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Recruitment patterns and processes of coastal fish species in a temperate rocky reef

Maria Klein

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Maria Klein

Thesis submitted for the degree of Doctor of Philosophy (Marine Sciences)

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Coordinators:
Dr. Rita Borges
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Dr. Jennifer Caselle

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Thesis title

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Declaration of authorship

This work has not previously been submitted for a degree in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

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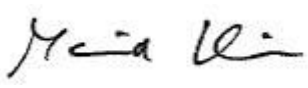

Maria Klein

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Short abstract

Recruitment of marine fish is composed of several steps, e.g. larval dispersal, metamorphosis to the juvenile stage, settlement and post-settlement. Each step is influenced by biotic and abiotic mechanisms, leading to a large variability of recruitment in space and time. Recruitment is a highly complex ecological process that has an essential impact on the demography, dynamics, persistence and overall genetic structure of a population and on the connectivity among populations. In this thesis, recruitment of a temperate nearshore fish assemblage was studied at various spatial and temporal scales by sampling fish with standardized collectors filled with artificial substrate. An analysis of recruitment patterns in relation to oceanographic processes and the study of early life history traits (ELHT) was undertaken. Additionally, population connectivity and larval dispersal of a common intertidal reef fish were investigated through an interdisciplinary approach by combining genetic tools with bio-physical modelling. The most abundant species sampled was a pelagic species, *Trachurus trachurus*, followed by various cryptobenthic and benthopelagic species. Recruitment varied highly between weeks within the summer recruitment season and between three consecutive years. Spatially, the recruitment pattern within the study area was highly patchy at a fine scale but less different at a larger scale. Recruitment patterns were related with environmental conditions. The weekly recruitment pattern of *T. trachurus* was related to the lunar cycle and the up-welling index. The inter-annual variation of the structure of the recruitment assemblage was related to the environmental variables up-welling, SST and surface current. Moreover, population differentiation and patterns of dispersal of the common intertidal reef fish *Lepadogaster lepadogaster* were highly variable between years and this could be explained by inter-annual variation in current patterns. These results highlight the important role of oceanographic conditions in mediating recruitment patterns and indicate the potential influence of recruitment on population dynamics and connectivity.

Long abstract

Recruitment of marine fish is composed of several steps, e.g. larval dispersal, metamorphosis to the juvenile stage, settlement and post-settlement. Each step is influenced by biotic and abiotic mechanisms, leading to a large variability of recruitment in space and time. Recruitment is a highly complex ecological process that has an essential impact on the demography, dynamics, persistence and overall genetic structure of a population and on the connectivity among populations. Regarding demersal and benthic fish species the dispersal of eggs and larvae describes the main form of gene flow among populations and facilitates connectivity among populations. Our level of knowledge on patterns of recruitment, dispersal and connectivity of fish differs between tropical and temperate marine systems, especially regarding the nearshore system. Most recruitment studies from temperate areas have analysed pelagic species and demersal species of sandy bottoms that are of commercial interest. While studies on recruitment patterns of fish inhabiting reef systems have been conducted mostly in tropical systems, in temperate areas such studies, in particular with a fine time scale are less common. One reason might be that traditional sampling methods (e.g. underwater visual census) are difficult to operate in the temperate nearshore due to harsher sea conditions (strong wind, waves, and currents and low visibility). Furthermore, several previous studies have highlighted the lack of knowledge on larval dispersal and population connectivity for temperate demersal fish. Therefore, the objectives of this thesis were i) to study temporal and spatial patterns of recruitment of rocky reef associated and coastal fish species at a nearshore rocky reef; ii) to analyse the influence of environmental processes on temperate fish recruitment at a small within season and a larger inter-annual scale; iii) to describe early life history traits that can be relevant to recruitment and iv) to estimate larval dispersal and population connectivity of an intertidal rocky reef fish species along the south west Iberian Peninsula. In this thesis, the main study area was the Arrábida Marina Park at the dynamic west coast of Portugal which is part of the Iberian Peninsula Upwelling System. Here, the recruitment of a temperate nearshore fish assemblage was studied at various spatial and temporal scales by sampling fish with standardized collectors filled with artificial substrate. This included the analysis of recruitment patterns in relation with oceanographic processes through generalized additive modelling and multivariate analysis and the study of early life history traits (ELHT). Moreover, population connectivity and larval dispersal of a common intertidal reef fish were investigated through an interdisciplinary approach combining indirect and direct genetic tools with bio-physical modelling. Standardized

recruitment collectors filled with plastic substrate and installed in the water column (SMURFs), performed better than other types investigated, mainly due to easier handling and replicability. This collector type was then used throughout the thesis and for the first time this type of recruit collector, registered as well the recruitment of a pelagic fish species, *Trachurus trachurus*. This was the most abundant species, followed by the benthopelagic *Serranus hepatus* and various other cryptobenthic and benthopelagic species. At a temporal scale, recruitment varied highly between weeks within the summer recruitment season and between three consecutive years of monitoring. Temporal variation of recruitment was found at a species level, but also on the structure of the recruitment assemblage and on the composition of biogeographic species groups. More temperate fish were collected in 2012 than in 2011 and 2013. On the other hand more warm-temperate fish were found in 2013. Within the study area, the recruitment pattern was highly patchy at a fine spatial scale (~20 m) but less different at a larger scale (1-10 km). Recruitment patterns were related with environmental conditions. The inter-annual variation of the structure of the recruitment assemblage was related to the environmental variables up-welling, SST and surface current. But, the interaction between abiotic factors and recruitment differed among species. Highest abundance of *T. trachurus* recruits was registered in 2011 and in this year recruitment peaks of *T. trachurus* occurred around the new moon phase. Additionally, recruitment was negatively correlated with upwelling. Four hatching cohorts were estimated through back-calculation of the hatching date and individuals from these cohorts differed regarding ELHTS. For example, the first cohort had a faster early fish growth. The larval dispersal and population connectivity study of this thesis indicated highly connected populations and potentially long-distance larval dispersal of the common rocky intertidal fish, *Lepadogaster lepadogaster*, despite its short pelagic larval duration. Still, the dispersal model revealed higher probabilities for larval retention and exponentially declining probabilities of dispersal with distance and the parentage analysis resulted in self-recruitment and short larval dispersal. These results indicate that both local recruitment and long-distance dispersal can play an important role in the replenishment of a population. Moreover, the population differentiation and patterns of larval dispersal of *L. lepadogaster* were highly variable between years and this could be explained by inter-annual variation in current patterns. In summary, in this thesis settlement (reef fish), nearshore recruitment (*T. trachurus*) and larval dispersal (*L. lepadogaster*), which can contribute to the overall patterns of recruitment of fish populations, exhibited clear temporal patterns. Furthermore, various interactions of these processes with abiotic factors were found, which highlight the important role of

oceanographic processes in mediating patterns of recruitment and larval dispersal. Results are discussed in relation to variation of the adult's reef fish assemblage of the Arrábida Marine Park and why the gained understanding is important for fishery and conservation management. Since knowledge on coastal fish recruitment, population connectivity and larval dispersal is available mostly for tropical coral reef systems, findings of this thesis can help to broaden our understanding of patterns and processes in temperate reef systems. Overall, this thesis demonstrates that a multidisciplinary study approach, combining ecology, oceanography and genetics, is necessary to improve our understanding of the highly complex, interactive process of recruitment.

Key words: recruitment, larval dispersal, population connectivity, coastal fish, rocky reef, oceanography

Resumo

O recrutamento dos peixes marinhos depende de várias fases e processos como a dispersão larvar, a metamorfose do estado larvar para juvenil, o assentamento para o habitat bentónico ou processos pós-assentamento. Cada uma destas etapas é influenciada por mecanismos bióticos e abióticos, podendo o recrutamento apresentar uma elevada variação espácio-temporal. É um processo ecologicamente complexo, com um impacto fundamental na demografia, dinâmica, persistência, conectividade e estrutura genética das populações. Em espécies de peixes bentónicos, a dispersão de ovos e larvas é o principal processo que potencialmente permite o fluxo genético e a conectividade entre populações. O conhecimento geral acerca dos padrões de recrutamento, dispersão e conectividade de espécies de peixes que vivem associadas a recifes costeiros difere entre sistemas tropicais e temperados; nestes últimos, a maioria dos estudos sobre recrutamento centra-se em espécies pelágicas e demersais de substratos arenosos com valor comercial, enquanto que nas regiões tropicais a maioria dos estudos centra-se em sistemas de recife e geralmente numa escala temporal mais fina. Os métodos de amostragem tradicionais (censos visuais), são difíceis de replicar em regiões temperadas devido às condições mais adversas destes sistemas junto à costa (vento, correntes e ondulação fortes e baixa visibilidade). Adicionalmente, há poucos trabalhos sobre processos de dispersão larvar e conectividade entre populações de peixe demersais nestes sistemas. Esta tese tem como objetivos: i) o estudo de padrões espácio-temporais de recrutamento de espécies de peixe costeiras e associadas a recifes rochosos; ii) a análise da possível influência de processos ambientais na variação temporal do recrutamento intrasazonal e interanual; iii) descrever características dos estados iniciais de desenvolvimento relevantes para o recrutamento; iv) estimar a dispersão larvar e conectividade entre populações de espécies de peixe dos recifes rochosos do intertidal, ao longo do sudoeste da Península Ibérica.

A principal área de estudo foi o Parque Marinho Professor Luiz Saldanha (costa oeste de Portugal), que pertence ao sistema de "Upwelling" da Península Ibérica. Estudou-se o recrutamento de peixes costeiros junto à costa, a várias escalas espácio-temporais, através de amostragem com coletores estandardizados com substratos artificiais (SMURFS). Analisou-se a relação entre os padrões de recrutamento e os processos oceanográficos através de modelos aditivos generalizados, análise multivariada e estudo das características dos estados iniciais (ELHT). Para além disto, investigaram-se os padrões de conectividade entre populações e de dispersão larvar de uma espécie comum no intertidal rochoso, através

de uma abordagem interdisciplinar combinando ferramentas genéticas diretas e indiretas com modelação biofísica. Os SMURFS testados com substrato plástico no seu interior, apresentaram maior facilidade de manuseamento e replicabilidade do que os SMURFS com outros materiais testados, pelo que foram utilizados como método principal. Este trabalho permitiu, pela primeira vez a monitorização do recrutamento de uma espécie de peixe pelágica com SMURFS, Trachurus trachurus (carapau); foi a espécie mais abundante ao longo de todo o estudo seguida da espécie bentopelágica Serranus hepatus e de outras espécies criptobênticas e bentopelágicas. O recrutamento apresentou uma variação elevada entre semanas e entre os três anos consecutivos de monitorização. Verificou-se igualmente uma variação temporal no recrutamento ao nível da espécie, da estrutura da assembleia de recrutas e nos grupos de espécies de acordo com a composição biogeográfica. Em 2012, registaram-se mais espécies de climas temperados do que em 2011 e 2013, sendo que em 2013 registaram-se mais espécies de climas temperados quentes. Quanto à variação espacial, o padrão de recrutamento foi altamente irregular a uma pequena escala mas pouco variável quando analisado a uma escala mais alargada. A variação interanual da estrutura da assembleia de recrutamento coincidiu com a variação dos padrões de "upwelling", temperatura e correntes superficiais. A interação entre fatores abióticos e recrutamento foi também distinta entre diferentes espécies. Em 2011, registou-se a maior abundância de recrutas de carapau, durante as fases de lua nova; o recrutamento desta espécie apresentou uma correlação negativa com o "upwelling". Foram estimadas quatro coortes à eclosão através de retrocálculo da data de eclosão, sendo que os indivíduos da primeira coorte apresentavam características (ELHT) distintas, como por exemplo um crescimento larvar mais rápido, do que os indivíduos das restantes coortes. O estudo da dispersão larvar e conectividade entre populações da espécie Lepadogaster lepadogaster revelou a existência de populações com elevado nível de conectividade e um potencial de dispersão larvar de longa distância, apesar da sua curta duração larvar pelágica. Apesar disto, o modelo de dispersão revelou uma probabilidade alta de ocorrência de retenção larvar e probabilidade exponencialmente decrescente de dispersão com o aumento da distância; do mesmo modo, os resultados da análise parental indicaram a existência de retenção larvar, com baixa dispersão. Assim sendo, tanto o recrutamento local como a dispersão de longa distância podem ter um papel importante na sustentabilidade destas populações. Contudo, a diferenciação de populações e padrões de dispersão larvar de L. lepadogaster apresentaram uma variação elevada entre anos, o que pode ser explicado pela variação interanual dos padrões das correntes. Em resumo, foram encontrados padrões temporais evidentes nos

processos de assentamento (nos peixes de recife), recrutamento (carapau) e dispersão larvar (L.lepadogaster), que podem contribuir para os padrões gerais de recrutamento das populações de peixe. Adicionalmente, as diferentes interações destes processos com os fatores abióticos encontradas, indicam uma importante influência das condições oceanográficas nos padrões de recrutamento e dispersão larvar. Os resultados são discutidos em relação à informação disponível sobre as assembleias de espécies que vivem associadas aos recifes na área de estudo e em relação à sua importância para o delineamento de estratégias de gestão de pesca e de conservação. Os resultados desta tese contribuem para aumentar o conhecimento e compreensão de padrões e processos que influenciam o recrutamento de peixes associados a recifes em regiões temperadas, uma vez que a informação disponível sobre os padrões de recrutamento costeiro, conectividade entre populações e dispersão larvar de espécies neste tipo de ambientes é menor do que a exsitente para recifes tropicais. Esta tese demostra a importância de uma abordagem interdisciplinar que combine aspectos da ecologia, oceanografia e genética, para aumentar a compreensão da complexidade e interacções ubjacentes aos processos de recrutameto dos peixes costeiros.

Palavras-chave: Recrutamento, dispersão larvar, conectividade, oceanografia, peixes costeiros, recife rochoso

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Chapter 1
General Introduction

1.1 Fish life cycle

The vast majority of marine fishes have a complex life cycle, in which the early life stages live in a different habitat than the adults. The life cycle is comprised of the following general developmental phases (Figure 1.1): embryo, larva, juvenile and adult. Eggs are released into the pelagic environment (pelagic eggs hereafter) or attached to demersal substrates (demersal eggs hereafter). Hatched larvae of most fish species develop and disperse in the water column until they metamorphose to the juvenile stage and recruit to the adult population (Figure 1.2).

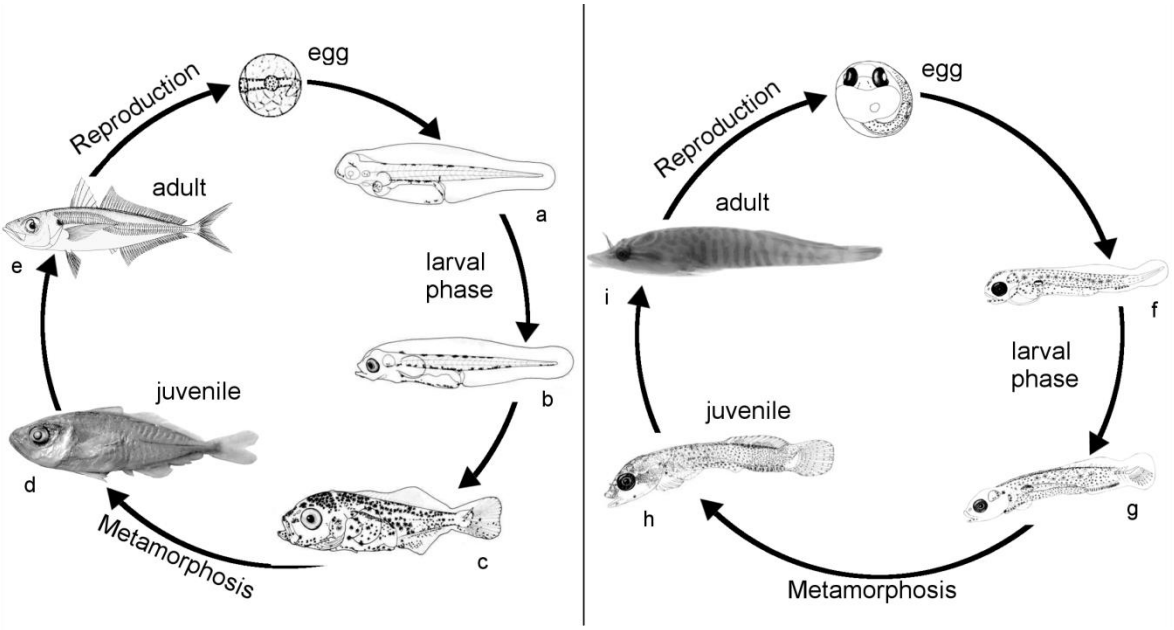

Figure 1.1 Life cycle of a pelagic (*Trachurus trachurus*) and a benthic fish (*Lepadogaster lepadogaster*), showing different developmental stages. Length of fish are 2.9 mm (a), 3.6 mm (b), 8.9 mm (c), ca. 25 mm (d), 15-40 mm (e; common adult fish range; Smith-Vaniz, 1986) for *T. trachurus* and 5.0-5.3 mm (f), 7.1-7.3 mm (g), 8.4–9.3 mm (h), <72 mm (i; max. length of adult fish, Hofrichter, 1995) for *L. lepadogaster*. Source of images: a-c (Brownell, 1979), d (chapter 4), e (www.fao.org), f-h (Tojeira *et al.*, 2012), and i (by P. Coelho).

The metamorphosis from the larval to the juvenile/adult stage often is accompanied by a transition in habitats such as from pelagic to benthic (settlement; Figure 1.2), offshore to nearshore (ca. 0 to 30 m water depth; Pineda, 2000) and/or marine to estuarine/riverine. This requires that the larvae reach and adapt to different water environments; for some species, ontogenetic transitions can occur between several environments before arriving to the adult habitat (e.g. Eggleston, 1995, Nagelkerken *et al.*, 2001, Chin *et al.*, 2013).

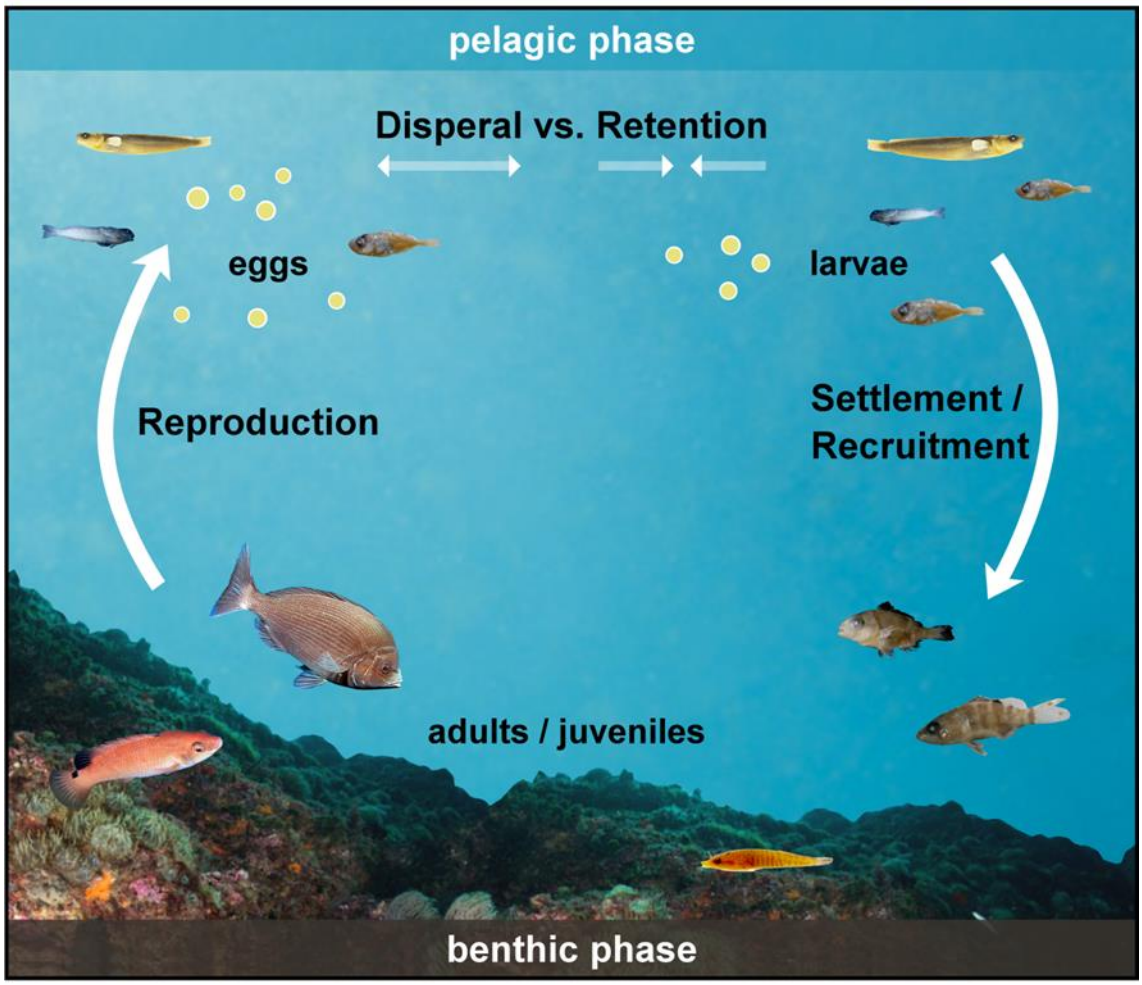

Figure 1.2 Reef fish life cycle. Different temperate fish species of the study area are presented.

The duration of the pelagic larval stage (pelagic larval duration, PLD) is species-specific and may last from days to months (Victor, 1986, Wellington and Victor, 1989, Macpherson and Raventós, 2006, Beldade *et al.*, 2007). For example some damselfishes (Pomacentridae) have a PLD of just a week (Thresher *et al.*, 1989a), but some porcupine fishes (Diodontidae) may stay in the pelagic stage for more than 64 weeks (Ogden and Quinn, 1984). Few species have been found that are lacking a pelagic stage (Leis and McCormick, 2002).

Eggs and larvae are typically very small (usually only few millimetres long) and more vulnerable to environmental processes than juvenile or adult fish. Hence mortality is in general very high during the larval stage (Houde and Zastrow, 1993, Ehrich *et al.*, 2007). Sources of mortality during these early stages include starvation, predation, parasitism and diseases (Keough and Swearer, 2007).

1.2 Recruitment definition

The term recruitment generally describes the addition of new fish to a population. It encompasses a range of phases and processes such as reproduction, the pelagic phase, larval mortality, transition to the juvenile stage and, in the case of reef fish, the settlement to adult habitat and post-settlement processes. Depending on which process is of interest and the type of sampling method used in a study, the designation "recruits" sometimes considers late stage larvae, new-settlers, post-settlers or juveniles. Using settlement interchangeably with recruitment can lead to a strong overestimation, as mortality during the post-settlement phase can be very high (Doherty *et al.*, 2004, Planes *et al.*, 2009b).

Moreover, fishery scientists often define recruits as young fish, which have attained a target size relevant to the fishery and in this case recruitment is often studied at a yearly scale. Furthermore, population modellers might consider as recruits only fish that have attained reproductive age and size. It is therefore important to describe in each study which type of process is being considered. Different biotic and abiotic factors may influence the multiple developmental stages and processes of the early life, making the process of recruitment highly complex and variable in space and time.

1.3 Recruitment hypotheses

Early in the 20th century fishery scientists began to relate fluctuations in the abundance of adult fish in a given year class (i.e. recruitment to the fishery) to processes happening during the early life stage (Hjort, 1914, Cushing, 1974). Since then, several hypotheses have been postulated to explain recruitment variability by linking larval fish survival with physical and biological processes during the larval stage (Keough and Swearer, 2007, Houde, 2008). The main recruitment hypotheses are detailed below:

"**Critical Period**" (Hjort, 1914): In most marine fish species larvae hatch with a yolk sack as an energy reserve and enter a critical period, from the yolk absorption until the first feeding. Hjort (1914) proposed that food availability during this critical period of first feeding would determine recruitment and year-class success.

"**Aberrant Drift**" (Hjort, 1914): In a hypothesis regarding hydrodynamics, Hjort (1914) suggested that larval survival would depend on ocean currents causing success or failure to reach favourable habitats at the end of the larval period (Doherty and Williams, 1988, Houde, 2008).

"**Match-Mismatch**" (Cushing, 1974): The critical period hypothesis was further refined by (Cushing, 1990), who suggested that a timing of the peak in fish larval abundance with a zooplankton bloom (larval food) would be critical for the survival of fish larvae. A mismatch between fish larval abundance and its food would lead to high larval mortality and thus low recruitment. This hypothesis found support particularly in temperate seas, where phyto- and zooplankton production are mostly seasonal, affecting the recruitment of fishes such as e.g. Atlantic cod (Ellertsen *et al.*, 1989) or Scott's weedfish (Thresher *et al.*, 1989b).

"**Stable Ocean**" (Lasker, 1978): Based on studies on the Northern anchovy (*Engraulis mordax*) Lasker (1978), (1981) proposed that under calm ocean conditions the water column becomes vertically stratified, which results in the aggregation of fish larvae and their prey at layer interfaces (e.g. thermoclines). This condition would increase larval survival, whereas turbulent water, due to for example upwelling or storms, could lead to high larval mortality (Lasker, 1981).

"**Optimal Environmental Window**" (Cury and Roy, 1989): Cury and Roy (1989) developed the stable ocean hypothesis further and suggested that in upwelling regions recruitment potential is dome-shaped and is highest at intermediate upwelling conditions. At intermediate levels of upwelling turbulent mixing is high enough to facilitate encounter of fish larvae with prey items and loss through offshore water advections is moderate. Several studies on small pelagic fish in upwelling areas have supported this hypothesis (Roy *et al.*, 1995, Waldron *et al.*, 1997, Serra *et al.*, 1998).

"**Larval Retention/Membership-Vagrancy**" (Sinclair, 1988): Building on the aberrant hypothesis of Hjort (1914) which suggested that recruitment variability might be as well a result of dispersal, Sinclair (1988) proposed the larval retention/membership-vagrancy hypothesis. By studying herring populations in the North Sea, he found that population size depended on the size of the retention area for the larvae. This means high recruitment would happen, when a large proportion of spawned larvae would be retained near the spawning

site, while low recruitment would occur in areas of limited retention with larvae being transported away from natal populations.

The above hypotheses focused on starvation and dispersal as causes of recruitment variability. However, predation can also be a major driver of larval mortality and thus an important factor controlling recruitment (Lasker, 1981, Bailey and Houde, 1989). Predation on fish early life stages is mostly size specific (Bailey and Houde, 1989). Therefore, the time needed to reach a size that is too big for potential predators and of larval development until the end of the planktonic phase can strongly affect recruitment success (Miller *et al.*, 1988, Leggett and Deblois, 1994).

"**Stage Duration**" (Houde, 1987) and "**Bigger is Better**" (Anderson, 1988): The stage duration and bigger is better hypotheses relate to two factors: food dependency and predation. These hypotheses state that fast growing larvae (shorter planktonic stage) and bigger larvae have a higher potential of escaping predation and getting food, thus have a higher rate of survival and recruitment success (e.g. Houde, 1987, Anderson, 1988). These hypotheses are often combined in the literature and called the "growth-mortality hypothesis".

"**Ocean Triad**" (Bakun, 1996): More recent approaches look not just at a single mechanism but rather at the interaction of several processes and their consequences on recruitment. With the ocean triad hypothesis, Bakun (1996) proposed that the combination of three important processes create a high potential of recruitment success: fish larval food production (e.g. upwelling), accumulation of food particles (e.g. convergences, fronts, water column stability) and transport to or retention within favourable habitat (reduced off-shore advection).

The above-mentioned hypotheses and early research on fish recruitment, derived from Hjort's hypothesis, have dealt mainly with pelagic commercially important fish species from temperate areas. In reef fish species the metamorphosis from the larval to the juvenile/adult stage usually occurs with the settlement to a demersal habitat e.g. a rocky or coral reef. Therefore, recruitment studies conducted in coral reef systems included the additional factor of the process of settlement and two major recruitment hypotheses were developed:

"**Lottery for living space**" (Sale, 1978, 1991): Observations of territorial damselfishes in the Great Barrier Reef revealed that coexisting species compete continuously for living space, but that this competition does not lead to niche-diversification and competitive exclusion (Sale, 1978, 1991). Sale's Lottery hypothesis postulated that randomly vacated living space is refilled quickly and randomly by recruits or adult fish. Coexisting species are all equally capable of invading vacated space and these interspecific lotteries may explain high local species richness. Due to the limitation and unpredictability of living space the optimal reproductive strategy of a reef fish would be to stay put in the gained space, breed often, and disperse numerous larvae in space and time in order to enhance the probability of getting offspring into suitable living sites as they get available (Sale, 1978, 1991). Thus, the lottery hypothesis relies on post settlement density-dependent mechanisms.

"Recruitment Limitation Hypothesis" (Doherty, 1981): In contrast to the "Lottery hypothesis", Doherty (1981) postulated that larval mortality would be very high and thus the number of settlers too low, for competition and other density-dependent processes to be relevant in regulating recruitment of reef fish. In that case, growth, mortality, settlement and reproduction would not depend on the local population size and populations would be limited by recruitment. A positive relationship between larval supply, generally measured as the abundance of late stage larval fish (i.e. pre-settlement), and recruitment has been found in several studies, giving support to the "Recruitment Limitation Hypothesis" (e.g. Milicich *et al.*, 1992, Hamer and Jenkins, 1996, Grorud-Colvert and Sponaugle, 2009).

The relative importance of post-settlement density dependent processes versus recruitment limitation in structuring reef fish populations was a huge debate (Hixon, 2011). Later it was accepted that in reef systems both pre- and/or post-settlement processes can operate and determine recruitment success (e.g. Doherty and Williams, 1988, Jones, 1991, Jenkins *et al.*, 1997, Doherty, 2002).

"**Settlement-linkage**" (Robertson *et al*., 1988): Regarding recruitment of reef fish, another hypothesis was suggested by Robertson *et al.* (1988, 1990), which argues that fish with a fixed larval duration and a settlement preference for specific time periods (e.g. lunar phases), are adapted to spawn at a time that allows most larvae to be competent at the favoured settlement time.

1.4 Recruitment and biotic factors

Recruitment of fish can vary on scales of days to years (e.g. Robertson *et al.*, 1999, Findlay and Allen, 2002, Lozano and Zapata, 2003, Vallès *et al.*, 2008, Fontes *et al.*, 2009a). Some of this temporal variability can be explained by spawning patterns (Robertson *et al.*, 1988, Meekan *et al.*, 1993, McIlwain, 2002), but most studies found no clear relationship between offspring production and recruitment (e.g. Robertson *et al.*, 1993, Srinivasan and Jones, 2006). As such, we can assume that biotic and abiotic processes affecting the pelagic, settlement and post-settlement phases can cause a great part of the temporal and also spatial variability in recruitment. The number of studies that have analyzed the relationship of biological and physical processes with recruitment is very high, thus examples given here are limited to coastal and reef fish species (the focus of this study), whose recruitment includes a settlement and post-settlement phase.

Biotic factors/processes affecting recruitment can be divided into three groups based on the period of life history affected: a) those related to the adult population, b) those influencing the larval stage from hatching until settlement (benthic fish) or recruitment (pelagic fish) and c) those affecting post-settlement processes of benthic fish.

The condition of parent fish may affect the performance of the early life stages and thus influence recruitment success. For example, the study of Green and McCormick (2005) on a tropical clownfish revealed that larval size at metamorphosis was related to **paternal and maternal conditions** and that larval growth to metamorphosis was affected mostly by the paternal condition. Raventos and Planes (2008) studied a temperate reef fish and observed that larger females produced larger eggs and larvae; from this, these authors concluded that larger females might enhance larval survival and thus recruitment success.

Well studied is the relationship between **early life history traits** (ELHT) such as size at hatching, larval growth and larval stage duration with recruitment success. For example Bergenius *et al.* (2002) found that larval **growth** 1-2 weeks after hatching was positively correlated with settlement and recruitment. Wilson and Meekan (2002) found that cohorts of faster growing larvae can have higher larval survival and can lead to higher levels of settlement. A positive correlation between larval growth and post-larval abundance was observed as well for a temperate reef fish (Jenkins and King, 2006). More studies exist that

found that larvae with bigger **size at hatching**, faster larval growth and shorter larval duration had higher survival of the early juvenile stages (e.g. Hare and Cowen, 1997, Searcy and Sponaugle, 2001, Vigliola and Meekan, 2002, Johnson, 2008, Samhouri *et al.*, 2009, Johnson *et al.*, 2012), providing support for the "**growth-mortality hypothesis**" (sensu Anderson, 1988; see section 1.2).

On the contrary, other studies show that bigger and faster-growing larvae can have a higher risk of predation than smaller, slow-growing ones (Sponaugle *et al.*, 2011). The study of Raventós and Macpherson (2005a) revealed that post-settlement survival (considered as recruitment success) was not always related to larval growth, nor to pelagic larval duration or size at settlement; only size at hatching was found to positively affect survival. Patterns of **selective mortality** can differ among species (D'Alessandro *et al.*, 2013), among different ontogenetic stages (Gagliano *et al.*, 2007, Murphy *et al.*, 2014), among populations of the same species (Searcy and Sponaugle, 2001) and among years with different levels of recruitment (Murphy *et al.*, 2014). The study of Murphy *et al.* (2014) stresses the importance of sampling the entire range of ontogenetic stages to obtain a complete view on how larval traits may be related to recruitment.

