.01
Engraulidae	*Engraulis encrasicolus*	P	C	21	0.3				
Gobiesocidae	*Lepadogaster* spp.	D	N	4	0.07				
Gobiidae	*Gobius paganellus*	D	N	1	0.02				
Haemulidae	*Parapristipoma octolineatum*	D	C					72	0.4
Labridae	*Coris julis*	D	N	55	0.9	658	4.02	1299	7.3
	Labrus merula	D	C			1	0.01	53	0.3
	Labrus viridis	D	C			1	0.01	24	0.1
	Symphodus cinereus	D	N			1	0.01	14	0.08
	Symphodus doderleine	D	N			4	0.02	169	0.9
	Symphodus mediterraneus	D	N			55	0.3	305	1.7
	Symphodus melanoceros	D	N					5	0.03
	Symphodus ocellatus	D	N			461	2.8	508	2.8
	Symphodus roissali	D	N	5	0.08	202	1.2	285	1.6
	Symphodus rostratus	D	N					157	0.9

Family	Species	Spatial	Econ.	Larvae	%	Juvenile	%	Adult	%
Labridae	*Symphodus* spp.	D	N	4	0.07				
	Symphodus tinca	D	N	1	0.02	729	4.5	1038	5.8
	Thalasoma pavo	D	N	6	0.1	585	3.6	78	0.4
Moronidae	*Dicentrachus labrax*	D	C					7	0.04
	Dicentrachus punctatus	D	N			259	1.6	18	0.1
Mugilidae	Mugilidae	P	C	7	0.1	195	1.2	100	0.6
Mullidae	*Mullus barbatus*	D	C	15	0.2				
	Mullus surmuletus	D	C	16	0.3	58	0.3	246	1.4
Muraenidae	*Muraena helena*	D	C					10	0.06
Myliobatidae	*Myliobatis aquila*	D	N					2	0.01
Pomacentridae	*Chromis chromis*	P	N	655	10.8	6213	38.0	2958	16.5
Sciaenidae	*Sciaena umbra*	D	C			2	0.01	254	1.4
Scombridae	*Auxis rochei*	P	C	1	0.02				
Scorpaenidae	*Scorpaena notata*	D	C					2	0.01
	Scorpaena porcus	D	C	4	0.07			1	0.01
	Scorpaena scrofa	D	C	7	0.1				
Serranidae	*Epinephelus costae*	D	C					1	0.01
	Epinephelus marginatus	D	C	1	0.02	9	0.06	17	0.1
	Serranus cabrilla	D	C	3	0.05	5	0.03	84	0.5
	Serranus hepatus	D	C	3	0.05				
	Serranus scriba	D	C			149	0.9	309	1.7
	Serranus spp.	D	C	1	0.02				
Sparidae	*Boops boops*	P	N	1136	18.7	779	4.8	1778	9.9
	Dentex dentex	D	C			52	0.3	45	0.2
	Diplodus annularis	D	C	186	3.1	22	0.1	567	3.2
	Diplodus cervinus	D	C			7	0.04	57	0.3
	Diplodus puntazzo	D	C			336	2.0	105	0.6
	Diplodus spp.	D	C	16	0.3	533	3.3	623	3.5
	Diplodus vulgaris	D	C	1	0.02	764	4.7	1672	9.4
	Litognathus mormyrus	D	C			7	0.04	7	0.04
	Oblada melanura	P	N	1273	20.9	1164	7.1	793	4.4
	Pagellus acarne	D	C	1	0.02				
	Pagellus bogaraveo	D	C	2	0.03				
	Pagrus pagrus	D	C	35	0.6			5	0.03
	Sarpa salpa	D	N			1909	11.7	3095	17.3
	Sparus aurata	D	C	1	0.02	9	0.06	24	0.1
	Spondyliosoma cantharus	D	C	39	0.6				

Family	Species	Spatial	Econ.	Larvae	%	Juvenile	%	Adult	%
Sphyraenidae	*Sphyraena viridensis*	D	C			6	0.04	147	0.8
Syngnathidae	*Syngnathus thyphle*	D	N	2	0.03				
Trachinidae	*Echiichthys vipera*	D	N	1	0.02				
	Total abundance			6078		16351		17868	
	Number of species/*taxa*			45		34		46	

Spicara maena, Myliobatis aquila, Scorpaena notata, Symphodus rostratus and *S. smaris* were only seen as adults (Table 4.1). A total of 14 species were observed in the three life history stages surveyed, they were *Bothus* spp., *Coris julis, Symphodus roissali, S. tinca, Thalassoma pavo, Chromis chromis,* Mugilidae*, Epinephelus marginatus, E. costae, Serranus cabrilla, Diplodus annularis, Boops boops, D. vulgaris, O. melamura* and *Sparus arata* (Table 4.1).

3.2 Effect of protection

The effect of protection measures was evident for post-larval and adult stages, but not for juveniles (Table 4.2, Fig. 4.2). Regarding post-larvae, total post-larval richness and the abundance of post-larvae of both pelagic and demersal commercial species responded significantly to the effect of the interaction term L×P, indicating that the effect of protection was not homogeneous among locations. Post-hoc comparisons indicate that post-larval species richness was found to be higher in unprotected zones except at PO location, where no differences were found. For its part, abundance of post-larvae of pelagic and demersal commercial species was higher in unprotected zones in all locations (Table 4.2, Fig. 4.2). In the case of adults, only the abundance and biomass of the demersal and commercial species responded significantly to protection (Table 2), with higher mean values inside protected sites in all locations, while non-commercial species presenting mean higher abundances outside reserves (Fig. 4.2). Additionally, significant variability among control sites within locations [C(L)] was found for demersal juveniles, demersal commercial adults and all adult species, both for abundance, biomass (adults' case) and number of species (Table 4.2, Fig. 4.2).

Table 4.2 Results of ANOVA on total abundance (N), species richness (S) and biomass[1] (B) of fish life stages – larvae (lar), juvenile (juv) and adult (ad) - for random factors Location (L), Protection (P: contrast of Protection vs Controls levels, P vs Cs), and the orthogonal and nested interactions LxP and C(L). [1] Biomass was only calculated for adult categories. Df-degrees of freedom, F-Fstatistic, *P*-significance: *** - P<0.001; ** - P<0.01; * - P<0.05; ns - not significant. Codes: lar – larvae, juv – juvenile, ad – adult, pel - pelagic, dem - demersal, com - commercial and nc - non-commercial.

		Larvae				**Lar pel com**				**Lar pel nc**				**Lar dem com**				**Lar dem nc**			
		N		**S**		**N**		**S**		**N**		**S**		**N**		**S**		**N**		**S**	
	Df	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Location	2	0.53	ns	5.01	ns	103.27	**	4.42	*	0.53	ns	0.29	ns	22.11	*	4.40	ns	8.52	ns	1.01	ns
Protect P vs C	1	6.25	ns	2.26	ns	0.82	ns	30.03	ns	3.33	ns	0.43	ns	0.18	ns	0.00	ns	2.59	ns	5.55	ns
LxP	2	0.04	ns	10.02	*	233.71	***	0.85	ns	0.97	ns	3.00	ns	25.50	*	1.85	ns	0.45	ns	1.06	ns
C(L)	3	4.65	*	0.20	ns	0.01	ns	0.19	ns	1.45	ns	2.63	ns	0.18	ns	0.61	ns	0.84	ns	0.48	ns
Res	18																				

		Juveniles				**Juv pel com**				**Juv pel nc**				**Juv dem com**				**Juv dem nc**			
		N		**S**		**N**		**S**		**N**		**S**		**N**		**S**		**N**		**S**	
	Df	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Location	2	1.24	ns	0.12	ns	9.50	ns	4.27	ns	0.69	ns	3.24	ns	1.08	ns	0.27	ns	0.11	ns	0.07	ns
Protect P vs C	1	16.35	ns	0.17	ns	2.23	ns	1.69	ns	8.03	ns	15.55	ns	0.00	ns	5.43	ns	1.51	ns	0.25	ns
LxP	2	0.42	ns	0.31	ns	5.68	ns	2.49	ns	0.24	ns	0.26	ns	1.00	ns	0.18	ns	0.57	ns	0.76	ns
C(L)	3	2.81	*	16.30	***	0.46	ns	1.12	ns	1.93	ns	1.34	ns	29.10	***	6.95	***	11.27	***	13.65	***
Res	126																				

		Adults						**Ad pel com**						**Ad pel nc**						**Ad dem com**						**Ad dem nc**					
		N		**S**		**B**		**N**		**S**		**B**		**N**		**S**		**B**		**N**		**S**		**B**		**N**		**S**		**B**	
	Df	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Location	2	0.08	ns	0.27	ns	0.91	ns	0.26	ns	3.44	ns	0.37	ns	3.86	ns	0.21	ns	5.20	ns	0.38	ns	0.34	ns	1.21	ns	0.46	ns	0.25	ns	1.55	ns
Protect P vs C	1	9.36	ns	25.17	ns	17.13	ns*	6.43	ns	4.40	ns	14.53	ns*	0.06	ns	0.39	ns	0.28	ns	30.13	*	61.28	*	36.46	*	2.74	ns	2.55	ns	2.14	ns
LxP	2	0.52	ns	0.13	ns	0.10	ns	2.85	ns	0.72	ns	0.52	ns	1.14	ns	1.82	ns	3.20	ns	0.15	ns	0.09	ns	0.18	ns	0.12	ns	0.30	ns	0.25	ns
C(L)	3	1.52	ns	7.46	***	2.99	*	0.58	ns	0.80	ns	1.24	ns	0.48	ns	1.26	ns	0.25	ns	4.60	**	6.07	**	3.89	*	2.39	ns	5.22	**	1.73	ns
Res	72																														

Moreover, juveniles of pelagic non-commercial species were more abundant inside no-take areas, commercial species varied greatly at location scales, and demersal non-commercial at zone scale, being always less abundant at northern control zone (Fig. 4.2).

Plots of nMDS analyses generally did not reveal differences among locations and protection levels for abundance of all species considered together or separated into commercial and spatial categories (Fig. 4.3); exceptions are those of adults of demersal non-commercial species, which responded significantly to protection factor, and post-larvae of demersal commercial and non-commercial species, as well as in the case of the whole post-larval assemblage, for which a significant differences among locations was detected, as detected by ANOSIM (Fig. 4.3, Table 4.3). SIMPER procedure (Table 4.4) revealed that differences in the adult assemblage between protected and unprotected sites was caused by the distinct occurrence of *S. salpa, A. imberbis, S. tinca* and *S. mediterraneus*, in this order of importance. For their part, differential abundances among locations of post-larvae of *B. boops, C. chromis, O. melanura,* Blennidae and *T. ovatus* were responsible for the significant effect of this large-scale spatial factor. Analogously, the abundance of post-larvae of demersal commercial species as *D. annularis, S. cantharus, P. pagrus* and *Diplodus* spp., and non–commercial species such as Blenniidae, *C. julis* and *C. argentatus* were the main responsible for the multivariate differences found among locations (Table 4.4).

3.3 Gradients of abundance across no-take zones limits

Gradients across MPA boundaries were only investigated in adult demersal commercial species as they were the only ones in which abundance and biomass was significantly higher inside the protected areas (see above). Significant fit of GAMs were obtained for all locations regarding species abundance, in which a clear reduction with increasing distances from MPA core was observed, except for Loma Pelada reserve, where the southern control zone harboured densities as high as the corresponding no-take area (Fig. 4.4). Although the effect of protection to increase fish

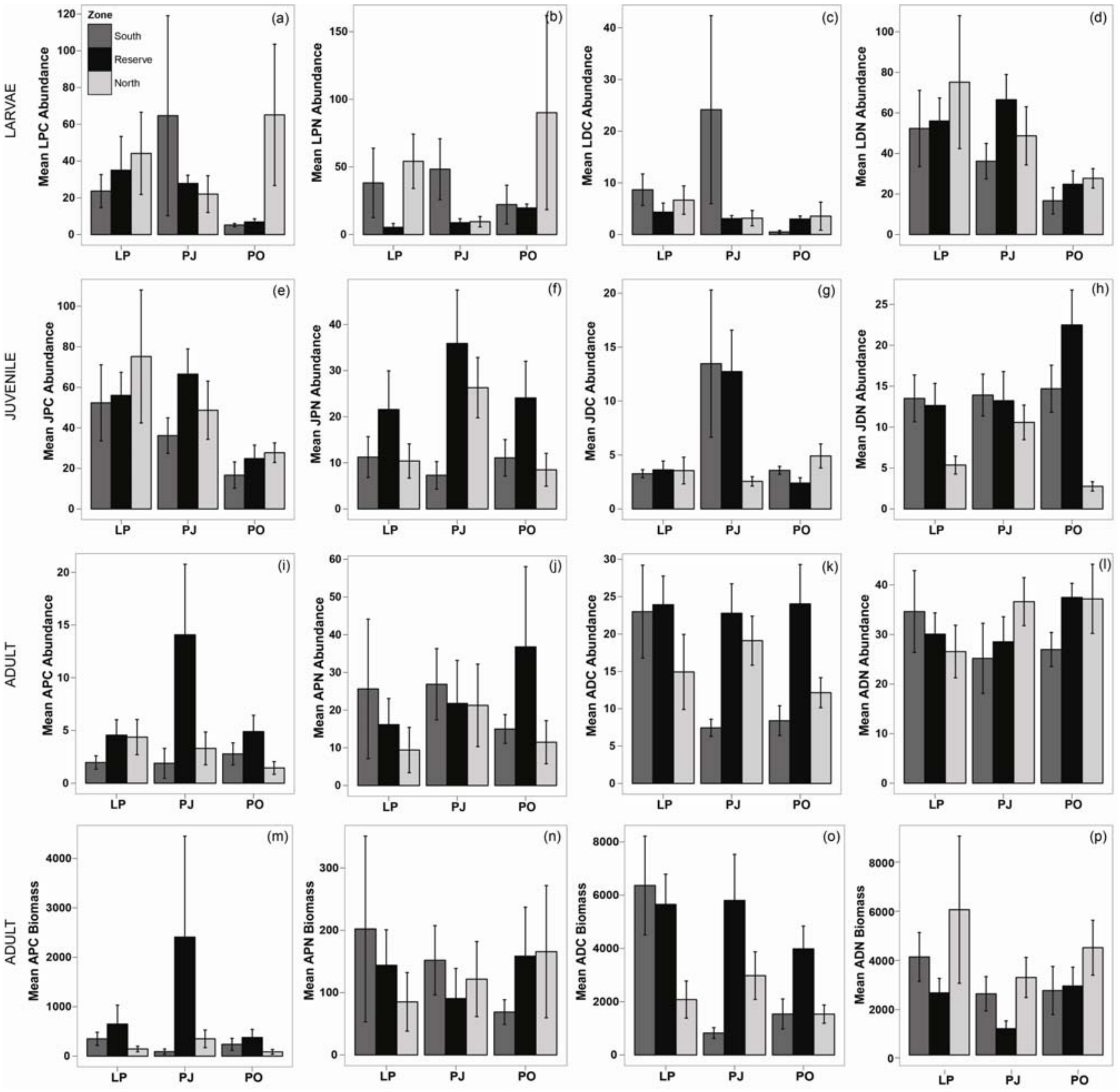

Figure 4.2 Mean abundance of larval (a-d), juvenile (e-h), adult (i-l) and biomass of adult (m-p) categories within zones inside reserves studied. LP-Loma Pelada, PJ-Punta Javana and PO-Polacra. Codes are composed by 3 digits, one for life stage: L-larvae, J-juvenile, A-adult; second for spatial category: P – pelagic, D – demersal; and third for economic importance: C – commercial, N – non-commercial.

abundance inside MPAs borders was supported by the description of gradients, it was not the case with biomass, for which a significant reduction was observed inside the limits of Loma Pelada reserve and increased beyond their boundaries. No significant differences were found for biomass values in other locations (Fig. 4.4). Determination coefficients of significant models were relatively

Table 4.3 ANOSIM results for main effects Location and Protectiont (Controls vs Protection) among species categories and life stages. R-Global R; Sig%-percentage of significance. Significant results are in bold (<5%). Codes as Table 2.

Source of variation	**Larvae**		**Lar pel com**		**Lar pel no com**		**Lar dem com**		**Lar dem no com**	
	R	**% Sig**	**R**	**% Sig**	**R**	**% Sig**	**R**	**% Sig**	**R**	**% Sig**
Location	0.288	**0.20**	0.109	10.5	0.081	15.1	0.44	**0.03**	0.207	**0.20**
Protection	-0.144	88.4	-0.23	98.7	0.00	47.5	0.195	5.3	-0.163	91.6
	Juvenile		**Juv pel com**		**Juv pel no com**		**Juv dem com**		**Juv dem no com**	
	R	**% Sig**	**R**	**% Sig**	**R**	**% Sig**	**R**	**% Sig**	**R**	**% Sig**
Location	0.031	30.3	0.109	10.4	-0.02	57	0.068	13.6	-0.006	49.2
Protection	0.012	44.2	0.138	12.4	0.08	23.1	-0.029	57.8	-0.078	73.5
	Adult		**Adu pel com**		**Adu pel no com**		**Adu dem com**		**Adu dem no com**	
	R	**% Sig**	**R**	**% Sig**	**R**	**% Sig**	**R**	**% Sig**	**R**	**% Sig**
Location	0.07	17.8	-0.029	58.2	0.09	11.8	0.04	28.1	0.01	40.8
Protection	0.191	6.2	0.128	14.1	-0.016	52.2	0.171	8.5	0.327	**0.50**

low, ranging from 0.18 – 0.24 (Table 4.5).