During the **settlement phase** some fish can return to the pelagic zone and search for better habitat and studies found that the settlement phase can extend from hours to weeks (delay of settlement; Kaufman *et al.*, 1992, McCormick and Makey, 1997). Furthermore, several studies have found that fish can delay metamorphosis to the juvenile stage, extending the competency period, which allows the search for adequate, unsaturated habitat, and/or favoured environmental conditions (Sponaugle and Cowen, 1994, McCormick, 1999, Schmitt and Holbrook, 1999, Findlay and Allen, 2002, Victor, 2007). Metamorphosis may happen before, during, or after the settlement phase (Kaufman *et al.*, 1992).

Both the settlement and **post-settlement phase** are related to a high rate of mortality (e.g. Caselle, 1999, Almany, 2004a, Doherty *et al.*, 2004, Planes *et al.*, 2009b), which is mainly caused by **predation**, especially within the first 48 hours of settlement (Taylor, 1990, Steele, 1997, Steele and Forrester, 2002, Webster, 2002, Holbrook and Schmitt, 2003, Almany and Webster, 2004, Doherty *et al.*, 2004). The presence of resident predators (including juveniles of predatory fishes that had already settled) reduced recruitment of fish studied (Shulman *et al.*, 1983, Webster, 2002, Almany, 2004b). Further, density-dependent processes such as

competition can also influence recruitment (Jones, 1991, Hixon and Jones, 2005). Competition for space, shelter and food can lead to the lower condition of individuals and thus result in higher mortality because of predation (Hixon and Menge, 1991, Holbrook and Schmitt, 2002, Almany, 2003, Almany, 2004a, Samhouri *et al.*, 2009).

However, the presence of older conspecifics (adult cues) in a reef can as well be used as a cue for fish to settle into that habitat (Steele, 1997). In a field experiment, Caselle (1999) studied the relationship between post-settlement mortality and recruitment density. The main findings showed that mortality correlated positively with recruit density and that the degree of this relationship varied strongly among sites; in few sites mortality was independent of recruit-density, since recruitment was too low. This study showed that even along the coastline of one island, populations can be recruitment-limited or affected by density-dependent post-settlement processes.

ELHTs such as larval growth and size at hatching mentioned in this section are key to understand recruitment patterns in marine fish. ELHTs can be studied through microstructure analysis of fish otoliths. Otoliths or "ear bones" are calcified structures in the head of the fish that assist in detecting sound and are used for balance and orientation (Campana and Neilson, 1985, Popper *et al.*, 2003). Otoliths grow along with the growth of a fish and thus can serve as a chronological recorder. With the finding of daily deposited increments in some fish otoliths by Pannella (1971), studies on early life history traits of fish have increased greatly. Among the ELHTs that can be investigated from otoliths, are patterns of early growth (e.g. Searcy and Sponaugle, 2000, Bergenius *et al.*, 2005, Fontes *et al.*, 2011), ontogenetic and habitat transition (Wilson and McCormick, 1999, Morales-Nin, 2000, Raventós and Macpherson, 2001), pelagic larval duration (Victor, 1986, Raventós and Macpherson, 2001), size at hatching and settlement (Vigliola and Meekan, 2002) and spawning or hatching patterns based on back-calculations (Xie and Watanabe, 2005, Palacios-Fuentes *et al.*, 2014).

1.5 Recruitment and abiotic factors

Environmental conditions such as oceanographic processes and habitat characteristics can greatly influence fish recruitment by affecting larval dispersal, larval condition, growth and survival and by determining settlement to the habitat. Relationships found between recruitment and physical factors often depend on the temporal and spatial scale examined. Most of the examples discussed below analysed recruitment as the delivery of pre-settlement stages, collected on artificial substrates or using light traps, or as abundance of settlers measured with underwater visual census (UVC).

In a study of diel patterns of reef fish settlement by Shenker *et al.* (1993) and Kingsford (2001) they found that significantly more fish settled during the night than during the day. At a daily to weekly time scale, many studies found strong relationships between recruitment and the **lunar cycle** (Victor, 1982, Robertson *et al.*, 1988, Robertson, 1992, Thorrold *et al.*, 1994, Sponaugle and Cowen, 1996a, Lozano and Zapata, 2003) and sometimes also with the tidal cycle (Sponaugle and Cowen, 1996b, Findlay and Allen, 2002, Sponaugle and Pinkard, 2004, D'Alessandro *et al.*, 2007, Vallès *et al.*, 2009, Sim-Smith *et al.*, 2013). Peaks of recruitment often coincided with the darker half of the lunar cycle (third quarter and New moon; Robertson *et al.*, 1988, Thorrold *et al.*, 1994, Sponaugle and Cowen, 1996a, b, Lozano and Zapata, 2003), but semi-lunar settlement has been also recorded (Thorrold *et al.*, 1994, Findlay and Allen, 2002, Lozano and Zapata, 2003). Settlement of the temperate reef fish *Paralabrax clathratus* correlated positively with **tidal amplitude** and negatively with sea surface temperature and Findlay and Allen (2002) suggested that internal tidal bores likely transport fish to the shore. Further correlations at fine time scales were found with water temperature and on-shore winds (Thorrold *et al.*, 1994, Raventos and Macpherson, 2005b, Lemberget *et al.*, 2009, Sim-Smith *et al.*, 2013).

Wind can relate to recruitment because of its influence on larval transport to the adult habitat (Lemberget *et al.*, 2009, Sim-Smith *et al.*, 2013) or due to wind-induced turbulence that can influence feeding conditions and thus larval survival (Cury and Roy, 1989, Lemberget *et al.*, 2009). Raventos and Macpherson (2005) found only a weak correlation between wind and settlement patterns and the pelagic larval period and these authors suggested that calm weather conditions during this period would commonly enhance settlement success. A relationship between recruitment and wind was found at an annual scale. Milicich (1994)

found at Lizard Island, that larval supply of reef fishes was positively correlated with the frequency of on-shore winds at the island's back reef habitats, but not at its fore reef habitats. A simple oceanographic proxy such as an **upwelling** index was found to reasonably explain annual nearshore settlement of rockfishes of the genus *Sebastes* (Caselle *et al.*, 2010a); these authors indicated the usefulness of such proxies for predicting annual recruitment.

Common, large-scale oceanographic processes such as tidally forced **internal waves** were found to aggregate fish in resulting slicks and potentially promote on-shore transport of pre-settlement fish (Kingsford and Choat, 1986). In addition, rare oceanographic events such as cyclones and other type of **storms** can lead to strong waves and currents and might explain mass-settlement events of, for example, the grouper species *Epinephelus merra* (Chabanet *et al.*, 2004) and *E. striatus* (Shenker *et al.*, 1993).

Different oceanographic features are predicted to influence recruitment at different spatial and temporal scales, even for a single species. For example, Caselle *et al.* (2010b) carried out an extensive study to understand the relationship of rockfish (genus *Sebastes*) settlement with physical processes acting at various temporal and spatial scales. The study revealed that at a small/local scale, wind stress during the arrival time of fish to the habitat (short time lag), and at a regional scale upwelling and downwelling processes operating during the early larval phase (long time lag) contributed to the variation of rocky fish settlement.

Environmental factors can also act on larval traits during the pelagic stage and by this can influence larval survival and recruitment, as described in the section 1.4. For example, **temperature** is often highly correlated with larval growth and other larval traits (McCormick *et al.*, 1995, Wilson and Meekan, 2002, Meekan *et al.*, 2003, Jenkins and King, 2006, Sponaugle *et al.*, 2006, Rankin and Sponaugle, 2011) and can, through increased larval growth, relate indirectly to higher recruitment success (Wilson and Meekan, 2002, Jenkins and King, 2006, Rankin and Sponaugle, 2011). Other factors such as solar radiation, alongshore winds, and rainfall (Bergenius *et al.*, 2005) or nearshore versus offshore water masses (Shima and Findlay, 2002, Hamilton *et al.*, 2008) have been observed to affect larval traits and subsequent patterns of recruitment.

Spatial variation of settlement and survival of settlers can be explained by **habitat characteristics** such as quality of corals (Öhman *et al.*, 1998), leeward vs. windward habitats

on islands (Schmitt and Holbrook, 1999), microhabitat characteristics (Tolimieri, 1995, Caselle and Warner, 1996, Félix-Hackradt *et al.*, 2014), density of shelters (Hixon and Beets, 1993) and density of macroalgae (Carr, 1991, Sponaugle *et al.*, 2012b). These differences in habitat characteristics can lead to species-specific spatial patterns of recruitment. The relationship between habitat features and recruitment likely depends on the spatial scale examined, as it might be important at a small spatial scale, but at a larger scale, oceanographic processes are likely more relevant (e.g. Tolimieri, 1995, Caselle and Warner, 1996). A detailed review on the influence of habitat characteristics on recruitment is provided by Adams and Ebersole (2009).

Oceanographic processes not only affect larval survival, - transport and settlement, but also can influence the survival of fish during the post-settlement phase. For example, the study of Jenkins *et al.* (1997) indicated that recruitment variation was caused by physical transport patterns affecting pre-settlement larvae and post-settlement mortality due to physical disturbance from **wave action**.

Overall, these studies show that links between biotic and abiotic factors with fish recruitment can vary at many spatial and temporal scales. Additionally, processes found to be important for recruitment at one time and location, might not be relevant at another time or study site (Milicich, 1994, Jenkins *et al.*, 1997, Caselle, 1999, Raventos and Macpherson, 2005). Finally, studies that have investigated multiple species mostly found that relationships of recruitment with physical processes were species-specific (e.g. Thorrold *et al.*, 1994, Lozano and Zapata, 2003, Raventos and Macpherson, 2005, Lemberget *et al.*, 2009, Caselle *et al.*, 2010b) and this could be due to different biological attributes (Caselle *et al.*, 2010b) and different bio-physical interactions.

1.6 Recruitment and population connectivity

In the marine environment individuals can disperse among habitats and populations in different ways. Dispersal is generally associated with the early life stages (eggs and larvae) when juvenile and adults are sedentary or associated with demersal habitat (Doherty *et al.*, 1995, Russ, 2002, Cowen *et al.*, 2007). In addition, dispersal can include migration of juvenile and adult fish among sites, when considering mobile species. As a result, marine fish species distribution range can be variable and extend over large geographic regions (Lester *et al.*, 2007). Nevertheless, since such habitats often exhibit a high spatial heterogeneity (e.g. islands, fragmented coral and rocky reefs) fish species are often distributed in geographically discrete populations (Hanski, 1998, Kritzer and Sale, 2004, Pinsky *et al.*, 2012). When several of these discrete populations are connected through some form of migration (ie. larval or adult or both), this assemblage can be thought of as a **metapopulation** (e.g. Cowen and Sponaugle, 2009). **Population connectivity** describes the exchange of individuals within a metapopulation and in the case of demersal fish species, population connectivity comprises the dispersal phase from reproduction through settlement (including metamorphosis and the post-settlement phase; Sale, 2004, Pineda *et al.*, 2007).

Successful dispersal means that it sufficiently influences the demographic rates of the sink population and regarding genetic connectivity, that dispersers contribute to the gene pool of the sink population (Wright, 1951, Crow and Kimura, 1970) and thus influences its genetic structure (Palumbi, 2003, Waples and Gaggiotti, 2006, Jones *et al.*, 2009). Larval dispersal affects the local population dynamics and that of connected populations (Fogarty and Botsford, 2007, Gaines *et al.*, 2007).

Depending on how connectivity influences population dynamics, evolutionary and demographic connectivity must be addressed separately. **Evolutionary connectivity** (i.e. genetic) describes how gene flow affects evolutionary processes among populations (Waples and Gaggiotti, 2006, Lowe and Allendorf, 2010). **Demographic connectivity** (ecological) defines how dispersal (immigration or emigration) influences population growth and vital rates (survival and birth rates) (Lowe and Allendorf, 2010). Generally, evolutionary connectivity occurs at a timescale of several generations, while demographic connectivity acts at a more contemporary timescale with ecological relevance (Leis *et al.*, 2011b). The magnitude of dispersal required to have ecological and thus demographic relevance is much

larger than the level needed to sustain genetic homogeneity among populations (Cowen and Sponaugle, 2009), which can be reached by dispersal of only a few individuals per generation (Slatkin, 1993).

1.6.1 Dispersal vs. retention

Initial research on fish larvae concentrated on temperate pelagic species of the order Clupeiformes, whose larvae hatch from pelagic eggs, with low swimming capabilities (Blaxter, 1986, Miller *et al.*, 1988) and poorly developed sensory systems. This lead to the initial conception of considering fish larvae as passive particles, vulnerable to large current systems and most studies found existence of long-distance dispersal of such species, with consequent highly connected **open populations** ('open population' paradigm; Williams *et al.*, 1984, Caley *et al.*, 1996, Roberts, 1997, Siegel *et al.*, 2003). Open populations are considered to be well mixed, with larvae from multiple sources mixing in the plankton.

However, a growing number of studies have revealed significant levels of **self-recruitment** (=self-replenishment, i.e. recruitment of fish to their natal population) of tropical reef fish species (Jones *et al.*, 1999, Taylor and Hellberg, 2003, Jones *et al.*, 2005, Almany *et al.*, 2007, Gerlach *et al.*, 2007). Recent studies have also found evidence of self-recruitment for temperate fish species (Carreras-Carbonell *et al.*, 2007, Galarza *et al.*, 2009, Swearer and Shima, 2010). These studies show that fish larvae may disperse more locally than initially thought and these findings led to an adjustment of the open population paradigm. Populations can range from a totally **closed population** (self-replenishing; e.g. endemic island species) to fully open populations that receive a large proportion of recruits from other populations.

Furthermore, studies finding active larval behaviour and specific oceanographic processes suggested that self-recruitment, reduced and directed larval dispersal can be relevant for several fish species (reviewed by Kingsford *et al.*, 2002, Sponaugle *et al.*, 2002). For example, restricted nearshore larval distribution and the recording of all larval developmental stages in the nearshore can indicate larval retention near the natal habitats (Leis *et al.*, 2003, Sabatés *et al.*, 2003, Borges *et al.*, 2007a, Borges *et al.*, 2009).

In general, spawning mode (here considered demersal eggs versus broadcast spawning of pelagic eggs) can have a clear effect on the dispersal potential (Bradbury *et al.*, 2008, Riginos

et al., 2011, 2014). Larvae from demersal eggs have been shown to hatch larger and more developed than larvae hatching from pelagic eggs (Figure 1.1; Blaxter, 1986, Miller *et al.*, 1988). These differences are expected to influence the degree of dispersal. For example Leis (1991) recorded that larvae from benthic eggs were closer to the reef than larvae of pelagic eggs. In addition, a multispecies analysis of marine benthic fish species revealed that potential of dispersal (measured as genetic differentiation) was significantly lower for fish laying demersal eggs than for those producing pelagic eggs (Riginos *et al.*, 2011, Riginos *et al.*, 2014).

Regarding active **larval behaviour**, several studies have found fish larvae to migrate vertically (with ontogenetic, diel, and tidal patterns), taking advantage of favourable currents to stay close to the natal reef or avoiding offshore currents (Paris and Cowen, 2004, Leis *et al.*, 2007). Furthermore, it was found through *in situ* and laboratory experiments that larvae of several perciforms have higher swimming speeds than expected, are able to swim faster than mean ambient current speed (Leis and Carson-Ewart, 2003, Fisher, 2005, Faria *et al.*, 2009, Leis, 2010, Faria *et al.*, 2014), can sustain swimming speed for several days and thus can swim over long distances (Dudley *et al.*, 2000, Fisher, 2005, Fisher and Leis, 2009, Leis, 2010). Observations of orientated swimming by changing swimming speed and direction have been recorded *in situ* for several coral reef fish species by following of fish larvae by SCUBA divers and by the use of drifting *in situ* chambers (Leis *et al.*, 2007, Paris *et al.*, 2013a, Leis *et al.*, 2014). Swimming speed and direction and vertical distribution of larvae is species-specific and can change ontogenetically (Leis *et al.*, 2009, Leis, 2010). Furthermore, it has been found that orientation behaviour is dependent or independent on the location (Stobutzki and Bellwood, 1998, Leis and Carson-Ewart, 2003, Leis *et al.*, 2009, Paris *et al.*, 2013a).

There is also recent evidence that coral reef fish larvae can detect and orient to environmental cues such as sound (Tolimieri *et al.*, 2000, Simpson *et al.*, 2004, Tolimieri *et al.*, 2004, Simpson *et al.*, 2010, Radford *et al.*, 2011) and odours (Atema *et al.*, 2002, Gerlach *et al.*, 2007, Leis *et al.*, 2011a, Paris *et al.*, 2013a). Furthermore it has been found, that larvae form groups and disperse cohesively early in their development (Ben-Tzvi *et al.*, 2012, Bernardi *et al.*, 2012). Groups of larvae have greater orientation skills and swim faster than isolated larvae at the stage of settlement (Irisson *et al.*, 2015). Overall, these findings suggest that

larvae are not passive particles but are able to detect, orient towards and actively select their recruitment habitats.

Ocean currents are an important factor influencing larval transport (e.g. Jenkins *et al.*, 1999, Simpson *et al.*, 2014), but local oceanographic conditions may as well favour reduced dispersal distances and larval retention, particularly in nearshore environments (reviews in Sponaugle *et al.*, 2002, Largier, 2003, Gawarkiewicz *et al.*, 2007, Cowen and Sponaugle, 2009). Possible processes are for example: tidal currents and related water circulation (Wolanski, 1994), eddies (Cowen and Castro, 1994, Swearer *et al.*, 1999, Largier, 2003, Sponaugle *et al.*, 2005, Hamilton *et al.*, 2006), upwelling "shadows" along a coast, benthic or coastal boundary layers with reduced flow (Nowell and Jumars, 1984, Largier, 2003, Pineda *et al.*, 2007), coastal areas near headlands with reduced flow and/or retention (Lipphardt *et al.*, 2006, Mace and Morgan, 2006), enclosed bays, lagoons and estuaries (Gawarkiewicz *et al.*, 2007) and shoreward moving of relaxation upwelling fronts (Shanks *et al.*, 2000).

Our knowledge on the physical oceanography of the shallow nearshore environment is still very limited but it is of high importance, since the nearshore describes the area where most coastal fish start and end their planktonic phase (Cowen and Sponaugle, 2009, Rubidge *et al.*, 2012). Even less understood, are the complex interactions between biological and oceanographic processes. Subsequently, larval dispersal can vary strongly at a spatial and temporal scale (Werner *et al.*, 2007, Botsford *et al.*, 2009, Jones *et al.*, 2009), making the estimation and prediction of population connectivity very hard. Yet, many methods have been developed to overcome this deficit (reviews by Hedgecock *et al.*, 2007, Leis *et al.*, 2011b, Calò *et al.*, 2013).

1.6.2 Methodology to estimate dispersal and connectivity

Due to the fact, that fish larvae are mostly tiny and transparent, direct tracking of larvae is extremely difficult and therefore many **indirect methods** have been developed to estimate larval dispersal and population connectivity.

PLD: The most common metric used to estimate larval dispersal distance is the duration of the pelagic phase (PLD) (Shanks *et al.*, 2003). PLD is species-specific and PLD can be also highly variable within and among populations (Wellington and Victor, 1992, Bay *et al.*,

2006, Di Franco *et al.*, 2013) and it can be affected by environmental conditions, such as temperature (McCormick *et al.*, 1995, O'Connor *et al.*, 2007). For these reasons, PLD estimates are mostly very poor estimators of dispersal distance. Regarding population connectivity, it has been shown by several studies that genetic differentiation (an estimate of population connectivity) was only weakly related or not related to PLD (Shulman and Bermingham, 1995, Bradbury *et al.*, 2008, Weersing and Toonen, 2009, Riginos *et al.*, 2011). And since PLD is not the only parameter that determines larval dispersal (see previous paragraph) it is imprecise to estimate larval dispersal, population connectivity or geographical range size directly from PLD (Leis *et al.*, 2011b, Mora *et al.*, 2012).

Genetic markers: Different kinds of genetic markers are used to study population connectivity depending on the temporal and spatial scale of interest. Allozymes and mitochondrial DNA (mtDNA) have slow mutation rates and are therefore primarily used to measure evolutionary connectivity, to detect events and estimate dispersal over the distant past (Selkoe and Toonen, 2006). For analysing population connectivity at a more contemporary timescale, microsatellites (or: simple sequence repeats, SSRs) are the most commonly used genetic markers (Selkoe and Toonen, 2006, Calò *et al.*, 2013). Microsatellites have high mutation rates, are neutral to selection and have a high genetic diversity (high polymorphism), which allows the study of the genetic structure of populations (genetic differentiation) at a small and fine spatial scale (Hauser and Carvalho, 2008) and over a more recent past (Selkoe and Toonen, 2006).

Recent connectivity studies of populations connected at moderate to fine spatial scale (e.g. 10 to several 100 km) have predominantly used microsatellites. Yet, microsatellites have several constraints, such as their low density in the genome, variable and complex patterns of mutation and a high genotyping error rate (reviewed by DeFaveri *et al.*, 2013, Putman and Carbone, 2014). A type of marker only recently used in molecular studies are SNPs (single nucleotide polymorphisms). These markers are expected to be increasingly used in population genetic studies (Putman and Carbone, 2014, Schunter *et al.*, 2014), due to various characteristics, such as high abundance throughout the genome, low genotyping error and low mutation rates (Liu *et al.*, 2005, Anderson and Garza, 2006).

Genetic studies estimate population connectivity by analysing the genetic differentiation among populations. Genetic differentiation, or genetic structure, reflects, in part, the degree

of gene flow, i.e. exchange of alleles (the alternative form of a gene), among populations. Thus, genetic differentiation among putative populations (i.e. locations) is estimated by comparing allelic frequencies. It is generally assumed that high connectivity among populations allows for consistent gene flow and leads to a homogenization of populations (i.e. low to no genetic structure). In contrast low connectivity restricts gene flow among populations and because of genetic drift and selection populations diverge and become genetically different (i.e. high genetic structure) (review by Hedgecock *et al.*, 2007, Lowe and Allendorf, 2010). Several methods are being applied to estimate population genetic structure at spatial and/or temporal scales, which commonly depend on theoretical models such as Wright's Island Model and apply Wright's F-statistics (Wright, 1931, 1965). A few common genetic tools are described here:

The most common measure to describe genetic differentiation is **F_{ST}**, defined by Wright (1943). F_{ST} ranges from 1 to 0, with lower values indicating less genetic differentiation. F_{ST} is based on several assumptions (e.g. populations have reached drift-gene flow equilibrium) which are often not realized. Associated measures such as G_{ST} and Jost's D have been compared to F_{ST} with different conclusions (Jost, 2008, Gerlach *et al.*, 2010, Whitlock, 2011). Another disadvantage of F_{ST} statistics is it neglects rare alleles. Generally, these indices (F_{ST}, G_{ST} and D) should be interpreted with caution especially in high gene flow systems (Hedgecock *et al.*, 2007, Lowe and Allendorf, 2010). For example the study of Knutsen *et al.* (2011) revealed that even weak genetic differentiation (average F_{ST}=0.0037) can be of high relevance and separate biologically meaningful populations. And Puebla *et al.* (2009) found by applying an **isolation by distance** analysis that larval dispersal is localized, despite low genetic differentiation.

The genetic isolation by distance analysis tests if genetic differentiation (e.g. F_{ST}) is related to geographic distance and can be used to estimate larval dispersal (Palumbi, 2003, Puebla *et al.*, 2009). The underlying assumption of this model is that nearer populations have higher levels of connectivity than populations that are farther away from each other (Wright, 1943). However, geographic distance may not explain patterns in the ocean as well as oceanographic distance based on currents (White *et al*., 2010).

In addition to F-statistics other more recent tools to study genetic population structure exist, such as Bayesian clustering methods (e.g. Pritchard *et al.*, 2000, Corander *et al.*, 2003) and

multivariate analyses (Jombart et al., 2010). One advantage of the multivariate analysis is that it does not rely on assumptions of Hardy-Weinberg equilibrium or linkage disequilibrium, in contrast to Bayesian clustering methods (Jombart et al., 2010).

These genetic methods introduced above are indirect methods to estimate population connectivity and larval dispersal. It is important to consider that these tools estimate evolutionary than demographic connectivity, since several generations are taken into account (Waples and Gaggiotti, 2006, Hedgecock *et al.*, 2007, Lowe and Allendorf, 2010). Therefore, these methods might overestimate larval dispersal and population connectivity on ecological timescales.

Geochemical markers: An alternative approach to population genetics is the use of otoliths as natural tags of the larval origin (e.g. Swearer *et al.*, 1999, Patterson and Swearer, 2007, Thorrold *et al.*, 2007). Throughout growth otoliths incorporate trace elements from the surrounding waters into their calcium-carbonate matrix, which creates a permanent geochemical signature since otoliths are metabolically inert (Campana and Neilson, 1985, Thorrold *et al.*, 2002). Critical to this approach is that water masses differ significantly in their chemistry so that this variation can be detected in the otoliths (Bath *et al.*, 2000, Thorrold *et al.*, 2002, Thorrold *et al.*, 2007, Leis *et al.*, 2011b). Application of this approach to study the dispersal history of fish is increasing, especially for riverine and anadromous species (Thorrold *et al.*, 2001, Tanner *et al.*, 2013, Macdonald and Crook, 2014, Martin *et al.*, 2015), but also studies on coastal and reef species are increasingly applying this method (Patterson *et al.*, 2005, Warner *et al.*, 2005, Patterson and Swearer, 2007, Ben-Tzvi *et al.*, 2008, Standish *et al.*, 2008, Fontes *et al.*, 2009b, Di Franco *et al.*, 2012).

Biophysical modelling: The first numerical modelling approaches to estimate larval dispersal were performed with solely physical models representing ocean currents from meteorological, topographic and hydrographic data. In these initial models larvae were tracked as passive particles, considering only PLD as a biological parameter and often concluded that larvae could disperse over large distances (10 to 100s of kms; e.g. Williams *et al.*, 1984, Roberts, 1997).

Most current models are Langrangian Individually Based Models (IBMs) that can track the path of individual particles for the whole planktonic duration (Black and Moran, 1991,

Werner *et al.*, 2007). The results of these models are often given in "dispersal kernels", which describe the probability of a larva to successfully disperse to a settlement location. Model complexity is increasing and represent better complex bio-physical interactions, as more biological parameters than just PLD are being incorporated. Biological variables included in bio-physical models are for example: vertical distribution (Paris and Cowen, 2004), mortality (Cowen *et al.*, 2000, Paris *et al.*, 2007), vertical migration (diel, ontogenetic; Paris *et al.*, 2007), sensory ability (James *et al.*, 2002, Staaterman *et al.*, 2012) and horizontal swimming (Staaterman *et al.*, 2012).

Including larval behaviour in modelling significantly changes the dispersal potential and the scale of connectivity (Cowen *et al.*, 2006). Moreover, including larval behaviour revealed results that matched better with empirical observations, than the outputs from models with passive particles (Wolanski *et al.*, 1997, Wolanski and Sarsenski, 1997). Biophysical models have a high potential to elucidate larval dispersal at a fine to large temporal and spatial scale and by this can provide crucial information on population connectivity and sink and source relationships (Andrello *et al.*, 2013, Holstein *et al.*, 2014).

The physical/hydrodynamic component of dispersal models has improved continuously as well, by increasing resolution and including small-scale oceanographic processes (North *et al.*, 2009). However, models having a resolution high enough to simulate coastal and nearshore processes relevant for larval fish are yet available only for few regions (e.g. Sponaugle *et al.*, 2012a, Paris *et al.*, 2013b).

Furthermore, there is a distinct lack of research that combines a larval dispersal model with empirical data collection to compare results and evaluate model outputs (Leis *et al.*, 2011b). The study by Schunter *et al.* (2011) on the coastal species *Serranus cabrilla* revealed a high correlation between genetic connectivity data and oceanographic data derived from a dispersal model. White *et al.* (2010) found that 50% of the variance of genetic differentiation was explained by larval exchange, when relative oceanographic distance derived from a dispersal model was used instead of the conventional Euclidean distance. These studies show how larval dispersal modelling can help to interpret empirical genetic population structure and indicate the importance of combining different approaches to study connectivity. Detailed reviews on physical and biophysical modelling are given by e.g. Werner *et al.*

(2007), North *et al.* (2009), Leis *et al.* (2011b), Willis (2011) and Staaterman and Paris (2013).

Tools to study connectivity described so far are indirect approaches, but over the last decade a few direct approaches have been developed, despite the difficulty of following small larvae in the field. These methods can assess dispersal and connectivity at a contemporary and ecologically meaningful time scale.

Mark and recapture: One of the first reef fish studies to provide direct evidence of self-recruitment used a mass marking method (Jones *et al.*, 1999). Over a three year period, otoliths of ~10 million embryos of the damselfish *Pomacentrus amboinensis*, were marked with the fluorescent tetracycline. More than 5000 juveniles were subsequently collected and 15 fish were marked. By considering the proportion of embryos marked (0.5-2%), a probability of self-recruitment of 15-60% was revealed (Jones *et al.*, 1999). Jones and colleagues (2005) repeated this methodology with the clownfish *Amphiprion polymnus*, using the stable isotope barium as an artificial tag (Jones *et al.*, 2005, Thorrold *et al.*, 2006, Almany *et al.*, 2007). The main drawback of this approach is that a great proportion of the total larval pool must be tagged to overcome the high mortality rate and advection of larvae (Thorrold *et al.*, 2007). Therefore, this method can be applied only at a very small spatial scale, since distant sources of larvae cannot be detected (Leis *et al.*, 2011b).

Assignment test: One genetic method to quantify ecological connectivity is to assign individuals to their natal population by estimating the probability that the individuals' multilocus genotype exists in one of the potential source populations (Manel *et al.*, 2005). Studies on marine fish that have used this method and/or parentage analysis are described in the next paragraph.

Parentage-Analysis: A second genetic method is the parentage analysis, which provides parent offspring relationships. Several statistical methods of parentage analysis exist. Jones and Ardren (2003), Jones *et al.* (2010) and Harrison *et al.* (2013) described and compared different statistical techniques and software for parentage analysis. Furthermore, these studies provide a guide, since choice of analyses and software will depend on the experimental design and study system.

Empirical studies on marine fish have started to apply these methods only recently and most are on coral reef fish species (e.g. Jones *et al.*, 2005, Planes *et al.*, 2009a, Christie *et al.*, 2010, Berumen *et al.*, 2012, Saenz-Agudelo *et al.*, 2012, Almany *et al.*, 2013, Madduppa *et al.*, 2014). One reason for the limited use of the parentage analysis might be that a large proportion of the adult population needs to be sampled. Therefore, most of these studies were made at a small spatial scale or with species with restricted habitats that allow sampling of a large proportion of the parent population (Planes *et al.*, 2009a, Saenz-Agudelo *et al.*, 2009). Assignment tests require less sampling but may be less successful in high gene flow conditions, which is often the case in marine fish populations. Several marine fish studies applying assignment tests to assign recruits to adult populations failed despite usage of highly polymorphic loci (~10) (Saenz-Agudelo *et al.*, 2009, Christie *et al.*, 2010, Félix-Hackradt *et al.*, 2013a).

Saenz-Agudelo et al. (2009) tested both assignment test and parentage analysis empirically on a high and low gene flow scenario for the panda clownfish (*Amphiprion polymnus*) in Papua New Guinea. In this study the assignment test had classified juveniles correctly to their source population when gene flow was low, but lacked power to assign recruits correctly in the high gene flow scenario, in contrast to the parentage analysis. This was confirmed by Christie et al. (2010a), which also had applied both types of methods on the bicolour damselfish (*Stegastes partitus*) collected at Exuma Sound, Bahamas. These results suggest that parentage analysis might perform better in a study system with high gene flow, while the assignment test might be more appropriate in a low gene flow system (Kane and King, 2009). However, it is clear that more empirical studies are needed that explicitly compare and evaluate both methods in varying scenarios, to confirm this assumption.

1.7 Why study recruitment, dispersal and connectivity?

The variable interaction of processes such as larval dispersal, settlement and post-settlement interactions with biotic and abiotic factors makes recruitment a highly complex ecological process. Therefore, recruitment variation has an essential impact on the demography, dynamics, persistence and genetic structure of a population and on the connectivity among populations (Doherty and Williams, 1988, Jones, 1990, Carr and Syms, 2006, Cowen and Sponaugle, 2009). Understanding processes and patterns of recruitment has been a long-

standing goal in marine ecology, fishery science and conservation. For example, analysing how environmental processes influence recruitment can improve the understanding of patterns, can allow for future predictions and for estimating the effect of future environmental changes (e.g. climate change) on recruitment and population dynamics. This is of great relevance especially for sustainable fishery management.

Since recruitment to a population is often not related to the local production of offspring due to the pelagic larval phase, investigating dispersal patterns and thus the connectivity among populations is essential. This implies that changes happening in one location/population might be a result of processes occurring at near or distant sites/populations.

Also the persistence and resilience of populations in the face of natural and anthropogenic disturbance are essentially related to the degree of dispersal and of population connectivity (Eckert, 2003, Botsford *et al.*, 2009, Hughes *et al.*, 2010, Burgess *et al.*, 2014). For this reason, fishery management and conservation programs have a strong interest in this research field, as it helps to understand the scales at which marine organism should be managed. This becomes highly apparent when designing Marine Protected Areas (MPAs) and other spatial management strategies (Wood *et al.*, 2008, Burgess *et al.*, 2014). Here, extensive knowledge of the extent and pattern of larval dispersal and population connectivity for a range of organisms is essential to successfully design the number, size and location of MPAs and the distances among MPAs in a network (e.g. Palumbi, 2003, Sale *et al.*, 2005, Botsford *et al.*, 2009, Green *et al.*, 2015). Furthermore, the knowledge of source-sink dynamics is also relevant for designing MPAs (Holstein *et al.*, 2014). Areas consistently supplying larvae indicate sources, while areas that mostly receive larvae are described as sinks.

Effective MPAs and networks might not only conserve the diversity and persistence of populations (by self-recruitment and dispersal) within the MPA but can also benefit fisheries in the surrounding area directly through "spill over" (export of fish) (McClanahan and Mangi, 2000, Goñi *et al.*, 2008, Stobart *et al.*, 2009, Harrison *et al.*, 2012). Furthermore, eggs and larvae can disperse out of the protected area, which is called the "recruitment subsidy" (Sale *et al.*, 2005). For example, Harrison *et al.* (2012) found by applying a parentage analysis within a MPA network on the Great Barrier Reef that a great proportion of the offspring produced within the protected area had recruited to unprotected reefs.

Another example of an empirical study of Almany *et al.* (2013) shows how understanding of larval dispersal can support local resource management and provide evidence on how fishing communities can benefit from protecting their spawning stock.

Our level of knowledge on recruitment patterns, dispersal and connectivity of fish differs between tropical and temperate marine systems, especially regarding the nearshore system. Most recruitment studies from temperate areas have analysed pelagic species and demersal species of sandy bottoms that are of commercial interest (e.g. Pepin and Myers, 1991, Planque and Fox, 1998, Le Pape *et al.*, 2003). Studies on recruitment patterns of fishes inhabiting reef systems have been conducted mostly in tropical systems and, especially, studies with a fine time scale are rare for temperate areas (but see Findlay and Allen, 2002). One reason might be that traditional sampling methods (e.g. underwater visual census) are difficult to conduct in the temperate nearshore due to harsher sea conditions (strong winds, currents and low visibility).

However, along the coast of California (USA) several studies investigated recruitment patterns of rockfish species (genus *Sebastes*) at different temporal and spatial scales, using Standard Monitoring Units for Recruitment of Fish (SMURF; White and Caselle, 2008, Wilson *et al.*, 2008, Caselle *et al.*, 2010a, Caselle *et al.*, 2010b). These standardised collectors have been used as well in New Zealand to assess recruitment patterns of the common rocky reef fish *Forsterygion lapillum* (Shima and Swearer, 2009), of the black rockfish *Sebastes melanops* in British Columbia (Canada; Lotterhos and Markel, 2012) and of the coral reef fish *Monacanthus tuckeri* at Turneffe Atoll (Belize; Ben-David and Kritzer, 2005).

In the Mediterranean Sea, recruitment of rocky reef fish has been investigated by using light traps to study the delivery of post-larval stage fish to the nearshore (Félix-Hackradt *et al.*, 2013b, Félix-Hackradt *et al.*, 2013c). Underwater census have been used also in temperate reefs to investigate fish recruitment, e.g. in the Mediterranean (García-Rubies and Macpherson, 1995, Vigliola *et al.*, 1998, Raventos, 2009), at the Azores (Fontes *et al.*, 2009a) and in California (Carr, 1991, Laidig *et al.*, 2007).

Leis *et al.* (2013) studied if larval dispersal and population connectivity of demersal fish differ between tropical and temperate areas, as expected, due to for example the effect of

temperature on PLD (O'Connor *et al.*, 2007, Bradbury *et al.*, 2008). The main conclusion of this study was that these processes cannot be compared meaningfully between temperate and tropical systems due to differences in taxonomic groups and spawning modes of fish examined (Leis *et al.*, 2013). And, this review highlighted the lack of studies on dispersal and connectivity for temperate demersal fish species.

Furthermore, only few studies exist for temperate reef fish that have revealed direct estimates of larval dispersal and connectivity. A study on the temperate reef fish *Trypterigion delaisi* showed high proportions of self-recruitment that were stable among the 3 years examined (Carreras-Carbonell *et al.*, 2007). For the same species, lower levels of self-recruitment were found in a more recent study, that sampled a finer spatial scale (Schunter *et al.*, 2014), demonstrating the importance of the scale of analysis. A low level of self-recruitment was found as well for the brown rockfish in Puget Sound (USA, Hauser *et al.*, 2007).

The Marine Strategy Framework Directive adopted by the European Union requires from its member states to achieve a "Good Environmental Status" of their waters by 2020 and one of the recommended measures is to develop a network of MPAs. Fenberg *et al.* (2012) highlighted in their review on European protected areas, that studies on dispersal and population connectivity are still in great need to accomplish this goal. Lacking knowledge on connectivity makes it difficult to design, manage and sustain effective MPAs over time.

1.8 Study area

The Portuguese coast is part of the Iberian Peninsula Upwelling System, which is part of the wind-driven Eastern Boundary Upwelling System. In winter and the beginning of spring, unsteady equatorward winds are related to local weak upwelling events along the coast. At the end of spring, in summer and autumn, lasting along-shore equatorward winds combined with an enhancing stratification of coastal water form a strong coastal upwelling of nutrient-rich waters (Wooster *et al.*, 1976, Fiúza *et al.*, 1982), followed by increased productivity of phyto- and zooplankton along the coast (Cunha, 1993, Moita, 2003, Cravo *et al.*, 2010). As a result of the upwelling process, a surface current to the south and southwest occurs (Relvas *et al.*, 2007). Inshore of the upwelling front over the inner-shelf, a poleward warm counter-

current often forms during relaxation of upwelling inducing winds (Peliz *et al.*, 2002, Relvas and Barton, 2002, Peliz *et al.*, 2007).

The main study area of this thesis is the Arrábida Marine Park (Prof. Luiz Saldanha Marine Park; AMP), which is located in the central region of Portugal (map in chapter 2). The area has been described as a biogeographic and oceanographic transition zone with a high diversity of marine species, with cold and warm temperate and sub-tropical affinities (Henriques *et al.*, 1999, Gonçalves *et al.*, 2003, Horta e Costa *et al.*, 2014). The park extends along 38 km of rocky shore and the disintegration of high calcareous cliffs forms highly heterogeneous nearshore rocky reefs. The AMP has a southern oriented steep coast and an adjacent mountain chain which protect the area from prevailing Northerly and Northwesterly winds and waves (map in the next chapter) and allows for nearshore studies. The marine park was established in 1998 to protect the high biodiversity of this area and to sustain its small-scale local artisanal fishery. Since 2009 the park is implemented and divided in several zones with three levels of protection and fishing restriction: fully protected area (no take), partial protected area and buffer zone.

1.8.1 Previous larval and adult fish studies

Studies on fish performed in the AMP so far have focused on the composition and distribution of larval and adult fish (Gonçalves *et al.*, 2003, Beldade *et al.*, 2006, Borges *et al.*, 2007b, Henriques *et al.*, 2007, Horta e Costa *et al.*, 2014). By studying the adult inshore fish assemblage over 11 (Henriques *et al.*, 2007) and 12 years (Horta e Costa *et al.*, 2014), these authors found that the areas' diverse composition of tropical, warm- and cold-temperate fish fluctuated inter-annually in relation with environmental winter conditions.

Highest abundance of fish larvae in the area was found to occur between May and July, indicating the peak of spawning activity of coastal fish species (Borges *et al.*, 2007b). A study of the horizontal structure of fish larval assemblages showed that larval abundance and diversity decreased with increasing distance to the reef (Borges *et al.*, 2007b). Further, a study on the vertical structure of the nearshore fish larval assemblage (Borges *et al.*, 2007a) revealed significant difference between the surface and bottom species composition. While the surface larval assemblage was more diverse, including coastal and shelf-dwelling species, with pelagic egg and demersal egg spawning species (e.g. clupeiformes, sparids, serranids, blennies, tripterygiids and labrids) and smaller larvae, the bottom assemblage was

comprised by fewer species of only reef-associated and demersal egg laying species (mainly gobies, trypterygiids and labrids).

Further results indicated that some coastal species might complete their pelagic phase near the adult habitat, assuming the possibility of nearshore retention (e.g. *Lepadgogaster lepadogaster*), whereas for other species dispersal seems to be more probable (e.g. *Parablennius pilicornis*; Borges *et al.*, 2007a). Two studies exist for the AMP regarding recruitment that investigated the natural settlement habitat of two common clingfish (Gobiesocidae). *Apletodon dentatus* settles preferably to algal turfs (Gonçalves *et al.*, 2002) and *Lepadogaster lepadogaster* to the underside of gravel (Beldade, 2006). Yet studies on recruitment patterns and dispersal are lacking for the Arrábida Marine Park. Importantly, it is also not known how this important rocky reef area might be connected to the nearest and distant rocky reefs.

1.9 Main objectives

- Investigating the settlement and metamorphosis stage of rocky reef associated and coastal fish species at a nearshore rocky reef
- Studying temporal and spatial patterns of recruitment
- Analysing the influence of environmental processes on temperate fish recruitment at a small within-season and inter-annual scale
- Describing early life history traits relevant to recruitment
- Estimating larval dispersal and population connectivity of an intertidal rocky reef fish species along the dynamic south west Iberian Peninsula

1.10 Thesis structure

The general introduction above introduced the thesis topic, described the study area and presented the main objectives. Besides this introduction, this thesis is comprised by five more chapters. In chapters 2-4, patterns of recruitment and the influence of abiotic and biotic factors on recruitment are described regarding the settlement phase and the metamorphosis to the juvenile stage (Figure 1.3). In chapter 5 larval dispersal and the consequential connectivity among populations is analysed (Figure 1.3).

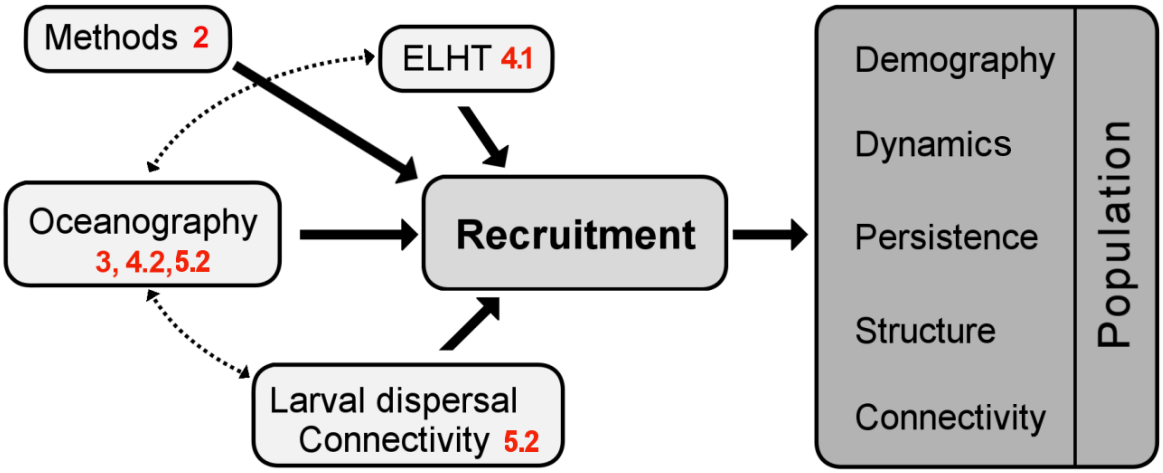

Figure 1.3 Scheme of the factors influencing fish recruitment, studied in this thesis and consequences for populations. Red numbers indicate thesis chapters.

In order to accomplish the thesis objective of studying recruitment patterns of reef associated fish species in the nearshore of the Arrábida Marine Park (AMP), we first tested which design of a standardized recruit collector is the most appropriate (**chapter 2)**. Two different collector designs and different artificial substrates were analysed in terms of recruit abundances, species diversity and effort regarding assembly and sampling of each type of collector. Furthermore, recruitment to water column collectors, which were sampled over a longer period, was studied regarding the factors depth, site and time. Results were compared with other studies that used standardized recruit collectors and with studies of the study area that have used other collection methods. The results from chapter 2 allowed to decide which type of recruit collector was used in chapters 3 and 4 of this thesis.

Most knowledge on recruitment patterns of coastal fish has been derived from studies on tropical reef systems. Only few temperate studies exist that have analyzed recruitment

patterns of various littoral species and especially cryptobenthic species are hardly represented.

Therefore, the objectives of **chapter 3** were to monitor spatial and temporal recruitment patterns of a coastal fish assemblage in a temperate nearshore rocky reef and to analyze the relationship between temporal patterns and environmental processes. Standardized collectors were installed at different sites of the AMP over three consecutive years and sampled during the summer recruitment season. A multivariate analysis was used to compare the structure of the recruit assemblage at an inter-annual, seasonal and spatial scale and between surface and bottom samples. Moreover, multivariate analyses were used to search for environmental variables and their combinations, that "best explain" the pattern of the recruit assemblage structure. Results were compared with previous studies that have analyzed relationships of recruitment and environment. Furthermore, results were also discussed with studies of the same area that have found inter-annual variation in the adult inshore fish assemblage and suggested recruitment variation as a possible cause.

Individuals from the most abundant species in chapter 3 (*Trachurus trachurus*) were studied in further detail regarding early life history traits (ELHT) and small-scale recruitment patterns within **chapter 4**. Knowledge on ELHTs is essential to understand patterns of fish recruitment. In **chapter 4.1** the microstructure of otoliths was analysed to study ELHTs such as early fish growth and age and size at metamorphosis. Moreover, ELHTs were compared between hatching cohorts, estimated from back-calculated hatching patterns. Chapter 4.1 was the first study that described the early life history of this highly commercial species.

Various environmental factors and processes can affect the rate of fish recruitment by influencing larval dispersal, larval condition, growth, survival and transport to the nearshore. Therefore, in **chapter 4.2** we analysed the relationship between small-scale temporal patterns of both hatching and recruitment with local environmental and oceanographic processes such as the moon cycle and upwelling. A generalized additive modelling approach was applied. Also the effect of oceanographic processes on ELHTs and subsequently on recruitment success was discussed.

The relationship of recruitment, larval dispersal and connectivity among populations is studied in chapter 5 by using the common cryptobenthic fish, *Lepadogaster lepadogaster*. Adults of this species are, as for most marine benthic and demersal fish, site-attached, which means that potentially only the dispersal of their larval stages ensures the connectivity among

populations. An analysis of genetic population structure is often used for estimating larval dispersal and population connectivity and microsatellites are most commonly used when studying demographic and ecological connectivity. For this reason, we developed several polymorphic microsatellites for *L. lepadogaster* in **chapter 5.1**, which were then used in the main study in **chapter 5.2**.

The AMP can be described as a continental island with rocky habitat, since sandy bottom extends for several kilometres till the next rocky shores to the north and south of the park. Moreover, the larvae of *L. lepadogaster* have a short pelagic duration (11-18 days) and all its developmental stages have previously been found near adult habitats. Based on this, we predicted high levels of self-recruitment for this species in the AMP and low population connectivity between the AMP and more northern and southern intertidal rocky reefs. In **chapter 5.2** an interdisciplinary approach with indirect and direct methods, including genetic methods based on microsatellites and simulations of oceanographic particle displacement, was applied to test this hypothesis. Results will be discussed considering biological factors of the species and oceanographic processes of the study area that could affect the larval dispersal and connectivity in this species.

Chapter 6, the general discussion, integrates and discusses the results of chapters 2 to 5 within the thesis overview. Moreover, this chapter includes implications to conservation and management, and suggestions for future research.

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Chapter 2

Testing standardized collectors to monitor fish recruitment in a shallow temperate rocky reef

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2.1 Abstract

Most research on reef fish recruitment has taken place in tropical systems and only fewer studies exist for temperate coastal environments, where it is difficult to regularly operate traditional sampling methods such as visual censuses nearshore. Standardized collectors created with artificial substrate have enabled the study of temporal and spatial patterns of reef fish recruitment and have been established as a standard monitoring tool for such studies in other regions. In this study, different designs of collectors and different artificial substrate types were tested in the summer of 2011 near a shallow rocky reef at the Portuguese west coast (Arrábida Marine Park). Collectors deployed in the water column filled with plastic substrate collected more recruits and had lower handling effort than water column collectors filled with cork and benthic collectors. Settlement to this type of collector differed significantly between surface and bottom samples. For the two most abundant fish species, *Trachurus trachurus* (Carangidae) and *Serranus hepatus* (Serranidae), a cyclic temporal pattern of settlement to water column collectors was found, indicating a possible relationship with the lunar cycle. The results suggest that water column collectors can be used in nearshore rocky systems to record temporal patterns of recruitment of certain species, at different spatial scales.

2.2 Introduction

Recruitment, the addition of new individuals to a population, can be highly variable and can have a crucial effect on the demography of fish populations. As such, understanding recruitment patterns is essential to describe population dynamics (reviewed by e.g. Doherty, 2002, Forrester *et al.*, 2002, Osenberg *et al.*, 2002, Carr and Syms, 2006). Recruitment is influenced by various biotic and abiotic factors at many spatial and temporal scales. Common drivers of recruitment include variation in larval supply, larval growth, survival, behavior, food availability (Milicich *et al.*, 1992, Kingsford *et al.*, 2002, Meekan *et al.*, 2003) and the interaction of these factors with, for example, ocean currents, tidal and wave systems, and up- or downwelling events (e.g. Milicich, 1994, Findlay and Allen, 2002, Raventos and Macpherson, 2005, Caselle *et al.*, 2010b).

Most research on reef fish recruitment has taken place in tropical systems and fewer studies exist for temperate coastal environments (e.g. Jenkins *et al.*, 1997, Carr and Syms, 2006, Shima and Swearer, 2009, Fontes *et al.*, 2011). The main reasons are likely the harsh sea

conditions (strong winds, waves, and currents and low visibility) along temperate coastlines almost all year around, making it difficult to regularly operate traditional sampling methods such as underwater visual census in the nearshore (e.g. Carr, 1991, Dixon *et al.*, 1999, Sponaugle *et al.*, 2012).

Besides visual census, various other methods exist to monitor recruitment, such as plankton tows, light traps, and collectors with artificial settlement substrate. All these methods differ in their efficiency of collecting different developmental stages and have advantages and disadvantages. For example, with underwater visual census, small and cryptic species that select hidden microhabitats and substratum for settlement might be underestimated (Robertson *et al.*, 1988, Ackermann and Bellwood, 2000). Moreover, mainly in temperate reefs, physical factors such as turbidity and currents might hinder a reliable estimation of recruitment and regular sampling with visual census, in particular of cryptic species. Furthermore, visual censuses are hard to perform at multiple locations simultaneously, yet simultaneous sampling replication is fundamental to monitor recruitment patterns that can be highly variable in time and space (Doherty, 1991, Gaines and Bertness, 1993, Meekan *et al.*, 2003).

This same disadvantage usually occurs when sampling with towed plankton nets, which have been used conventionally to collect fish larvae (larval supply; e.g. Borges *et al.*, 2007a). For some species, towed nets might underestimate late stage larvae prior to recruitment, possibly due to net avoidance (Leis, 1982, Choat *et al.*, 2003, Borges *et al.*, 2007a, Catalán *et al.*, 2014). Fixed plankton nets installed at different sites might overcome the problem of a continuous sampling, but only if currents remain to direct larvae into the net (Anderson *et al.*, 2002).

Another technique to collect late stage fish larvae, which is since the last two decades commonly used in coral reefs and increasingly used in temperate reefs, is the use of light traps (e.g. Doherty, 1987, Milicich *et al.*, 1992, Félix-Hackradt *et al.*, 2013). Light traps can monitor larval supply at several sites simultaneously, but these devices are selective towards phototactic fish species or developmental stages and its efficiency can be affected by water visibility (Steele *et al.*, 2002).

The affinity of post-larval fish of many species to physical structures (thigmotaxis; Kingsford, 1993) can be taken as an advantage and allow the use of artificial substrates to collect ready-to-settle fish. Compared to light traps, these collectors are simpler and cheaper to design, to construct and to maintain, facilitating their usage over a large geographic area. Furthermore, these collectors might be less affected by environmental factors, such as turbidity (McClean, 1999, Steele *et al.*, 2002). Standardized collectors, as Standard Monitoring Units for the Recruitment of Fish (SMURF) have been developed by Ammann (2004) for temperate rocky reef systems with kelp forests. Filled with artificial kelp-like substrate (usually plastic fencing), these collectors are deployed in the water column. For monitoring fish recruitment in coral reef systems, Vallès *et al.* (2006) designed benthic SMURFs with coral rubble that are installed on the sea floor. When deployed off the reef, both types of collectors can index relative estimates of settlement of competent larvae, as they don't depend on the presence and characteristics of nearby habitat (Steele *et al.*, 2002). And settlement estimates are potentially unbiased by post-settlement processes since predation and emigration are reduced, when samples are collected regularly (Ammann, 2004, Carr and Syms, 2006, Vallès *et al.*, 2006).

Along the Californian coast, water column SMURFs have been used successfully in several fish recruitment studies (White and Caselle, 2008, Wilson *et al.*, 2008, Caselle *et al.*, 2010a). In these studies, the majority of fish settling to collectors belonged to the genus *Sebastes* (rockfish) and recruits of these species are known to be attracted by algal structure (e.g. kelp forest; Carr, 1991). Other rockfish species from the genus *Sebastes* with a preference of settling to rocky habitat were not collected with water column collectors (Ammann, 2004). Benthic collectors of the study by Vallès *et al.* (2006) collected species that settle to coral rubbles. In our study area at the west coast of the Iberian Peninsula abundant canopy-forming brown algae *(e.g. Laminaria ochroleuca*, *Sacchoriza polishides*, *Fucus vesiculosus*; Saldanha, 1974, Santos, 1993), have disappeared likely caused by increasing temperature (Assis *et al.*, 2013, Nicastro *et al.*, 2013). Furthermore, the natural settlement habitat, (e.g. algal turfs and the underside of gravel) has been established for only a few species based on underwater observation in this area (Gonçalves *et al.*, 2002, E. Gonçalves, unpublished data, Beldade, 2006). Beyond those observations, studies on temporal or spatial patterns of reef fish recruitment in this region are lacking.

The aim of this study was to test the suitability and efficiency of types of standardized collectors for monitoring patterns of fish recruitment in a temperate rocky reef.

For this reason, collectors as used in the water column by Ammann (2004) were tested with two different artificial substrate filling materials and compared to benthic collectors fixed on the sea bed (e.g. Steele *et al.*, 2002, Vallès *et al.*, 2006). Besides the traditional plastic fence, water column collectors were filled with bark pieces of the cork oak (*Quercus suber*) to imitate the surface structure of a rocky habitat. A second objective was to study the factors depth, location and time on fish settlement to water column collectors.

2.3 Material and Methods

2.3.1 Study area

The Luiz Saldanha Marine Park (Arrábida Marine Park, hereafter AMP) is located at the highly dynamic west coast of Portugal (Figure 2.1). But due to its steep coast oriented southward and an adjacent mountain chain, Arrábida's waters are protected from prevailing northerly and northwesterly winds and waves. The AMP is characterized by heterogeneous rocky habitats that host a highly diverse fish community (Henriques *et al.*, 1999, Gonçalves *et al.*, 2003). The park area is divided in several zones with three levels of protection (Figure 2.1); a fully protected area (no-take, except for research), partially protected areas and buffer zones (see more details on implementation and zoning in Horta e Costa *et al.*, 2013).

2.3.2 Sampling

Sampling was performed in the fully protected area of the AMP (Figure 2.1), during summer of 2011, between the last week of June and the last week of September (Table 2.1). The aim was to cover the recruitment season of most reef fish species, as previous studies in Arrábida reported highest larval diversity from May-July (Gonçalves *et al.*, 2003, Borges *et al.*, 2007a).

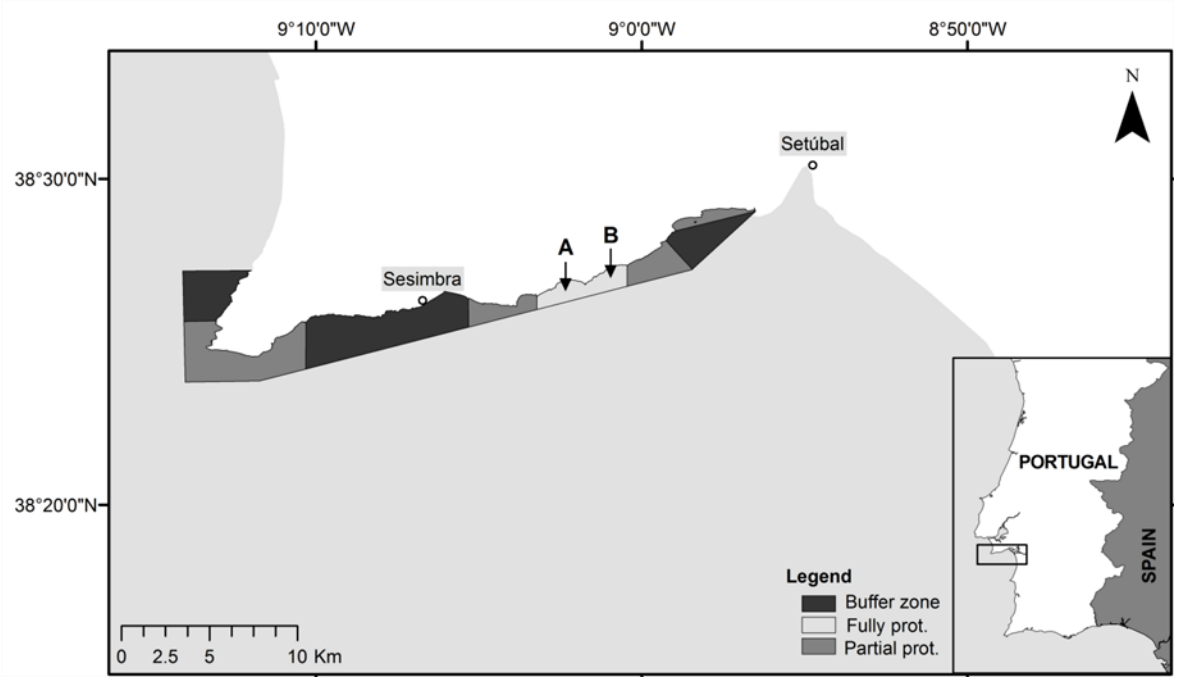

Figure 2.1 Map of the study site (Arrábida Marine Park), with location of the protection zones and the two sampling sites (A and B). Country maps were provided by the Portuguese Institute of Hydrography (www.hidrografico.pt).

Two different types of standardized collectors (Figure 2.2 and 2.3), were used: i) water column collectors (Steele *et al.*, 2002, Ammann, 2004) and ii) benthic collectors (adapted from Steele *et al.*, 2002, Vallès *et al.*, 2006). Water column collectors, a cylindrical frame of green gardening fence (1.0x0.35 m diameter), were filled with two different types of materials: i) plastic fencing to imitate algae, as applied in several recruitment studies in California (Ammann, 2004, Wilson *et al.*, 2008, Caselle *et al.*, 2010a), or ii) broken pieces of cork as artificial rocky substrate. We used natural cork as it exhibits many shapes, cracks and crevices and it is much lighter than rocks.

Table 2.1 Sampling design of different recruit collector types for summer and autumn of 2011.

		29-Jun	5-Jul	13-Jul	20-Jul	27-Jul	3-Aug	10-Aug	17-Aug	24-Aug	31-Aug	7-Sep	14-Sep	21-Sep	27-Sep	Sampling date +
SMURF	Substrate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	week
water column	plastic															
	cork															
benthic	cork+rock															

Benthic collectors built of green gardening fence covering a metal frame (1.0x1.0x0.4 m) contained light lava rocks and broken pieces of cork. These collectors were placed on top of a net (1 mm mesh size) and were placed ~5 m towards shore from water column collectors (Figure 2.2 and 2.3).

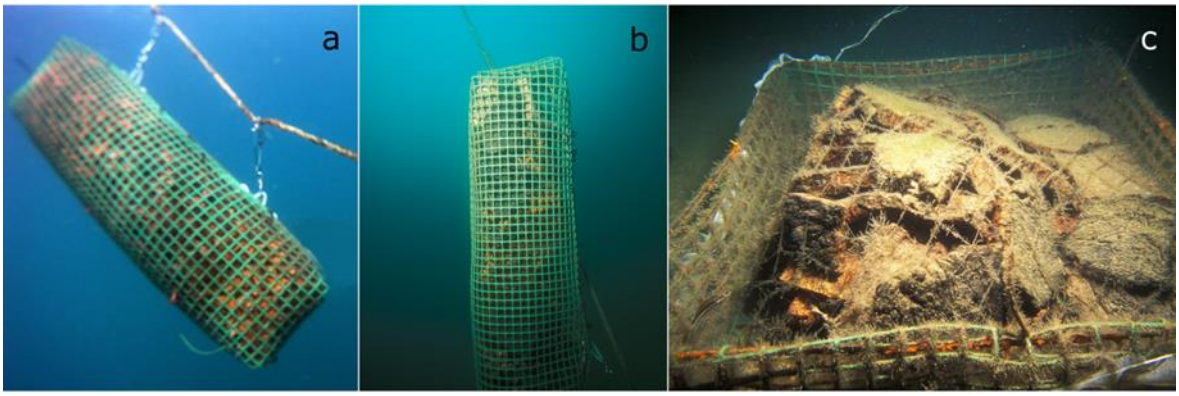

Figure 2.2 Underwater images of water column collectors filled with plastic (a), and cork (b) and benthic collectors filled with cork and lava rock (c).

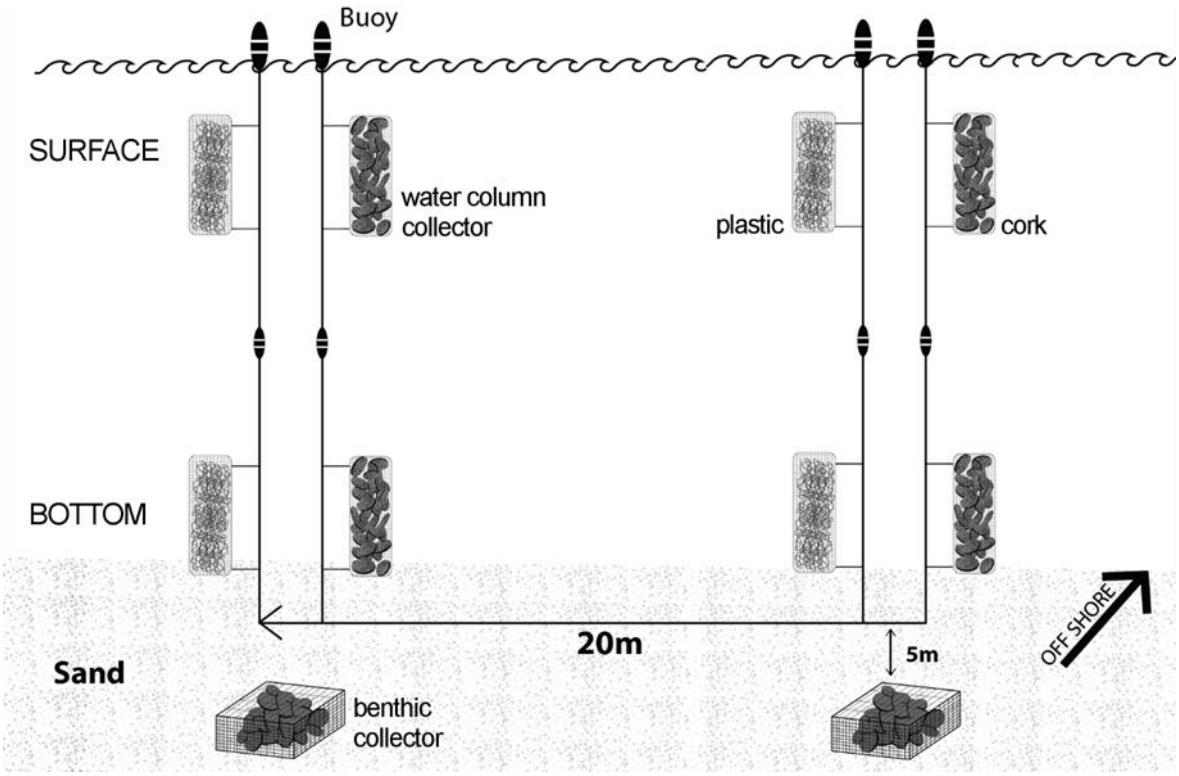

Figure 2.3 Diagram of the mooring system displaying the position of water column and benthic collectors at site A. Two of 3 replicates are displayed.

Water column collectors were attached to mooring lines close to the bottom (ca. 40 cm above the bottom) and approximately 3 m below the surface (Figures 2.2 and 2.3). Three mooring lines with plastic collectors or cork collectors and three benthic collectors were placed 100 m off-shore from the rocky reef, at a water depth of around 12 m over sandy bottom in a bay called "Derrocada" (site A, Figure 2.1). Furthermore, in order to create spatial (site) replication, around 1 km east in a bay with similar habitat called "Risco" (site B, Figure 2.1), three mooring lines with plastic collectors at the bottom and below the surface were deployed at around 15 m water depth.