3.4 Relationship between life stages

Among all combination of species tested, significant linear relationships were observed only between post-larval supply and the abundance of juveniles of Mugilidae and only inside marine reserves ($R^2 = 0.41$, $p < 0.05$), and between the abundance of juveniles and adults of *C. julis* considering all locations together ($R^2 = 0.125$, $p < 0.05$).

4. Discussion

A growing number of studies highlight the benefits of MPAs on adult population by evidencing increases in abundance and mean size as well as density dependent effects (see García-Charton *et al.*, 2008 for a review). Since fecundity is exponentially related to fish size, increased production of eggs and larvae within MPAs should result in enhanced recruitment both inside and outside protected areas. However, few empirical studies have addressed recruitment success in MPAs

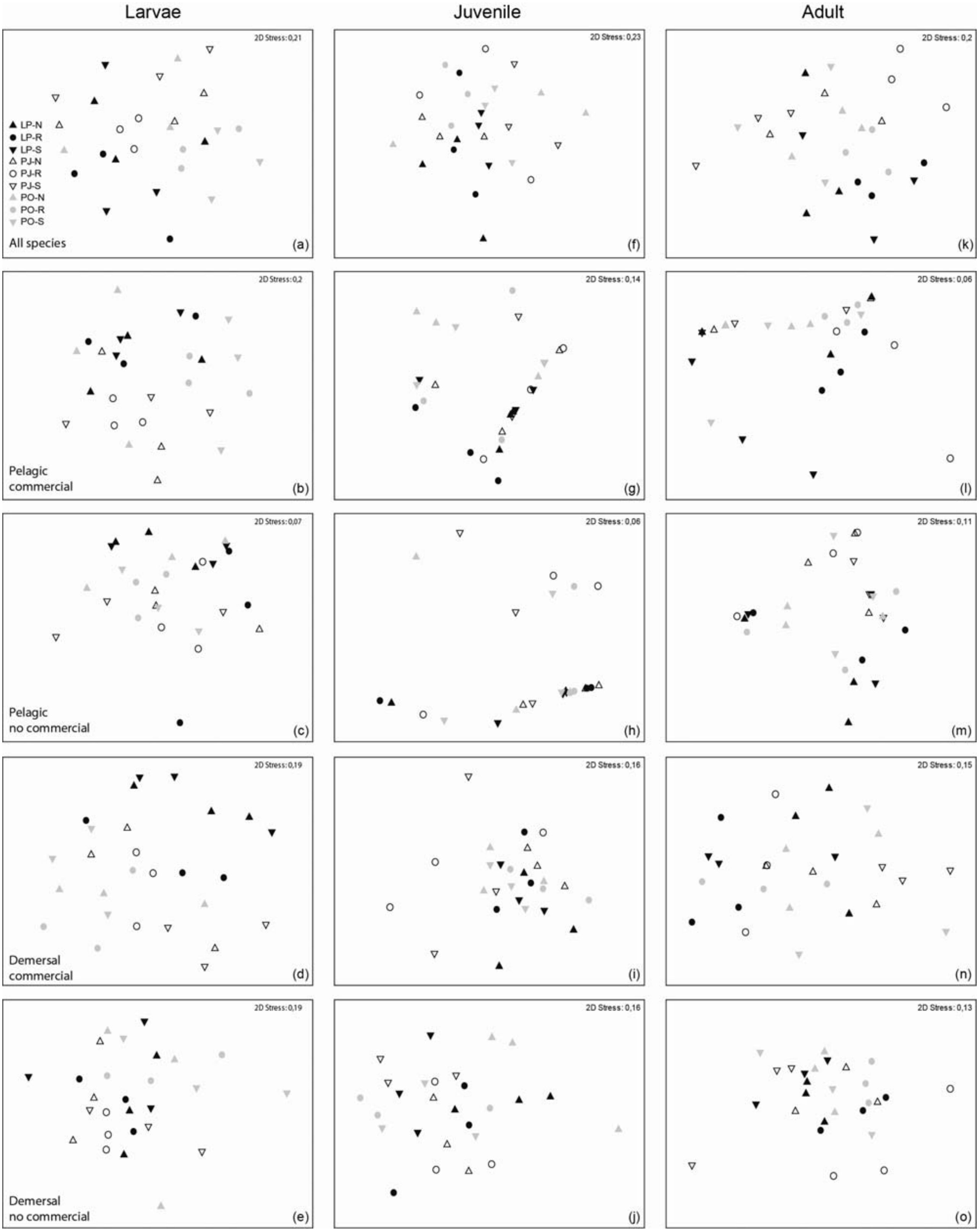

Figure 4.3 Multi-dimensional scaling (MDS) ordination plots of categorized larvae (a-e), juvenile (f-j) and adult (k-o) species across treatments and locations studied.

Table 4.4 Species contribution (in percentage) to dissimilarities between localities and treatments for significant response groups in ANOSIM analysis. Factor Location, 3 levels: LP – Loma Pelada, PJ – Punta Javana, PO – Polacra and factor Treatment, 2 levels: Controls and Reserve.

All larvae species	**LP (65.37%)**	**PJ (61.09%)**	**PO (56.06%)**	**LP vs PJ (43.61%)**	**LP vs PO (44.26%)**	**PJ vs PO (44.47%)**
Blenniidae	27.62	31.06	26.56		7.45	7.10
Chromis chromis	17.66		20.83	10.21	7.32	6.12
Oblada melanura	13.86	12.42		9.62	11.06	10.85
Boops boops		9.65	15.12	10.10	12.76	12.18
Trachinotus ovatus				8.15		8.18
Diplodus annularis				6.52		6.66
Spondyliosoma cantharus				6.35	6.70	
Sardina pilchardus					6.03	
Larvae of demersal commercial species	**LP (49.79%)**	**PJ (35.70%)**	**PO (14.47%)**	**LP vs PJ (73.57%)**	**LP vs PO (83.42%)**	**PJ vs PO (74.97%)**
Spondyliosoma cantharus	49.24			23.83	31.45	
Pagrus pagrus	28.89					15.09
Diplodus annularis		37.78	38.69	24.12	19.53	30.57
Diplodus spp.		26.78		12.82		18.93
Mullus barbatus			31.40			
Larvae of demersal no commercial species	**LP (76.25%)**	**PJ (75.47%)**	**PO (65.66%)**	**LP vs PJ (26.78%)**	**LP vs PO (32.07%)**	**PJ vs PO (33.95%)**
Blenniidae	76.67	85.08	73.35	20,53	33.62	29.97
Coris julis				18,74	18.58	15.75
Clinitrachus argentatus				18,59		
Ariosoma balearicum						18.09
Adult demersal no commercial species	**Controls (78.37%)**	**Protection (78.56%)**	**Controls vs Protection (25.48%)**			
Sarpa salpa	28.87	13.48	20,64			
Apogon imberbis			18,83			
Sympodus tinca	15.03	18.09	9,15			
Symphodus mediterraneus			8,83			
Coris julis	20.64	19.24				

(Planes *et al.*, 2000). On the other hand, sampling design is considered the major limiting factor to fully evaluate MPAs effect (García-Charton and Pérez-Ruzafa, 1999; Guidetti, 2002). Lack of proper replication, pseudo-replication, and uncontrolled confounding factors (such as habitat

Table 4.5 GAM results of the relationship adult demersal commercial species for each studied marine reserve – Loma Pelada, Polacra and Punta Javana. F-statistic (F), degrees of freedom (Df), coefficient of determination (R^2), *P* - significance at α=0.05, * - *P*<0.05, ** - 0.05<*P*<0.001, *** - *P*<0.001

Variable	Location	Df	F	*P*	Deviance	R^2
Abundance	Loma Pelada	76	3.772	*	11.624	0.18
	Polacra	76	6.373	***	9.913	0.23
	Punta Javana	76	3.108	*	11.547	0.19
Biomass	Loma Pelada	76	7.904	***	25.237	0.24
	Polacra	76	2.578	ns	32.942	0.19
	Punta Javana	76	1.938	ns	32.617	0.25

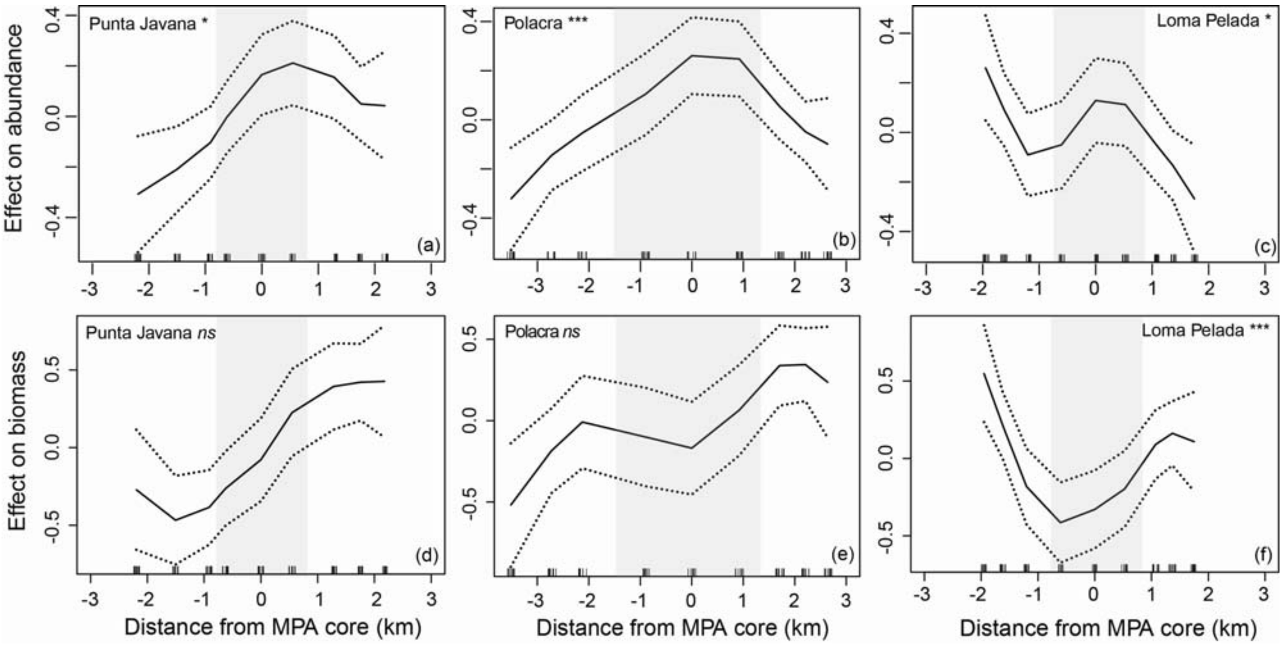

Figure 4.4 GAMs output for the relationship abundance and biomass of adult demersal commercial species to distance from MPA core of studied reserves; Punta Javana (a,d), Polacra (b,e) and Loma Pelada (c,f). Shaded area delimit no take area. * - significant, *ns* – not significant.

features) are the main obstacles to accurately assess the effects of protection (García-Charton *et al.,* 2000b; Russ *et al.,* 2004; Williams *et al.,* 2009; Goñi *et al.,* 2010).

In this work we assessed the effect of protection by using a beyond-ACI design (Underwood, 1991, 1992, 1993; Glasby, 1997) incorporating a replication of the effect of protection (in 3 distinct locations) and their contrast with multiple nearby controls, as well as fine-scale spatial replication, in order to control for habitat heterogeneity, thus allowing comparative and reliable estimates of MPA effects. Moreover, this is the first contribution to date that evaluates simultaneously the response of different life stages – post-larvae, juveniles and adults – to protection measures.

Only adults belonging to commercial and demersal categories were positively affected by protection. Higher abundance, biomass and species richness was found inside no-take zone in all reserves studied. In a recent meta-analytical study of European marine reserves, Claudet *et al.* (2010) found that the effect of protection on fish will depend firstly on their commercial value and to a lesser extent to their habitat preference. It is expected that target species will be the first to respond to a fishing ban by an abundance increase inside reserve limits, and the greater is the relationship of a species with the bottom, the greater and faster will be the response to protection; this was the case of demersal economically important species in this work.

On the other hand, a clear reduction in densities gradients with increase distances were consistent at both southern and northern boundaries for all reserves analysed, with the exception of Loma Pelada, in which similar abundances were detected within the no-take area and across the southern boundary. Spillover could be the result of random movements of individuals from MPAs to outside their borders (Kramer and Chapman, 1999) or by ontogenetic habitat shifts (Nagelkerken and van der Velde, 2002) or also by density-dependent interaction with other individuals (Abesamis and Russ, 2005), and resulting diffusive process (Pérez-Ruzafa *et al.*, 2008). In any case, the patchy nature of the marine environment can act as barriers to fish movement and will vary according to species and their relationship with habitat, thus influencing spillover effect (Rowley, 1994). Indeed, a significant variation among controls within locations was detected for adult species, mainly for demersal ones. This heterogeneous distribution could be related to habitat preferences and availability in the area. This habitat effect could be also related to adult biomass distribution across Loma Pelada reserve boundaries which was the opposite to one found for abundance. As species composing commercial and demersal category might present distinct habitat affinities, biomass gradients could reflect differential occupancy of habitat by different species with distinct sizes, masking biomass gradients. We could not reject the possibility that the increase in biomass observed outside this no-take area was mainly due to the accumulation of small-sized fish inside the no-take area, rather than an increase in mean size of protected population. Recently, the Ministry of Environment has strengthen the conditions of use inside the Cabo de Gata Natural Park due to cases of transgression of park rules as illegal spearfishing, professional fishing inside park limits, etc., as well as the enforcement of monitoring and *in situ* vigilance. It is well known that without enforcement positive effects of marine reserves could not be distinguished from fished areas (Guidetti *et al.,* 2008). Considering the selective effect of poaching on larger individuals is probable that their continued removal from inside no-take would have prevent population to attain bigger sizes, explaining why only small sized fish was found inside the reserve.

Another expected benefit of MPAs is the increase production of eggs and larvae by increasing abundance and age of target organisms and as consequence their fecundity (García-Charton *et al.,* 2008). The larval export is one of the major theoretical benefits of MPAs, and increased empirical data have been supporting their effectiveness in achieve this goal (Almany *et al.*, 2007; Cudney-Bueno *et al.,* 2009; Harrison *et al.,* 2012). Nevertheless, the impact of MPA in enhancing recruitment is still an open question.

The results of the present study indicate a negative correlation to protection on post-larval species. Two not-mutually exclusive hypotheses could be called on to explain such findings. With the increased evidence of self-recruiting mechanisms for a number of different species (see Jones *et al.*, 2009 for a review) higher abundance in control areas could be reflecting the enhancement of production of larvae by adjacent no-take zones. Additionally, unique configuration of local currents could also cause larval accumulation at control zones, thus contributing to larval retention (Jones *et al.,* 2009); however, identification of larval source is fundamental to support the hypothesis of self-replenishment from neighbouring no-take areas. Regardless of the origin of larvae, studied no-take zones were all located around protruding projections of the rocky coastline highly exposed to prevailing winds and currents (Fig. 5.1). For their part, adjacent control zones were usually situated in sheltered embayments; wave's refraction on protruding coast produce littoral currents which could have contributed to the concentration of post-larvae at these zones, specially the pelagic ones. Embayment areas are often dominated by seagrass patches formed mainly by *P. oceanica* (see Appendix III), which is considered the nursery habitat for a number of Mediterranean species (Francour, 1997) such as sparids (*D. annularis, S. cantharus, P. bogaraveo*), syngnatids (*S. thyphle, H. hippocampus*), labrids (*S. rostratus*), among others (Félix-Hackradt *et al., in prep.* Chapter III). As larvae are capable to perceive reef habitat from distances of at least 1 km (Leis *et al.,* 1996), they could have actively chosen unprotected areas to settle due to the fact that these zones may harbour optimal settlement habitats than protected zones. One might ask why only larvae of commercially important species were negatively correlated to protection, the answer could be because the species composing the non-commercial category are rocky reef-associated species (Blennids, most *Symphodus*, *C. chromis,* etc.) and this habitat is as well represented inside as outside reserve boundaries, and thus it is not directly related to protection.

Most studies regarding larval fish assemblages in MPAs focused to now on the description of distribution patterns of larval species at increasing distances from MPA core, evidencing the potential of MPAs as source of eggs and larvae (e.g. Vidal-Peñas *et al.,* 2001; Sabatés *et al.,* 2003; Crec'hriou *et al.,* 2010; López-Sanz *et al.,* 2011). The works of Valles *et al.* (2001) and Grorud-Colvert and Sponaugle (2009) go further by comparing the effect of larval supply abundance and diversity between protected and unprotected areas. Both studies were unable to find any differences between protected and unprotected areas regarding larval abundances, although the low spatial replication would have limited their conclusions. In our case, spatial configuration and habitat characteristics apparently accounted for a great part of the spatial distribution of fish early life-history stages, suggesting that only MPAs harbouring the maximum habitat diversity will be effective on protect initial stages by providing high quality habitats for settlement (Lindholm *et al.,* 2001).

Abundance of fish juveniles did not vary significantly between levels of protection. Instead, high spatial variability on juvenile abundance and species richness between control sites within locations was observed for demersal species and all species together. The heterogeneous distribution and low densities of many species may have contributed to reduce the power of statistical comparisons of individual species over the small spatial scale (~3 km) studied (Chapman and Kramer, 1999). It is known that variation in recruitment, survival, competition, and habitat structure all result in high spatial variability in fish density (Williams, 1991). For instance, studies which investigated the effect of protection on juveniles of *Diplodus* species have found high spatial and temporal variability at both small and large scales, but no differences in settlement intensity and mortality rates inside MPAs compared to unprotected areas was found (Macpherson *et al.,* 1997; Vigliola *et al.,* 1998).