Due to logistical constraints, plastic, cork and benthic collectors were sampled in different time periods (Table 2.1). However, the two types of water column collectors overlapped for 4 weeks and the benthic and plastic collectors overlapped for 4 weeks. All three types overlapped for 2 weeks.

Water column collectors were collected weekly by SCUBA divers using a Benthic Ichthyofaunal Net for Coral/Kelp environments (BINCKE, Figure 2.4.1) (Anderson and Carr, 1998, Ammann, 2004). Once on board the boat, water column collectors were cleared over the net with repeated washings (Figure 2.4.2) and the sample was transferred first to a bucket and then into a sieve (Figure 2.4.3) in order to separate the fish recruits (Figure 2.4.4).

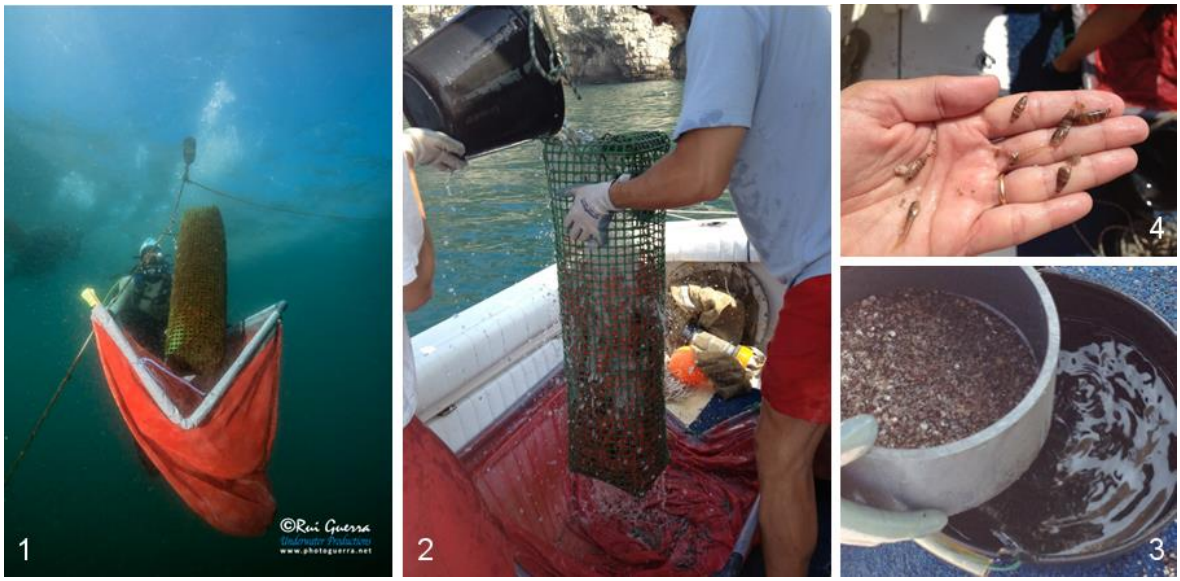

Figure 2.4 Images of the sampling process. 1: retrieval of a water column collector with a BINCKE net, 2-4: on board process; clearing of collector and sample fixation.

For benthic collectors a clove oil anesthetic solution was sprayed into the artificial substrates and afterwards the collectors was shaken above the net (1 mm mesh size) to collect all organisms. All samples were immediately fixed in 80% Ethanol.

In the laboratory, all fish were identified to the species level and measured by recording total and standard length (TL and SL) with a caliper to the nearest 0.01 mm. Sagittal and lapilli otoliths were extracted for detection of settlement marks (Wilson and McCormick, 1999). The number of rings following the settlement mark, was used to distinguish between newly settlement and post-settlement stage fishes.

During the sampling period a few inevitable gaps occurred at both sites and depths. In the first sampling week in site B one bottom collector was not sampled, in the second week at site A three surface collectors were missing and in the third week two bottom and two surface collectors were not sampled in site B. Such sampling problems were cautiously considered in the statistical analysis.

2.3.3 Data analysis

Total and average abundance of all fish (newly- and post-settlers) and recruits (newly settlers), species richness and handling effort (including assembling, deployment and underwater sampling) were compared between different types of collectors. Underwater sampling time was estimated as person dive minute, the bottom dive time of one diver for sampling one collector (Vallès *et al.*, 2006).

Of all three type of collectors, water column collectors filled with plastic were sampled over the longest period (14 sample times at the surface, bottom and in both sites A and B). Thus, only this type of collector was used to statistically analyse temporal patterns and the effect of depth (surface and bottom) and location (sites A and B). After testing for parametric assumptions, the non-parametric Kruskal-Wallis rank sum test was applied with the package ‘stats’ in the software R (v3.1.0, R-Core-Team, 2009).

2.4 Results

In total, 2627 individual fish from 11 species and 7 families were sampled throughout the entire study (Table 2.2). The majority of the species collected were cryptobenthic (63%) and belonged to the families Gobiesocidae, Gobiidae and Scophthalmidae. Benthopelagic species (27%) belonged to the families Gadidae and Serranidae and one pelagic-neritic species of the family Carangidae was collected.

Table 2.2 Mean number (M) and standard deviation (SD) of fish per collector of type benthic or water column, filled with different substrates and at two depth levels (surface vs. bottom). Species listed use a cryptobenthic (C), benthopelagic (B) or pelagic-neritic (P) habitat (Hab.).

	Collector type		water column		water column		benthic
	Substrate		plastic	plastic	cork	cork	cork + rock
	Depth		surface	bottom	surface	bottom	bottom
Family	**Species**	**Hab**	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
Carangidae	*Trachurus trachurus*	P	31.77 ± 86.91	0.27 ± 0.79	0.92 ± 1.73	0 ± 0	0 ± 0
Gadidae	*Trisopterus luscus*	B	0 ± 0	0.01 ± 0.10	0 ± 0	0 ± 0	0 ± 0
Gobiesocidae	*Apletodon incognitus*	C	0 ± 0	0.02 ± 0.15	0 ± 0	0 ± 0	0.13 ± 0.35
Gobiesocidae	*Apletodon pellegrini*	C	0 ± 0	0.05 ± 0.27	0 ± 0	0 ± 0	0.20 ± 0.41
Gobiesocidae	*Lepadogaster candolii*	C	0 ± 0	0.02 ± 0.15	0 ± 0	0 ± 0	0 ± 0
Gobiidae	*Gobius gasteveni*	C	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.20 ± 0.56
Gobiidae	*Gobius paganellus*	C	0 ± 0	0.01 ± 0.10	0.08 ± 0.29	0 ± 0	0.20 ± 0.77
Gobiidae	*Parablennius pilicornis*	C	0 ± 0	0.01 ± 0.10	0 ± 0	0.08 ± 0.29	0.20 ± 0.41
Scophthalmidae	*Zeugopterus regius*	C	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.07 ± 0.26
Serranidae	*Serranus cabrilla*	B	0.03 ± 0.16	0.13 ± 0.43	0 ± 0	0 ± 0	0.13 ± 0.35
Serranidae	*Serranus hepatus*	B	0 ± 0	0.32 ± 0.96	0 ± 0	0 ± 0	0.47 ± 0.74

2.4.1 Type of collectors

The three types of collectors used in this study attracted similar fish species (Tables 2.2 and 2.3). A pelagic-neritic species was sampled with surface collectors, while bottom collectors caught mainly benthopelagic and cryptobenthic fish species, regardless of the filling materials. However, the number of fish per species differed strongly between types of collector, considering the respective sampling efforts (Table 2.3). Few fish from only three species settled to cork filled collectors both at the surface and bottom. In contrast, plastic ones collected twice as many species and over 14 times more fish in total (Tables 2.2 and 2.3). Benthic collectors sampled on average more fish than water column collectors, but

proportionally fewer recruits (Table 2.3). Two species, *Gobius gasteveni* and *Zeugopterus regius* were collected only with benthic collectors (Table 2.2). Time spent on the assembling, deployment and sample retrieval varied distinctly between the three types of collector (Table 2.3). Benthic collectors were the most laborious and two divers needed ca 10 min to collect fish from one unit (equals 20 person-minutes). Deployment of water column collectors filled with cork was more demanding than plastic filled collectors, due to the floating strength of cork. Thus plastic filled water column collectors had the least handling effort.

Table 2.3 Comparison of type of collectors and depth (surface=sur; bottom=bot) in regard to total fish and recruit abundance, number of species and handling effort (person dive minute= bottom dive time of one diver for sampling one collector).

Sampling period		17.08. - 8.09.				31.08 - 8.09.			31.08. - 28.09.	
Type of collector		water column				water column		benthic	water column	benthic
Artificial substrat		plastic		cork		plastic	cork	cork, rock	plastic	cork, rock
Depth		sur	bot	sur	bot	bot	bot	bot	bot	bot
Nr. of replicates		12	12	12	12	6	6	6	15	15
Total Nr. of fish		181	6	12	1	3	1	12	11	18
Total fish / collector		15.1	0.5	1.0	0.1	0.5	0.2	2.0	0.7	1.2
Nr. species		1	5	2	1	3	1	7	6	8
Nr. reef fish species		0	4	0	1	3	1	7	5	8
Nr. of recruits		163	4	7	1	1	1	0	2	3
% of recruits		90	67	58	100	33	100	0	18	17
EFFORT	assembling, deployment	low	low	med-ium	high	low	med-ium	high	low	high
	sampling (person dive minute)	10	10	12	10	10	12	20	10	20

2.4.2 Depth and location

In each of the two sites and at each depth strata no significant differences in total fish and recruit abundance were found among the three replicates of plastic filled collectors (Kruskal-Wallis test: X^2=0.07 to 3.85, p=n.s.). Between sites A and B, a significant difference in total fish and recruit abundance was found at the bottom (total: X^2=7.837, p<0.01; recruits:

X^2=8.125, p<0.01), but not at the surface (total: X^2=2.397, p=n.s.; recruits: X^2=2.151, p=n.s.; Figure 2.5). At the bottom, approximately 21% more recruits settled to collectors in site A than in site B. The most significant difference, both when considering total fish and recruit abundance, was found between surface and bottom collectors in both sites (Kruskal-Wallis test: X^2=27.29 to 39.36, p=<0.001).

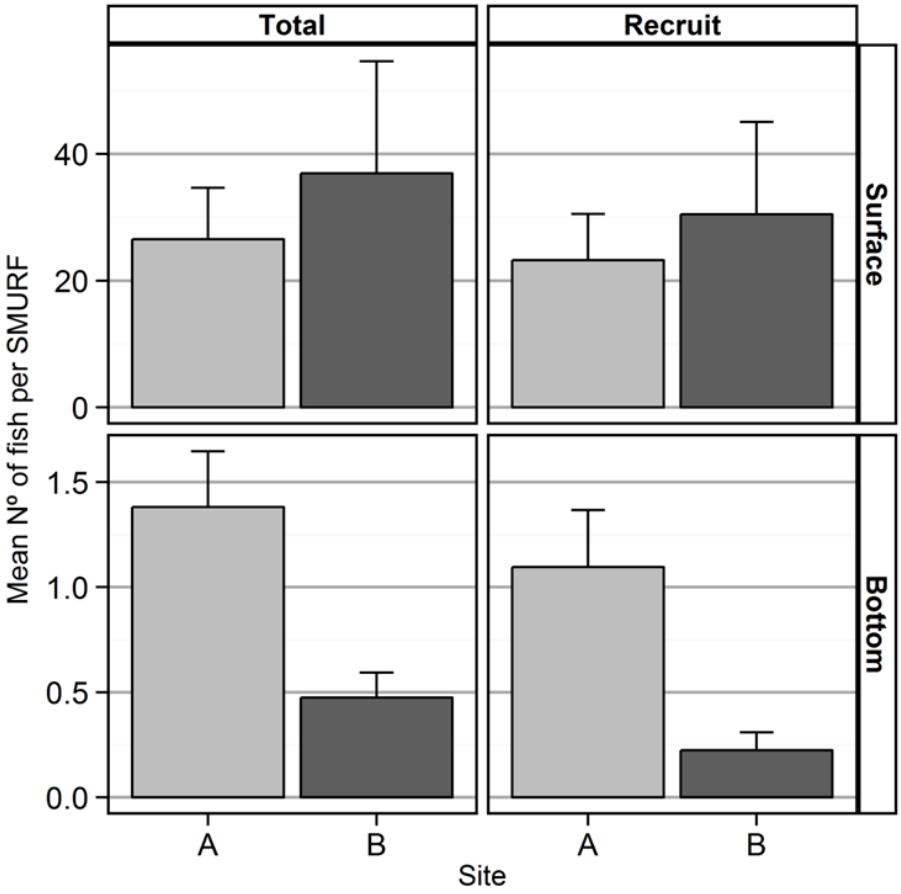

Figure 2.5 Mean total fish and recruit abundance in plastic filled water column collectors at the surface and bottom in site A and B. Error-bars indicate standard error.

Over the entire sampling period, for plastic-filled water column collectors, a total of 2512 fish had settled to surface collectors and 77 to bottom collectors. At the surface, 99.9% of the fish belonged to the pelagic species *Trachurus trachurus*, whereas 9 fish species settled to bottom collectors (Table 2.2 and 2.3). The most abundant fish species in bottom collectors were the bentho-pelagic *Serranus hepatus* (38%) and also *T. trachurus* (33%) and from these fish 83% and 100%, respectively, were newly settled fish (Table 2.4). The significant difference in fish abundance in bottom collectors found between sites was due to more *T. trachurus* and *S. hepatus* recruits settled to bottom collectors in site A than in site B (X^2=4.744, p<0.05 and X^2= 5.170, p<0.05, respectively; Figure 2.6).

Table 2.4 Total fish (N) recruit abundance (Recruits; Nr. (%)) and range of standard fish length (SL in mm) of fish species settled to bottom collectors (plastic filled). Described per species are habitat use (C=cryptobenthic, B=benthopelagic, P=pelagic-neritic) and egg type.

Species	Habitat	Egg type	N	SL	Recruits
Apletodon incognitus	C	demersal	1	19.2	0 (0%)
Apletodon pellegrini	C	demersal	5	14.0-24.5	0 (0%)
Gobius paganellus	C	demersal	1	22.9	0 (0%)
Lepadogaster candolii	C	demersal	2	6.1-7.4	2 (100%)
Parablennius pilicornis	C	demersal	1	22.3	1 (100%)
Serranus cabrilla	B	pelagic	12	21.1-67.7	2 (17%)
Serranus hepatus	B	pelagic	29	11.0-47.5	24 (83%)
Trachurus trachurus	P	pelagic	25	5.9-22.4	25 (100%)
Trisopterus luscus	B	pelagic	1	11.3	1 (100%)

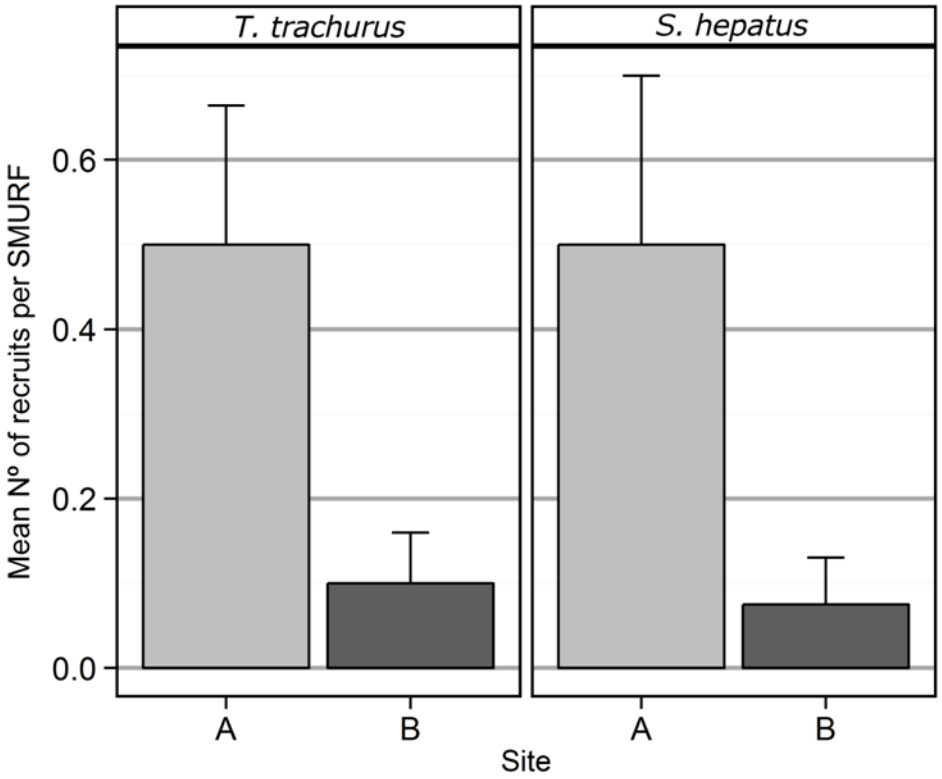

Figure 2.6 Mean recruit abundance of *T. trachurus* and *S. hepatus* in plastic filled bottom collectors. Error-bars indicate standard error.

2.4.3 Temporal settlement pattern

In plastic filled water column collectors at the surface, where most fish were collected, 72% of *T. trachurus* recruited during the first two sampling weeks. Three further peaks of settlement followed and peaks occurred at intervals of around 30 days (Figure 2.7). At the bottom, fewer *T. trachurus* recruits occurred as well throughout the sampling period but most had settled at the beginning of the sampling period. The second most abundant species, *S. hepatus* settled to bottom collectors during the whole sampling period, but recruits of this species occurred only until mid-August. Three peaks of *S. hepatus* recruitment were recorded at an interval of around 15 days (Figure 2.7).

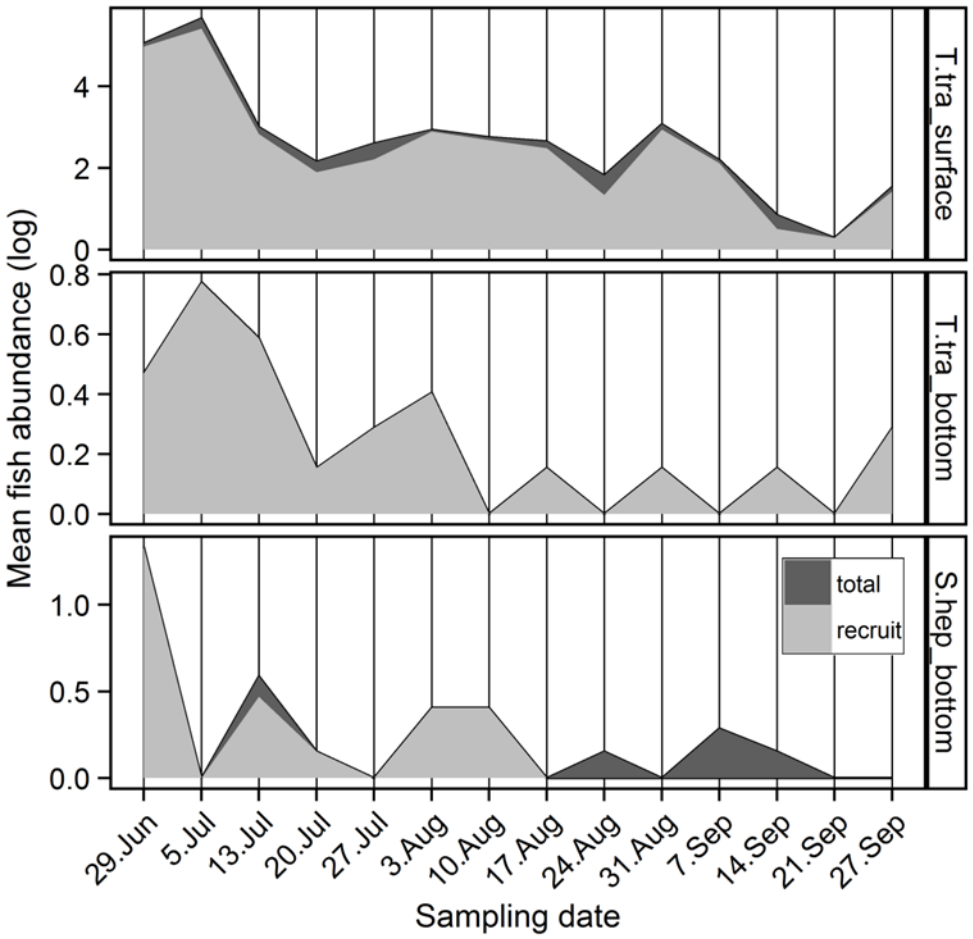

Figure 2.7 Weekly mean total fish and recruit abundance of *T. trachurus* (T.tra) at the surface and bottom and of *S. hepatus* (S.hep) at the bottom, collected with plastic water column collectors. Mean abundances are log-transformed and y-axes have independent scales.

2.5 Discussion

The different type of standardized collectors for fish recruitment used in this study collected similar species, such as a pelagic neritic species, of the family Carangidae on surface collectors and cryptobenthic (Gobiesocidae, Gobiidae, and Scophthalmidae) and benthopelagic species (Serranidae and Gadidae) on bottom collectors, regardless of the artificial substrate used. However, differences in total fish and recruit abundance and handling effort between types of collectors were evident. A statistical comparison was however not feasible due to the restricted number of weeks with simultaneous sampling and the overall low number of fish settling to collectors at the bottom. Yet, regarding water column collectors, the ones filled with plastic sampled 15 times more fish at the surface and 6 times more at the bottom compared to collectors filled with cork. At the bottom, more fish had settled to benthic than to water column collectors, but most fish that had settled to benthic collectors were too large and had too many rings after the settlement mark to be considered as newly settled. These fish might have migrated from nearby rocky reefs or might have settled before to drifting seaweed or other habitat (e.g. ontogenetic habitat shifts) (Ammann, 2004), but this needs to be clarified in future research.

The handling effort including assembling, deployment and underwater sampling of collectors varied significantly among collector types. Benthic collectors were the most labour intensive and water column collectors filled with plastic were the least laborious. The floating attribute of cork makes the deployment and sampling more difficult than with plastic. This short pilot study revealed that collectors with plastic fencing as artificial substrate performed the best among the ones tested and should be the choice when aiming for a continuous monitoring of nearshore fish recruitment at several sites at the AMP. Moreover, plastic fencing is widely available, which allows for a globally standardized utilization, whereas cork might be hard to find in many places.

In the AMP the distribution of larval fish of different developmental stages had been previously analysed with respect to spatial (horizontal and vertical) and temporal patterns (Beldade *et al.*, 2006, Borges *et al.*, 2007a, 2007b). Fish species collected here with collectors had been sampled as well in these larval studies. Nevertheless, higher species diversity was recorded in the prior fish larval studies, which might underline the selectiveness of collectors towards species with thigmotactic behaviour. Furthermore, Carr

(1991) and Ammann (2004) suggested that collectors are attracting fish that will settle to algal habitats. In our study area, only one species collected in this study, *Lepadogaster candolii*, had been found to settle to algal turfs in the AMP (E. Gonçalves, unpublished data). Thus, too little information on the natural settlement habitat is available, to make further assumptions.

Plankton nets pulled from the boat or with underwater scooters used in larval studies collected mostly small and undeveloped larvae (pre-and flexion stages; Borges *et al.*, 2007a, Borges *et al.*, 2007b). Light-traps used at the same study site and year collected predominantly fish larvae in older developmental stages (flexion and post-flexion stages; R. Borges unpublished data) than plankton net samples but these were still smaller and less well-developed than fish sampled in this study. Differences in fish size at catch among sampling methods has been shown before (Carassou *et al.*, 2008, Catalán *et al.*, 2014) suggesting that the method of choice depends on the process of recruitment and the early life stage that will be studied (larval supply, settlement, etc.).

The depth at which water column collectors were installed had a large effect on the species composition. Except for two fish (0.01%) all fish settling to surface collectors belonged exclusively to the species *Trachurus trachurus*, a pelagic-neritic fish that produces pelagic eggs. *T. trachurus* larvae have been mostly collected in offshore plankton sampling above the shelf edge (e.g. Farinha and Borges, 1994, Fives *et al.*, 2001, Álvarez *et al.*, 2012) and only few studies had recorded the occurrence of its early stages in the nearshore environment (Sabatés *et al.*, 2003, Borges *et al.*, 2007a, 2007b). In contrast, bottom collectors attracted, besides *T. trachurus*, eight other fish species, which are all reef associated when adults. Of these, the most abundant was *Serranus hepatus*, a benthopelagic reef fish that produces pelagic eggs. This difference in species composition between surface and bottom collectors likely reflects the preferred depth of the natural settlement habitat (Ammann, 2001, Findlay and Allen, 2002). Considering *T. trachurus*, this species has a pelagic life style but might use the nearshore reef area as a nursery for its post-larval and early-juvenile growth (chapter 4.2).

Besides this study, Ammann (2004) is the only other study that used water column collectors at different depths and described which species had settled. Contrasting to our results, in that study at the Californian coast only reef fish species were collected and the majority of

species, belonging to the genus *Sebastes*, had settled primarily to surface collectors (Ammann, 2004). The only two families shared with our study were Gobiesocidae and Gobiidae. These differences might be due to general differences in the reef fish assemblages and in the depth and type of the natural settlement habitats (e.g. kelp canopy) between both systems.

Regarding spatial variation, differences in recruit abundance between the two sampling sites were found only in bottom samples. However, bottom samples in general had very low fish abundances, thus a very low power to detect differences. Therefore, no definite conclusions are possible. Further sampling with more sites is required to evaluate the spatial difference in fish recruitment at the bottom. Previous studies revealed variation in reef fish settlement at both large spatial scales (100s of km) and at small scales (1-10s of km) (Caselle and Warner, 1996, White and Caselle, 2008, Fontes *et al.*, 2009). Both variable (Vigliola *et al.*, 1998, Wilson *et al.*, 2008) and consistent settlement patterns have been described (e.g. Sponaugle and Cowen, 1996, Fontes *et al.*, 2009). Various reasons might lead to variation in settlement at a spatial scale, with for example oceanographic and hydrodynamic processes being found to act at a large (Caselle and Warner, 1996, White and Caselle, 2008, Fontes *et al.*, 2009) and small scale (Breitburg *et al.*, 1995) and habitat characteristics influencing recruitment as well at a small spatial scale (Tolimieri, 1995, Caselle and Warner, 1996, White and Caselle, 2008).

For the two most abundant fish species, *T. trachurus* and *S. hepatus*, a temporal pattern of settlement to water column collectors was clearly observed. For *T. trachurus* higher settlement rates occurred with approximately 30 day intervals and for *S. hepatus* this interval was approximately 15 days. These cyclic patterns might indicate a lunar or a semi-lunar periodicity of settlement (chapter 4.2). For many fish species, lunar periodicity in recruitment has been established, particularly in coral reef species (Robertson *et al.*, 1988, Tricklebank *et al.*, 1992, Lozano and Zapata, 2003). The exact lunar pattern is often species-specific (Lozano and Zapata, 2003) and further investigation is required to understand what is driving the correlation between recruitment and moon phase. For both species, highest settlement was found at the beginning of the sampling period, suggesting that sampling earlier in the season might be important to cover the full settlement period in this location. This could allow for a more precise analysis of within season patterns at a finer scale and potentially increase the number of species settling to collectors. Temporal variation of

nearshore fish recruitment has been recorded to occur at different time scales: inter-annual (Vigliola *et al.*, 1998, Fontes *et al.*, 2009, Caselle *et al.*, 2010a, Félix-Hackradt *et al.*, 2013) and within season (e.g. Findlay and Allen, 2002, Vallès *et al.*, 2009, Rankin and Sponaugle, 2014). As for spatial patterns in recruitment, several studies have looked at the possible mechanisms explaining the temporal variation of fish recruitment and found that reproductive timing (Robertson *et al.*, 1988), survival and duration of the pelagic phase potentially influenced by climatological and oceanographic processes can cause temporal variation in recruitment (Raventos and Macpherson, 2005, Ben-Tzvi *et al.*, 2007, D'Alessandro *et al.*, 2007). In order to further understand such patterns, the use of replicated units such as the water column collectors used in this study will be of great value.

2.6 Conclusion

Here, we found that water column collectors filled with plastic substrate performed the best. This type of collector has been used to date in rocky reefs with (White and Caselle, 2008, Caselle *et al.*, 2010a, Lotterhos and Markel, 2012) and without kelp forest (this study), and in coral reefs (Ben-David and Kritzer, 2005), suggesting that this type of standardized recruit collector is suitable for various habitat types and comparative studies. When extending sampling for the whole major recruitment period, this type of collector potentially can reveal specific temporal patterns of several common species of the nearby rocky reefs. Its practical handling gives the opportunity to investigate settlement patterns at different spatial scales. Furthermore, the use of such collectors can allow studies to investigate the relationship between recruitment patterns and oceanographic features.

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Chapter 3

Patterns of nearshore fish recruitment at a temperate reef matching oceanographic processes

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3.1 Abstract

Variation in recruitment can substantially affect the demography and size of populations, making it a highly important topic in marine ecology, fishery science and conservation. Recruitment of most coastal fishes follows a transition from a pelagic larval phase to a benthic phase. Most knowledge on recruitment patterns of coastal fish has been derived from studies on tropical reef systems, whereas less is known for species associated to temperate reefs. In this study we used standardized artificial substrates to analyze recruitment patterns of a fish assemblage in a temperate nearshore rocky reef. During three recruitment seasons a total of 3649 fish from 30 species and 12 families settled to artificial collectors. Except for one pelagic-neritic species that settled primarily to surface collectors, all other species, mainly cryptobenthic and benthopelagic reef fish, settled to the collectors on the bottom. High temporal variation of recruitment occurred at the species and assemblage level, both at a seasonal and inter-annual scale. The recruitment assemblage structure was related with the environmental variables upwelling, SST and ocean currents. Species-specific relationships between recruitment and environment were observed. Results are also discussed in relation to annual variation of the adult's reef fish assemblage.

3.2 Introduction

Most coastal reef fish undergo a complex life cycle that includes the transition from a pelagic larval phase to a benthic or demersal phase at the time of recruitment to the reefs. Rates of recruitment (i.e. delivery of young fish to the adult habitat) can fluctuate highly at a variety of spatial and temporal scales, resulting in substantial variation in population size and dynamics (Roughgarden *et al.*, 1988, Jones, 1990, Caley *et al.*, 1996, Doherty, 2002). Therefore, a long-standing goal in marine ecology, fishery science and conservation is to understand the mechanisms explaining recruitment variation.

Among the main biological factors that can influence recruitment are adult spawning timing and location (Robertson *et al.*, 1988, Meekan *et al.*, 1993), predation (Bailey and Houde, 1989, Hixon, 2011), larval sensory and behavioral capabilities (Paris and Cowen, 2004, Leis *et al.*, 2007) and habitat choice (e.g. the presence of conspecifics) (Steele, 1999, Holbrook *et al.*, 2000, Félix-Hackradt *et al.*, 2014). Additionally, various environmental factors and processes can directly or indirectly affect the rate and patterns of onshore recruitment. These

include temperature (Findlay and Allen, 2002, Meekan *et al.*, 2003, Jenkins and King, 2006), upper ocean stability (including upwelling, wind and current speed and direction or tidal movement (Milicich, 1994, D'Alessandro *et al.*, 2007, Vallès *et al.*, 2009, Caselle *et al.*, 2010b), food availability (Meekan *et al.*, 2003, Caselle *et al.*, 2010a) and the lunar phase (Victor, 1982, Sponaugle and Cowen, 1994, D'Alessandro *et al.*, 2007).

Most knowledge on recruitment patterns of coastal fish has been derived from studies on tropical reef systems, which might be due to the usually challenging and unstable conditions found in temperate nearshore shallow environments, which limit sampling of early life stages of fish. However, underwater census has been used successfully in temperate reefs to investigate fish recruitment, e.g. in the Mediterranean (García-Rubies and Macpherson, 1995, Vigliola *et al.*, 1998, Macpherson and Zika, 1999, Raventos and Macpherson, 2005, Félix-Hackradt *et al.*, 2013a), at the Azores (Fontes *et al.*, 2009) and in California (Carr, 1991, Laidig *et al.*, 2007). While underwater surveys are possible in temperate regions, light traps and artificial collectors have gained traction in these systems, since these sampling methods are less affected by weather and water conditions and allow simultaneous sampling at various sites (Steele *et al.*, 2002, Félix-Hackradt *et al.*, 2013a).

In the Mediterranean Sea recruitment of rocky reef fish has been investigated by using light traps to study the delivery of late larval stage fish to nearshore environments (Félix-Hackradt *et al.*, 2013a, Félix-Hackradt *et al.*, 2013b). Along the west coast of North America, several studies investigated recruitment patterns of rockfish species (genus *Sebastes*) at different temporal and spatial scales, using standardized collectors (Standard Monitoring Units for Recruitment of Fish; SMURF) (White and Caselle, 2008, Wilson *et al.*, 2008, Caselle *et al.*, 2010a, Caselle *et al.*, 2010b, Lotterhos and Markel, 2012). For example, the study of Caselle *et al.* (2010b) revealed that small/local scale processes such as wind stress during settlement (at short time lags), and regional scale processes such as upwelling and downwelling operating during the early larval phase (at longer time lags), correlated with the variation of rocky fish settlement. In New Zealand, Shima and Swearer (2009) found that the recruitment intensity of the common triplefin (*Forsterygion lapillum)* to SMURFS was negatively or positively correlated to fish condition depending on the site sampled.