In marine open populations, larval supply is spatially and temporally unpredictable, resulting in the uncoupling of the relative abundances of the incoming larvae and local recruitment (Caley *et al.,* 1996; Almany, 2003); however, growing evidences on local retention could alter this classical view (Jones *et al.,* 2009). A number of studies have shown that settling larvae show strong habitat preferences that determine new recruit distribution (Booth, 1992; Tolimieri, 1995; Booth and Wellington, 1998), yet these preferences could be further altered by differential mortality caused by residents-recruits interactions (Almany, 2003). In our case we were able to detect a direct relationship between Mugilid larval abundance and recruits inside marine reserves boundaries. As larvae are more abundant outside reserve limits, the attenuation of density dependent mortality relationship due to low densities inside (Macpherson *et al*., 1997; Shima, 1999, 2001; Johnson, 2007) would have contributed for the direct relation between both life stages.

Besides larval habitat preferences, juvenile habitat-mediated survival can be important for shaping fish community structure (Shulman and Ogden, 1987; Danilowicz, 1996; Almany, 2003). Much of the post-settlement mortality is attributed to predation (Doherty *et al.,* 2004) and may be strongly affected by local habitat features such as structural complexity (Hixon and Carr, 1997; Johnson, 2007; Juanes, 2007). In that sense, MPAs could not only enhance recruit mortality by the higher abundance of predators but also increase recruit survival probability by providing high-quality habitat (Planes *et al.,* 2000). The absence of recruit-adult relationships inside no-take areas depicted by our data may indicate a tendency of higher mortality on the mortality-survival trade-off, therefore decoupling recruit-adult relationship (Caley *et al.,* 1996), or even juvenile displacement such as ontogenetic shifts (Félix-Hackradt *et al., in prep.*, Chapter III), however further studies are needed to completely solve this question.

Although recruitment patterns can explain much of community structure (Victor, 1986; Ault and Johnson, 1998), to predict recruitment from local population size is not an easy task (Axenrot and Hansson, 2003). Long larval planktonic duration (Caley *et al.,* 1996), large distances between spawning and recruitment sites (Fogarty *et al.,* 1991), and larval mortality can wipe out the association between stock size and production of larvae (Houde, 1987; Sparholt, 1996). However these inconsistencies may be explained by a scale problem. As many of recruitment studies were done in coral reef environments at patch reefs, the high local variability may contribute to obscure such relationships (Caley *et al.,* 1996), therefore at larger scales stock-recruitment should exist (Hughes *et al.,* 2000). It was not the scope of this article to measure or discuss these relations, but instead to highlight the importance of fish early life-history stages to determine population dynamics, and therefore on the design of MPA networks, in order not only to protect adult stocks but also to maintain nursery habitats safe from degradation and promote population replenishment in an ecosystem-based perspective (Pikitch *et al.,* 2004).

5. Conclusions

In this work we assessed the effects of a network of no-take areas on different stages of Mediterranean reef fish life cycle – post-larvae, juveniles and adults - by using asymmetrical beyond-ACI approach. We found that not only adults but also post-larvae were affected by spatial protection measures. Adults of commercial and demersal species were more abundant inside reserve limits, as well as their biomass. However, as no clear biomass gradient was observed across reserve boundaries we conclude that higher abundance values were caused by an increase in small-sized fishes, which did not contribute with proportionally high biomass values. Lack of enforcement is suggested as the principal cause for these results, highlighting the need of surveillance to fulfil MPA objectives. On the other hand, post-larvae of commercially important species (both pelagic and demersal) were negatively related to MPA. Active selection of settlement preferred habitats as well as larval retention favoured by the geomorphological configuration of the coast (i.e. predominance of embayments outside no-take areas, and local currents produced by wave's refraction) has been proposed to explain such findings. High spatial variability in recruit distribution accounted for the lack of response to fishing protection measures. Relationships between fish life-history stages indicate that MPAs could change demographic rates and the way in which population are being regulated. By changing mortality rates, increasing habitat complexity, enhance predator number, and other community/ecosystem effects, protection can exert huge influence of population dynamics. Nevertheless, much more research has to be undertaken in order to understand the factors explaining larval spatial distribution, their reflection on year-class strength and therefore on adult stock. We strongly suggest the inclusion of knowledge on early life-history stages of reef fishes on the design of MPA networks, due to the direct implications of these phases on the successful achievement of MPA objectives.

Chapter V

Félix-Hackradt FC, Planes S, Hackradt CW, Pérez-Ruzafa A, García-Charton JA. Discordant patterns of genetic connectivity between two sympatric species, *Mullus barbatus* (Linnaeus, 1758) and *Mullus surmuletus* (Linnaeus, 1758), in South-western Mediterranean Sea.

Manuscript

Discordant patterns of genetic connectivity between two sympatric species, *Mullus barbatus* ***(Linnaeus, 1758) and*** *Mullus surmuletus* ***(Linnaeus, 1758), in South-western Mediterranean Sea***

Abstract

Scales of population connecvity on marine environments are important tools for managers to prevent endangered populations to be locally extinct as soon as a adjacent source of individuals could be identified. Mullets are among the most important commercial species in Mediterranean and they are heavily exploited by trawnling fishery. By using 10 microsaltellites marker we sampled *M. barbatus* and *M. surmulletus* across a small portion of Spanish coast (~400km) to determine at which scale genetic heterogeneity could be found. In addition we sampled larvae of both species from marine protected areas to test for larval exportation. Surprisingly, we found divergent patternsof genetic connectivity for both sympatric species, from complete homogeneity (*M. barbatus*) to high genetic structure (*M. surmuletus*). Habitat partitioning, geomorphological characteristics of continental shelf and oceanographic patterns are discussed as potential causes of patterns observed. Although we were unable to verify the population origin of larvae collected inside the marine reserves, we could detect higher heterozigosities values inside cabo de Palos marine reserve for *M. surmuletus* individuals, and on the vicinities, in the case of *M. barbatus*. Our results indicate that for two co-occurring species scales of connectivity may be completely discordant, and that life history traits such as PLD may not have huge effects on determining dispersal capability of a fish species.

Key-words: mullets; population connectivity; isolation by distance; geomorphological features; currents

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1. Introduction

Stripped red mullet (*Mullus surmuletus*, Linnaeus, 1758) and red mullet (*Mullus barbatus*, Linnaeus, 1758) are distributed in the Eastern Atlantic Sea from North Sea to Senegal as well as throughout the Mediterranean and Black seas (Fischer *et al*., 1987; Whitehead *et al*., 1986). Both are demersal fishes and share very similar biological characteristics (Mamuris *et al*., 1998b); both species spawn in spring, and their larvae and juveniles are pelagic. Notwithstanding, they differ in their habitat preference: while *M. surmuletus* is mostly found on rough bottoms, its congener, *M. barbatus*, prefers sandy or/and muddy habitats (Tserpes *et al*., 2002). Although both species occur at depths ranging from 10 to 300 m, Lombarte *et al*. (2000) described a preference of *M. sumuletus* to occur in narrow continental shelf areas with rocky or sandy bottoms, while *M. barbatus* shows a clear preference for areas with a wider continental shelf.

Both species are considered flagship species for coastal Mediterranean demersal fisheries (Longepierre *et al*., 2005) as they are among the most valuable species in commercial landings. They are caught mainly by trawling fleet (Tserpes *et al*., 2002) and thus subject to intense fishing pressure (Reñones *et al*., 1995). Trawl catches are composed mainly of individuals 15 to 20-cm in length which corresponds to 1st and 2nd year classes (Reñones *et al*., 1995), meaning that mullet stocks are composed mainly by young fish (Tserpes *et al*., 2002) and thus they are highly susceptible to overfishing.

Due to their economic importance, considerable knowledge of mullet biology has been acquired in the past years. Studies on growth and reproduction (Tursi *et al*., 1994; Reñones *et al*., 1995; Özbilgin *et al*., 2004), distribution patterns (Lombarte *et al*., 2000; Tserpes *et al*., 2002), feeding and ontogenetic behaviour (Vassilopoulou and Papaconstantinou, 1993; Labropoulou *et al*., 1997) and movement strategies (Machias *et al*., 1998) have contributed significantly to the knowledge of both species. Also, a number of comparative works between the two species added substantial information about genetic population structure and variability across Mediterranean localities. RAPD, allozymes and mtDNA showed that *M. surmuletus* population of Eastern Mediterranean were more heterogeneous and genetically structured than *M. barbatus* in accordance with geographic distances (Mamuris *et al*., 1998a, 2001). However, microsatellite data at a larger spatial scale reveal a slightly different pattern from those described above. Although a weak genetic structure was found among Eastern Mediterranean populations of *M. barbatus* (Garoia *et al*., 2004; Maggio *et al*., 2009), a metapopulation structure was observed for the same species at Mediterranean scale, and a reduction of gene flow between Mediterranean and Atlantic Ocean, indicating separate stocks units (Galarza *et al*., 2009). Therefore, a scale effect could be the cause of the distinct patterns observed. Analogously, allozyme analysis for the white seabream, *Diplodus sargus* in Western Mediterranean Sea showed genetically heterogeneous structure within spatial a scale of 10^2 to 10^3 km (González-Wangüemert *et al*., 2004), but at larger scales, mtDNA and nuclear markers were not able to find any differences for Atlantic and Mediterranean white seabream populations (González-Wangüemert *et al*., 2010).

Connectivity between populations is achieved both by adult movements and/or larval dispersal. Due to limitations of adult mobility, often observed in benthic species, habitat availability and predation risk, it is assumed that the greater contribution to genetic exchanges comes from pelagic stages (Schunter *et al*., 2011). Spawning season of both mullets occurs in spring (Tursi *et al*., 1994; Reñones *et al*., 1995) at deep sites (70-150 m depth, Machias *et al*., 1998). Eggs and larvae are pelagic (Sabatés, 1990) and larvae can remain 25-35 days in the water column until finding a suitable habitat to settle (Macpherson and Raventos, 2006), all constituting important sources of genetic homogenisation. Nevertheless, while connectivity through larval dispersal is an assumption made by most genetic studies, no studies have assessed to date the genetic variability of larval individuals and the degree to which larval patterns match those of the species adult populations.

Here we investigate the fine-scale genetic structure of *Mullus surmuletus* and *M. barbatus* populations in the Western Mediterranean Sea by using 10 microsatelittes markers. Also, we examined the genetic variation of larval individuals and the most probable population source and discuss the possible causes of the patterns found.

2. Material and Methods

2.1 Sample collection

During the period of 2010 to 2012, a total of 416 adult individuals of striped red mullet, *Mullus surmuletus* were collected at 13 locations encompassing a geographic range of about 400 km of Spanish continental Mediterranean coast (Fig. 5.1, Table 5.1). Mean distance between localities was 50 km. Also, a total of 213 post-larval individuals were collected inside the limits of two Marine Reserves: Cabo de Palos-Islas Hormigas Marine Reserve and Natural Park of Cabo de Gata-Nijar (Fig. 5.1, Table 5.1). Larvae from Cabo de Palos were collected during settlement periods of 2010 and 2011, while those of Cabo de Gata were collected only during 2011. Adult specimens were obtained directly from fishing landings of local artisanal or/and trawling gears, and all post-larvae were collected using light-traps (CARE®, Ecocean). All fishes were measured before genetic samples were collected for analysis.

2.2 Extraction, amplification and scoring

DNA extractions were done from the anal fin (in the case of adults) and muscle tissue (for post-larvae), which were dissolved using lysis buffer solution with proteinase K, and total genomic DNA was isolated by protein precipitation and final precipitation with ethanol (Sambrook *et al.*, 1989). Ten dinucleotid microsatellite of *M. surmuletus* describe by Galarza *et al.* (2007) were amplified by polymerase chain reaction (PCR). PCRs were performed in 10-µl total volume, which included 50 ng of DNA, 2 mM of $MgCl_2$, 0.2 µm of each primer, 0.3 mM dNTP's, 1× reaction buffer [75 mm Tris-HCl, 20 mm $(NH_4)2SO_4$] and 0.75 U Taq polymerase (BIOTAQ). Reaction conditions were as follows: an initial denaturation step of 5 min at 95 °C, 30 cycles consisting of 30 s at 92 °C for denaturation, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s followed by a final extension at 72 ºC by 30 s. A 2-µl sample of each PCR product was run on 1.5% agarose gel stained with safeDNA® before being viewed under UV light. Amplified products were resolved on an ABI Prism 3130 automated genetic analyser (Applied Biosystems). Allele scoring was done using GeneMapper v. 3.5 software (Applied Biosystems, Foster City, California).

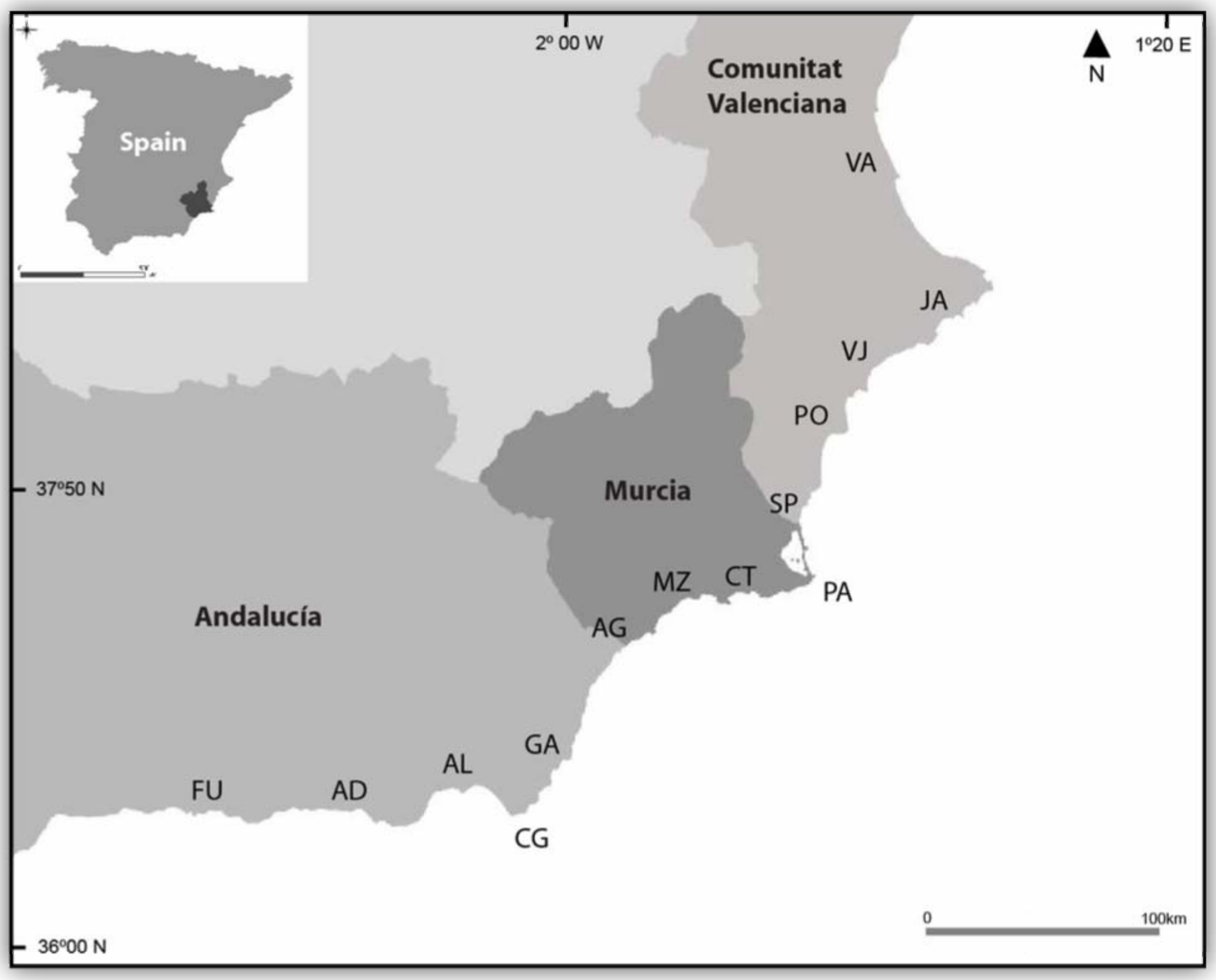

Figure 5.1. Sampling localities at Western Mediterranean Sea. VA: Valencia, JA: Javea,VJ: Villajoyosa, PO: Santa Pola, SP: San Pedro, PA: Cape of Palos MPA, CT: Cartagena, MZ: Mazarrón, AG: Águilas, GA: Garrucha, AL: Almeria, CG: Cape of Gata MPA, AD: Adra and FU: Fuengirola.

Preliminary results indicate that both *Mullus* species were represented in adult samples. To confirm it, 10 individuals of each supposed species were analysed using mitochondrial DNA using

COI-1 universal primers (FF2d, FR1d). Extraction was done as described before. PCR were performed in 25-µl total volume, which included 50ng of DNA, 1.25 mM of $MgCl_2$, 0.16 µm of each primer, 0.3 mM dNTP's, 1× reaction buffer [75 mm Tris-HCl, 20 mm $(NH_4)2SO_4$] and 0.75 U Taq polymerase (BIOTAQ). Reaction conditions were as follows: an initial denaturation step of 4 min at 94 °C, 35 cycles consisting of 25 s at 94 °C for denaturation, annealing at 52 °C for 25 s, and extension at 72 °C for 60 s followed by a final extension at 72 ºC by 60 s. A 2-µl sample of each PCR product was run on 1.5% agarose gel stained with safeDNA® before being viewed under UV light. Amplified products were resolved on a ABI Prism 3130 automated genetic analyser (Applied Biosystems). Sequences were aligned using Bioedit (Hall, 1999), and consensus sequences were introduced on Bold (Barcoding of life) database to check for species identification.