Most of these recruitment studies in temperate waters, investigated one or few species from the same genus and with similar early life histories. Multispecies settlement patterns were

recorded in the Mediterranean Sea (García-Rubies and Macpherson, 1995, Félix-Hackradt *et al.*, 2013a, 2014), but cryptobentic species, although very abundant in the nearshore (Hofrichter and Patzner, 2000, Beldade *et al.*, 2006, La Mesa *et al.*, 2006) were represented by very few species or not included. However, multispecies studies are important since recruitment patterns often differ between species; furthermore, the relationship of recruitment with physical processes can be species-specific (e.g. Thorrold *et al.*, 1994, Lozano and Zapata, 2003, Raventos and Macpherson, 2005, Lemberget *et al.*, 2009, Caselle *et al.*, 2010b).

In the Arrábida Marine Park, located in the central region of Portugal (Figure 3.1), Henriques *et al.* (2007) and Horta e Costa *et al.* (2014) studied the adult inshore fish assemblage over more than a decade and found that the diverse composition of species with tropical, warm- and cold-temperate affinity fluctuated inter-annually in relation with environmental winter conditions. Both studies suggested recruitment variation to be a cause, but more research is required to understand if this is the case. Knowing if recruitment is affecting the adult fish assemblage is important, as recruitment is generally highly variable in space and time and often related to oceanographic processes (see section above). Due to this relationship, recruitment is likely influenced by climate change affecting environmental conditions, for example water temperature and upwelling (Lima and Wethey, 2012, Miranda *et al.*, 2012, Bakun *et al.*, 2015).

Highest abundance and diversity of fish larvae in the area was found between May and July, indicating the peak of spawning activity of coastal fish species in this period; moreover, larval abundance and diversity decrease with increasing distance from the reef (Borges *et al.*, 2007b). Despite this knowledge of adult and larval fish assemblages in the Marine Park, little is known regarding patterns and processes of recruitment in this area (except for Gonçalves *et al.*, 2002, Beldade, 2006).

Therefore, the aim of this study was: i) to monitor the patterns of nearshore delivery of fish; ii) to compare the structure of the settler assemblage at an inter-annual, seasonal and spatial scale and iii) to analyze the relationship between the obtained patterns with environmental factors.

3.3 Material and Methods

3.3.1 Sampling

Over three years (2011 to 2013) recruitment of fish was monitored at a temperate rocky reef in the extreme nearshore of the Arrábida Marine Park (AMP; Figure 3.1), composed of areas with three different levels of protection (i.e. fully protected, partially protected and buffer area). Fish were sampled with standardized collectors filled with artificial substrate (SMURF [Standard Monitoring Units for Recruitment of Fish] (Chapter 2; Ammann, 2004, Wilson *et al.*, 2008). Earlier studies have shown that the rate of settlement to SMURFs can serve as an estimate of nearshore delivery of competent larvae and pelagic juveniles that is not affected by the presence and quality of close by natural settlement habitat and that can be related to the actual recruitment to the nearby reef (Ammann, 2001, Steele *et al.*, 2002, Ammann, 2004). Thus, throughout this study, the term settlement is used as synonym of the appearance of young fish on the collectors, regardless of the species, and is an estimate of delivery of young fish to the nearshore sites, where collectors were placed.

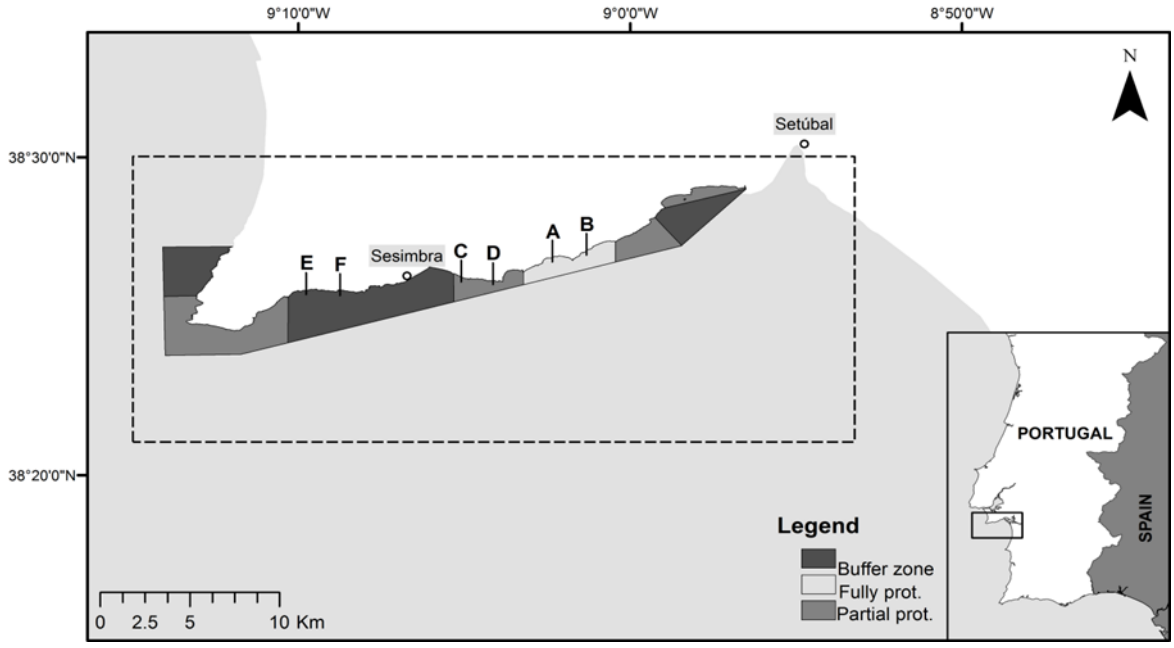

Figure 3.1. Map of the study site (Arrábida Marine Park), with indication of the protection zones, the six sampling sites A - F, and the area (dashed frame) for which satellite data were averaged.

In this study collectors were deployed in several sites of similar rocky reef habitat and depth within the AMP during the three-year period (Figure 3.1). Although the study was not designed to test MPA effects, the three areas sampled are located each in one of the three

protection zones of the AMP, in order to monitor recruitment in several sites of similar rocky reef habitat that are separated by different distances (approximately 1 km between sites and up to 10 km between areas). In 2011 sampling was conducted only in sites A and B; in 2012 additional sites C, D, E and F were sampled and in 2013 only sites in the fully and partial protected areas (A-D) were monitored, due to frequent inevitable sampling gaps in the buffer zone in the previous year.

Sampling was done weekly in 2011 from late June until late September. In 2012 and 2013, collectors were retrieved and replaced bi-weekly from April to October. Biweekly sampling was chosen as it is frequent enough to reduce post-settlement mortality on the SMURF (Steele *et al.*, 2002, Ammann, 2004).

In each area of protection, collectors were deployed at two sites, approximately 1 km apart and approximately 100 m seaward from the nearest reef (Figure 3.1). In each site, six (in 2011) or eight (in 2012 and 2013) collectors were attached to mooring lines located 20 m apart. Half the collectors were placed just below the surface (1-3.5 m depending on the tide) and the other half close to the bottom (~40 cm). Collectors were retrieved by SCUBA divers using a Benthic Ichthyofaunal Net for Coral/Kelp environments (BINCKE; Anderson and Carr, 1998; Ammann, 2004). On board the vessel, collectors were cleared over the net and samples were preserved in 80% Ethanol. Fish were identified to the lowest possible taxonomic level (species for the majority of the collections) under a stereomicroscope and total (TL) and standard length (SL) were measured to the nearest 0.01 cm.

3.3.2 Data analysis

Assemblage structure: Spatial and temporal patterns

For a spatial and temporal comparison of the fish assemblage structure, the abundance of each species in each sample was used to calculate a triangular matrix of zero-adjusted Bray-Curtis similarities. Differences in the assemblage structure among various spatial and temporal factors were tested with a permutational ANOVA (PERMANOVA) with a type III (partial) sums of squares, given its most conservative nature for unbalanced designs, and 9999 permutations of residuals under a reduced model. Dispersion of significant factors was investigated with PERMDISP, analyzing deviation from centroid and repeating 9999 runs.

Differences among areas and sites (nested in areas) were tested for the 2012 samples, since it was the only year in which sampling was performed in all 3 areas.

To compare fish recruitment among the three years, the 2011 fish abundance per collector was summed up over every two sampling weeks respectively, to match the biweekly sampling of the subsequent years. A PERMANOVA test was used to analyze temporal variance in the multivariate assemblage structure among all samples from July, August and September, since this period was sampled in all three years. Three factors were considered: depth (surface vs. bottom), site and year. The inter-annual structure of fish assemblages was graphically displayed with a nonmetric multidimensional scaling (MDS) two-dimensional plot (Clarke and Gorley, 2006).

To determine the species that most contributed to the similarity of the assemblage structure of each year, a similarity percentages analysis (SIMPER) was applied, assuming a cut off at 95%. For this analysis only bottom samples were used, since surface samples contained almost exclusively just one species.

The AMP is located within an important temperate biogeographic transition zone (Henriques *et al.*, 1999, Horta e Costa *et al.*, 2014) and in order to analyze if the biogeographic affinity of settlers changed among sampling years, species were classified by their climatic affinity (temperate [T], cold- [CT] and warm-temperate [WT], tropical [Tr] and eurythermic [E]) according to Henrique et al. (2007). And in order to test possible differences of the assemblage structure across these affinity groups, a PERMANOVA test with the factors site and year was made with bottom samples from July, August and September, since settlers of surface collectors belonged to just one biogeographic group.

Linking assemblage structure to environment

For July, August and September of each year, daily data were compiled for the following environmental parameters: sea surface temperature (SST), concentration of Chlorophyll a (Chla), eastward and northward current velocity (U- and V-component, respectively), wind speed and direction, Northern Atlantic Oscillation (NAO) index and up-welling index (Table 3.1). For spatial data (SST, Chla and currents), a daily average was calculated for the study region (Figure 3.1; extent: -9.25, -8.50, 38.50, 38.35). The upwelling index was calculated as the difference between offshore SST measured from an offshore area of similar size

located two degrees further offshore at the same latitude, and the SST in the AMP area, (Santos et al., 2005; Relvas et al., 2009). Positive values relate to upwelling conditions and negative values describe relaxation from upwelling.

Table 3.1. Environmental variables. Listed are the unit, spatial and temporal resolution (Spa. and Temp. res.), the type (RS=remote sensing, DO=direct observation, MO=Model), source of the dataset and reference.

Variable	Unit	Spa. res.	Temp. res.	Type	Dataset/ Source	Reference
SST	°C	~6 km	daily	RS + DO	Copernicus Marine environment monitoring service (marine.copernicus.eu)	OSTIA[a]; Donlon *et al*., 2012
CHLa	mg/m^3	~4 km	daily	RS		Gohin *et al*., 2008
U and V current	m/sec	~3 km	daily	MO		IBI[b]; Sotillo *et al*., 2015
NAO	index	NA	daily	RS	NOAA Center for Weather and Climate Prediction (cpc.ncep.noaa.gov)	Chen and van den Dool, 2003
Wind speed	m/sec	NA	hourly	DO	Local weather station	NA
Wind direction	°	NA	hourly			

[a]OSTIA (Operational Sea Surface Temperature and Sea Ice Analysis) run by the UK Met Office combines remote sensed satellite data provided by the GHRSST project with in-situ observations, to determine through an Optimal Interpolation procedure global sea surface temperature (SST; Donlon *et al*., 2012).
[b]IBI (Iberian Biscay Irish) Ocean Analysis and Forecasting system run by Puertos del Estado and Mercator Ocean is based on an eddy-resolving NEMO model application driven by meteorological and oceanographical forcing (Sotillo et al., 2007 and 2015, Madec, 2008).

For a comparison of the recruitment and environmental data the latter were averaged over the two weeks preceding sampling, to capture the time during which fish settled to the collectors. Both a multiple pairwise scatterplot with Pearson correlations and a Variance Inflation Factor (VIF) analysis were used to look for collinearity among environmental variables (Zuur et al., 2010). A conservative Pearson correlation of R^2=0.6 was used as a threshold. The eastward current component (U) was highly correlated to the northward current component (V; Pearson R^2 = 0.87) and so only the first one was used in the analysis, from now on referred as current. Similarly, the NAO index and current were also highly correlated (Pearson R^2 = 0.63). Thus, current was used in the analysis, as it is a local variable that is assumed to have a more direct effect than the climatic index NAO. Accordingly, explanatory variables that were included in the analysis were SST, upwelling index, current and Wind speed.

Biweekly recruitment (response variable) was calculated using the average abundance per sampling date for each species. We used data from bottom samples only in the fully protected area, in order to have a balanced design in the linkage analysis. The Bio-Env analysis in PRIMER6 was applied to search for the environmental variables and their combinations that best "explain" the pattern of settlement to the collectors. For this, the function searches for the highest rank correlation (coefficient is ρ) between the fixed biotic triangular similarity matrix (Bray-Curtis) and the variable abiotic matrix (Euclidean distance), a varying subset of normalized environmental variables (Clarke and Gorley, 2006, Clarke *et al.*, 2008). The Spearman rank correlation method was used and 9999 permutations were run.

The subset of environmental variables selected from the Bio-Env analysis was then used in a Linkage Tree analysis (Clarke and Gorley, 2006, Clarke *et al.*, 2008), to divide the fish assemblage samples into successive smaller groups, by binary divisions that are explained each by a threshold of one or more of the selected environmental variables. The Linkage Tree analysis is an extension of the Multivariate Regression Tree (MRT; De'Ath, 2002, Clarke *et al.*, 2008), to allow for a non-parametric and non-additive procedure (Clarke *et al.*, 2008). Only ranks are used from the triangular matrices and ANOSIM R statistic (0-1) is increased between the two groups of samples at each separation and matrices are re-ranked at each successive division (Clarke *et al.*, 2008). A similarity profile test (SIMPROF) was added to the Linkage Tree analysis to test the significance of the derived subdivisions.

The multivariate analysis was performed with the software PRIMER v6 (Clarke and Gorley, 2006) with PERMANOVA add-on (Anderson, 2001) and the software R (v3.1.2; R-Core-Team, 2009) was used for the exploration of the environmental data.

3.4 Results

3.4.1 Species composition

During the course of the study, a total of 3649 fish from 28 species and 12 families settled to the collectors. Of all species collected, 53% were cryptobenthic and 43% benthopelagic (Table 3.2). Most (70%) of these species inhabit the rocky reef, 24% occur in the rocky reef and also in sandy areas between rocks in the nearshore and only two species occur as well further offshore over the continental shelf (Table 3.2). A similar proportion of species spawning pelagic or demersal eggs was found. Considering morphology and size, most of the settled fish ranged from competent larvae to juveniles. Nevertheless, for some species, a few larger fish also settled to the collectors (see size range in Table 3.2). The species composition differed greatly between surface and bottom collectors (Table 3.3), as 98% of fish from surface collectors belonged to the pelagic neritic species *Trachurus trachurus* (Carangidae). All the other recorded species settled to bottom collectors (including *T. trachurus*).

3.4.2 Species structure: Spatial and temporal patterns

The settlement assemblage structure was characterized by strong interannual variation, as well as both within-season and spatial variation.

In 2012, the assemblage structure was not significantly different between the three areas, but differed between sites within areas (Table 3.3). However, the Permdisp results revealed that dispersion between sites was also significantly different ($F=4.7829$, $df1=5$, $df2=528$, $P<0.05$). When analysing all three years, significant differences between sites were found only in 2012 (pairwise PERMANOVA: $P_{perm} <0.05$) and not in 2011 and 2013 (pairwise PERMANOVA: $P_{perm}>0.05$).

Table 3.2. Species information regarding commercial value (€; 0-non, 1-minor, 2-medium, 3-high), biogeographic group (T-temperate, CT-cold-, and WT-warm-temperate, Tr-tropical, E-eurythemic), habitat (1- rocky reef, 2-coastal, 3-coastal and shelf area, 4-rocky reef and sea grass, 5-rocky reef and coastal , 6-rocky reef, coastal and sea grass), habitat use (C-cryptobenthic , B-benthopelagic, P-pelagic-neritic) , egg type (D-demersal, P-pelagic), spawning period (Borges, R., unpublished data; Macpherson, E., pers. communication), pelagic larval duration (PLD; Raventos and Macpherson, 2001; Macpherson and Raventos, 2006; Beldade et al., 2007; Borges, 2006; Klein, M., unpublished data), and from this study: total abundance (N), yearly (2011-2013) relative recruitment per collector, recruitment period (REC), settlement proportion at surface (SUR) and bottom (BOT) , mean and range of standard length (SL).

Relative recruitment: 0 | <0.2 | <0.4 | <0.6 | <0.8 | ≤1

Family	Species	€	Biog.	Hab.	H. use	Egg	PLD	Spawning	Year N	11	12	13	REC	Depth SUR	BOT	SL (mm) Mean	Range
Blenniidae	*Parablennius gattorugine*	0	WT	1	C	D		Mar - Jul	41				Apr - Sep	0.3	0.7	18.1	13.1 - 24.7
Blenniidae	*Parablennius pilicornis*	0	Tr	1	C	D	28-45	Dec - Sep	63				Jul - Aug	0.1	0.9	26.7	16.2 - 51.3
Carangidae	*Trachurus trachurus*	3	T	3	P	P	NA	Apr - Sep	2439				Apr - Oct	1.0	0.0	17.7	5.9 - 65.4
Gadidae	*Trisopterus luscus*	1	T	3	B	P			2				Jul - Aug	0.0	1.0	34.7	11.3 - 58.2
Gobiesocidae	*Apletodon dentatus*	0	WT	1	C	D	12 - 17		23				Jul - Aug	0.0	1.0	18.1	8.5 - 28.9
Gobiesocidae	*Apletodon incognitus*	0	WT	4	C	D	12-24		5				Aug - Sep	0.0	1.0	15.1	11.3 - 19.2
Gobiesocidae	*Apletodon pellegrini*	0	Tr	1	C	D	17-20		22				Apr - Oct	0.0	1.0	21.2	14.4 - 30.1
Gobiesocidae	*Diplecogaster bimaculata*	0	T	1	C	D	10 - 14		20				Apr - Sep	0.0	1.0	18.3	12.6 - 28.9
Gobiesocidae	*Lepadogaster candolii*	0	WT	1	C	D	12 - 15		28				Jun - Oct	0.0	1.0	16.1	6.1 - 24.6
Gobiidae	*Gobius gasteveni*	0	WT	2	C	D	14 - 23		9				Jul - Sep	0.1	0.9	34.9	20.9 - 52.1
Gobiidae	*Gobius paganellus*	0	WT	2	C	D	22 - 29		4				Jun - Sep	0.3	0.7	20.2	15.2 - 25.3
Gobiidae	*Gobius xanthocephalus*	0	WT	2	C	D	24 - 42	Aug - Jul	7				Jul - Oct	0.0	1.0	33.2	19.0 - 39.9
Gobiidae	*Pomatoschistus pictus*	0	T	2	C	D	30-43	Apr - Nov	117				Apr - Sep	0.0	1.0	17.8	12.8 - 33.5
Labridae	*Ctenolabrus exoletus*	0	CT	4	B	D			2				Jul - Sep	0.0	1.0	30.9	20.0 - 41.7
Labridae	*Ctenolabrus rupestris*	0	T	1	B	P	18-37	Apr - Sep	136				Apr - Oct	0.0	1.0	27.4	8.8 - 56.9
Labridae	*Labrus bergylta*	0	CT	1	B	D			1				Jun	0.0	1.0	33.8	33.8 - 33.8
Labridae	*Labrus mixtus*	0	T	1	B	D			26				May - Sep	0.0	1.0	37.7	14.2 - 98.5
Labridae	*Symphodus melops*	0	CT	1	B	D	15	year round	8				Jul - Sep	0.0	1.0	28.0	13.4 - 41.0
Lotidae	*Ciliata mustela*	0	CT	5	C	P			6				Apr - Jul	0.2	0.8	46.5	26.8 - 72.5
Lotidae	*Gaidropsarus mediterraneus*	1	T	1	C	P	43	Apr - Jul	116				Apr - Aug	0.1	0.9	39.7	24.7 - 98.7
Scophthalmidae	*Zeugopterus regius*	0	T	5	B	P			5				Jul - Sep	0.2	0.8	18.5	10.6 - 32.9
Scorpaenidae	*Scorpaena notata*	1	WT	1	C	P			13				Aug - Sep	0.0	1.0	14.9	11.7 - 19.6
Serranidae	*Serranus cabrilla*	1	Tr	6	B	P	21 - 28	Apr - Sep	22				Jul - Oct	0.1	0.9	37.8	19.9 - 67.7
Serranidae	*Serranus hepatus*	1	WT	6	B	P	16-29	Apr - Sep	370				Jun - Oct	0.0	1.0	18.8	5.8 - 47.5
Sparidae	*Boops boops*	3	E	2	B	P	16 - 20	Apr - Sep	2				Apr - May	0.5	0.5	14.5	11.3 - 17.8
Sparidae	*Pagrus pagrus*	3	WT	2	B	P	38		3					0.0	1.0	18.2	16.5 - 20.4
Sparidae	*Spondyliosoma cantharus*	2	T	2	B	D	29 - 38	Apr - Aug	157				Apr - Aug	0.0	1.0	16.9	8.9 - 35.9
Tripterygiidae	*Tripterygion delaisi*	0	WT	1	C	D	16 - 21	Mar - Sep	2				Aug - Sep	0.0	1.0	15.7	15.5 - 15.9

Table 3.3. PERMANOVA results of (a) spatial (only 2012), (b) inter-annual analysis of settler and (c) biogeographic species group composition with factors Area (AR), Site (SI nested in Area), Year (YE) and Depth (DE).

	Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms	P(MC)
(a) Spatial	AR	2	6181.5	3090.7	0.72245	0.7937	90	0.7342
	SI(AR)	3	12957	4318.9	3.3521	0.0001	9906	0.0001
	Res	528	6.80E+05	1288.4				
	Total	533	7.00E+05					
(b) Temporal & vertical	YE	2	1.12E+05	56011	23.593	0.0002	9958	0.0001
	DE	1	66091	66091	30.274	0.0011	9875	0.0001
	SI	5	15412	3082.4	2.8033	0.0001	9889	0.0001
	YExDE	2	88163	44081	14.74	0.0005	9971	0.0001
	YExSI**	4	9794.9	2448.7	2.227	0.0012	9914	0.0007
	DExSI	5	15408	3081.5	2.8025	0.0001	9871	0.0001
	YExDExSI**	4	12406	3101.5	2.8207	0.0001	9889	0.0001
	Res	589	6.48E+05	1099.6				
	Total	612	9.91E+05					
(c) Biogeo. Groups	YE	2	5.25E+04	26236	6.8114	0.0402	9963	0.0033
	SI	5	22631	4526.1	4.0377	0.0001	9899	0.0001
	YExSI**	4	15975	3993.8	3.5628	0.0001	9927	0.0003
	Res	294	3.30E+05	1121				
	Total	305	4.30E+05					

The temporal analysis revealed that the settlement assemblage was significantly different between the three years (Table 3.3b). The settlement assemblage from bottom collectors at all sites was significantly different between all three years (pairwise PERMANOVA: $P_{perm}<0.01$). At the surface, only at some sites (of the FP area), the structure of the assemblages was different between the three years (pairwise PERMANOVA: $P_{perm}<0.01$); in the other sites, surface samples were not significantly different between 2012 and 2013 (pairwise PERMANOVA: P_{perm}= n.s.). Significantly different dispersion was also found between the three years ($F=89.411$, $df1=2$, $df2=610$, $P_{perm}<0.001$), but the MDS graphic reveals a good spatial separation between samples of the three years (Figure 3.2).

Average similarities between bottom samples from 2011, 2012 and 2013 were low (12.3%, 4.9% and 24.4%, respectively), but different species groups contributed to explain similarities of samples within each year. In 2011 *Serranus hepatus* (36%), *Serranus cabrilla* (33%) and *T. trachurus* (28%) explained most of the similarity; in 2012 *Ctenolabrus rupestris* (50%) and *Parablennius pilicornis* (21%) explained the low similarity; in 2013 the similarity between samples was higher, with *S. hepatus* explaining 91% of such similarity.

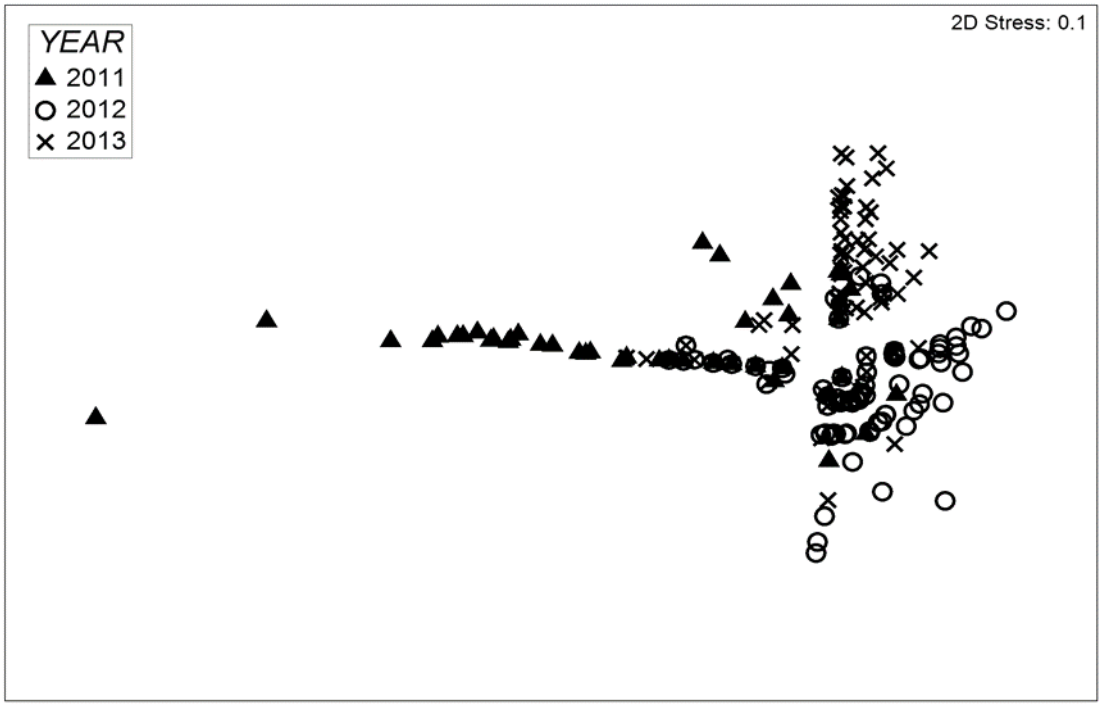

Figure 3.2. Non-metric multidimensional scaling (MDS) plot showing SMURF samples for each year (indicated by symbols). The spatial segregation of samples from the three years reflects differences in the structure of the settler assemblages.

Settlement patterns within the sampling season varied between species and within species between years (Figure 3.3). The most abundant fish *T. trachurus* was nine and seven times more abundant in 2011 than in 2012 and 2013, respectively, despite a shorter sampling season in 2011. *Gaidropsarus mediterraneus* and *Spondyliosoma cantharus* were abundant in 2012, much less in 2013 and were absent in 2011 (Table 3.2, Figure 3.3). Peaks of settlement varied between years as peaks of *S. hepatus*, *P. pilicornis*, occurred later in 2012 compared to the two other years. In contrast, the peak of settlement for *Lepadogaster candolii* occurred at a similar time between years (Figure 3.3).

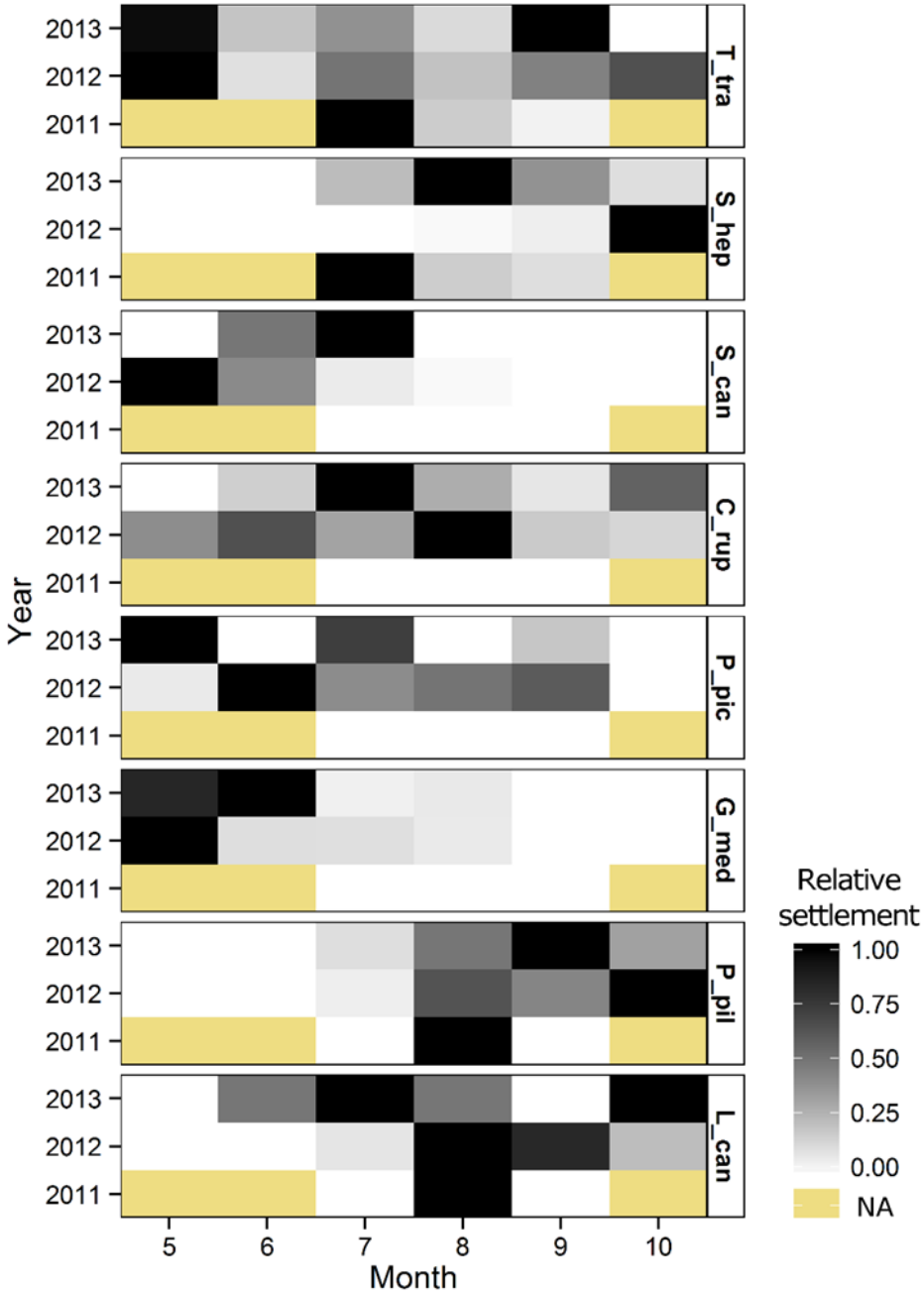

Figure 3.3. Relative monthly patterns of settlement in 2011, 2012, and 2013. Most abundant species are plotted in decreasing order from top to bottom: *Ctenolabrus rupestris* (C. rup), *Gaidropsarus mediterraneus* (G. med), *Lepadogaster candolii* (L. can), *Parablennius pilicornis* (P. pil), *Spondyliosoma cantharus* (S. can), *Serranus hepatus* (S. hep) and *Trachurus trachurus* (T. tra). Relative settlement values equals the mean abundance of settlers per collector for each species/month/year combination divided by the highest abundance for this species in that year. No samples (NA) were available for May, June and October in 2011.

3.4.3 Biogeographic group structure

The structure of biogeographic affinity groups was also significantly different between years at all sites except of site A (PERMANOVA, $P_{perm}<0.005$, Table 3.3). On average, more temperate fish settled to collectors in 2012 than in 2011 and 2013, while settlement of warm temperate species was strongest in 2013 (Figure 3.4).