2.3 Population genetic analysis

As the result of mtDNA analysis was positive for the presence of both *Mullus* species, we reorganized the samples and further analyses were done considering each species separately.

The total number of alleles and exclusives alleles as well observed and expected heterozygosity for each locus and locality were obtained with GeneAlex v.6 (Peakall and Smouse, 2006). Allele richness was calculated by FSTAT v. 2.9.3 (Goudet, 2002) and deviations from Hardy-Weinberg equilibrium and estimates of linkage-disequilibrium were estimated by means of 100,000 Markov chain Monte Carlo (MCMC) iterations using GENEPOP v 3.4 (Raymond and Rousset, 1995). F-statistics and their estimated probability were calculated via 10,000 random permutation using Arlequin v 2.0 according to Weir and Cockerham (1984) method.

A pairwise genetic distance matrix was computed using F_{ST} values among sampling localities and then was used to evaluate the population structure by a principal coordinate analysis (PCoA) of all adult samples within species using GeneAlex v. 6 (Peakall and Smouse, 2006). Additionally, the Bayesian approach implemented on STRUCTURE v. 2.3 (Pritchard *et al*., 2009) was used to identify the clustered populations based on individual genotype at multiple loci. The highest mean posterior probability of K [P(X/K)], is defined as the selected K. However, as the posterior probabilities increase there is an overestimation of K due an increase in the genetic clusters selected, and therefore we used the method suggested by Evanno *et al*. (2005) to obtain the true K. This method evaluates the rate in change of the logarithm probability between different K values as function of K, and the modal value of the distribution is elected as the real K (Evanno *et al* 2005). Finally, the variance among genetic clusters depicted from previous analysis was assessed by hierarchical analysis of molecular variance (AMOVA) in Arlequin software v.3.5.1.2 (Excoffier *et al*., 1992; 2005). Also, F-statistics were calculated from genotypic frequencies, partitioning molecular variance among populations (F_{ST}), among individuals within populations (F_{IS}), and within individuals (F_{IT}).

Genetic distance (D; Nei, 1978) between pairwise sample localities was computed with GeneAlex v.6 and used for testing the isolation by distance hypothesis by the correlation of Nei's D values and a distance matrix of geographical reference of each sampling locality. Probabilities were estimated through Mantel's test (Mantel, 1967) by running 9,999 random permutations with GeneAlex v.6 (Peakall and Smouse, 2006).

Gene flow was estimated by the number of migrants exchanged between population pairs using N_em. Values of N_em were derived from pairwise Fst values, following the island model of Wright (1951), where $N_em = [(1 / F_{ST}) - 1] / 4$.

2.4 Post-larvae identification and assignment

Since we discovered both *Mullus* species in our samples, and considering that post-larvae identification is even more challenging than adult specimens, we decided to first assign larvae to each of the two species and then estimate the most probable source population of larvae individuals. Notwithstanding, the search for the origin of larvae among potential sources only makes sense if sampled populations ("sources") constitutes genetically distinct unities. If sources populations do not reveal any genetic heterogeneity, assignment was not performed. Otherwise, tests were done following Paetkau *et al*. (2004) algorithm and estimated probability calculated by 100,000 simulations using GeneClass v. 2.0 (Piry *et al*., 2004).

3. Results

3.1 Genetic variability

The genetic variation at 10 microsatellite loci was high in both species (Tables 5.1-5.2). The number of alleles per locus ranged from 2 to 24 for *M. surmuletus* and 2 to 27 for *M. barbatus*. Mean expected heterozygosities were similar for *M. barbatus* (0.709-0.749) and *M. surmuletus* (0.691-0.710) while observed heterozygosity was higher for *M. barbatus* (0.603-0.706) compared to 0.521-0.619 in *M. surmuletus* samples. Mean allelic richness was slightly higher in *M. surmuletus* (10.08) than *M. barbatus* (9.95) however greater number of alleles was found among *M. barbatus* samples (Table 5.1-5.2).

Within *M. surmuletus* samples, higher number of alleles, thus higher allelic richness and expected heterozygosity, was found in Adra, however samples from the Cabo de Palos – Islas Hormigas Marine Reserve presented the highest observed heterozygosity. This MPA also harboured the lowest inbreeding coefficient, while the highest was found in Jávea locality (Table 5.1). In the case of *M. barbatus,* higher richness, expected and observed heterozygosity was found in Cartagena samples, as well as the lowest F_{IS} value. Garrucha was the locality with lower number of alleles, and heterozygosity levels and though highest inbreeding coefficient (Table 5.2). About 25% and 45% from a total of 60 and 70 single locus tests for *M. barbatus* and *M. surmuletus*, respectively, revealed significant departure from Hardy-Weinberg equilibrium. After applying the Bonferroni correction, 15% and 28% of single-locus tests were still significant ($p<0.00083$ and 0.00071, for *M. barbatus* and *M. sumuletus*, respectively). Although we found some evidence of linkage disequilibrium between locus pairs for both species, they represented only 2 and 4% over all possible combinations, i.e. less than would be expected by chance (P=0.05), so we decided to perform further population analysis, including all locus, although results will be discussed

Table 5.1 Summary statistics of 10 microsatellite loci and overall mean among 7 sampling location of stripped red mullet, *Mullus surmuletus*. (n=number of samples, Na=number of alleles, Ne=number of exclusive alleles, Rs=Allelic richness, Ho=observed heterozygosity, He=expected heterozygosity, Fis=inbreeding coefficient of an individual relative to the subpopulation.)

	Locus										
	M11	**M130**	**M132**	**M133**	**M14**	**M28**	**M3**	**M46**	**M55**	**M63**	**All loci**
Adra (AD)											
N	34	35	35	35	34	31	32	35	35	34	34.0
Na	10	18	12	14	24	5	6	4	6	15	11.4
Ne	1		1		1	1			1		0.5
Rs	9.736	16.806	10.986	13.102	21.786	4.71	5.749	3.793	5.793	14.049	10.651
Ho	0.294	0.486	0.686	0.629	0.882	0.387	0.406	0.457	0.714	0.824	0.576
He	0.776	0.881	0.819	0.813	0.924	0.405	0.496	0.406	0.718	0.857	0.710
Fis	**0.630**	**0.460**	0.177	0.241	**0.060**	**0.060**	0.196	**-0.111**	**0.020**	**0.054**	0.165
Aguilas (AG)											
N	34	32	35	35	35	35	35	35	35	35	34.6
Na	12	18	10	11	17	3	7	6	5	13	10.2
Ne	2							1			0.3
Rs	11.104	16.953	9.68	10.871	16.354	2.962	6.361	5.724	4.962	12.285	9.726
Ho	0.412	0.469	0.629	0.657	0.857	0.314	0.371	0.543	0.714	0.657	0.562
He	0.723	0.856	0.803	0.833	0.893	0.272	0.390	0.446	0.720	0.838	0.677
Fis	**0.442**	**0.465**	0.231	0.225	**0.055**	**-0.140**	**0.063**	-0.204	**0.023**	0.230	0.125
Cape of Palos (PA)											
N	33	33	33	33	33	33	32	33	32	33	32.8
Na	9	16	10	14	23	3	7	4	8	17	11.1
Ne	1					1			1	1	0.4
Rs	8.373	14.915	9.65	13.067	21.113	2.848	6.846	3.697	7.611	15.871	10.399
Ho	0.576	0.424	0.667	0.606	0.970	0.212	0.594	0.545	0.688	0.909	0.619
He	0.750	0.880	0.808	0.853	0.911	0.282	0.503	0.463	0.741	0.854	0.705
Fis	0.247	**0.529**	0.189	0.303	**-0.049**	0.263	**-0.164**	**-0.162**	**0.088**	**-0.049**	0.105
San Pedro (SP)											
N	34	31	34	34	34	33	34	33	34	34	33.5
Na	18	12	11	13	19	5	5	4	8	14	10.9
Ne	5				1	1			1		0.8
Rs	16.468	11.709	10.433	12.563	17.844	4.828	4.618	3.846	7.589	13.517	10.342
Ho	0.588	0.258	0.794	0.765	0.882	0.121	0.265	0.515	0.765	0.647	0.560
He	0.866	0.856	0.820	0.838	0.908	0.425	0.263	0.453	0.715	0.833	0.698
Fis	**0.334**	**0.707**	**0.047**	0.102	**0.043**	**0.722**	**0.010**	**-0.122**	**-0.055**	0.238	0.189
Santa Pola (PO)											
N	28	30	30	29	30	29	30	30	30	30	29.6
Na	13	13	10	11	20	3	5	4	6	14	9.9
Ne	2				1						0.3
Rs	13	12.913	9.863	10.895	19.329	2.966	4.867	3.997	5.933	13.786	9.755
Ho	0.321	0.267	0.833	0.724	0.900	0.276	0.400	0.433	0.700	0.767	0.562
He	0.842	0.846	0.801	0.851	0.907	0.242	0.369	0.496	0.733	0.865	0.695
Fis	**0.630**	**0.694**	**-0.024**	0.166	**0.024**	**-0.123**	**-0.067**	0.142	**0.062**	0.130	0.148

	Locus										
	M11	**M130**	**M132**	**M133**	**M14**	**M28**	**M3**	**M46**	**M55**	**M63**	**All loci**
Javea (JA)											
N	30	33	33	32	33	31	33	33	33	33	32.4
Na	9	13	9	14	20	3	8	6	7	14	10.3
Ne	1	1		1		1		1	1		0.6
Rs	8.86	12.848	8.822	13.554	18.98	2.903	7.221	5.543	6.524	13.698	9.895
Ho	0.300	0.333	0.606	0.625	0.879	0.226	0.394	0.485	0.606	0.758	0.521
He	0.772	0.815	0.760	0.833	0.911	0.252	0.458	0.580	0.669	0.857	0.691
Fis	**0.622**	**0.601**	0.218	0.265	**0.051**	0.121	0.154	0.179	0.109	0.131	0.231
Valencia (VA)											
N	30	30	30	30	30	30	30	30	30	30	30.0
Na	11	16	11	12	18	2	7	5	7	11	10.0
Ne	1		1	1			1		1	1	0.6
Rs	10.86	15.463	10.797	11.723	17.586	2.000	6.796	4.933	6.923	10.923	9.800
Ho	0.133	0.533	0.800	0.767	0.867	0.233	0.367	0.300	0.533	0.867	0.540
He	0.792	0.848	0.869	0.771	0.901	0.206	0.459	0.501	0.733	0.873	0.695
Fis	**0.837**	**0.385**	0.097	**0.023**	**0.055**	**-0.115**	0.218	**0.415**	0.288	**0.025**	0.208

cautiously.

3.2 Adult population structure

F_{ST} and the permutation tests for homogeneity of allelic frequencies indicated low genetic structure in *M. barbatus* samples, except for the weak but significant difference found between Mazarrón and Almería localities (Table 5.3). Instead, a significant genetic heterogeneity was evidenced for *M. surmuletus* samples (Table 5.4). Jávea and Valencia were the most genetically divergent localities among others showing moderate values of F_{ST} (>0.05), while samples from San Pedro and Águilas were only slightly different, although significant, from remaining localities (Table 5.4). The representation of genetic distances using the principal coordinates (PCoA) analysis graphically describe genetic divergence computed in pairwise F_{ST}. With the exception of Mazarrón locality, *M. barbatus* samples are widespread at the ordination space, showing no genetic structure (Fig. 5.2a). Meanwhile, those of *M. surmuletus* presented a clear segregation of Javea and Valencia, and also a further separation of San Pedro and Santa Pola from the remaining localities, evidencing genetic divergence amongst them (Fig. 5.2b).

Table 5.2 Summary statistics of 10 microsatellite loci and overall mean among 6 sampling location of red mullet, *Mullus barbatus*. (Codes as in Table 5.1)

	Locus										
	M11	**M130**	**M132**	**M133**	**M14**	**M28**	**M3**	**M46**	**M55**	**M63**	**All loci**
Fuengirola (FU)											
N	26	26	26	26	26	26	26	26	26	26	26
Na	9	10	8	13	23	2	18	8	11	11	11.3
Ne	1	2			2		2	1		1	0.9
Rs	6.926	8.151	7.206	10.095	17.718	1.615	14.267	7.277	9.935	9.292	9.248
Ho	0.538	0.231	0.577	0.885	0.962	0.038	0.808	0.846	0.462	0.731	0.608
He	0.547	0.723	0.615	0.813	0.930	0.038	0.888	0.811	0.883	0.842	0.709
Fis	**0.034**	**0.691**	0.082	**-0.069**	**-0.015**	**0.000**	0.109	**-0.024**	**0.492**	0.152	0.145
Almeria (AL)											
N	32	31	32	32	32	32	31	32	32	32	31.8
Na	13	10	10	15	27	2	19	8	15	12	13.1
Ne	1		1	2	1		2		1		0.8
Rs	9.213	7.922	8.618	10.457	18.807	1.943	14.374	7.22	11.449	9.891	9.989
Ho	0.719	0.452	0.656	0.813	0.844	0.125	0.742	0.750	0.688	0.594	0.638
He	0.693	0.710	0.781	0.787	0.943	0.117	0.894	0.821	0.884	0.862	0.749
Fis	**-0.037**	**0.364**	0.160	**-0.032**	0.106	**-0.067**	0.170	0.087	**0.223**	**0.311**	0.144
Garrucha (GA)											
N	19	17	19	20	19	20	20	19	20	19	19.2
Na	11	11	8	12	19	2	13	6	13	11	10.6
Ne		1		4	1				1		0.7
Rs	9.873	10.643	7.347	10.599	17.347	1.8	11.717	5.84	12.05	10.345	9.756
Ho	0.842	0.294	0.789	0.750	0.842	0.050	0.550	0.579	0.700	0.632	0.603
He	0.723	0.737	0.666	0.834	0.924	0.049	0.836	0.762	0.865	0.868	0.726
Fis	**-0.138**	**0.620**	**-0.159**	0.126	0.115	**0.000**	**0.365**	**0.265**	0.215	**0.298**	0.171
Mazarrón (MZ)											
N	21	21	21	21	21	21	21	21	21	21	21
Na	9	11	8	14	22	2	18	7	13	12	11.6
Ne		1		2	1		1			1	0.6
Rs	8.365	9.889	7.584	11.98	19.322	1.99	15.451	6.503	12.055	10.87	10.401
Ho	0.714	0.667	0.571	0.762	0.952	0.143	0.810	0.571	0.667	0.619	0.648
He	0.744	0.831	0.662	0.833	0.938	0.133	0.906	0.722	0.880	0.846	0.749
Fis	**0.064**	**0.221**	0.161	0.110	**0.009**	**-0.053**	0.130	**0.232**	**0.265**	**0.291**	0.143
Cartagena (CT)											
N	18	16	18	18	18	18	18	18	18	18	17.8
Na	10	10	9	8	22	2	15	8	14	12	11
Ne	1		1	1				1	1	2	0.7
Rs	9.425	10	8.638	7.555	20.7	1.99	14.285	7.87	13.304	11.526	10.529
Ho	0.722	0.563	0.778	0.667	1.000	0.111	0.889	0.778	0.833	0.722	0.706
He	0.722	0.791	0.707	0.736	0.946	0.105	0.892	0.833	0.883	0.875	0.749
Fis	**0.029**	**0.318**	**-0.072**	0.123	**-0.029**	**-0.030**	**0.032**	0.095	0.084	0.202	**0.075**
Villajoyosa (VJ)											
N	28	25	27	28	28	28	28	28	28	28	27.6
Na	11	14	10	13	22	2	15	8	12	11	11.8
Ne		1	1	1	2			1		2	0.8
Rs	8.814	11.922	8.185	10.668	17.718	1.571	12.285	7.136	10.639	9.143	9.808
Ho	0.786	0.560	0.630	0.857	0.964	0.036	0.821	0.571	0.786	0.679	0.669
He	0.722	0.856	0.584	0.821	0.941	0.035	0.879	0.830	0.880	0.830	0.738
Fis	**-0.070**	**0.364**	**-0.059**	**-0.026**	**-0.006**	**0.000**	0.083	**0.328**	0.125	0.200	0.094

Table 5.3 Estimates of N_em (above diagonal) and population pairwise Fst values (below diagonal) among samples of *M. surmuletus*. Bold numbers are significant results after 10,000 permutation tests. See table 5.1 for localities code.