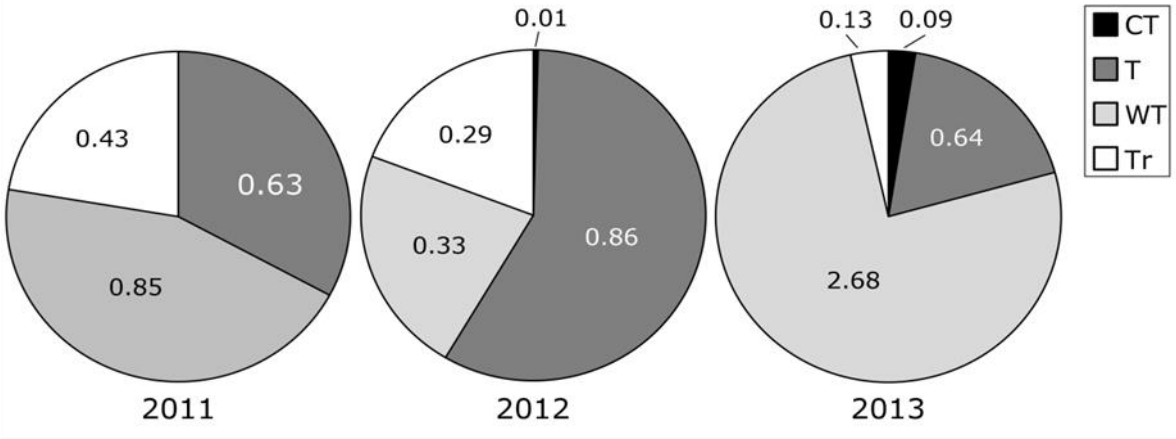

Figure 3.4. Yearly composition of biogeographic species groups at the bottom (CT: cold-temperate, T: temperate, WT: warm-temperate, Tr: tropical). Numbers in pie diagrams represent average settlement per SMURF sample.

3.4.4 Linking species assemblage to environment

All environmental variables differed between years (Figure 3.5). The best combination of environmental variables found to match the pattern of the settlement assemblage was the upwelling-index and current (ρ =0.302, $P_{perm}<0.01$). The second highest correlation was found with just the upwelling index (ρ =0.297) and the third highest with upwelling index, current and SST (ρ =0.278).

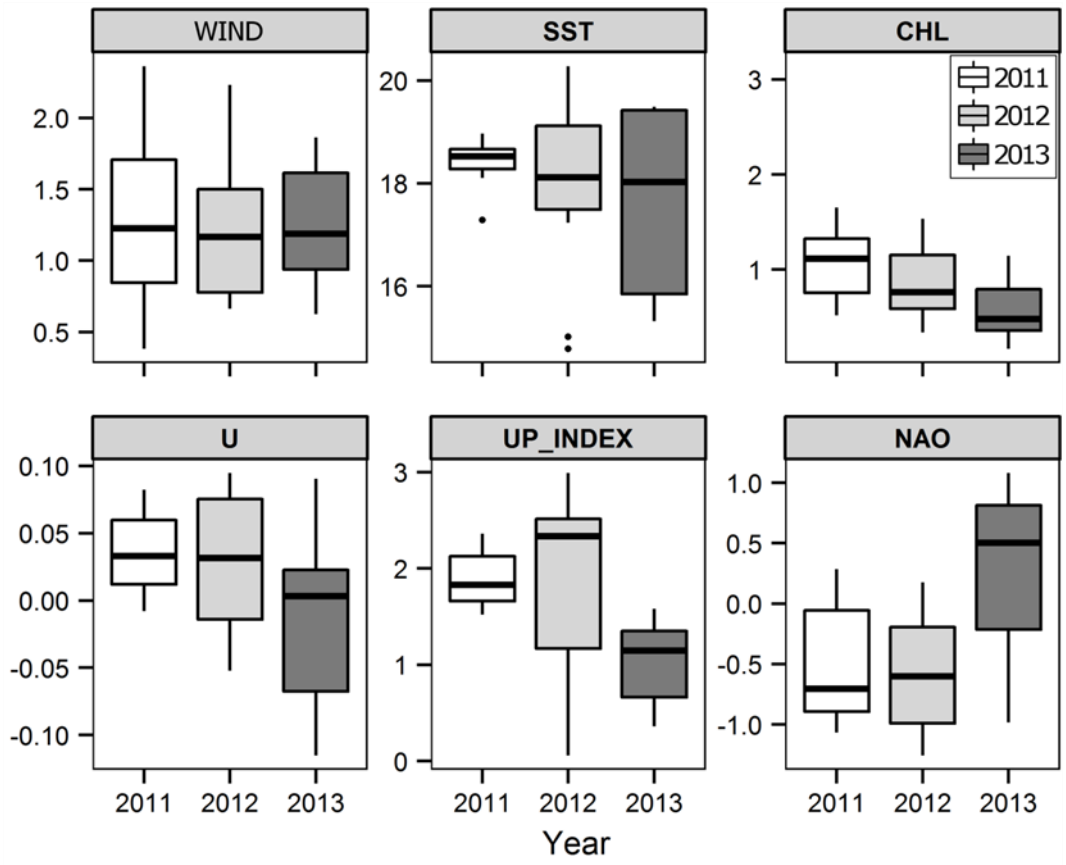

Figure 3.5. Yearly boxplots of environmental variables: Wind speed (WIND; m/sec;), SST (°C), Chla (mg/m3), U current component (U; m/sec), up-welling index (UP_INDEX) and NAO index. Data shown were two week averages restricted to the sampling period. Legend in top right graph corresponds to all graphs.

The Linkage Tree analysis using these three variables with high correlations identified three major clusters (Figure 3.6). The first split (A) divided almost all samples from 2012 from the samples of 2011 and 2013. The samples of 2012 had a higher upwelling index (>2.22) than the samples from 2011 and 2013 (<2.22) and the separation had an optimal ANOSIM R of 0.52. The second split (B) divided 2011 samples from 2013 samples by a SST threshold of >19.2 for 2011 samples and <19.1 for 2013 samples. This split had a degree of separation of R=0.61. Splits defined by the linkage tree analysis were not statistically significant (SIMPROF: π=1.52, p= n.s.).

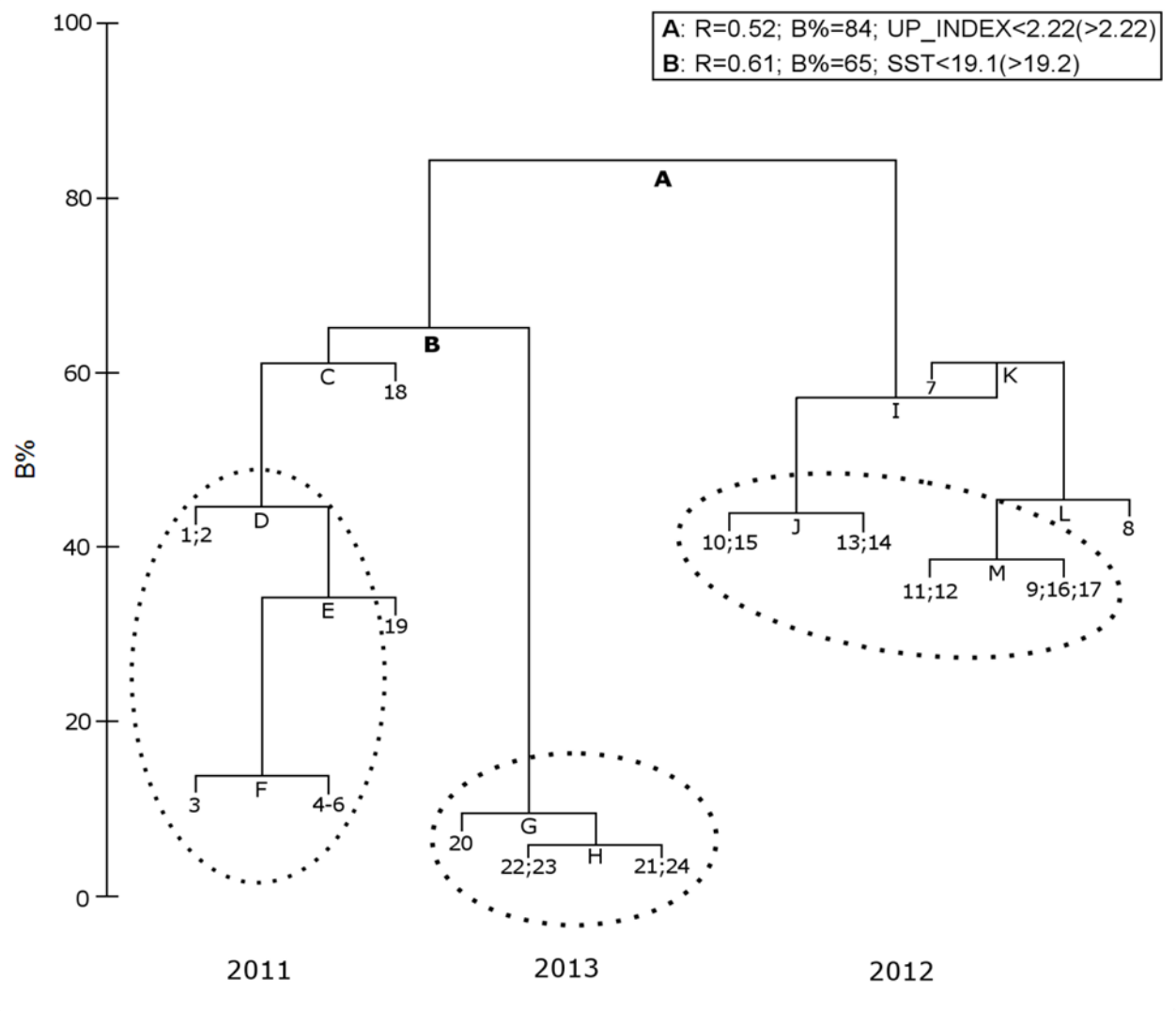

Figure 3.6. Linkage Tree analysis showing clear clustering of years from settlement composition, due to inequalities in environmental variables upwelling-index and SST. Top-right box shows for the major split A and B the optimal ANOSIM R value (relative subdivision separation) and B% (absolute measure of subdivision separation). Both values range from 0 (no separation) to 1 (total separation). For each split first inequality describes division to the left and second inequality (in brackets) division to the right.

3.5 Discussion

Here we demonstrate high temporal variation of fish settlement in a temperate rocky reef system. Variation occurred at both species- and assemblage-levels and at seasonal and inter-annual scales. The inter-annual pattern of the structure of the settlement assemblage matched with the pattern of local environmental variables such as upwelling, current and SST. The structure of cold-temperate, temperate, warm-temperate and tropical biogeographic affinity groups also varied between years and was related with oceanographic conditions.

The strong effect of depth on the composition of species settling to the collectors was consistent over time and space. Surface collectors were preferred almost exclusively by one species, *T. trachurus*, a pelagic-neritic fish that inhabits the coastal and shelf area as older fish. In contrast, bottom samples were dominated by cryptobenthic and bentho-pelagic reef fish. This difference in species composition between the surface and the bottom likely reflects the preferred depth and features of the natural settlement habitat (Ammann, 2001, Findlay and Allen, 2002). Other species that settled to the surface, although in very low numbers, were *P. gattorugine*, *S. hepatus* and *G. mediterraneus*. Settlers of these species were smaller and much less abundant at the surface than in bottom collectors and *G. mediterraneus* individuals still had their pelagic countershading coloration (silver at the ventral side and blue at the dorsal side, turning to brown when settling to the bottom). We therefore assume that these fish were late stage competent larvae and pelagic juvenile (in case of *G. mediterraneus*; Demir, 1982), starting to migrate to the bottom.

Most of the species sampled throughout this study are cryptobenthic as juveniles and adults. This shows the advantage of using standardized artificial substrates to monitor recruitment, such as SMURFs, for this kind of species, since in visual census cryptobenthic species are normally overlooked. Many of the species collected in this study were not recorded in recruitment studies using visual census and light traps in the Mediterranean Sea, although many species are shared between both systems (García-Rubies and Macpherson, 1995, Félix-Hackradt *et al.*, 2013a, 2014); this difference is likely caused by differences in the sampling methods.

The most abundant species in this study after *T. trachurus*, from highest to lowest were *S. hepatus*, *S. cantharus*, *C. rupestris*, *Pomatoschistus pictus*, *G. mediterraneus* and

P. pilicornis. As adult fish most of these species were common and abundant in underwater visual census of the shallow rocky reef in the study area (Gonçalves *et al.*, 2003, Horta e Costa, unpublished data, Beldade *et al.*, 2006). *S. hepatus*, *S. cantharus* and *G. mediterraneus* were rare or variable in these studies, which in case of *S. hepatus* could be due to the lack of analysing the near-reef sandy bottom.

In larval surveys *T. trachurus*, *C. rupestris*, *P. pictus* and *P. pilicornis* were abundant, but larvae of *S. hepatus*, *S. cantharus* and *G. mediterraneus* were rare (Borges *et al.*, 2007a, Borges *et al.*, 2007b). In contrast, *Tripterygion delaisi* that is a very common cryptobenthic species in the study area (Gonçalves *et al.*, 2003, Beldade *et al.*, 2006) and had very high abundance in larval surveys (Borges *et al.*, 2007a), were rare in our collectors. The clingfishes (Gobiesocidae) *Apletodon dentatus*, *A. incognitus*, *A. pellegrini* and *Diplecogaster bimaculata* recorded here have not been sampled in fish larval surveys. These differences between previous larval studies and this study are likely explained by the different methods used. The collectors used here might be more selective and attract only fish settling to algal habitats (Ammann, 2004). However, for only 6 of the settled species (clingfish and labrids) settlement to algal turfs has been recorded in the study area (Gonçalves, E., unpublished data), thus more *in situ* observations are needed to confirm this hypothesis. Another possible explanation could be that SMURFs might have been installed too far offshore from the nearest rocky reef, thus missing species that can be retained in the very nearshore near the rocky reef throughout their larval phase (e.g. *T. delaisi* and *L. lepadogaster*; Borges et al., 2007a, Borges et al., 2007b). The latter is a common clingfish which was absent from collectors, but that commonly settles in the intertidal (Beldade, R., unpublished data). However, PLDs of collected species ranged from a few days (10 days for *D. bimaculata*) to over a month (45 days for *P. pilicornis*), suggesting that species with different early life history traits, e.g. different natural settlement habitats, had settled to the collectors.

A few settlers of some species collected in this study were larger than settled fish observed in the natural habitat (own observations). This is especially curious for the cryptobenthic species that are reported to have very high site fidelity such as clingfish and blennies (Gonçalves *et al.*, 1998, Gonçalves *et al.*, 2002) and thus would not be expected to move from the rocky reef to the collectors. Our results indicate that some of these fish may move more than what has been assumed, leaving the rocky reef after settlement and migrating to

distances as far as at least 100 m off the reef on sandy bottoms, where the collectors were deployed. Another hypothesis is that some of these fish may have already settled to the bottom prior to finding a suitable reef, and where in search of adequate habitat for settlement. The ability to delay settlement has been described before and in most cases metamorphosis was delayed as well (Cowen, 1991, McCormick, 1999, Victor, 2007). But Kaufman *et al.* (1992) found that metamorphosis may happen before, during, or after the settlement phase. Another explanation could be that some of these species move between settlement habitats or could have settled to floating material such as rafts of wood or plastics and found the collectors when approaching the shore. Ontogenetic habitat shifts have been reported for tropical and temperate reef fish (Eggleston, 1995, Nagelkerken *et al.*, 2001, Gonçalves *et al.*, 2002, Félix-Hackradt *et al.*, 2014). Future otolith microstructure analysis might provide crucial information on the actual timing of settlement and more accurate relationships between settlement and environmental processes could be achieved.

Spatial variation in settlement occurred at a very fine scale (~20 m between collectors). No difference was found between the three areas and the significant difference between sites found only in 2012 might have been caused by the high dispersion between samples. This suggests that the settlement pattern within the study area is highly patchy at a fine scale but less variable at a larger scale (1000-10000 m). Vallès *et al.* (2008), using standardized benthic collectors, found that recruitment of *Stegastes partitus* varied highly at a fine scale (~9 m) and less at a larger scale (~2 km), suggesting a possible influence by small-scale flow patterns (Breitburg *et al.*, 1995) and resident fishes (Lecchini *et al.*, 2005).

Temporal variation in settlement was found between the three sampling years as well as within years. This difference was found to occur at the species level, as for example *T. trachurus* settlers were highly abundant in 2011 but recruitment was much weaker in the two following years.
Settlement of the more abundant species *C. rupestris*, *G. mediterraneus*, *S. cantharus* and *P. pictus* appeared to have failed in 2011. While this might have been, in part due to the later sampling start date in 2011 (i.e. July), these same species did settle in months later than June during the two following years. Furthermore, it can be assumed that settlement of the sparid *S. cantharus* failed as well in 2013, since only four settlers were counted (40 times less than in 2012).

Such failures in settlement could be due to either unfavorable oceanographic processes leading to the mortality of the larval stages or affecting transport and delivery of larvae to the coast (Fontes *et al.*, 2009). Another explanation could be a lack of an essential abundance of larvae due to restricted larval production. Studies performing underwater visual census in the area, also recorded highly variable abundances of adult *S. cantharus* between years (Horta e Costa, unpublished data).

Annual failures ("year-class failures") in settlement have been recorded before for rockfish species in a temperate, nearshore environment in California (Wilson *et al.*, 2008, Caselle *et al.*, 2010a). In these studies, settlement failure occurred at a larger spatial scale (100s of kilometers) than in our rather small-scale study. Possible causes could be a failing in larval production, survival, transport and delivery due to anomalous and/or unfavorable environmental conditions (e.g. late upwelling; Caselle *et al.*, 2010a). Large climatic events, such as El Ninõ-Southern Oscillation and extreme anomalies of NAO can strongly affect larval survival over large scales (Bailey *et al.*, 1995, Stenseth *et al.*, 2002). To identify at which phase (larval production, transport, etc.) and over which spatial scale environmental processes act is still very difficult. One of the reason is the difficulty of tracking the source of settlers (Warner and Cowen, 2002, Siegel *et al.*, 2008, Caselle *et al.*, 2010b).

Annual differences in the structure of the settlement assemblage and differences in settlement timing found here for some species may be due to oceanographic processes affecting larval production, transport and/or survival. The inter-annual pattern of the settlement assemblage was best related with the pattern of upwelling, currents and SST. Current co-varied strongly with the NAO index, thus we could not separate which of the two variables (or their combination) had an effect on the settlement assemblage. However, in contrast to the climatic index NAO that may indirectly affect the assemblages, water currents can have a direct influence on the transport of fish early stages.

Although the divisions of the Linkage Tree were not statistically significant, the results give an indication of how annual variation in these oceanographic variables can relate with differences found in the settlement assemblage structure. The upwelling index separated the settlement assemblage of 2012 with the two other years. These results indicate that upwelling had a positive effect on settlement as in 2012 upwelling intensity and the number of species was highest and with most species recording highest mean abundances. However, the

relationship between upwelling and the two most abundant species, *T. trachurus* and *S. hepatus* differed from the other species, as *T. trachurus* was most abundant at medium upwelling intensity (2011) and *S. hepatus* settlement was highest in 2013 when average upwelling intensity was the lowest. A within season study of *T. trachurus* recruitment patterns in 2011 revealed a negative correlation between upwelling and recruitment, confirming a strong influence by this oceanographic process (chapter 4.2).

A study on rockfishes (genus *Sebastes*) in California, that used the same sampling method, also found a high correlation between nearshore settlement and upwelling (Caselle *et al.*, 2010a). The positive correlation found here for most species, might be due to the higher production of phyto-and zooplankton caused by upwelling (Cunha, 1993, Moita, 2003) and possible higher rate of larval survival. However, the interaction with oceanographic processes such as upwelling seems to be species specific as settlement of *S. hepatus* appears to be negatively affected, which might be due to related increased offshore transport or low coastal water temperature. Furthermore, the timing of settlement varied between years for some of the more abundant species. For example settlement of *S. hepatus* and *P. pilicornis* occurred later in 2012, possibly resulting from a delay in larval production or longer PLD, likely related to environmental conditions (Green and Fisher, 2004, Sponaugle *et al.*, 2006). Species-specific analyses are required for a deeper understanding of how and which oceanographic processes may affect settlement patterns.

In the same area, studies on the adult rocky reef fish assemblage revealed inter-annual variation of the biogeographic composition, which correlated with winter sea conditions (Henriques *et al.*, 2007, Horta e Costa *et al.*, 2014). The inter-annual variation of adult fish assemblages was influenced by SST at a regional scale and by the large-scale climatic variable NAO (Henriques *et al.*, 2007). However, the study by Horta e Costa *et al.* (2014) found a better correlation with local-scale oceanographic variables such as winter Wind Stress and SST. Both studies suggested that the rapid variation of the fish assemblage between cold and warm years was caused primarily by larval transport and recruitment success however, neither study tested this. Here we confirm that the rocky reef fish assemblage at the settlement stage was also correlated with oceanographic variables. Furthermore, when considering the structure of the settlement assemblage by biogeographic groups (as in Henriques *et al.*, 2007, Horta e Costa *et al.*, 2014), inter-annual variation was also found. For example, in 2013 three and eight times more fish with warm-temperate

affinities had settled compared to 2011 and 2012, respectively. This increase in settlement of warm-temperate species could be a result of low upwelling conditions, higher summer SST (Figure S3.1 in the supplements) and/or the occurrence of northward current during some weeks in 2013 (Figure S3.2 in the supplements). Current direction in 2011 and 2012 was exclusively southwards. Moreover, the 2013 settlement season followed a winter with negative NAO values, different to the two previous years, which in general indicates warm years in southwest Europe (Hurrel, 1995). Similar conditions of SST and NAO revealed as well a higher proportion of adult fish with warm-water affinities (Henriques *et al.*, 2007, Horta e Costa *et al.*, 2014), but in these studies winter conditions were primarily relevant. A longer time series of recruitment in the study region is important in order to bolster the results here showing that oceanographic processes are a driver of recruitment variation.

3.6 Acknowledgements

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3.8 Supporting information

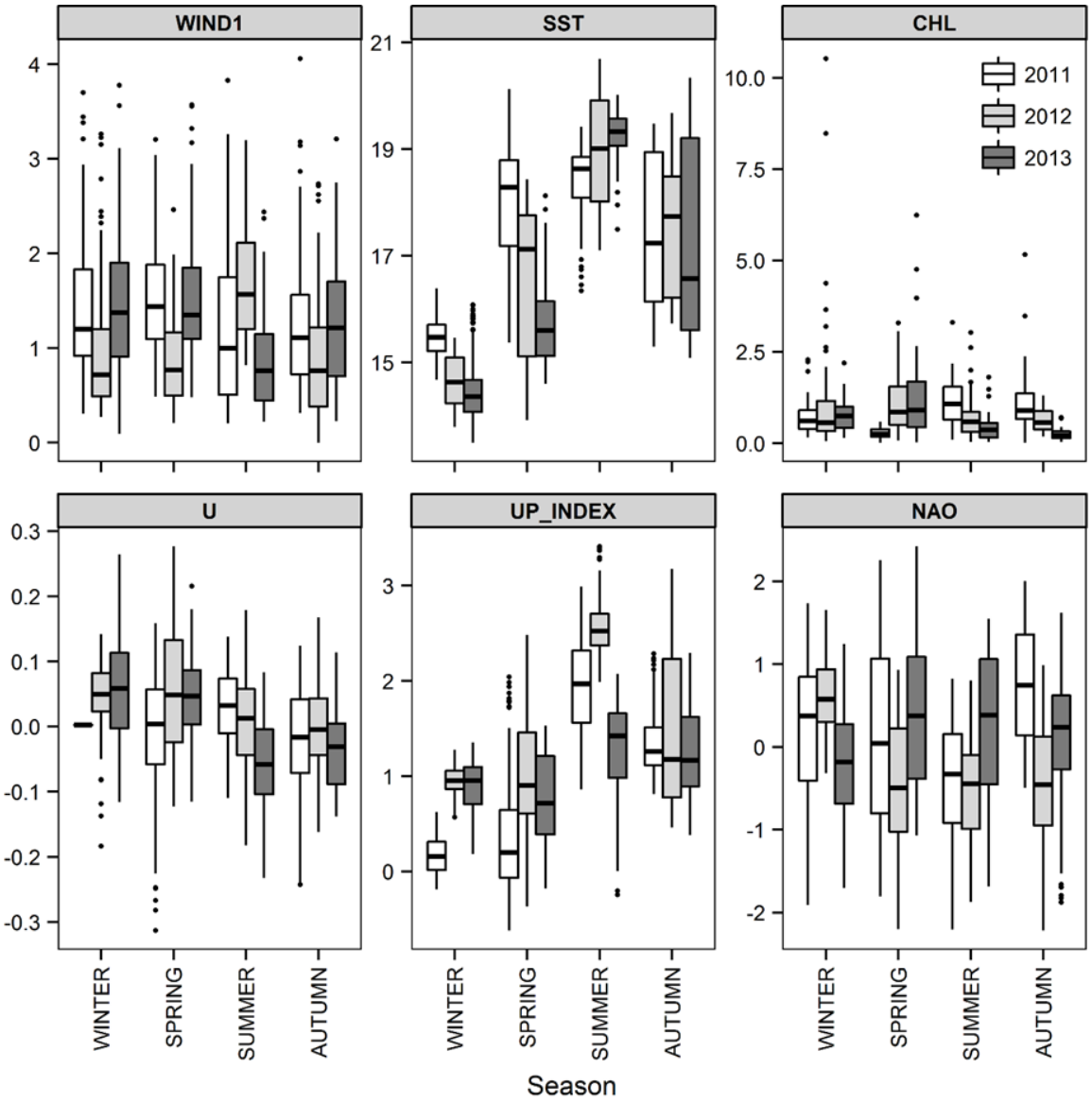


Figure S3.1. Seasonal boxplots of wind (m/s), SST (ºC), Chl a concentration (mg/m^3), current (U-component; m/s), upwelling index (UP_INDEX) and NAO index. Winter includes values from January, February and March; spring from April, May and June; summer from July, August and September and autumn from October, November and December.

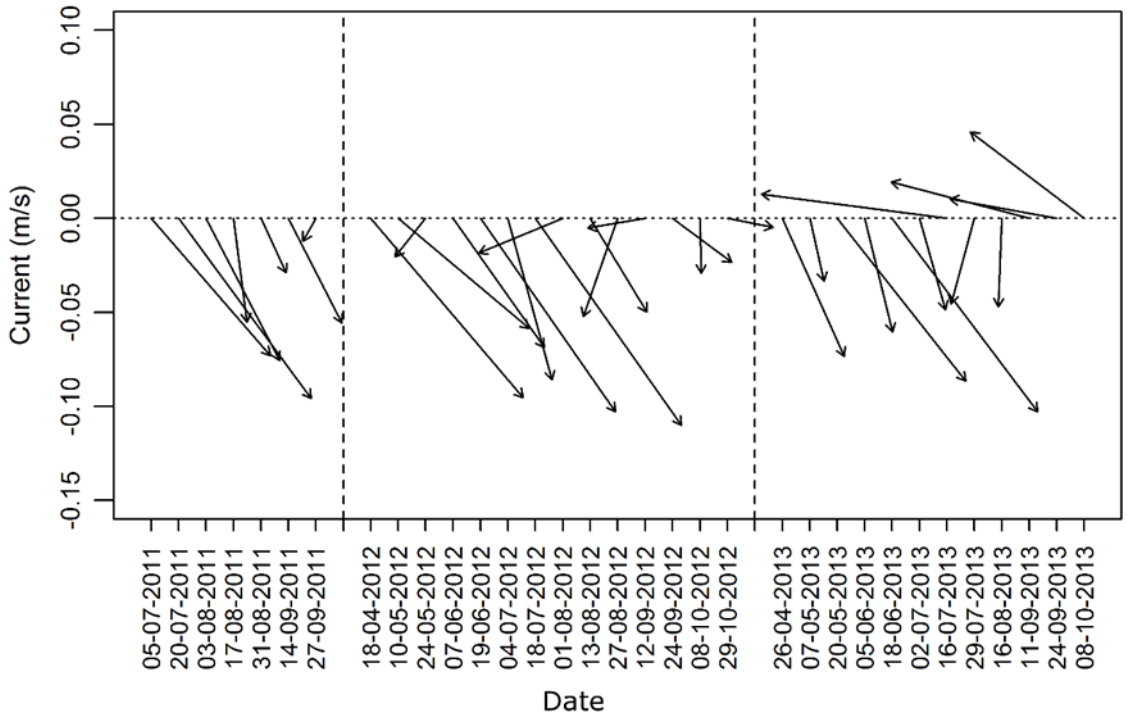
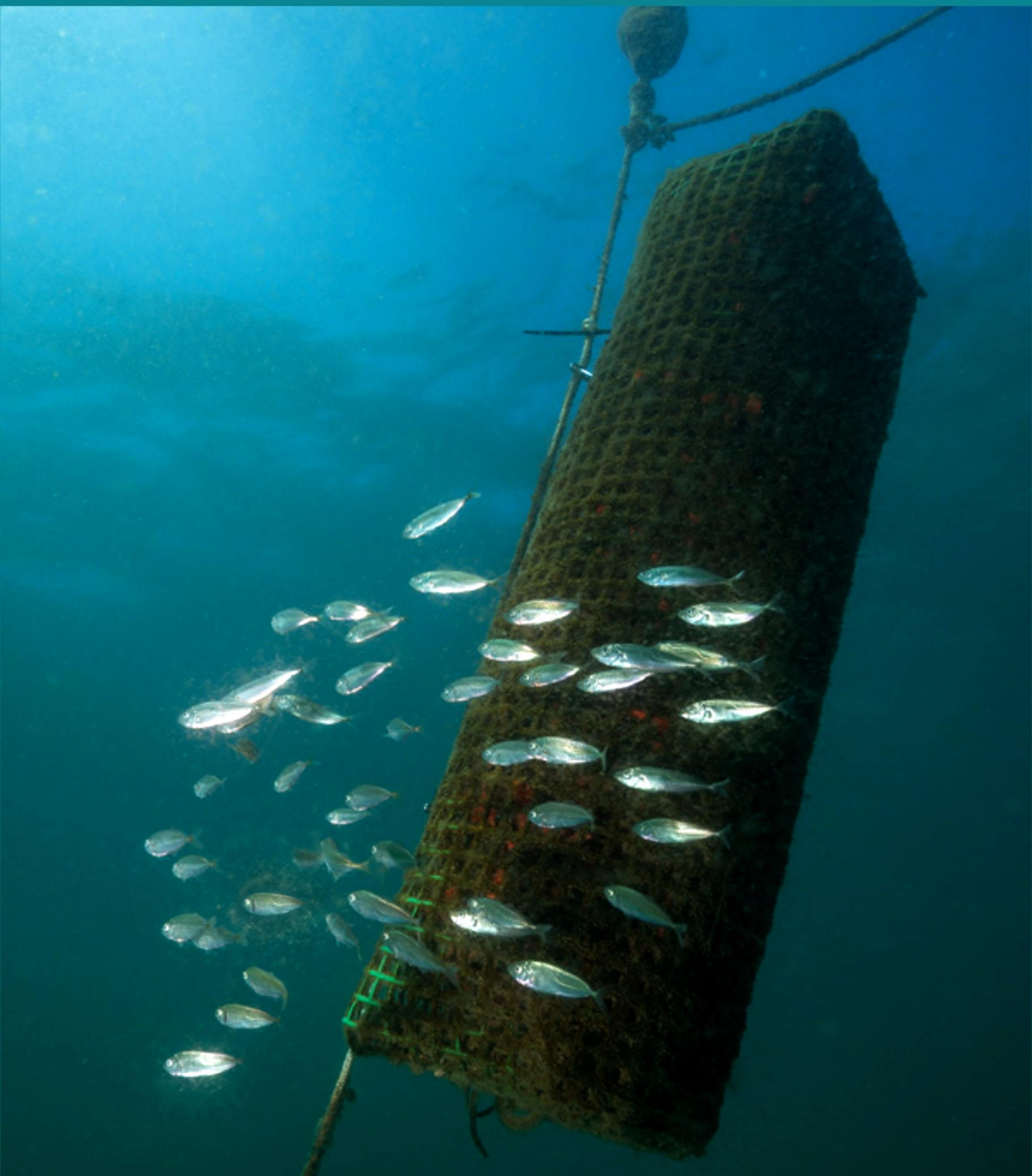


Figure S3.2. Surface currents averaged over two weeks prior to recruit sampling. Arrows point in the compass direction of the current flow. Length of arrows represent the current speed; the longer the arrow, the faster the current.

Chapter 4.1

Early life history of larvae and early juvenile Atlantic horse mackerel, *Trachurus trachurus*, off the Portuguese west coast



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Photograph of previous page was taken by Rui Guerra.

4.1.1 Abstract

Early life history traits (ELHTs) are key to understand recruitment patterns in marine animals. However, for reef fishes, studies on ELHTs are mainly focused on tropical systems and little is known for temperate reefs. In this study we used SMURFs (Standard Monitoring Units for the Recruitment of Reef Fishes) to collect fish in a temperate rocky reef system (Arrábida Marine Park, Portugal) on a weekly basis for three months during the recruitment period. Six sub-surface SMURFs sampled 2490 Atlantic horse mackerel (*Trachurus trachurus*) postlarvae and juveniles. Sagittal and lapilli otoliths were extracted from a subsample of 296 fish and ELHTs, such as size and age at metamorphosis, growth rate and age at first secondary growth formation were examined. Additionally, we tested three growth curves and selected the best suited to back-calculate the hatching pattern based on the lengths of all sampled fish. Standard length ranged from 6.13 mm to 48.56 mm and subsampled fish were aged between 19 days to 44 days. Age and size at settlement were estimated between 19 days and 36 days for individuals of 6.13 mm and 24.95 mm, respectively. Otolith shape changed clearly with increasing age and, on average, secondary growth started to form on day 33 (±3 days). Age/length relationship was well described by a Gompertz growth model which was used to back-calculate hatching dates. Four distinct hatching cohorts were identified with fish of the earliest cohort showing a faster body and otolith growth. This study indicates that the nearshore environment might have an important role in the early growth, development and hence recruitment of Atlantic horse mackerel. Information on the early life history of Atlantic horse mackerel is key to understand recruitment processes for this economically and biologically important species.

Keywords: Carangidae, metamorphosis, hatching cohort, otolith microstructure, secondary growth, early growth

4.1.2 Introduction

The dynamics of adult fish populations depend highly on the recruitment patterns of juvenile fish (Houde, 1987, Campana, 1996, Jenkins, 2005). In turn, recruitment is influenced by interactive biological and physical processes acting upon the early life stages. These processes can cause extreme levels of mortality, hence triggering large population fluctuations (e.g. Roughgarden *et al.*, 1988, Caley *et al.*, 1996, Hixon, 2011). The importance of the early life stage on population dynamics was emphasised for the first time by Hjort (1914) and was followed by other seminal studies (Cushing, 1974, Iles and Sinclair, 1982, Bakun, 1996). Early life history traits (ELHT) such as larval growth, pelagic larval duration, and size at settlement, are variable at spatial and temporal scales and this variability can greatly influence post-settlement survival and recruitment success (e.g. Shima and Findlay, 2002, Macpherson and Raventos, 2005, Rankin and Sponaugle, 2014).

Of particular relevance for the study of ELHT was the development of microstructural techniques for the analysis of larval or juvenile otoliths (Pannella, 1971). By counting and measuring daily rings it is possible to retrospectively examine the early growth rate, size at hatching and settlement, back-calculated hatching date, and potential ontogenetic or environmental transitions, such as from the larval to the juvenile stage (Campana, 2005).

Species of the genus *Trachurus* are of major economic importance for the world fisheries. In the Northeast Atlantic the most abundant species of this genus is the Atlantic horse mackerel, *Trachurus trachurus* (*T. trachurus;* Linnaeus, 1758), which is one of the most important species in the European fishing industry with 200207 tonnes captured in 2013 (FAO, 2015). In Portugal, it was the third most important fish species in 2014 totalling 15% of the landings, caught over the shelf. Only sardine (*Sardina pilchardus*, 28%) and chub mackerel (*Scomber scombrus*, 27%) had higher landings (Instituto Nacional de Estatística, Instituto Nacional de Estatística de Portugal, 2014).

Although research on Atlantic horse mackerel is abundant (see e.g. the review of Abaunza, 2003), most studies were conducted in deeper off-shore waters and were directed to the adult phase. However, there is evidence that eggs and larvae concentrate near the Portuguese shelf-edge (Farinha and Borges, 1994, Farinha and Lopes, 1996) and that immature fish can be distributed over the shelf closer to the coast, at least in Southwest Portugal (Borges and

Gordo, 1991). Furthermore, early stages of *T. trachurus* have been observed several times in nearshore environments (Massutí *et al.*, 1999, Beldade *et al.*, 2006, Borges *et al.*, 2007, Borges *et al.*, 2009), but little is known about their occurrence in these areas. Some studies report spawning to extend from January to August in the northern and central part of Portugal (Barraca, 1964, Borges *et al.*, 1977, Arruda, 1984) and the whole year in the southern coast (Arruda, 1984), while another estimated the spawning season to last from December until June for the entire Portuguese coast (Borges and Gordo, 1991). An ichthyoplankton survey at the central Portuguese coast recorded larvae from April to September (R. Borges, unpublished data).

Additionally, age and growth studies from otolith analyses have so far only focused on annual patterns (e.g. Waldron and Kerstan, 2001). Although for some other *Trachurus* species, otolith isotope composition or growth were investigated (Hewitt *et al.*, 1985 , Jordan, 1994, Syahailatua *et al.*, 2011), the otolith microstructure of postlarvae and early juveniles is only described for *T. japonicus* (Xie and Watanabe, 2005, Xie *et al.*, 2005). For *T. trachurus* there is also no information available on the variability of ELHTs, which is essential for understanding population dynamics.

Here, we describe and analyse the otolith microstructure and ELHTs of *T. trachurus* postlarvae and juveniles caught in a nearshore reef. Size and age at settlement, early growth and age at first secondary growth formation are described. The estimated hatching patterns were back-calculated from the otoliths and information from the transition from larva to juvenile is provided.

4.1.3 Material and Methods

4.1.3.1 Study site and sampling

From 29/6/2011 to 27/09/2011, weekly samples were taken at two sites with similar habitat and species composition within the fully protected area of the Arrábida Marine Park, located at the Portuguese west coast (Figure 4.1.1). This area is part of the North Atlantic Upwelling region and is characterised by a predominantly southwards current, with the occurrence of an occasional inshore warm counter current arriving from southern Portugal (Relvas et al., 2007). Also, the inner shelf dynamics, which prevail in the sampling zone can boost the

retention of e.g. fish larvae (Relvas et al., 2007). The sampling sites (A and B, Figure 4.1.1.) were 1 km apart and at a 100 m distance from the nearest reef. In each site, three Standard Monitoring Units for the Recruitment of Reef Fishes (SMURFs), artificial settlement substrates that were first developed to monitor recruitment of temperate coastal rocky reef fish in California (Ammann, 2004), were deployed. Due to sabotage on the second and third week of sampling, respectively, two SMURFS were missing at site A and three at site B, and no fish could be sampled.

The SMURFs were attached vertically along a mooring line, approximately 2-3 m below the surface and in each site mooring lines were about 20 m apart (chapter 2). SMURFs were retrieved by scuba-divers using a BINCKE net (Anderson and Carr, 1998). On board the vessel, the traps were rinsed over the net to remove all animals before they were directly re-used. Samples were preserved in 80% ethanol and all individuals were identified to the species level under a stereomicroscope. The standard length (SL) of all undamaged *Trachurus trachurus* (88.5%, N=2208) were measured to the nearest 0.01 mm. The term "settlement" refers in this study to the arrival of *T. trachurus* to the nearshore and the aggregation to SMURFs, i.e. settlement to floating habitat.

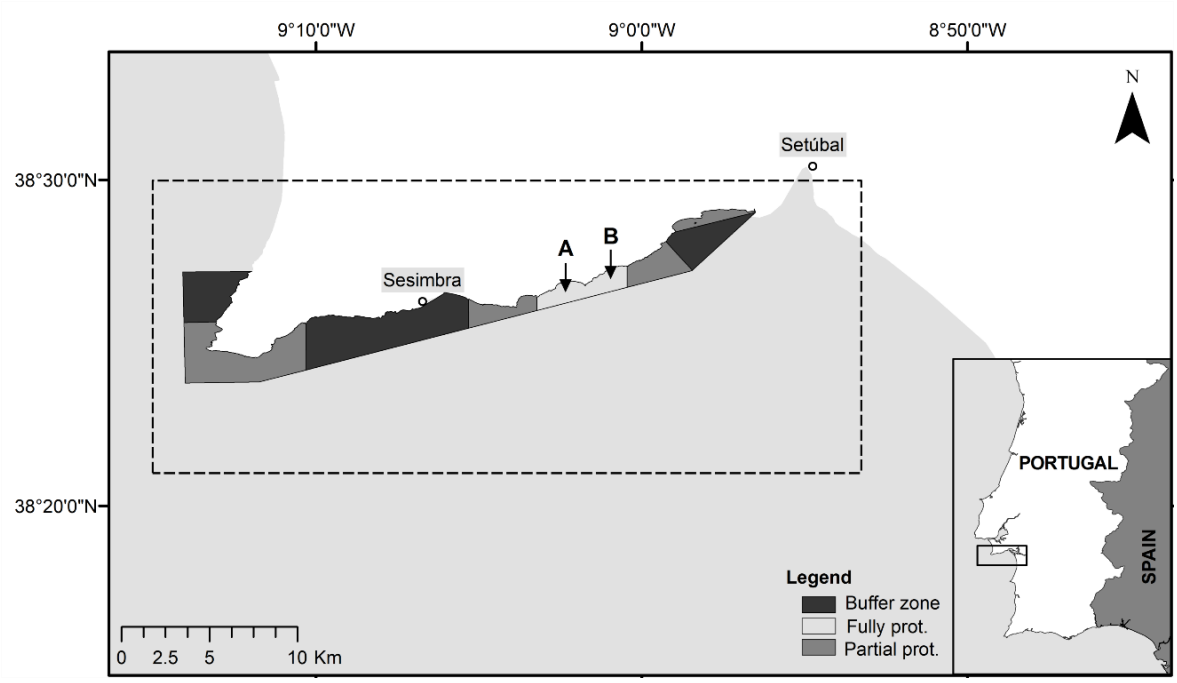

Figure 4.1.1. Map of the study site (Arrábida Marine Park), with indication of the protection zones and the two sampling sites (A and B) within the fully protected area. Country maps were provided by the Portuguese Institute of Hydrography (Instituto Hidrografico de Portugal).

4.1.3.2 Otolith preparation and analysis

Otolith analyses were performed according to common procedures (Secor *et al.*, 1991, Stevenson and Campana, 1992, Green *et al.*, 2009) for a subsample of 296 fish (this sample size was assessed by applying a coefficient of variation: Som, 1995). For otolith analyses of recruits, up to 8 of the smallest individuals were selected from each sample, as for older individuals it was highly uncertain when they arrived at the sampling site (hampering estimation of certain life history traits). However the occurrence of larger fish could not be ignored and hence we provided information on their length and dates of occurrence. Left and right sagittae were extracted with a binocular microscope (Zeiss Discovery.V8, magnifications 10x to 80x) employing 2 methods; without (Secor *et al.*, 1991, Geffen *et al.*, 2011) or with (Green *et al.*, 2009) the use of bleach. To test the effect of bleach, left sagittae of 10 fish were immersed for 15 minutes before being further processed and compared (statistically and visually) to the corresponding right untreated sagittae.

All extracted sagittae were cleaned in distilled water to remove any remaining tissue. Next, they were submerged in immersion oil to make rings clear and reduce cracking, before being mounted on a separate glass slide by embedding them in thermoplastic resin (CrystalbondTM). Otoliths were positioned with their proximal (curved) side facing up. After mounting both sagittae separately, the right one was polished gently in the sagittal plane with successively finer gridded diamond abrasive lapping film (15µm, 9µm and 3µm grain size, respectively). The left sagitta was only polished when the right one was not recovered or was unreadable (16% of the samples).

For the microstructure analyses, increments were enumerated at least 3 times and if possible in different directions, until an equal number of rings was obtained (c.f. Campana, 1992). We assumed daily deposition rates as Waldron and Kerstan (2001) counted daily rings for *T. trachurus* up to the first annulus and concluded that the numbers found showed daily periodicity. Furthermore, daily increment formation was already shown for two other species of the same genus (Jordan, 1994, Xie *et al.*, 2005). At the start of the study, 30 otoliths (9%) were read independently by two observers, for which the results matched by plus or minus one ring (93%, N=28). Afterwards, analyses were continued by one observer. To start measurements, a picture was taken from the otolith core at the highest magnification (1000x; Zeiss Axio Observer D1) and from the complete otolith at a lower magnification (100x to 500x). Images were analysed with ImageJ (and the ObjectJ plug-in). The core diameter, the

post-rostrum and rostrum length were measured. Increment width measurements were made along the post-rostrum axis. When secondary growth cores (SG) were present, their age of formation and position on the otolith were also registered.

4.1.3.3 Data analysis

Otolith microstructure

Possible differences between bleached (N=10) and unbleached (N=10) otoliths in terms of core diameter and radial length (RL) of the rostrum ($RL_{rostrum}$) and post-rostrum ($RL_{post\text{-}rostrum}$) were analysed with a paired t-test and a Wilcoxon test, respectively (as the assumptions for parametric analysis were not met in the later). The relation between age and the ratio of $RL_{rostrum}$ to $RL_{post\text{-}rostrum}$ was explored with a Spearman's correlation test.

Early life history traits

The relationship between otolith and fish size was analysed with a Spearman's rank correlation. To investigate fish growth trajectories (age vs. SL), three models that were used in previous studies dealing with larval/juvenile *Trachurus* species (Hewitt *et al.*, 1985 , Jordan, 1994, Xie *et al.*, 2005) were compared: I) the Gompertz curve $SL_t = SL_\infty e^{-e^{-K\left(t-t_f\right)}}$, II) an exponential growth model $SL_t = e^{(a+b*t)}$ and III) a linear model $SL_t = a + b * t$. In these equations t indicates the age in days; SL_t the standard length at age t; t_f the age at the inflexion point of the curve; K the instantaneous rate of growth when t = t_f; SL_∞ the asymptotic SL; a the y-intercept; and b the slope. Age t was calculated as the ring number plus 2 (see Xie *et al.*, 2005 for *T. japonicus*), since rings do not always form directly after birth in *Trachurus* species (Hewitt *et al.*, 1985 , Jordan, 1994, Xie *et al.*, 2005, Syahailatua *et al.*, 2011, Takahashi *et al.*, 2012). Since otoliths were analysed only for a subsample of fish, age of all fish smaller than 25 mm was estimated from length at capture by using the Gompertz growth model, as it showed a good fit and is the most common model used to estimate juvenile growth (Campana and Jones, 1992). The estimated age was then subtracted from the date of capture to calculate the date of hatching for each individual. The distribution of these hatching dates was used to identify hatching cohorts.

Hatching cohorts

ELHT were compared between the first three cohorts of the four identified cohorts, which where visually established, after other methods proved unsatisfying (intersections of normal distributions determined with a mixture model, dates with the lowest frequencies and dates

halfway between the peak frequencies). Fish growth (somatic growth) and otolith growth were compared using an ANCOVA (including the interaction between age and cohort). For fish growth, the dependent variable (ring width) was log transformed to obtain a linear relation with ring number, so that the assumptions were met. Differences in the abundance of SG cores and age of first SG formation among cohorts were assessed using an ANOVA and Kruskal-Wallis test, respectively. A Kruskal-Wallis test was also used to evaluate differences in the otolith core diameter among juveniles from the different cohorts.

4.1.4 Results

The overall standard length of sampled fish ranged from 6.13 mm to 48.56 mm with an average of 15.48 ± 4.63 mm (Figure 4.1.2a). Most individuals (96%) had a SL below 25 mm and these were selected for the otolith analysis (Figure 4.1.2b), with sizes ranging between 6.51 mm and 24.85 mm (13.24 ± 3.19 mm). Colouration and morphological characteristics match those from postlarvae to juvenile (Di Padoa, 1956, Russell, 1976).

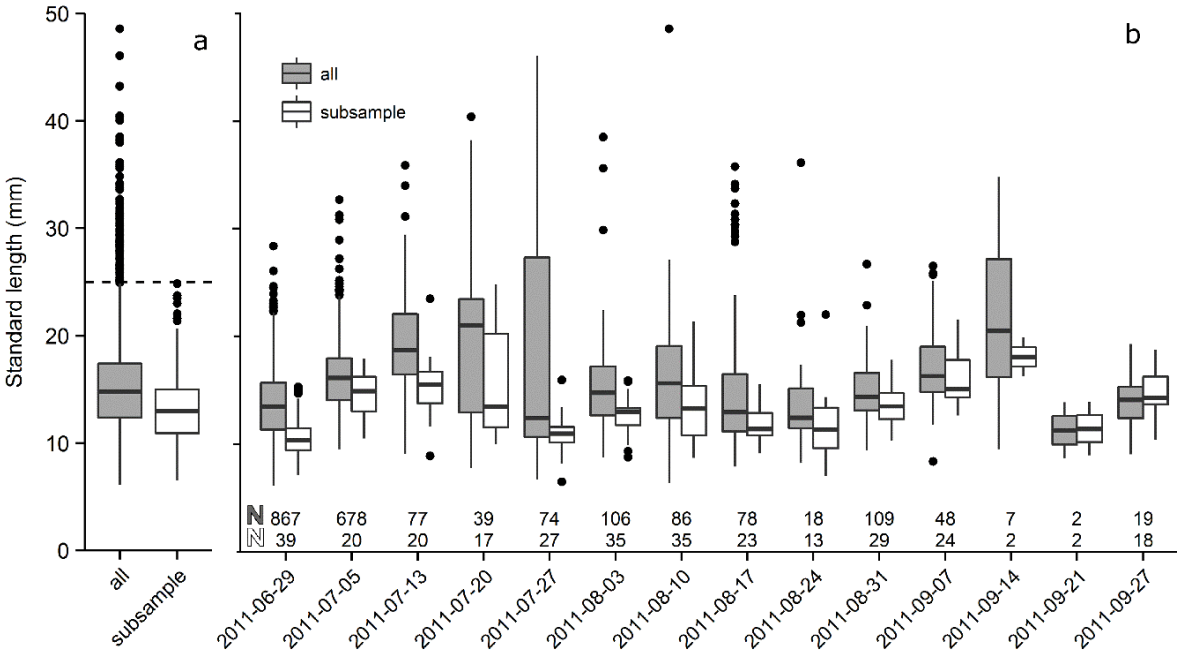

Figure 4.1.2 Length distribution of all sampled fish (dark grey) and the fish used for otolith analysis (white) over the total sampling period (a) and per sampling week (b). Points indicate outiers (values more than 1.5 times the length of the box away from the box quantiles).

4.1.4.1 Otolith microstructure

No significant difference was found between otoliths treated with and without bleach in terms of age (Wilcoxon, $p>0.05$, $Z=0.91$, $N=20$) and core diameter (paired t-test, $p>0.05$, $t(9)=0.72$, $N=20$), or $RL_{rostrum}$ (Wilcoxon, $p>0.05$, $Z=0.66$, $N=20$) and $RL_{post\text{-}rostrum}$ relationships (Wilcoxon, $p>0.05$, $Z=0.66$, $N=20$), validating further analyses with all individuals pooled together.

Daily growth rings of otoliths were clear and relatively easy to read (Figure 4.1.3), although sometimes fissures formed which increased reading difficulty. Overall, age ranged from 19 to 44 days (30.25±5.01 days). In sampling weeks 1, 5, 9 and 13, the average standard length was smaller, increasing in the following weeks (Figure 4.1.2b). Age at capture during these weeks (1, 5, 9 and 13) ranged from 19 to 36 days.

Overall, a clear change in the shape of the otoliths with increasing age was observed (Figure 4.1.3). In young fish, sagittal otoliths were somewhat spherical (Figure 4.1.3a); with growth, otoliths started to elongate along the rostrum – post-rostrum axis and SG formed along the otolith periphery (Figure 4.1.3c, d).

Elongation along the rostrum – post-rostrum axis occurred evenly, as the ratio between $RL_{post\text{-}rostrum}$ and $RL_{rostrum}$ was only weakly correlated with age (Spearman's rank correlation, $\rho_s = 0.35$, $p<0.001$, $N=302$, Figure S4.1.1a) and was reasonably constant (1.09±0.08 µm). The $RL_{post\text{-}rostrum}$ ranged from 61.43 µm to 510.20 µm (mean = 205.53±77.11 µm), while for the $RL_{rostrum}$ the range was between 63.04 µm to 463.04 µm (mean=187.92±64.55 µm).

SG was first recorded at the age of 27 days, while the oldest formation occurred at 41 days (mean=33.2±3.1 days). From fish older than 27 days, 32.9% (N=72) had SG, of which 42% (N=30) had SG at the post-rostrum, hindering reading. The number of SG cores (maximum 7) was positively correlated with age (Spearman's rank correlation, $\rho_s =0.59$, $p<0.001$, $N=298$, Figure S4.1.1b).

The core of the otolith (diameter=22.03±2.29 µm) was slightly elongated and rarely circular. Rings were in general clearly distinct from the core to around the 8th day. From there onwards sub-daily rings (increments formed with a subdaily periodicity) start forming and their number gradually increased towards the otolith periphery.

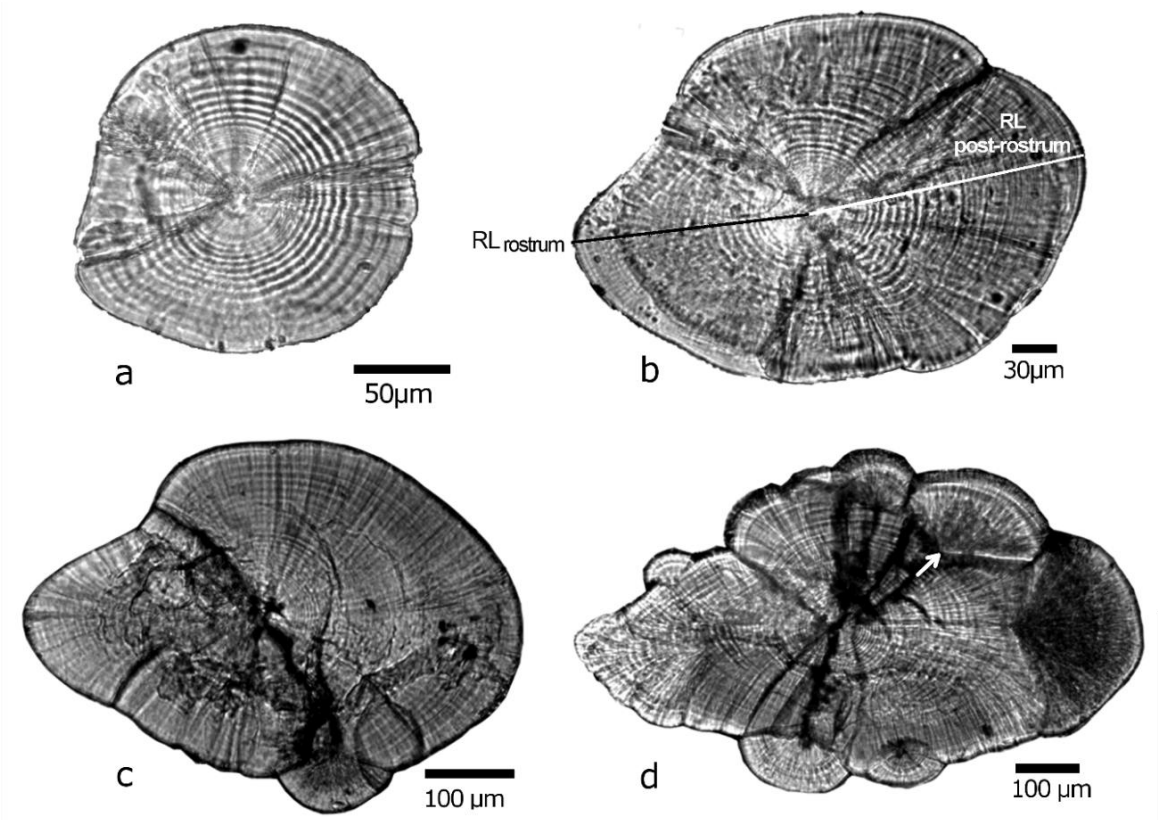

Figure 4.1.3. Development of *T. trachurus* otoliths: 20 days (a), 27 days (b), 36 days (c) and 43 days (d). The radial length (RL) towards the rostrum and post-rostrum are shown on figure (b) and on figure (d), one secondary growth core is indicated with a white arrow.

At approximately day 18, the distinction between sub-daily and daily rings become more subtle. Increment width of rings increased towards the otolith edge with a variable relationship: linear until around day 25, exponential afterwards (Figure 4.1.4a) with a decrease from day 40 onwards in some fish. No settlement or recruitment mark as defined in Wilson and McCormick (1997) could be detected.

Ring width increased from an average of 1.51±0.30 µm on day 1 to 12.36±3.27 µm on day 30. A strong and significant linear correlation was found between SL and $RL_{rostrum}$ (Figure S4.1.1c, Spearman's rank correlation, ρ_s =0.95, p<0.001, N=295) and $RL_{post\text{-}rostrum}$ (Figure 4.1.5a, Spearman's rank correlation, ρ_s =0.95, p<0.001, N=295), showing that increment formation is proportional to fish growth.

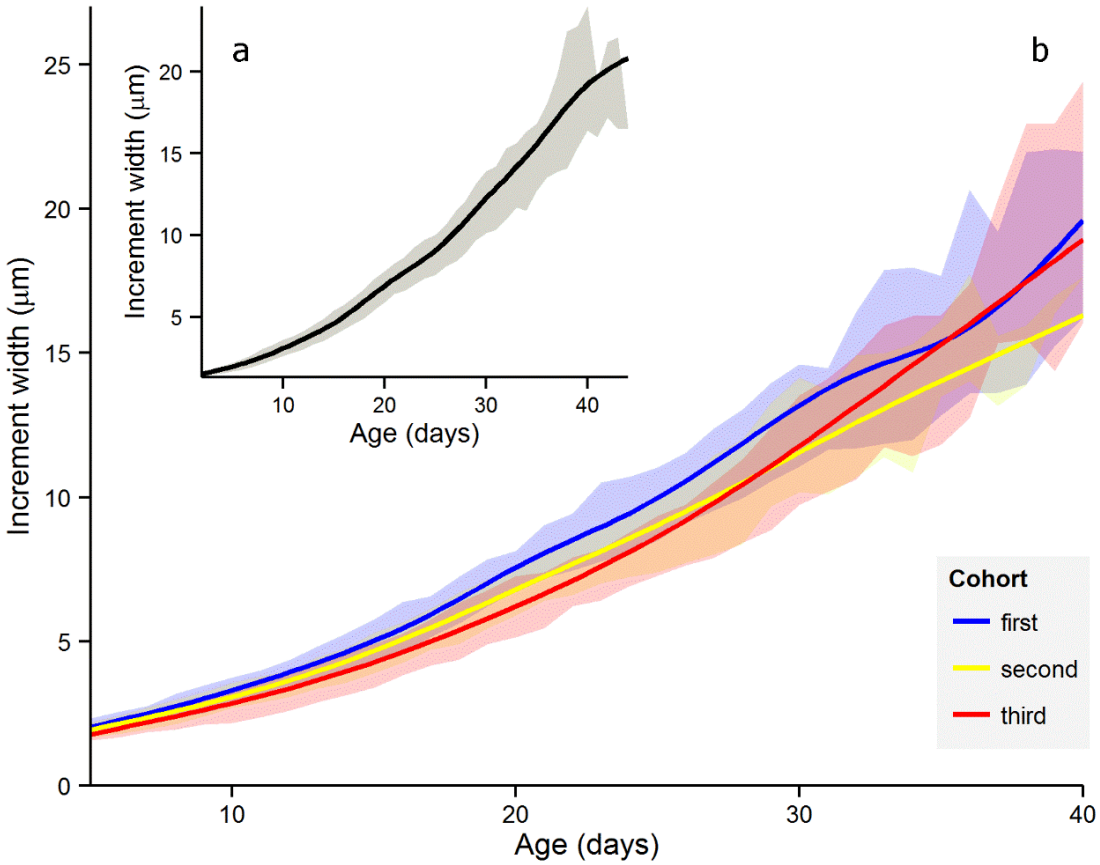

Figure 4.1.4. Otolith growth rate of all analysed otoliths (a) and of individuals from the first three hatching cohorts (b). The shaded areas indicate the range between the 1st and 3th quartile.

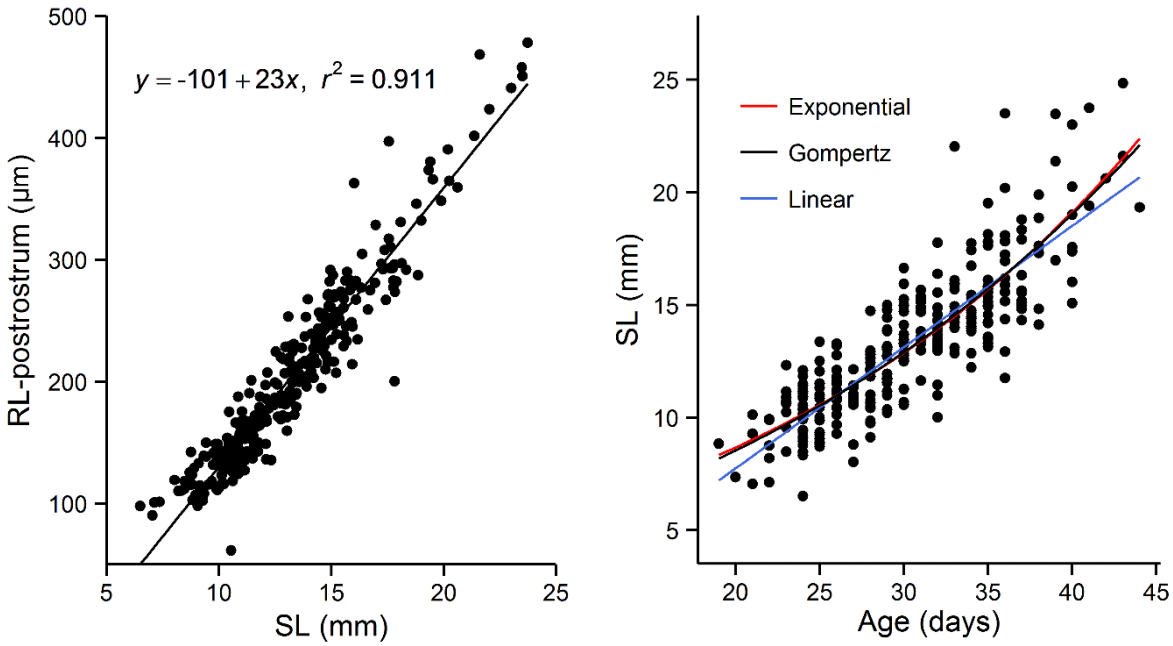

Figure 4.1.5. Linear relationship between otolith radius ($RL_{post\text{-}rostrum}$) and standard length (a) and relationship between fish standard length (SL) and age (days), with three fitted growth models (b).

4.1.4.2 Early life history traits

All three applied fish growth models (Figure 4.1.5b) significantly explained the length at age relationship. The Gompertz (SL_∞=245.55 ,K=0.01, t=109.34, R^2=0.72, p<0.05, N=297), the linear (a=-2.79, b=0.53, R^2=0.71, p<0.05, N=297) and the exponential model (a=1.39, b=0.04, R^2=0.72, p<0.05, N=297) showed a highly similar fit to the data. Fish growth rate (SL/age) was positively correlated with the number of SG cores (maximum=7; Figure S4.1.1d, Spearman's rank correlation, ρ_s=0.66, p<0.001, N=297). In contrast, the age at which the first SG core was formed was unaffected by fish growth rate (Spearman's rank correlation, p>0.05, ρ_s =-0.09, N=79).

4.1.4.3 Hatching cohorts

Four hatching cohorts were detected from the estimated hatching dates (Figure 4.1.6): 10/5/2011 – 12/6/2011, 14/6/2011 – 12/7/2011, 13/7/2011 – 8/8/2011 and 15/8/2011 – 3/9/2011. This last one had very few individuals and was excluded for subsequent comparisons.

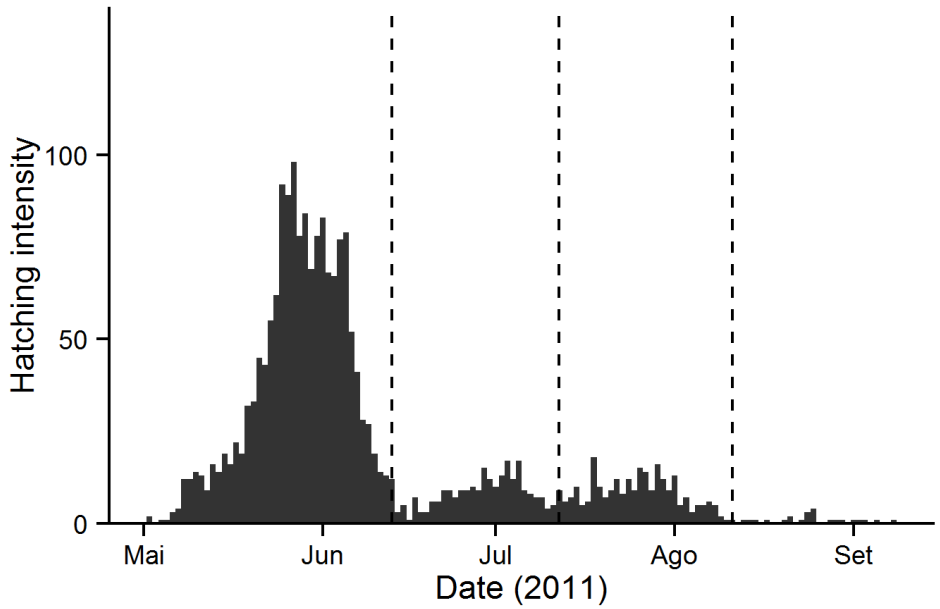

Figure 4.1.6. Back-calculated hatching intensity (back-calculated number of individuals estimated to have hatched on each date). Dashed lines separate the four hatching cohorts.