	AD	AG	PA	SP	PO	JA	VA
AD	-	15.280	30.814	11.375	19.714	5.318	4.808
AG	**0.0198**	-	12.636	13.288	15.670	5.189	6.067
PA	0.0016	0.0268	-	13.544	27.951	5.767	4.915
SP	**0.0275**	**0.0225**	**0.0193**	-	17.519	4.357	4.591
PO	0.0099	0.0188	0.0032	0.0101	-	5.711	5.428
JA	**0.0744**	**0.0782**	**0.0698**	**0.0871**	**0.0711**	-	11.385
VA	**0.0783**	**0.0613**	**0.0792**	**0.0782**	**0.0706**	**0.0281**	-

Table 5.4 Estimates of N_em (above diagonal) and population pairwise Fst values (below diagonal) among samples of *M. barbatus*. Bold numbers are significant results after 10,000 permutation tests. See table 5.2 for localities code

	FU	AL	GA	MZ	CT	VJ
FU	-	21.295	17.351	13.368	16.551	19.287
AL	0.0050	-	20.814	13.145	20.430	22.974
GA	0.0101	0.0031	-	12.269	15.169	17.027
MZ	0.0133	**0.0172**	0.0144	-	13.489	16.246
CT	0.0052	-0.0010	0.0041	0.0123	-	19.868
VJ	0.0075	0.0003	0.0071	0.0074	0.0016	-

The Bayesian approach implemented in "Structure" also sustained previous results. Evanno *et al*. (2005) method calculates the rate of change of mean posterior probability between 2 different clusters (ΔK) in function of K, and the resulting mode values of K distribution is considered the best number of clusters. However, to calculate a ΔK it is necessary a minimum of 2 clusters, and thus a complete homogeneous population is never selected by this method. This was the case of *M. barbatus* samples (Fig. 5.3a-b). Although the ΔK distribution revealed a K=2 (Fig. 5.3a), the mean posterior probability plot indicates that individuals belong to a unique homogeneous group (Fig 5.3b). Regarding *M. surmuletus,* two different clusters, K=3 and K=5, with the first exhibiting a higher ΔK, were selected by both approaches, indicating samples originates from genetically heterogeneous populations (Fig. 5.3b-c, Table 5.5). Using K=3 case study, samples within localities split according to geographic unities with: a) Group 1 was formed by individuals of Adra, Águilas and part of Cape of Palos samples, the southern localities; b) the second cluster was mainly made by San Pedro, Santa Pola and the remaining samples of Cape of Palos, located in an intermediate geographic position; c) and the third group was mainly composed by samples of the northernmost localities of Jávea and Valencia (see Fig. 5.1). When considering K=5 case study, clusters were made of admixtures of individuals of different sources composes each formed group, with only Javea and Valencia individuals building a single genetic group.

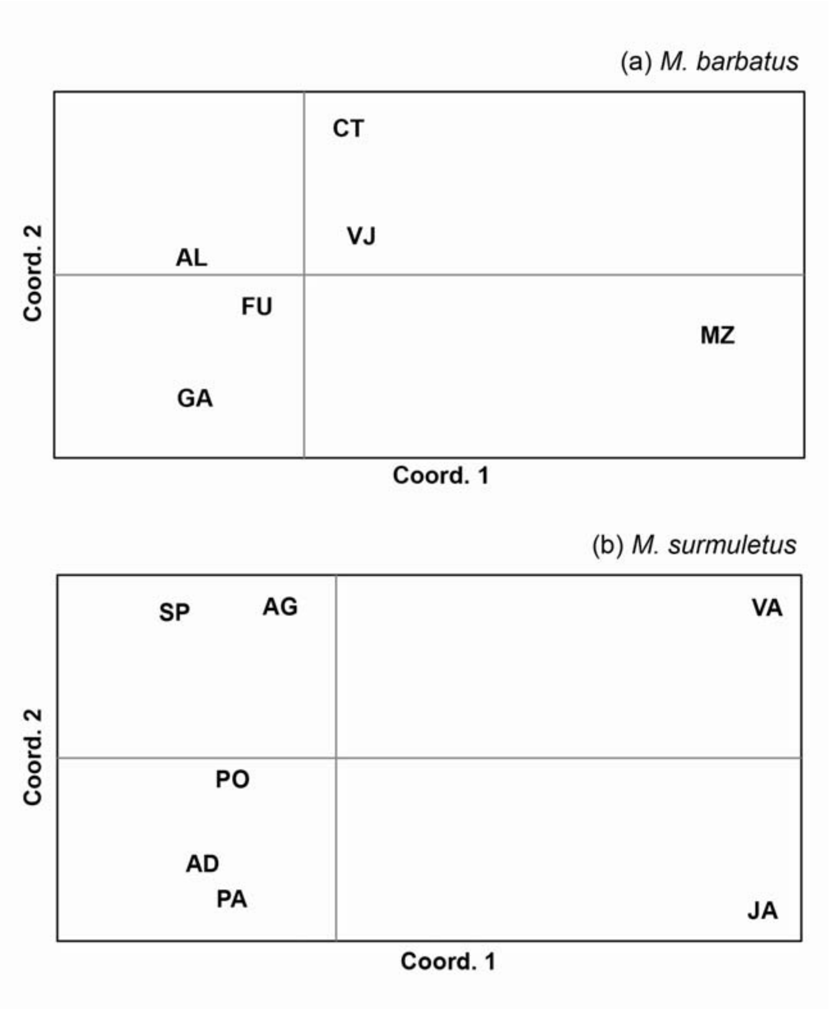

Figure 5.2 Principal Coordinates Analysis for *M. barbatus* (a) and *M. surmuletus* (b) samples. First two axis explain 52.36% and 86.25% of data variance, respectively. See table 5.1 for localities codes.

Analysis of molecular variance was conducted for both species, but in different ways. For *M. barbatus* all samples were clustered together while the genetic groups of *M. surmuletus* individuals defined by Structure analysis was used as criteria for grouping samples into 3 and 5 groups. The partitioning of variance among samples of *M. barbatus* yielded a significant but low

Table 5.5 Bayesian clustering results of Structure, where K is the number of genetic clusters.

				Proportion of membership of subpopulation cluster				
K	**Mean LnP(K)**	**Stdev LnP(K)**	**Delta K**	**1**	**2**	**3**	**4**	**5**
1	-8835.99	0.5782						
2	-8686.51	24.004	0.307					
3	-8544.41	8.1158	13.927	0.716	0.762	0.746		
4	-8515.34	36.9466	0.115					
5	-8482.03	22.985	11.641	0.550	0.626	0.599	0.629	0.590
6	-8716.29	175.4299	0.044					
7	-8958.29	403.5698						

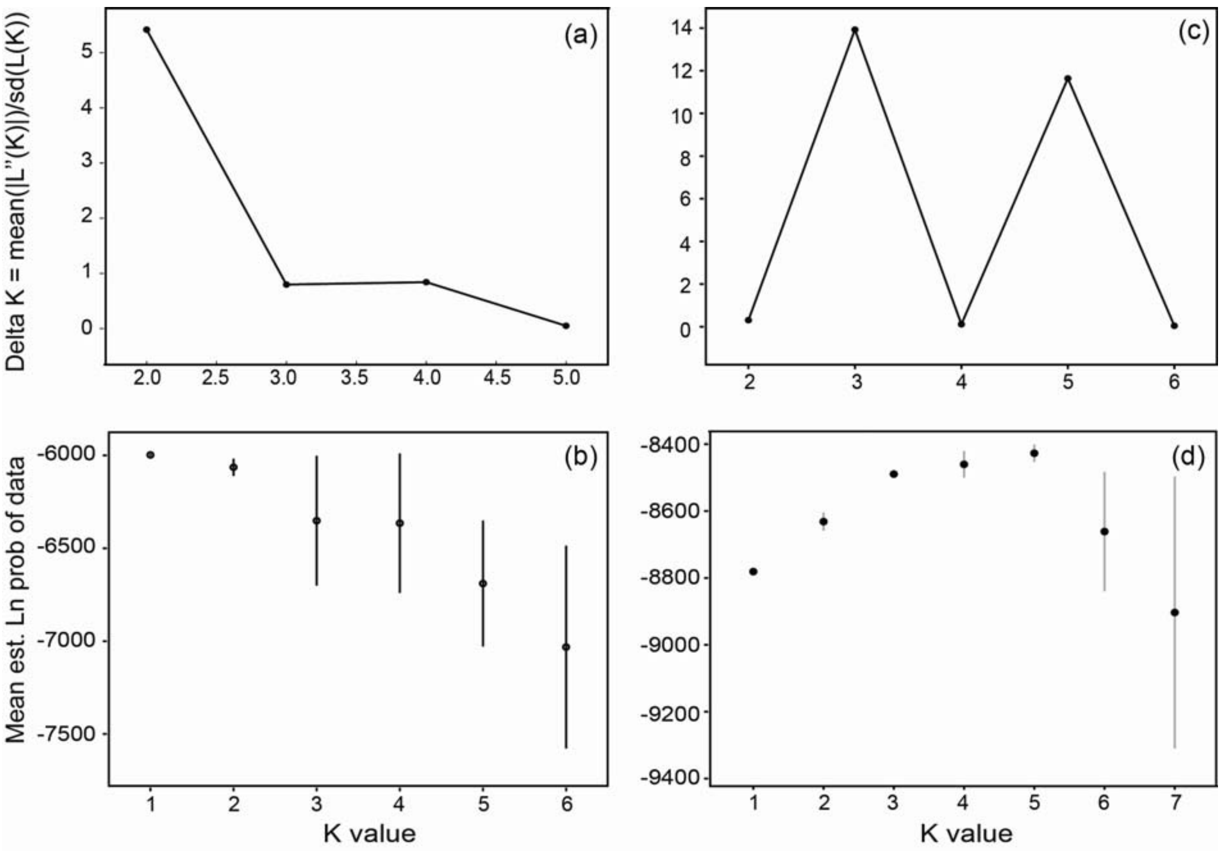

Figure 5.3 *M. barbatus* (a-b) and *M. surmuletus* (c-d) delta K and mean posterior probability (K) plots suggested by Evanno et al (2005) to elect the number of genetic clusters.

mean F_{ST} value of 0.004, indicating very low genetic structure or complete homogeneity as 88% of total genetic variance was within individuals (Table 5.6). On the other hand, the comparison of genetic clusters for K=3 and K=5 resulted in very similar results, but with K=5 they performing slightly better, explaining a larger proportion of variance among populations, 6 *vs* 5%, for K=5 and K=3 respectively. The higher explanation power considering K=5 indicates a hierarchical substructuring within K=3 groups, causing the reduction of variance among individuals and increasing explanation among populations. As usually in AMOVA greatest part of variance (76%) is carried between individuals, indicating a high genetic diversity on samples (Table 5.6).

Table 5.6 Results of hierarchical AMOVA conducted on *M. barbatus* and *M. surmuletus* samples according to genetic clusters evidenced by Structure.

Source	df	Sum of Squares	Estimation of Variance	Variance (%)	Fixation indices
M. barbatus					
Among Pops	5	22.13	0.015	0.45	$F_{ST} = 0.004$*
Among Ind.	139	514.68	0.365	10.89	$F_{IS} = 0.109$*
Within Ind.	145	431.00	2.972	88.66	$F_{IT} = 0.113$*
Total	289	967.80	3.353		
M. surmuletus					
K=3					
Among Pops	2	70.78	0.206	5.64	$F_{ST} = 0.056$*
Among Ind.	225	926.77	0.669	18.30	$F_{IS} = 0.194$*
Within Ind.	228	634.00	2.780	76.05	$F_{IT} = 0.239$*
Total	455	1.631.55	365.621		
K=5					
Among Pops	4	95.80	0.221	6.10	$F_{ST} = 0.061$*
Among Ind.	221	893.43	0.626	17.22	$F_{IS} = 0.183$*
Within Ind.	226	630.50	2.789	76.69	$F_{IT} = 0.233$*
Total	451	1.619.73	363.801		

* Fixation indices significant at $p<0.001$

The indication of geographical structure highlighted by Bayesian approach for *M. sumuletus* was tested for isolation by distance (Fig. 5.4). The regression between corresponding matrices was low ($R^2 = 0.15$), but the same analysis excluding Adra locality improved the explanation power of the linear model to 49% and evidenced an increase in Nei's D genetic with distance (Fig. 5.4). All combined samples resulted in a non significant Pearson coefficient (r=0.39, P=0.11) as indicated by Mantel's test, while extracting Adra locality increased the correlation (r=0.70) and its significance (P=0.04).

Finally, the mean number of migrants per generation using F_{ST} as an estimation of the degree of gene flow between sampling localities was greater for *M. barbatus* than for *M. surmuletus,* this value ranging from 13.1 to 22.9 individuals per generation (Table 5.3). On the contrary *M. sumuletus* showed a larger variability of gene exchange levels, but a lower mean number of migrants (Table 5.4).

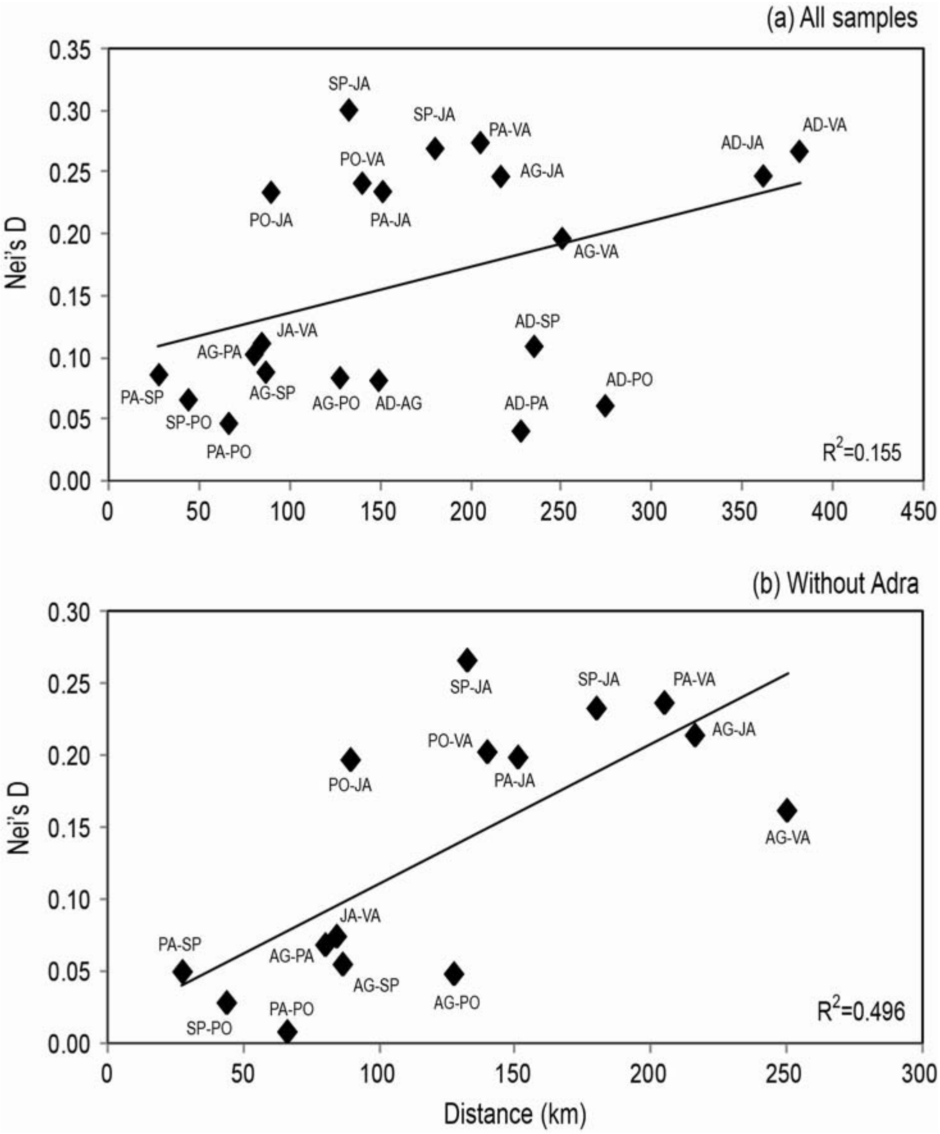

Figure 5.4 Relationship between genetic (Nei's D) and geographical distance of all *M. surmuletus* sampling localities (a) and excluding Adra samples (b).

3.3 Larvae assemblage structure and assignment

Post-larvae samples were also represented by the two species of *Mullus* (Fig. 5.5). Most post-larval individuals were identified as *M. barbatus* (as much as 90% of all post-larvae captured with light-traps) and therefore, due to absence of genetic structure of adult population, assignment tests were not performed. For their part, few *M. surmuletus* larvae (n=20) were caught in Cabo de Gata Natural Park, but assignment tests failed to identify a source population between sampled localities and therefore these individuals were considered as immigrants.

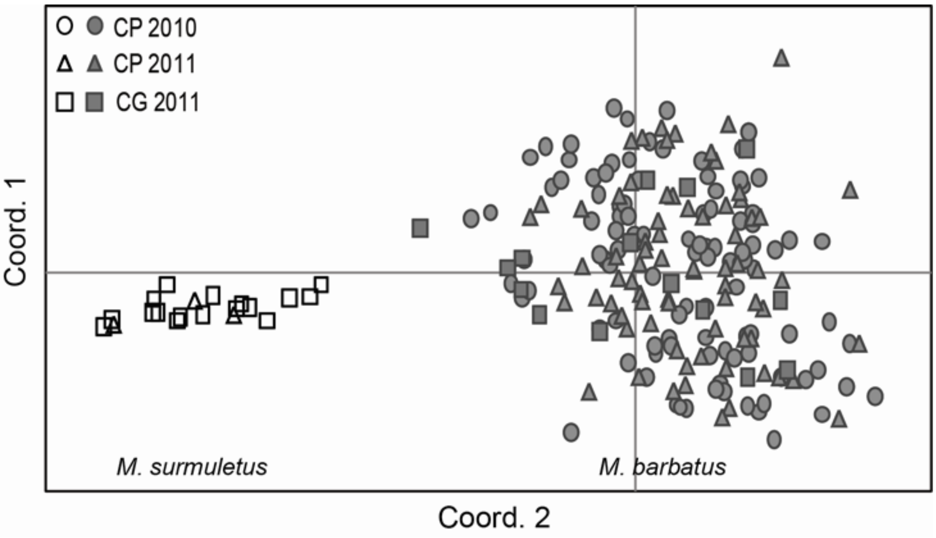

Figure 5.5 Principal Coordinates Analysis indicating the separation of larval samples into the 2 species. First two axis explain 46.12% of data variance. Species are indicated by colours: *M. surmuletus* in white and *M. barbatus* in gray; Location are indicated by shapes: larvae from Cabo de Palos in 2010 as circles, larvae from Cabo de Palos in 2011 as triangles, and larvae from Cabo de Gata from 2011 as squares.

4. Discussion

Mullets are considered important economic resources in the Mediterranean Sea, and in consequence, much effort has been devoted to understand mullet biology and distribution. Past studies have revealed a high degree of intraspecific variability, mainly during juvenile phase (Mamuris *et al.*, 2001), making their taxonomic classification by biometric parameters a difficult task (Cammarata *et al.*, 1991). In the course of the present study we came to the fact that adult samples were composed of a mix of the two species, *M. surmuletus* and *M. barbatus,* and this circumstance obliged us to restructure our objectives and data analyses.

Generally speaking, spatial scales of dispersal and resulting connectivity of coastal fish populations resulted to be smaller than previously thought (Planes *et al*., 1998; Pineda *et al*., 2007). Several studies have evaluated the population genetic diversity of both mullet species across de Mediterranean Sea, finding different levels of genetic connectivity at different spatial resolutions (Mamuris *et al*., 1999; Garoia *et al*., 2004; Galarza *et al*., 2009; Maggio *et al*., 2009). Recently, Di Franco and Guidetti (2011) evidenced that early life-history traits of a Mediterranean coastal fish species could be spatially more variable at a scale of kilometres than at a scale of tens to hundreds of kilometres. These results highlight the need to ascertain at which spatial scale genetic heterogeneity can be detected in marine populations. Bearing in mind these precedents, the present work focused on describing the genetic structure of two important commercial species, *M. surmuletus* and *M. barbatus,* at a small spatial scale, covering ~400 km of coastline in the Spanish Mediterranean Sea. High levels of genetic variability were observed for both species, in agreement with other previous works using microsatellite techniques (Garoia *et al*., 2004; Maggio *et al*., 2009; Galarza *et al.,* 2009). Overall, genetic variability was very similar between species, nonetheless, *M. surmuletus* showed a slightly higher allele richness and as well higher mean expected and observed heterozygosity, as found by Galarza *et al*. (2009), which employed the same microsatellite markers as this study.

Significant departures from Hardy-Weinberg equilibrium were found and a generalized trend to heterozygosity deficiency was observed. This deficit is usually generated by the presence of null alleles, population substructuring or selection (Garoia *et al*., 2004; González-Wangüemert and Pérez-Ruzafa, 2011). The lack of null amplifications in the totality of individuals analysed suggests that the presence of null alleles could not be the cause of the observed HW departures. The heterozygosity deficit could be a result of an important inbreeding or mating among relatives, as moderate and significant values of F_{IS} statistic were found in each population of both species. Also, the Wahlund effect could have contributed to the observed heterozygosity deficiencies. Although more evident for *M. sumuletus* samples, in which a clear substructuring of samples was observed, both species presented low-to-moderate levels of admixture of individuals from genetically different populations (see below), which might have contributed to the departures found (Garoia *et al*., 2004; van Open *et al*., 2009). Additionally, the unbalanced and lower number of *M. barbatus* samples may also have played a part.

Fisheries can reduce heterozygosity levels and also produce a strong selection on mean sizes, growth, and maturity, among others characteristics (González-Wangüemert *et al*., 2004). Marine protected areas could act as a genetic reservoir of high allelic richness and thus improve fitness under adverse conditions (Pérez-Ruzafa *et al*., 2006). In this study we registered higher allelic richness and observed heterozygosity levels of stripped red mullet samples as well as lower values of inbreeding coefficient (F_{IS}) within the Cabo de Palos-Islas Hormigas Marine Reserve. Although *M. barbatus* was not sampled inside the marine reserve, the highest values of observed and expected heterozygosity and lowest F_{IS} was recorded in Cartagena, the area closest to the MPA. These results might indicate that Cabo de Palos Marine Reserve could have been benefiting nearby areas by promoting the admixture of individuals with high allelic diversity.

Both species showed very different patterns of population structure. A greater level of structuring was found in stripped red mullet when compared to its congener. Ten times more significant F_{ST} comparisons was observed in *M. surmuletus* samples in contrast to *M. barbatus,* in which Mazarrón-Almeria was the only significant comparison (F_{ST}=0.017). Although overall F_{ST}=0.04 indicate a subtle but significant heterogeneity amongst *M. barbatus* samples, other methods such as Bayesian approach failed to depict any genetic difference among samples. Other works such as those of Garoia *et al*. (2004) and Maggio *et al*. (2009) have detected low but significant genetic heterogeneity on *M. barbatus* samples across localities; however the heterogeneity of red mullet samples was not related to geographic distances. For their part, Mamuris *et al*. (2001) found no genetic structure for this species in Greek waters.

Conversely, we detected a high degree of genetic heterogeneity among stripped red mullet samples and a clear substructuring among localities sampled. Ordination analysis highlighted the high genetic distance between *M. surmuletus* samples of Jávea and Valencia and the other localities and also the existence of a second subdivision between San Pedro and Águilas samples, indicating a geographic pattern in the genetic groups. The division of *M. surmuletus* samples into 3 genetically distinct clusters was supported by Structure analysis with K=3, which separates the northern (Jávea and Valencia), from intermediate (San Pedro, Santa Pola and part of Cape of Palos samples) and southern localities (Águilas, Adra and the remaining Cape of Palos samples). A second possible real number of clusters (K=5) was also depicted by Bayesian analysis. According to Evanno *et al.* (2005) the presence of two possible true K's for a given dataset indicates the existence of relative genetic isolation between the three groups of populations and sometimes also a pattern of isolation within each group, named ”contact zone” model. The existence of a contact zone may be further evidenced by the membership values of clusters formed. For K=3, namely northern, intermediate and southern groups, mean membership was q=0.74, much higher than q=0.598 for K=5 (northern and within remaining groups), meaning that admixture of individuals between different genetic clusters are occurring more strongly when K=5. Maggio *et al.* (2009) described the same situation for *M. barbatus* samples on Eastern Mediterranean Sea, revealing an isolation of Adriatic samples and further segregation of remaining sites.

The isolation-by-distance model applied using Nei's genetic distance and geographic distances for *M. surmuletus* dataset revealed no geographic trends in the genetic structure of this species (R^2=0.06; p>0.05). But, noticeably, when Adra samples were removed from this analysis, a stronger, significant relation with distance appeared (R^2=0.49; p<0.04). This result suggests that complex gene flow patterns could be found even at reduced spatial scales. For instance, Santa Pola was not significantly different from all southern localities and neither was Cape of Palos to Águilas and Adra. However adjacent localities such as San Pedro – Cape of Palos could present high gene flow (F_{ST}=0.04) or more restricted ones such is the case of the Santa Pola – Jávea pair (F_{ST}=0.07).

Gene flow, as estimated by the mean number of migrants per generation, was 17 for *M. barbatus* and 11 for *M. surmuletus*. Although these values were higher than those found by other authors for *M. barbatus* (N_em = 5.6, Mamuris *et al*., 1998) and *M. sumuletus* (N_em = 6.9, Mamuris *et al*., 1999), they probably reflect the spatial scale in which the different works have been conducted. Nevertheless, Allendorf and Phelps (1981) stated that significant divergence in allele frequency could be maintained with up to 10 migrants per generation which is the case of stripped red mullet samples.

Gene flow is the result of larval dispersal, adult movement, or both. High gene exchange was observed between intermediate and southern localities, being the northernmost localities, i.e. Jávea and Santa Pola, the most isolated, with in average 5 migrants per generation with the others. This restriction to gene flow could be a result of both biological features and hydrodynamic conditions of the studied region. The area surrounding the Cape of Palos is a transition zone where an abrupt change of the coastline occurs; the north region is mainly exposed to East, and the southern one to South-east. Additionally, the northern area is characterized by a continental shelf of gentle slope which extends until 35 km from coastline. Instead, in the southern area the continental shelf is narrow and steep, reaching 1000 m deep just 6 km offshore. In addition, the abrupt submerged relief and the presence of deep canyons produce local upwelling of complex dynamics (Taxon, 2009). The geomorphological differences between northern and southern areas produce dissimilar hydrodynamics. To the north of Cape of Palos, hydrodynamism is mainly influenced by the very saline deep Mediterranean water (MWP) current, originated from Ligurian Sea and Balear sea while the southernmost area receives the surface Atlantic-Mediterranean waters (MAW), which are richer in nutrients originated from the Alboran Sea (Millot, 2005). When wind blows from the south, a temporary cyclonic gyre is established moving surface waters from Argelia to Spanish coast. This mass bifurcates at the Cape of Palos to the north, towards the Balearic Sea, and the remaining is deflected and re-circulates onto the Alboran Sea.

According to Tserpes *et al*. (2002) and Katsanevakis and Maravelias (2009), both *Mullus* species differ in their habitat requirements. Stripped red mullet *M. sumuletus* prefers rough bottoms and narrow and deep continental shelves, while red mullet *M. barbatus* is most commonly found in wider continental shelves where sandy or muddy bottom predominate. The geomorphological feature of southern areas of Cape of Palos could enhance the exchange of both adults and larvae of *M. surmuletus.* The continuous rocky fringe existing in this part could favour adult movements, while the Alboran current could drive larvae to the region of Cape of Palos, in which such current is deflected. The current deflection could impede the communication by larval dispersal between northern and southern areas, and the unfavourable habitat conditions (shallow and sandy/muddy shelf) of northernmost areas could restrict the flow of adult individuals and recruitment, thus isolating both northern and southern populations. However, as we could observe, gene flow is maintained to some extent in the contact zone between neighbouring sub-populations, creating the isolation-by-distance effect. Notwithstanding, there is an important flux between Cape of Palos and Adra, one of the most southernmost localities, which could be achieved by larval transport of Algerian current gyre, originated at Alboran sea, which reaches Cape of Palos region when south winds blows. Offshore transport had been also suggested as a linking mechanism in the coastal species *Diplodus sargus* in the same studied region; González-Wangüemert *et al*. (2004) observed higher genetic similarity between the farthest populations of *D. sargus* (Águilas and Tabarca) while the closest ones presented reduced gene flow, being Cape of Palos the most isolated from all. These authors suggested that this pattern may have been produced by an offshore larval pathway that interconnected populations at extreme geographic sites. Although limited, our results indicate that mechanisms others than larval dispersal could explain the homogeneity and moderate heterogeneity of both *M. barbatus* and *M. surmuletus* samples, respectively. Probably a coupled effect of adult movements and larval pathways could be a better explanation to the different patterns observed at such fine spatial scale. Owing that both mullet species have similar pelagic larval duration (28-35 days; Macpherson and Raventós, 2006; Galarza *et al*., 2009b), and that larvae and juvenile stages are pelagic, and also that juveniles can retard the settlement period (Machias *et al*., 1998), it is expected that both species can be equally affected by circulation patterns in the region. Given the uneven distribution of adults over the continental shelf, as well as the higher natural abundance of

M. barbatus and the different habitat preferences that restrict *M. surmuletus* to more coastal zones than *M. barbatus*, larvae of both species could be subjected to different local hydrodynamic regimes, thus contributing to the observed differences between both species. While larvae of stripped red mullet may be more affected by local wind driven currents, those of red mullet could be more exposed to offshore currents. A more oriented study involving modelling local oceanographic features as well as finer spatial scale data collection is needed to solve this puzzle.

Finally most larvae collected in both MPAs belonged to *M. barbatus* species, however due to the absence of genetic structure on adult populations sampled we were unable to identify the possible origin for *M. barbatus* larvae. However, the few identified larvae of *M. sumuletus* were not assigned to any of the populations sampled, indicating that scales of genetic connectivity can be very large (more than ~400km) for this species. In a similar work using recruits of *Tripterygion delaisi* collected in Blanes (Spain), authors found high self-recruitment rates to Costa Brava localities, however 25% of recruits were left unassigned and other 5% corresponded to distinct populations across Spanish coast (Carreras-Carbonnel *et al.,* 2007). These results indicate that significant levels of unasignment could arise even for a species with limit adult and larval dispersal capabilities. Although significant structuration can be maintained under low gene flux (Carreras-Carbonnel *et al.,* 2006), as evidenced by *M. surmuletus*, more larval samples would be necessary to conclude about dispersal capability of this species.

5. Conclusions

In two sympatric species as *Mullus surmuletus* and *Mullus barbatus*, which have similar life history traits as same pelagic larval duration and spawning and settlement season, we found divergent patternsof genetic connectivity in a small spatial scale (~400 km). While a complete homogeneity was found for *M. barbatus*, high genetic structure was depicted in *M. surmuletus* samples. We suggest that adult habitat partitioning together with geomorphological characteristics of continental shelf and oceanographic patterns, as inshore and offshore currents, must have influenced on patterns found in the studied region. Although we were unable to verify the population origin of larvae collected inside the marine reserves, we could detect higher heterozigosities values inside cabo de Palos marine reserve for *M. surmuletus* individuals, and on the vicinities, in the case of *M. barbatus*. Our results indicate that for two co-occurring species scales of connectivity may be completely discordant, and that life history traits such as PLD may not have huge effects on determining dispersal capability of a fish species.

General Discussion

General discussion

1.1 Thesis overview

Marine populations are considered demographically open owing to the external (but not exclusive) source of eggs and larvae that will be incorporated into the local population (Caley *et al*., 1996). These eggs and larvae are produced in reproductive events predictable in time defined by fish life histories and are dispersed by oceanographic mechanisms. In temperate regions, as the Mediterranean Sea, there are two well defined reproductive seasons – summer and winter – however the vast majority of species reproduce during the warmer period (Tsikliras *et al*., 2010). Thus, a great number of fish larvae reach nearshore habitats during spring and summer months, when generally high local phytoplankton productivity occurs (Sabatés *et al*., 2007, Chapter I). Winds and currents are the main drivers in determining larval distribution at nearshore areas (Chapter I). However at local scales, in the reef vicinities, larvae are capable to orient themselves (Kingsford *et al*., 2002; Staaterman *et al*., 2012) and actively choose the preferred habitat to settle (Booth and Wellington, 1998; Jenkins, 2005) as evidenced by a high fine-scale variability in abundance of post-larvae (Chapter I). Yet, we have found strong indications that habitat selection rather than differential larval supply were responsible for larvae distribution mainly because we found an uneven distribution of fish species abundance among locations under the same environmental influence and not distant from each other by more than 10 km (Chapter I).

We also observed that settlement season is very predictable for each species as usually there is high temporal correlation between settlement seasons of successive years. Nevertheless, due to variation in reproductive success, there is also high variability in settlement intensity (Chapter II). Besides the variable input of post-larvae from the plankton, there are also post-settlement events such as mortality that can exert strong effects on population demography (Johnson *et al.,* 2009). Benthic losses can reach more than 90% of settler abundance (~80% in average), and if a threshold value of abundance is reached, that mortality can be qualified as density-dependent (Osenberg *et* *al.*, 2002; Chapter II). This high initial mortality just after settlement contributes to the decoupling between abundance of post-larvae and settlers. Conversely, for most species recruit abundances reflect those of settlers, indicating that year-class strength regulation is likely to occur during the first fish life-history stages (Houde, 1987; Chapter II). This would mean that demography of local populations is established at the beginning of fish life, and that recruitment patterns will be reflected on adult stock. Moreover, stronger settlers ~ recruits relationships were found in fish nekto-benthic species with strong habitat relationships (Chapter III), indicating that habitat could have played an important role in reducing mortality by the presence of more refuges due to a higher complexity (Juanes, 2007).

Recruitment was concentrated in the coastal zone, where species habitat requirements are probably the principal cause of its important spatial variability (Sale *et al.*, 2005b; Johnshon, 2007; Chapter III). Fine-scale variability of fish abundance corresponded to a spatially composed mosaic of habitats, reinforcing the idea that active selection by larvae at settlement plays an important role (Chapter III). Normally, young fish do not settle in the same habitat than that occupied as adult, to avoid competition by spatially partitioning the available resources (Eggleston, 1995). As recruits grow and optimize their feeding abilities, while increasing their capability of defence against predators and competitors, they undergo ontogenetic shifts to explore new habitats and resources and incorporate themselves into the adult local population (Bailey, 1994; Chapter III).

Due to their constant removal by fisheries, the effects of protection are more prone to be seen in adult populations, by enhancing their abundance, biomass and consequently their fecundity, especially in the case of economically important species, and species with low mobility or/and demersal habits (Chapter IV). We also corroborated that the lack of enforcement in a marine reserve impedes to fully accomplish with their potential benefits (Guidetti *et al.*, 2008): although the effects of protection was detected in abundance terms, they were not accompanied by an increase in average size of individuals, likely due to poaching of big-sized ones. Therefore, we were not able to verify clear gradients of adult biomass across reserves boundaries although gradients of abundances were present (Chapter IV). On the other hand, we were able to measure a negative effect of protection on larval abundances, which could be attributed to either local retention of larvae in unprotected sites due to current patterns (Jones *et al*., 2009) or/and the presence of singular habitat characteristics, the latter meaning a strong habitat selection by larvae. Although apparently marine reserves do not affect early life-history stages of fishes, by influencing mortality rates, increasing habitat complexity, enhancing predator abundance, etc., protection can exert huge influence on population dynamics. Much research needs to be done to verify these expectations.

By studying the genetic structure of two commercially important species – stripped red mullet, *Mullus surmuletus*, and red mullet, *Mullus barbatus,* we could observe that these two sympatric species with similar life history traits, with the same pelagic larval duration and spawning and settlement season, have very distinct patterns of genetic connectivity (Chapter V), one with a complete homogeneity (*M. barbatus*), and the other presenting structured populations (*M. surmuletus*). Habitat preferences of adult individuals of both species (Lombarte *et al*., 2000; Tserpes *et al*., 2002) together with regional geomorphological characteristics, such as the topography of the continental shelf, may be responsible for the divergent patterns observed. Although our intention was to be able to assign larvae to potential source populations, due to the absence of population structure in *M. barbatus* samples we could not found any relationship between larval pool and adult population (Chapter V). Despite of the impossibility to conclude something about larval exportation from marine reserves we could verify a positive effect of marine reserves in conserving genetic diversity and also the effect of such reservoir of alleles (Pérez-Ruzafa *et al*., 2006) on the bottoms surrounding an MPA. Recent tools, apart from assignment tests, are available to test larval exportation (e.g. parentage analysis, otolith microchemistry) and must be employed in conjunction with oceanographic models to better understand the patterns of connectivity of marine fish populations by larval dispersal.

1.2 Implications to fisheries management

In the case of the Mediterranean Sea most MPAs are small sized and designed to a common objective, to become a fishery reserve (Abdulla *et al*., 2008; García-Charton *et al*., 2008). In our approach we were able to work in two distinct MPAs: one small sized (1,898 ha) with a insular no-take area, several submerged rocky reefs inside buffer zone, and a small coastal zone within reserve limits (Cabo de Palos – Islas Hormigas Marine Reserve); and one MPA network of 5 coastal no-take areas encompassing 4,653 ha (Cabo de Gata – Níjar Natural Park). Although harbouring distinct designs both have their pros and cons.

Cabo de Palos – Islas Hormigas Marine Reserve has revealed a very effective MPA for increasing biomass of heavily fished populations as top-predators such as *Epinephelus marginatus* and *Dentex dentex*, among others (García-Charton *et al*., 2010). It does provide biomass export to vicinities and thus perform well according to its main objectives. However, the patchy organization of the rocky reefs together with the isolation of the no-take zone, limit their effects to populations that are capable to move between patches and that can recruit within reserve limits. Many Mediterranean species depends on coastal areas as nursery habitats (Planes *et al*., 2000) but a minimal portion of these habitats are contained within reserve boundaries. We observed a higher recruitment rate outside MPA borders where habitats for young fish are threatened by anthropogenic effects such as pollution, fishing, construction, tourism, etc.

For its part, Cabo de Gata Natural Park is a very extensive marine park entirely located at coastal zone encompassing the mosaic of habitats that are used by most species as young. Thus, as neashore habitats are inside the park limits they are in some way protected down to 60 m deep. The five no-take zones are located on purely rocky areas focusing on areas previously known by local fishermen as good places to fish. However, the major problem of Cabo de Gata reserve is the deficiency of its surveillance. There is no effective enforcement and illegal fishing is occurring inside the no-take areas, hindering positive effects to occur. This is probably the reason why we did not found differences in biomass between inside and outside the integral reserves, but only in abundance, indicating that the numerous fish individuals visually censused within no-take boundaries were generally very small-sized fishes.

The main ecological goals of MPAs are restoring community structure, protecting biodiversity, and increasing ecosystem resilience and stability. These broad objectives can only be achieved if the implementation of the MPA is oriented to cover a broad scale, large enough to encompass all habitats used by the whole set of target species to complete their life cycle. This areas, known as Essential Fish Habitats (EFH), can be defined as "...those waters and substrate necessary to fish for spawning, breeding, feeding, or growth to maturity" (NOAA, 1999). The application of this concept to MPA design guarantee the protection of the entire life cycle of target species and many others that live in the same areas and use the same zones. By this mean MPA can be fully effective and be even more beneficial to surrounding areas.

The example of Cabo de Palos, albeit being a very effective reserve, does not fulfil the requirements to guarantee that the entire life cycle of many reef species is achieved. As many of them depend on coastal areas as nursery grounds to provide juveniles to reserve and adjacent zones, they will be exposed to low habitat quality, fishing pressure and disturbed environment during the most vulnerable period of life. For example, sparids, that include a group of very economically important species, settle mainly in gravel dominated beaches or *Posidonia oceanica* meadows, habitats which is not present inside Cabo de Palos-Islas Hormigas Marine Reserve. Therefore, ESH assessments should be included in MPAs implementation studies in order to effectively assure that the beneficial effects of MPAs are fully accomplished.

Since larval period is the dispersive phase for most fish species, it is responsible for the exchange of individuals (i.e. connectivity) among local populations (Almany, 2009). As the distance between two populations is lower, the intensity of the connections between them increases. Thus, the persistence of a population will depend of how much it is connected to adjacent ones. This has important consequences for fisheries management. Actually, no MPAs or MPA networks have incorporated estimates of larval dispersal in their design to now, and consequently they may fail to guarantee persistence, resilience and protection of biodiversity (Almany *et al*., 2009).

This is probably the case of Cabo de Gata Natural Park, since although its design is very well constructed and it is one of the few Spanish marine reserves incorporating a network of no-take zones inside their limits, its performance could be suboptimal, so that further knowledge on population connectivity could significantly improve its design. Spacing between reserves is ~7 km; considering that in average studies of larval dispersal have observed scales from 10-100's km, larger but more spaced no-take areas could improve the net results of all MPAs within and around the park in terms of enhancing the abundance and fecundity of reproductive stocks.

Further research on the ecology of fish ELHS and their effect on population dynamics is critical to optimize the use of MPAs as a tool for fisheries management and, ultimately, for the conservation of marine coastal ecosystem goods and services.

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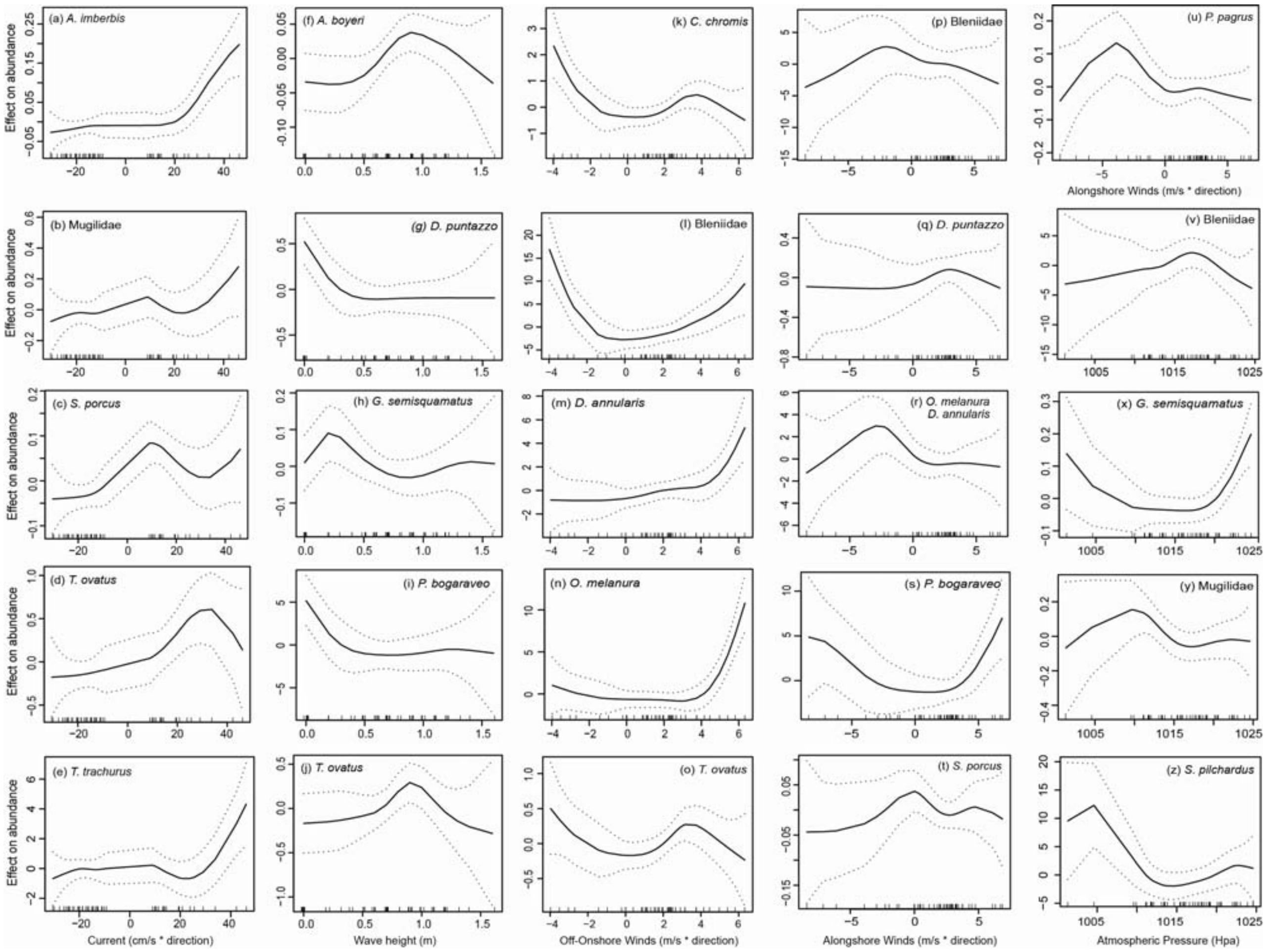
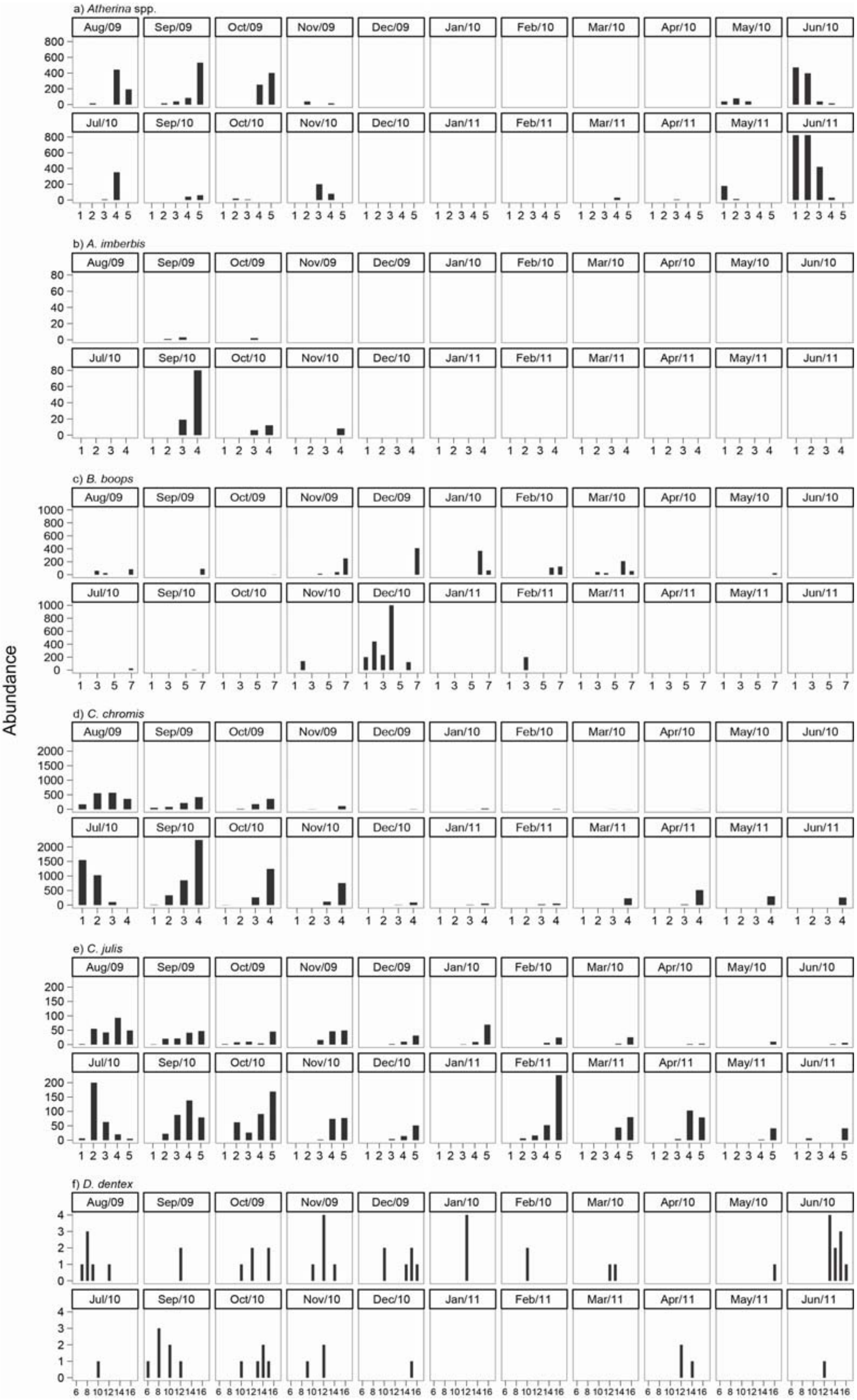
Appendix

Appendix I

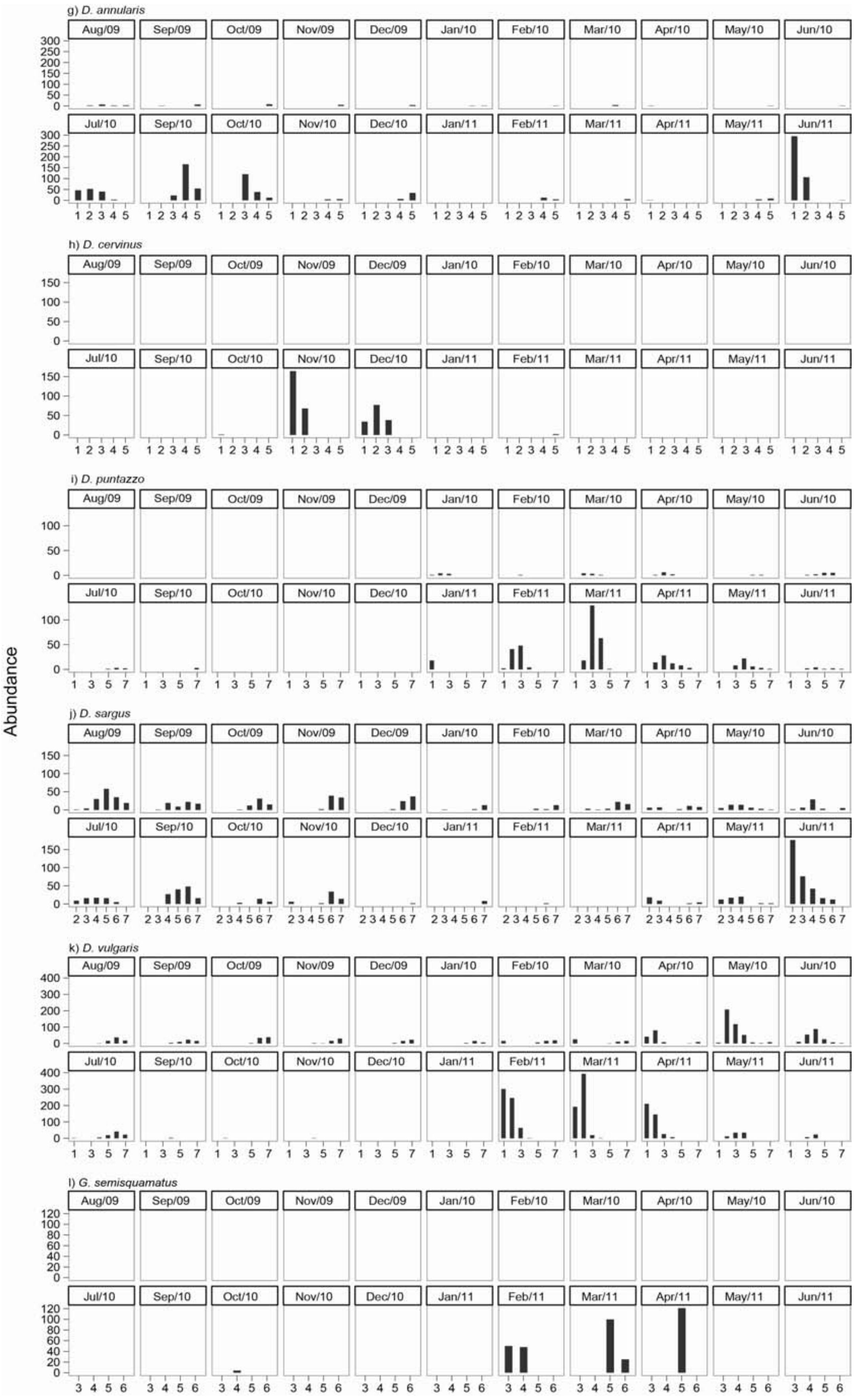


Appendix I GAM plots of species response to environmental variables tested. a) *A. imberbis*, b) Mugilidae, c) *S. porcus*, d) *T. ovatus*, e) *T. trachurus*, f) *A. boyeri*, g) *D. puntazzo*, h) G. *semisquamatus*, i) *P. bogaraveo*, j) *T. ovatus*, k) *C. chromis*, l) Blennidae, m) *D. annularis*, n) *O. melanura*, o) *T. ovatus*, p) Bleniidae, q) *D. puntazzo*, r*) O. melanura* and *D. annularis*, s) *P. boagaraveo*, t) *S. porcus*, u) *P. pagrus*, v) Blennidae, x) *G. semiaquamatus*, y) Mugilidae and z*) S. pilchardus*.

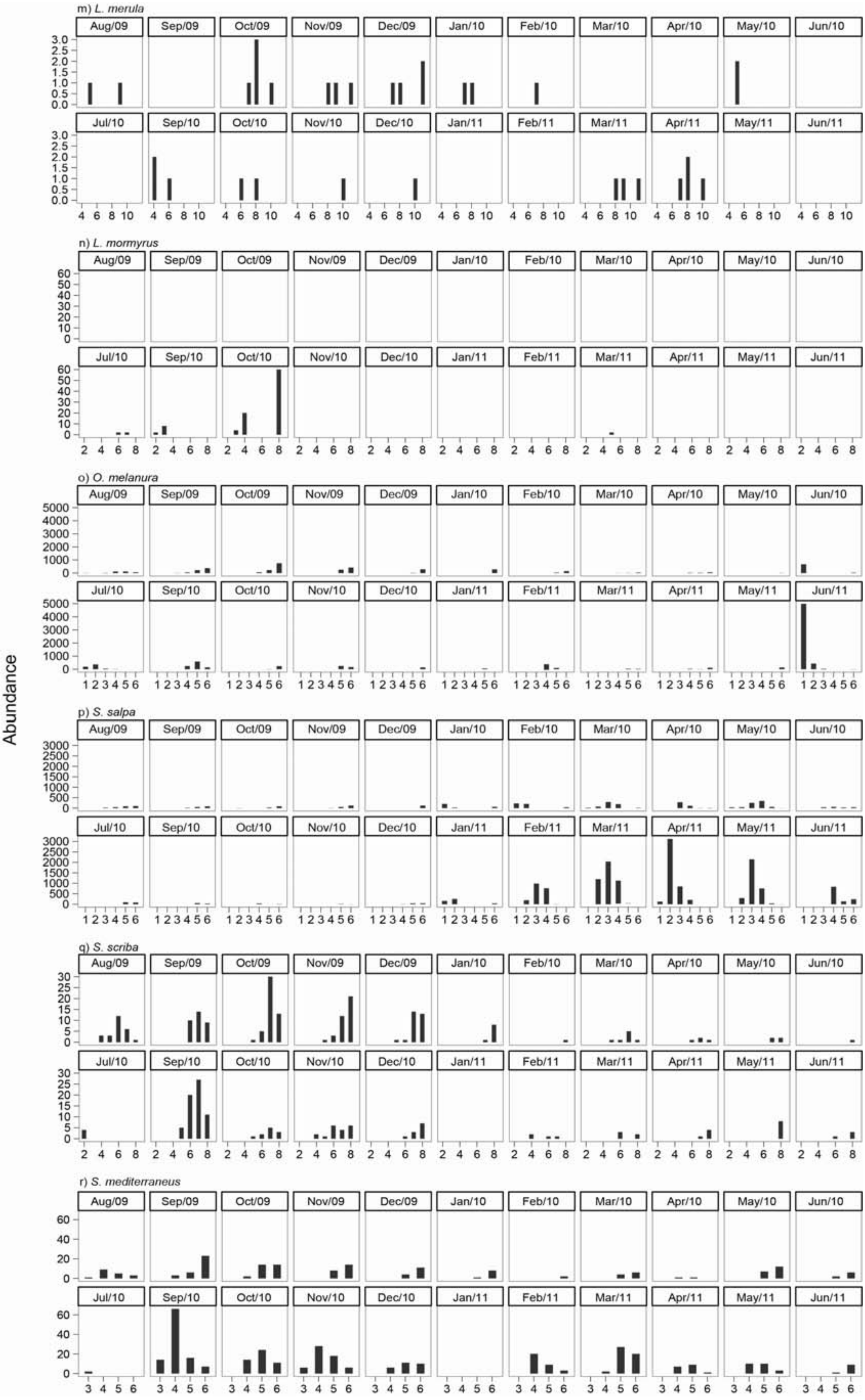
Appendix II



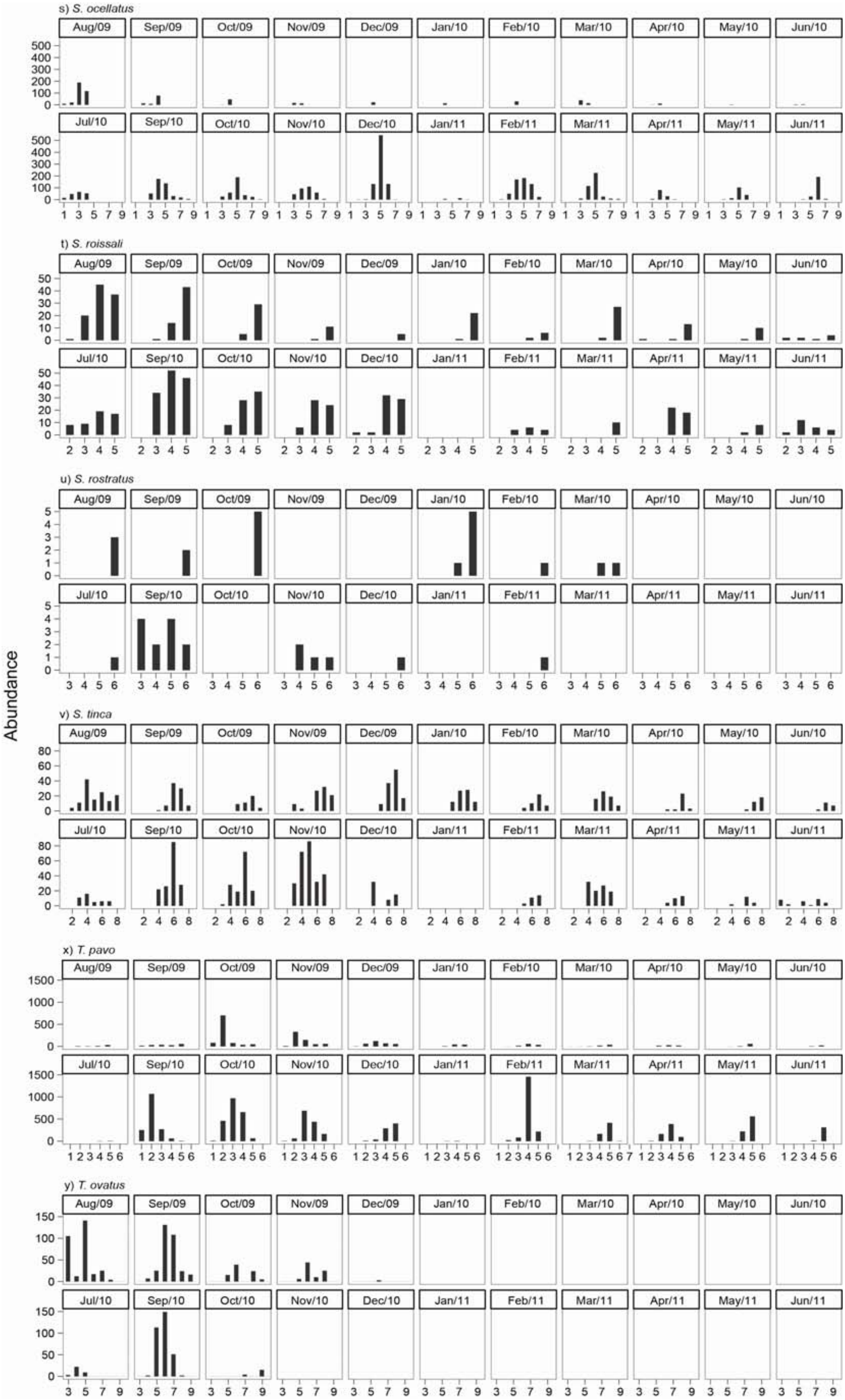
Appendix II Temporal variability of species size class. a) *Atherina* spp., b) *A. imberbis*, c) *B. boops*, d) *C. chromis*, e) *C. julis*, f) *D. dentex*.



Appendix II Temporal variability of species size class. g) *D. annularis*, h) *D. cervinus*, i) *D. puntazzo*, j) *D. sargus*, k) *D. vulgaris*, l) *G. semisquamatus*



Appendix II Temporal variability of species size class. m) *L. merula*, n) *L. mormyrus*, o) *O. melanura*, p) *S. salpa*, q) *S. scriba*, r) *S. mediterraneus*



Appendix II Temporal variability of species size class. s) *S. ocellatus*, t) *S. roissali*, u) *S. rostratus*, v) *S. tinca*, x) *T. pavo* and z) *T. ovatus*

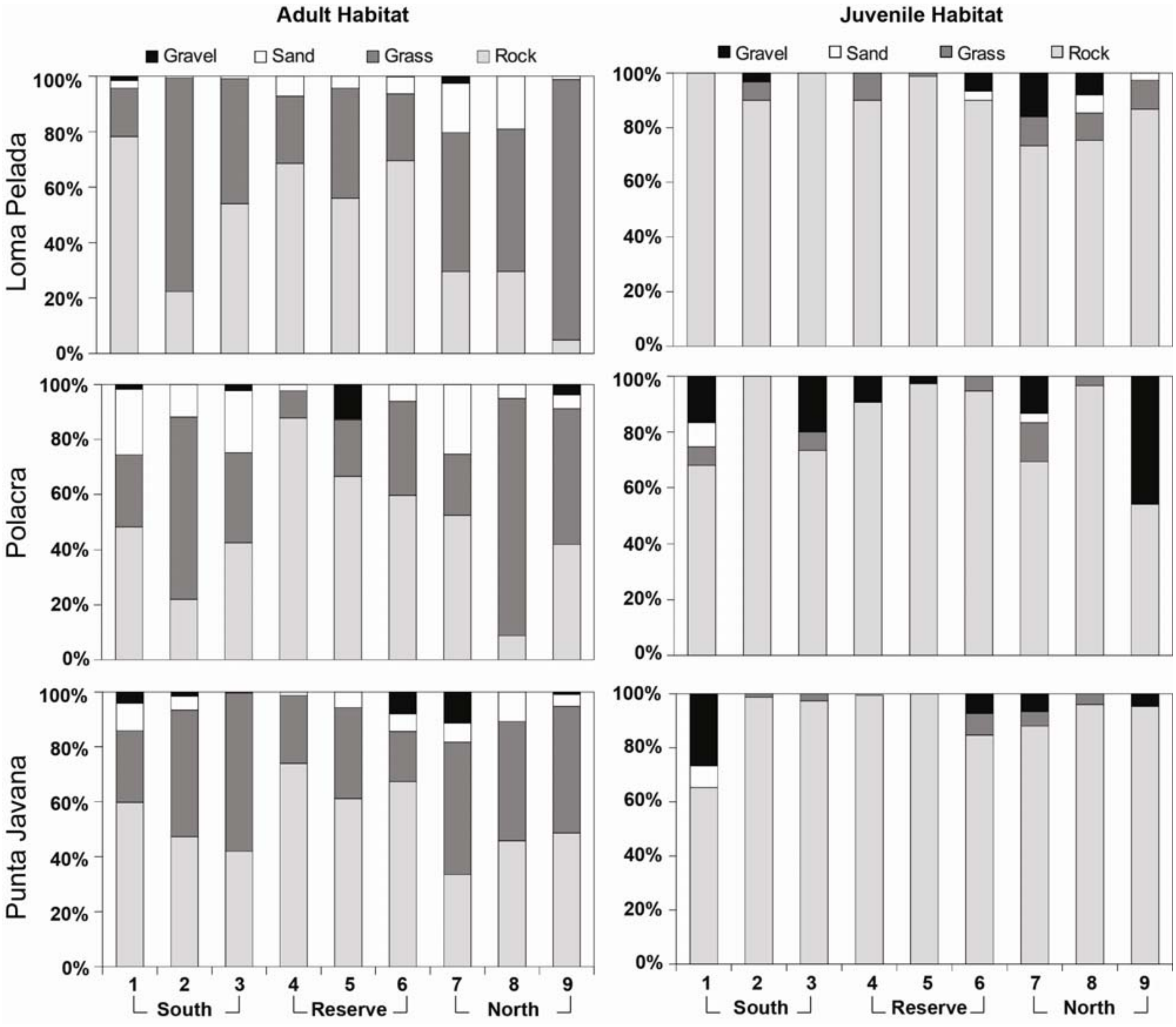
Appendix III

Appendix III. Results of best model selection for community descriptors (abundance, richness and Shannon-Wiener diversity) and fish species belonging to YOY and juvenile size stage with habitat categories ((PC1s for complexity – com, heterogeneity – het, and total cover – cov, and depth). AIC – Akaike information criteria value, w_i – Akaike weights, Intercept and ß coefficient – linear model. Symbols "+" and "-" indicate slope, "*" pelagic and rare species excluded.

Variables	**Best model**	**AIC**	**wi**	**intercept**	**ß cofficient**
Young-of-year					
Abundance	-com-het-depth	14743.574	0.369	32.656	-6.699 -8.867 -2.014
Abundance*	-het+depth	13812.212	0.298	12.682	-3.639+0.951
Richness	-het-cov	5850.016	0.204	2.327	-0.298-0.380
Richness*	-cov-het+depth	5603.009	0.233	1.669	-0.227-0.262+0.12
Diversity	-cov	3039.979	0.206	0.673	-0.165
Diversity*	-com-cov-depth	2181.944	0.406	0.356	-0.175-0.092+0.029
Atherina spp.	com+cov+depth	12135.383	0.301	5.518	3.281-2.364-0.964
B. boops	depth	10465.019	0.330	0.377	0.318
C. chromis	het+depth	12515.374	0.523	-1.562	-3.906+1.734
C. julis	depth	6988.283	0.330	-0.231	0.297
D. annularis	het+depth	5258.887	0.423	-0.045	0.4100+0.056
D. dentex	cov+depth	-42.188	0.277	-0.007	-0.032+0.012
D. puntazzo	het+depth	865.823	0.391	0.084	0.048-0.015
D. sargus	com+cov+depth	5989.709	0.533	0.834	-0.411-0.745-0.109
D. vulgaris	het+depth	7771.266	0.444	1.513	1.647-0.204
M. surmuletus	com+het+cov+depth	5479.676	0.328	0.358	-0.284+0.464+0.246-0.070
Mugilidae	het+depth	12466.520	0.479	12.441	-4.714-2.086
O. melanura	depth	11635.948	0.188	4.781	-0.428
Pagellus spp.	cov+het+depth	5530.312	0.393	0.316	0.350+0.214-0.063
S. maena	het+cov	7278.167	0.188	0.064	0.416+0.442
S. mediterraneus	cov+het+depth	2158.263	0.555	-0.060	0.401-0.142+0.055
S. ocelatus	cov+depth	7205.094	0.308	-0.072	0.754+0.200
S. roissali	com+het+depth	3566.520	0.441	0.345	-0.203-0.161-0.0291
S. rostratus	cov+depth	-1722.046	0.319	-0.004	-0.027+0.006
S. salpa	cov+het+depth	11032.806	0.323	5.994	1.848+1.272-1.080
S. scriba	cov+depth	1708.776	0.354	0.047	-0.072+0.029
S. viridensis	depth	1847.076	0.264	0.082	-0.0151
S. tinca	com	5645.377	0.242	0.558	-0.737
T. ovatus	cov+het+depth	8598.123	0.465	1.179	-0.972-0.657-0.188
T. pavo	com+het+cov+depth	8830.812	0.771	0.131	1.351-1.948+1.155+0.503
Juveniles					
Abundance	com-het+cov+depth	13813.579	0.375	13.080	5.006-6.849+4.636+1.577
Abundance*	com-het+cov+depth	1886.766	0.340	7.752	2.434
Richness	-het+depth	5706.253	0.325	1.533	-0.241+0.225
Richness*	-het+depth	5645.849	0.332	1.398	-0.219+0.241
Diversity	depth	2945.303	0.294	0.468	0.066

Variables	**Best model**	**AIC**	**wi**	**intercept**	**ß cofficient**
Juveniles					
Diversity*	depth	13437.039	0.747	0.315	-0.054-0.023-0.008+0.046
A. imberbis	depth	-6382.217	0.185	-0.001	0.0004
Atherina spp.	cov+depth	11551.045	0.270	4.760	-1.633-0.838
B. boops	het	8839.338	0.284	0.348	-0.610
C. chromis	com+het+cov+depth	12623.921	0.611	-0.036	3.835-3.944+6.927+2.404
C. julis	com+depth	6857.797	0.264	-0.117	-0.452+0.431
D. annularis	com+het+depth	2290.781	0.467	0.000	-0.060+0.249+0.031
D. puntazzo	het	-663.894	0.183	0.030	-0.012
D. sargus	com+het+cov	4926.245	0.403	0.402	-0.376-0.215-0.476
D. vulgaris	cov+depth	4254.715	0.364	0.092	-0.345+0.093
Mugilidae	com+depth	4084.021	0.239	0.217	-0.161-0.042
O. melanura	com+het+depth	10658.314	0.198	2.847	1.48-0.951-0.376
S. mediterraneus	cov+het+depth	-38.814	0.619	-0.029	0.134-0.044+0.022
S. ocelatus	depth	7375.819	0.326	0.066	0.181
S. roissali	com	2922.217	0.139	0.295	0.066
S. rostratus	cov+het+depth	-424.598	0.687	-0.010	-0.067+0.038+0.014
S. salpa	cov+het+depth	9741.328	0.342	2.896	-2.582-0.699-0.357
S. scriba	cov+depth	3099.424	0.287	0.010	0.098+0.023
S. tinca	depth	2634.391	0.232	0.088	0.021
T. pavo	com+het+cov+depth	7526.299	0.480	1.216	0.859-0.739+1.217-0.065

Appendix IV



Appendix IV Adult and juvenile habitat characterisitics within the three marine reserves and adjacent zones across Cabo de Gata Natural Park network.