The identified cohorts had a similar mean age distribution: cohort 1 = 29.98 ± 5.62 days, cohort 2 = 29.25 ± 4.32 days, and cohort 3 = 31.08 ± 5.17 days. The fish of the first hatching cohort grew significantly faster (0.65 mm/day; R^2=0.82) than fish from the second (0.49

mm/day; R^2=0.68) or third (0.47 mm/day; R^2=0.65) cohorts (ANCOVA, $p<0.01$, N=279), between which no difference was found (Tukey post hoc test, $p>0.05$, N=279). A significant difference was also found on otolith growth (ANCOVA, $p<0.01$, N=181 with the first cohort showing a faster growth rate than the third cohort (Tukey post hoc test, $p<0.05$, N=181). The second and third cohort did again not differ significantly from each other, neither the first and second cohort (Tukey post hoc test, $p>0.05$, N=181). However, otolith growth during the early age was faster in the second cohort than in the third, but around 30 days this pattern was reversed (Figure 4.1.4b). SG was significantly different among cohorts (ANOVA, $p>0.05$, N=279, F=3.64), with the first cohort presenting significantly more SG cores than the second one. On average the first cohort formed SG slightly sooner (mean age = 32.19 ± 2.93, mean size= 13.77 ± 4.00) but this was not significantly different among cohorts (Kruskal-Wallis test, $p>0.05$, N=279, χ^2=5.13). The second and third cohort had the same onset of SG development (second: mean age=34.00±2.98, mean size=12.61±2.56 and third: mean age= 34.00±3.51, mean size=13.27±3.00). Core diameter did not differ between cohorts (Kruskal-Wallis test, $p>0.05$, N=279, χ^2=2.91).

4.1.5 Discussion

Here we described otolith microstructure and early life history traits of postlarval and early juvenile *T. trachurus* sampled off the western Portuguese coast (part of the distinct Iberian horse mackerel spawning population, see Abaunza et al., 2003). Otolith microstructure followed the general pattern previously found for *T. declivis* (Jordan, 1994) and *T. japonicus* (Xie *et al.*, 2005). This included otolith elongation with aging, width of the first increment and the presence of sub-daily rings. Nevertheless, in contrast to *T. declivis*, the first ring was not always clear. However, the average core radius of *T. trachurus* (11.02±1.15 µm) was very similar to the one of *T. japonicus* (10.4±1.6µm, Xie *et al.*, 2005) and *T. declivis* (11.90±0.77 µm, Jordan, 1994), indicating that all species hatch with similar otolith sizes. At the core, the ring shape was not always circular what is in contrast with the pattern found in most fish species (Morales-Nin, 2000), including *T. declivis* (Jordan, 1994).

Increments increased in width from day 25 onwards in most otoliths, but a distinct check mark was not detected, which hinders to define an exact age of metamorphosis from the larval to the juvenile stage and/or a change from the pelagic to nearshore environments.

However, since SMURFs were collected weekly, age at capture can be a good estimate of larval duration. The majority of fish analysed ranged from around 6 to 25 mm standard length, were aged from 19 to 44 days and ranged from late stage larvae to juveniles according to their coloration and morphological features. These ranges indicate that age and size upon arrival to the nearshore and/or metamorphosis is variable. Xie et al. 2005 recorded for *T. japonicus* a mid-age of 34 days and a range of 30 to 37 days for the transition from post-flexion larva to early juvenile. Furthermore, Xie and Watanabe (2005) observed a stronger increase in width of otolith increments until day ~40, followed by narrowing and stabilizing of ring width (Figure 6 in Xie and Watanabe, 2005). The turning point in increment growth was associated by these authors to the end of metamorphosis. They suggested that the increased growth rate before this turning point could be caused by a dietary shift to a higher trophic level. In our study, the otoliths of the few *T. trachurus* analysed that were aged 40 days or older, displayed a similar increment width profile, but most fish were too young to derive any definitive conclusions. It would be interesting in the future to investigate the relationship between otolith microstructure and diet to compare with the results obtained for *T. japonicus*.

The formation of secondary growth (SG) in *T. trachurus* can be associated with environmental, habitat or ontogenetic dietary transitions (e.g. Campana, 1984, Marks and Conover, 1993, Morales-Nin, 2000). Formation of accessory cores was demonstrated to be synchronised with metamorphosis in several species such as the Japanese horse mackerel (between the age of 19 and 54 days: Xie and Watanabe, 2005, Xie *et al.*, 2005), Dover sole (Toole *et al.*, 1993), European hake (Morales-Nin and Aldebert, 1997) and Raitts sand eel (Wright, 1993). In our study, not all otoliths had SG, but when such formations were present they started to form from day 27 to 41, supporting the above mentioned larval duration and metamorphosis to the juvenile stage.

The Gompertz growth curve was well-suited to fit the length at age relationship in juvenile *T. trachurus*, but the linear and exponential functions were equally appropriate. The only study investigating growth of the genus *Trachurus* in the early juvenile stage also applied the Gompertz curve (Xie *et al.*, 2005). The studies of Kanaji *et al.* (2009, 2010) using the biological intercept method (Campana, 1990) and the formula of Lleonart *et al.* (2000) are not compared here, since the juveniles examined in these studies were far larger than the

ones collected by us. Other studies focusing on post-larval and juvenile *Trachurus* used linear and exponential models (Table 4.1.1), but studied fish with smaller SL.

Table 4.1.1. Comparison of growth models used in studies of larval and juvenile *Trachurus*. SL = Standard Length.

Scientific name	English name	SL (mm)	growth model	Author, Year
T. declivis	Greenback horse mackerel	3-12	exponential	Jordan, 1994
T. japonicus	Japanese jack mackerel	3-49	Gompertz	Xie, 2005
T. novaezelandiae	Yellowtail horse mackerel	2-16	linear	Syahailatua, 2011
T. symmetricus	Jack mackerel	2-7	linear	Hewitt, 1985
T. trachurus	Atlantic horse mackerel	6-25	Gompertz	This study, 2015

The back-calculation of hatching dates identified four cohorts, occurring from about May to September. These cohorts should be considered in light of the protracted spawning season of horse mackerel. For central Portugal, the spawning period might last from December to October (Borges and Gordo, 1991), although shorter time spans have been reported as well (e.g. Arruda, 1984). Thus, almost year-round sampling should be performed to capture all cohorts. Here, the first cohort contained by far more individuals than the subsequent cohorts, potentially indicating the decrease in a seasonal peak and the need for earlier sampling.

The first cohort showed also the fastest growth and a higher number of SG cores. This is an interesting result as it suggests that growth is higher early in the season, and potentially when hatching intensity was stronger. This difference might be caused by different environmental conditions and/or different parental investment on egg or larval quality. However, since size at hatching was similar among the different cohorts, differential egg quality and condition of newly hatched larvae might be a less probable explanation. A variation in number of SG cores and otolith growth with hatch date was also observed for *T. japonicus* by Xie and Watanabe (2005) with these authors suggesting differences in water temperature as a plausible cause. However, for *T. trachurus* off western Portugal, such relationships are yet to be established. To our knowledge, only a link between the environment (upwelling in specific) and recruitment has already been made (Santos et al., 2001). More detailed studies are necessary to unravel the relationship between ELHTs and environmental conditions, as well as on how this relationship might influence survival and recruitment success of *T. trachurus*.

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We wish to thank D. Rodrigues, G. Franco, H. Folhas, and F. Pessanha for help during sampling campaigns and J. Afonso and F. Pessanha for helping in the otolith microstructure analyses. We thank the Portuguese Foundation for Science and Technology (FCT) for funding this study through a PhD grant (SFRH/BD/72970/2010) for MK, a post doc fellowship (SFRH / BPD / 23743 / 2005) for RB and through the following project grants: Matrix (PTDC/MAR/115226/2009), Reefish (PTDC/MAR-EST/4356/2012), CCMAR/Multi/04326/2013 and PEst-OE/MAR/UI0331/2011.

4.1.7 References

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4.1.8 Supporting information

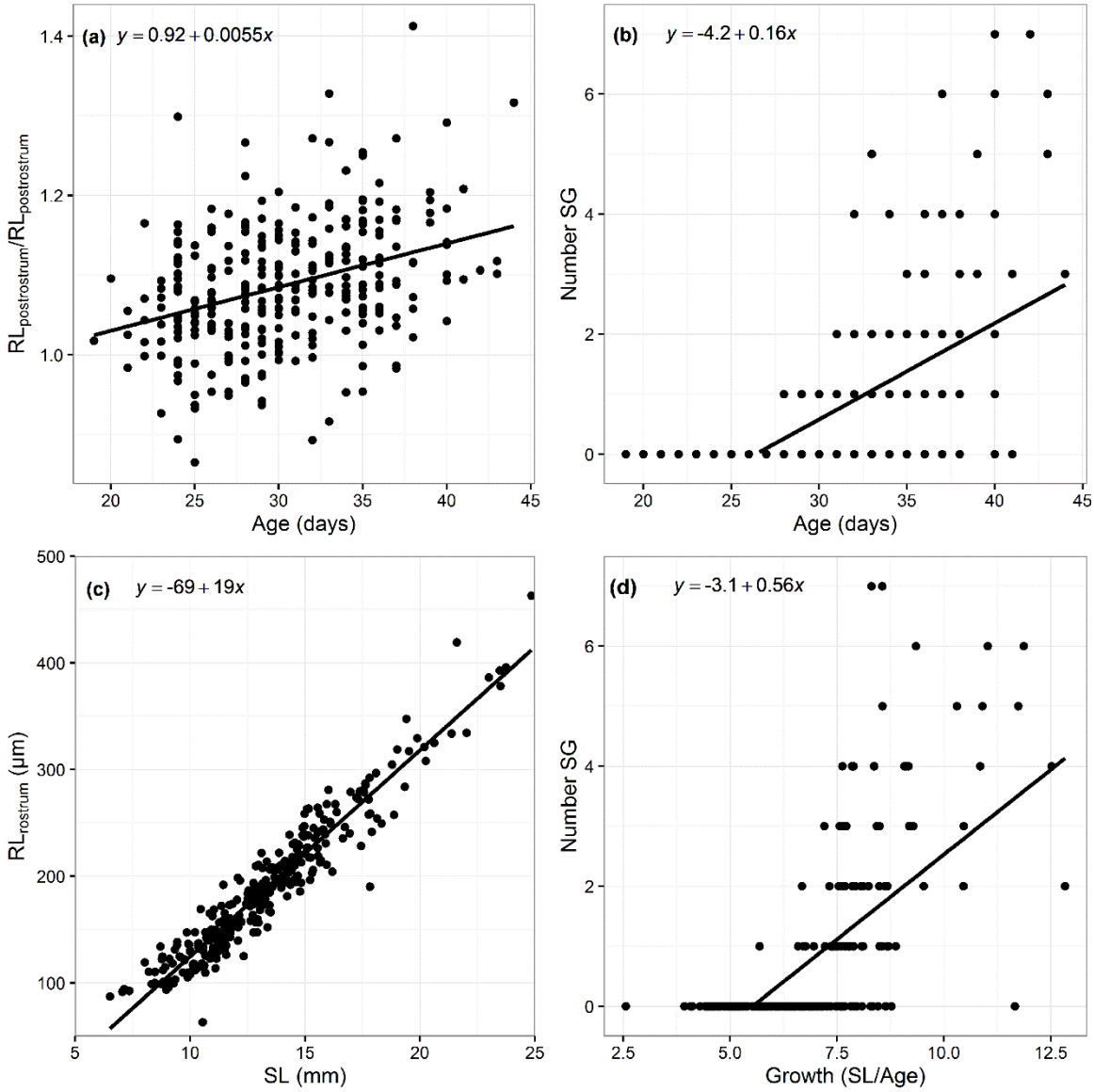

Figure S 4.1.1 Linear relationship between (a) age and the ratio of $RL_{postrostrum}$ to $RL_{rostrum}$ (RL=Radial Length), (b) age and the number of secondary growth (SG) cores, (c) standard length (SL) and $RL_{rostrum}$ and (d) fish growth (defined as SL over age) and the number of SG. The linear relationships are given in the upper left corner.

Chapter 4.2

Small-scale temporal patterns of recruitment and hatching in Atlantic horse mackerel (L.) at a nearshore reef area

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Status: in preparation to be submitted to a scientific journal.

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4.2.1 Abstract

Atlantic horse mackerel (*Trachurus trachurus*, Linnaeus, 1758) is a highly exploited species, common throughout the North-East Atlantic. As a pelagic-neritic fish it typically occurs over the shelf from the surface to 100-200 m deep on sandy bottoms. Most research has focused on adults or early life stages (eggs and larvae) caught in these waters and only a few have reported the occurrence of early stages near the coast. However, these nearshore environments might be important for the early growth and survival of the Atlantic horse mackerel. In addition, little is known on how environmental processes might affect the early stages of this species. Here, we monitored weekly recruitment of horse mackerel to Standard Monitoring Units for the Recruitment of Fish (SMURF) deployed near the coast at both the surface and near the bottom. Possible environmental factors influencing recruitment patterns and back-calculated hatching cohorts were analysed. From the 2515 fish collected in the SMURFS, 99% recruited to those at the surface. A GAM and GAMM analysis of the recruitment and hatching patterns, respectively, revealed a strong relationship with the lunar cycle and upwelling events. Both recruitment and hatching showed a periodic pattern with peaks near the new moon and revealed a negative impact of upwelling. This study suggests that the nearshore environment might be an important nursery area for post-larval and early juvenile Atlantic horse mackerel.

Keywords: *Trachurus trachurus*, lunar pattern, upwelling, juvenile, recruitment, hatching

4.2.2 Introduction

Marine fish can have highly variable recruitment patterns, which can strongly affect population dynamics (reviewed by e.g. Doherty, 2002, Forrester *et al.*, 2002, Osenberg *et al.*, 2002, Carr and Syms, 2006). Various interacting biotic and abiotic processes at multiple scales can cause this variability. The former may include adult fecundity, spawning location and timing, larval behaviour, development and survival, and/or density-dependent processes such as competition for habitat and food. Abiotic factors include for example sea surface temperature (SST; Daskalov, 1999, Jenkins and King, 2006), upper ocean stability (including wind and current speeds and direction or tides; Roy *et al.*, 1989, Milicich, 1994, D'Alessandro *et al.*, 2007, Caselle *et al.*, 2010b), nutrient availability (Meekan *et al.*, 2003, Caselle *et al.*, 2010a) or the lunar phase (Victor, 1982, Sponaugle and Cowen, 1994, D'Alessandro *et al.*, 2007).

Most research on fish recruitment in nearshore areas has been conducted on tropical systems (e.g. Victor, 1986, Meekan *et al.*, 2003, Rankin and Sponaugle, 2014) and only relatively few studies on nearshore and reef environments exist in temperate regions (Jenkins *et al.*, 1997, Carr and Syms, 2006, Shima and Swearer, 2009, Fontes *et al.*, 2011). The reason for this might be the usually turbulent conditions found in temperate nearshore shallow environments, which limit sampling possibilities for the early life stages of fish. This limitation might lead to an underestimation of the importance of temperate nearshore systems as nursery and/or recruitment areas for species known to spawn in more offshore waters.

Atlantic horse mackerel (*Trachurus trachurus*, Linnaeus, 1758) is a highly exploited species common throughout the North-East Atlantic, but occurs from the Norwegian Fjords to the southern tip of Africa (Lockwood and Johnson, 1977, Smith-Vanith, 1986). In 2013, 200,207 tonnes of Atlantic Horse Mackerel were captured by the European fishing industry (FAO, 2015). In Portugal, it was the third most important marine fish species in 2014 regarding landed biomass. Given its importance, this species has been the subject of intensive research (e.g. Macer, 1977, Smith-Vanith, 1986, Abaunza *et al.*, 2008) revealing its pelagic-neritic distribution, typically occurring over the shelf from the surface to 100-200 m depth over sandy bottoms. Still, it has been reported to depths of 500 m (Smith-Vanith, 1986), or from the surface to 1050 m depths in the Mediterranean (Lloris and Moreno, 1995). Most research

has focused on deeper off-shore waters directed at the adult phase, with few exceptions that aimed at the distribution of larvae and eggs (Farinha and Borges, 1994, Farinha and Lopes, 1996).

Some studies report spawning to extend from January to August in the northern and central part of Portugal (Barraca, 1964, Borges *et al.*, 1977, Arruda, 1984) and the whole year in the southern coast (Arruda, 1984), while another estimated the spawning season to last from December until June for the entire Portuguese coast (Borges and Gordo, 1991). An ichthyoplankton survey at the central Portuguese coast recorded larvae from April to September (R. Borges, unpublished data).

There is evidence that eggs and larvae concentrate near the shelf-edge (Farinha and Borges, 1994, Farinha and Lopes, 1996) and that immature fish can be distributed over the shelf closer to the coast, at least in Southwest Portugal (Borges and Gordo, 1991). Despite the absence of studies focusing on nearshore environments and although literature about the post-larval phase of this species is scarce, at the Arrábida Marine Park (AMP, Portugal) larvae and early stages of *Trachurus* species have been observed several times in nearshore rocky reefs (Beldade *et al.*, 2006a, Borges *et al.*, 2007b, Borges *et al.*, 2009), indicating the potential importance of this habitat to the early life of this pelagic fish.

Additionally, little is known on how environmental processes might affect early life stages of Atlantic horse mackerel (Trenkel *et al.*, 2014). The few published studies found: i) no significant correlation between larval abundance and SST in the Celtic Sea (Fives *et al.*, 2001); ii) a negative correlation between upwelling during the winter spawning season and the yearly recruitment to the fishery in Portugal (Santos *et al.*, 2001); iii) a positive relationship between lower coastal SST (which indicate upwelling and milder weather patterns) during spring and summer and higher yearly recruitment in the whole Iberian Peninsula (Lavín *et al.*, 2007).

The latter two studies used as a proxy for recruitment an annual estimate of the number of recruits based on fisheries data. However, Atlantic horse mackerel recruitment (i.e. the transition from the larval to the juvenile stage) has not yet been directly investigated. Moreover, the influence of environmental variability on recruitment is unknown, although

this relationship is of major importance to understand recruitment and population dynamics and/or to infer mechanisms of larval transport.

The present study aimed at: i) studying the occurrence and recruitment patterns of Atlantic horse mackerel at small temporal resolution (weekly) in nearshore environments; and ii) investigating the impact of environmental processes on recruitment and back-calculated hatching patterns.

4.2.3 Material and Methods

4.2.3.1 Study area

The sampling sites were located at the Arrábida Marine Park (hereafter AMP) in central Portugal (Figure 4.2.1), which is part of the Western Iberian upwelling ecosystem, a wind-driven eastern boundary upwelling system. In winter and early spring unsteady equatorward winds are related to local weak upwelling events, and in late spring, summer and autumn, lasting along-shore equatorward winds combined with enhanced stratification of coastal waters, form a strong coastal upwelling of nutrient-rich waters (Wooster *et al.*, 1976, Fiúza *et al.*, 1982), followed by an increased coastal productivity (Cunha, 1993, Moita, 2003). An alongshore equatorward current occurs associated to the upwelling process (Relvas and Barton, 2002).This area has been described as a biogeographic and oceanographic transition zone with a high diversity of habitats and marine species (Henriques *et al.*, 1999, Gonçalves *et al.*, 2003). The AMP has a southern oriented steep coast and an adjacent mountain chain that protects the area from prevailing northerly and north-westerly winds and waves (Beldade *et al.*, 2006b), allowing frequent sampling in the nearshore.

4.2.3.2 Sampling

In this study, recruitment refers to the arrival of fish to the nearshore and the approximately concurrent metamorphosis to the juvenile stage. Given that juvenile Atlantic horse mackerel show thigmotactic behaviour (Deudero *et al.*, 1999, Massutí *et al.*, 1999), standard monitoring units for recruitment of fish (SMURFs; chapter 2; Ammann, 2004, Wilson *et al.*, 2008) were used here to monitor nearshore aggregation of this pelagic species. SMURFs can successfully sample ready to settle fish in the nearshore (White and Caselle, 2008, Tavernetti *et al.*, 2009, Caselle *et al.*, 2010b). These collectors were deployed in two bays (A and B,

Figure 4.2.1) with a similar rocky reef habitat within the fully protected area of the AMP, around 1 km apart and around 100 m seaward from the nearest reef (Figure 4.2.1).

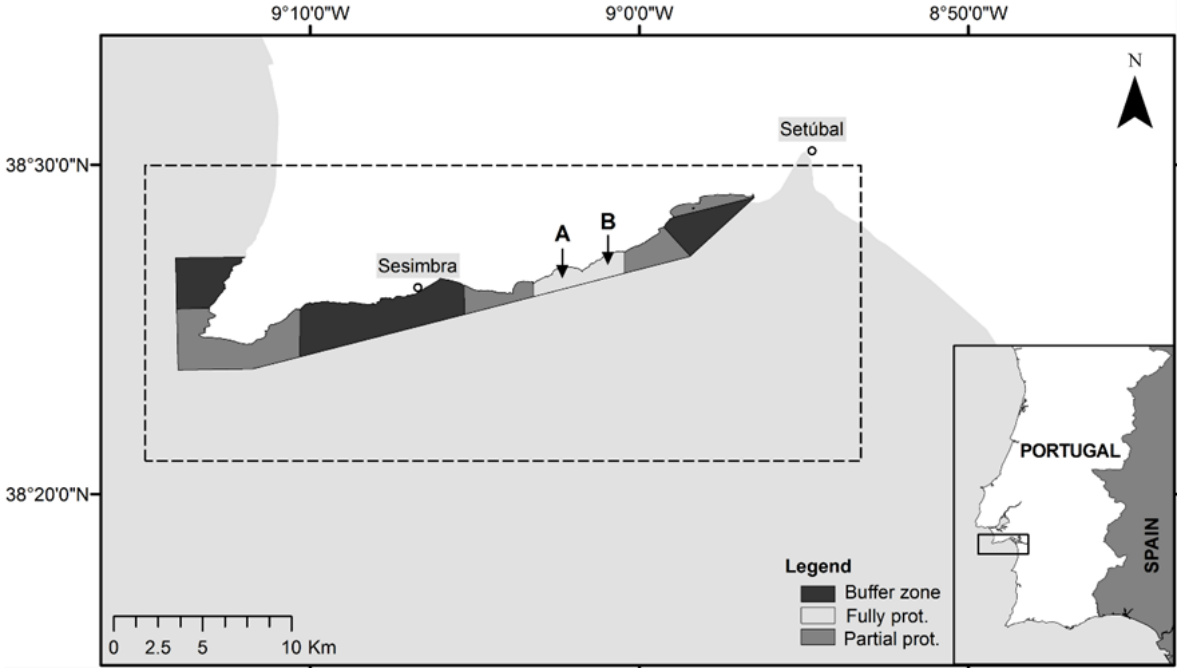

Figure 4.2.1. Map of the study site (Arrábida Marine Park), with indication of the protection zones, the two sampling sites A and B, and the area (dashed frame) for which satellite data was averaged. Country maps were provided by the Portuguese Institute of Hydrography (Instituto Hidrografico de Portugal).

In each site, six SMURFS were attached to three mooring lines; three below the surface (1-3.5 m depending on the tide) and three close (~40 cm) to the bottom. Sampling was performed weekly between the last week of June and the last week of September in 2011, comprising 14 weeks of sampling. SMURFs were collected by scuba-divers using a benthic ichthyofaunal net for coral/kelp environments (BINCKE net; Anderson and Carr, 1998, Ammann, 2004). On board the vessel, samples were preserved in 80% Ethanol. After species identification, total (TL) and standard length (SL) were measured to the nearest 0.01 cm. A subsample of 296 fish (13% of the collected fish) was aged from sagittal otolith analysis to develop a length-age relationship which was well described by the Gompertz growth function (R^2=0.72; unpublished data), which was then used to back-calculate the date of hatching.

4.2.3.3 Environmental factors

For the time period between the earliest back-calculated hatching date and the end of the recruitment sampling period (from 10/05/2011 until 27/09/2011; Figure 4.2.2 A, B), daily

data were compiled for the following environmental parameters: sea surface temperature (SST), concentration of Chlorophyll a (Chla), eastward and northward current velocity (U- and V-component, respectively), wind speed and direction, an up-welling index, moon phase and tidal amplitude (Table 4.2.1). For spatial data (SST, Chla and currents), a daily average was calculated for the study region (Figure 4.2.1; extent: -9.25, -8.50, 38.50, 38.35). The upwelling index was calculated as the difference between offshore SST measured from an offshore area of similar size located two degrees further offshore at the same latitude, and the SST in the sampling area (Santos *et al.*, 2001, Relvas *et al.*, 2009). Positive values relate to upwelling conditions and negative values describe relaxation from upwelling. For the model analysis percentage of moon illumination was categorized in order to distinguish between 1st and 3rd quarter moon: full moon (75-100%), new moon (0-25%) and 1st and 3rd quarter moon shared 25-75% illumination.

Table 4.2.1. Environmental variables. Listed are the unit, spatial and temporal resolution (Spa. and Temp. res.), the type (RS=remote sensing, DO=direct observation, MO=Model), source of the dataset and reference.

Variable	Unit	Spa. res.	Temp. res.	Type	Dataset/ Source	Reference
SST	°C	~6 km	daily	RS + DO	Copernicus Marine environment monitoring service (marine.copernicus.eu)	OSTIA[a]; Donlon *et al*., 2012
CHLa	mg/m^3	~4 km	daily	RS		Gohin *et al*., 2008
U and V current	m/sec	~3 km	daily	MO		IBI[b]; Sotillo *et al*., 2015
Wind speed	m/sec	NA	hourly	DO	Local weather station	NA
Wind direction	°	NA	hourly			
Moon phase	%	NA	daily	MO	Portuguese calender	NA
Tidal amplitude	m	NA	daily	MO	Hydrographic Insitute	NA

[a]OSTIA (Operational Sea Surface Temperature and Sea Ice Analysis) run by the UK Met Office combines remote sensed satellite data provided by the GHRSST project with in-situ observations, to determine through an Optimal Interpolation procedure global sea surface temperature (SST; Donlon *et al.*, 2012).

[b]IBI (Iberian Biscay Irish) Ocean Analysis and Forecasting system run by Puertos del Estado and Mercator Ocean is based on an eddy-resolving NEMO model application driven by meteorological and oceanographical forcing (Sotillo et al., 2007 and 2015, Madec, 2008).

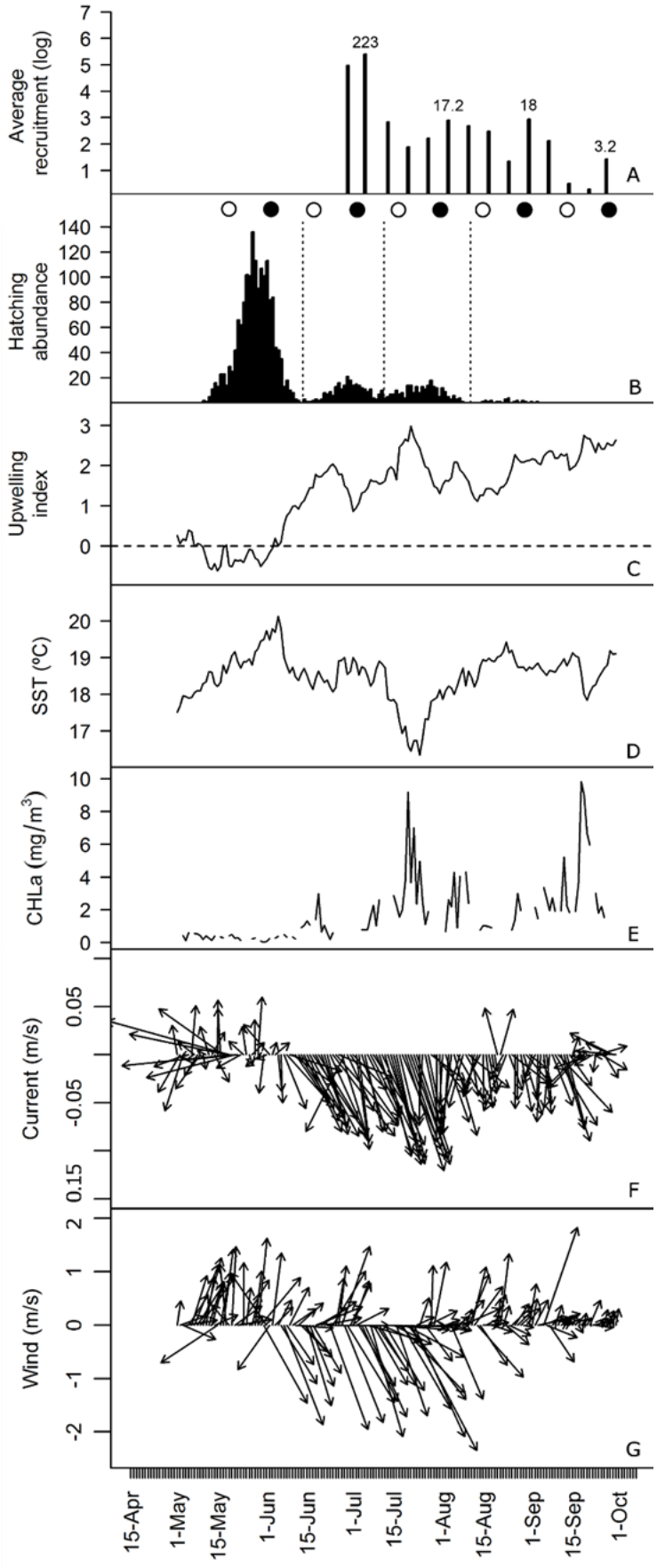

Figure 4.2.2. Weekly averaged recruit abundance of *Trachurus trachurus* (A: log-transformed with real numbers on peaks); estimated daily hatching intensity (B) with dashed lines separating hatching cohorts and circles indicate phases of full moon (white) and new moon (black); daily time series of the upwelling index (C), SST (D), Chla concentration (E) surface currents (F) and wind (G). See Table 4.2.1 for units and source of environmental variables.

4.2.3.4 Data Analysis

Fish sampled with surface SMURFs and with an SL< 25 mm (Figure S4.2.1 in supporting information; chapter 4.1) were considered recruits and selected for data analysis. A Kruskal-Wallis test was used to compare the non-normally distributed abundance of recruits among SMURF replicates in each site (1-3, west to east). A Wilcoxon rank sum test was used for site (A, B) comparisons. To analyse the weekly recruitment patterns as a function of daily environmental parameters, the latter were averaged over the seven days preceding sampling, to capture the timing during which fish arrived to SMURFs (due to the weekly nature of sampling).

Data exploration of the response variables (recruitment and hatching abundance) and of the explanatory variables was performed by following the protocol of Zuur *et al.* (2010). Both a multiple pairwise scatterplot with Pearson correlation and a Variance Inflation Factor (VIF) analysis was used to test for collinearity and to choose which explanatory variables were included in the models (Zuur *et al.*, 2010). A Pearson correlation of R^2=0.6 was used as a threshold. Accordingly, wind variables were correlated with current components, which were highly correlated between each other and Chla was correlated with the upwelling index. The modelling included as continuous variables Chla, SST and the upwelling index and U current, due to their higher correlation with the response variables compared to the wind variables. Selected categorical variables were moon phase, site (only in recruitment models) and hatching cohort (only in hatching models).

For the recruitment dataset, a Zero Inflation Model was tested first, since the dataset contained a significant number of zeros (Zuur *et al.*, 2009). However, model validation was not satisfactory and thus a generalized additive model (GAM) was chosen instead. Since we worked with time series, an auto-correlation analysis (ACF) was performed on the model residuals to check for temporal independence.

For hatching data, a generalized additive mixed model (GAMM; Wood, 2006, Zuur *et al.*, 2009) was used, to deal with temporal dependence. A dependence structure was included by using the factor cohort as a random intercept. Models were set up using a negative-binomial error structure and a log link function (Wood, 2006). The Akaike Information Criterion (AIC) and a graphical validation of model residuals were used to select the best model (Zuur *et al.*, 2009). The data exploration and all statistical analyses were carried out with R

(version 3.1.0; R-Core-Team, 2009) and test results were evaluated at the $p<0.05$ level. The GAM and GAMM models were analysed using the mgcv library (version 1.8-1; Wood, 2011) in R.

4.2.4 Results

A total of 2515 Atlantic horse mackerel were collected, 2490 at the surface and 25 at the bottom SMURFs. At the bottom, all fish had a SL of less than 25 mm and at the surface 2126 out of 2209 measureable fish were recruits (96.2%; 6.1 mm - 25.0 mm SL). In total, standard length ranged from 6.1 mm to 48.56 (mean= 15.5 mm; Figure S4.2.1). Fish morphology ranged from late larvae to the juvenile stage (Di Padoa, 1956, Russell, 1976). In several weeks the great majority of fish aggregated just to one or two out of the six SMURFs, but no spatial preference was detected (Figure S4.2.2). Abundance of recruits was not significantly different among replicate SMURFs in site A and B (Kruskal-Wallis test, X^2= 0.38, p-value = n.s., n=42 and X^2 = 1.99, p-value = n.s., n=42, respectively), neither between the two sites (Wilcoxon rank sum test, W = 893, p-value = n.s., n=84).

Fish recruited every week on both sites but the majority of recruitment (72%) occurred during the first two sampling weeks (Figure 4.2.2 A). Despite a sharp decrease in recruitment after the first two weeks, three later smaller peaks were observed on the 3rd and 31st of August and 27th of September (Figure 4.2.2 A). Standard length of fish collected during recruitment peaks was very similar, but on subsequent weeks samples tended to have older and larger fish. Sampling dates during which fish were generally small (samples with lowest median and smaller variability) likely indicate the arrival of a new recruitment cohort. Four recruitment groups were detected during the sampling period (Figure 4.2.3).

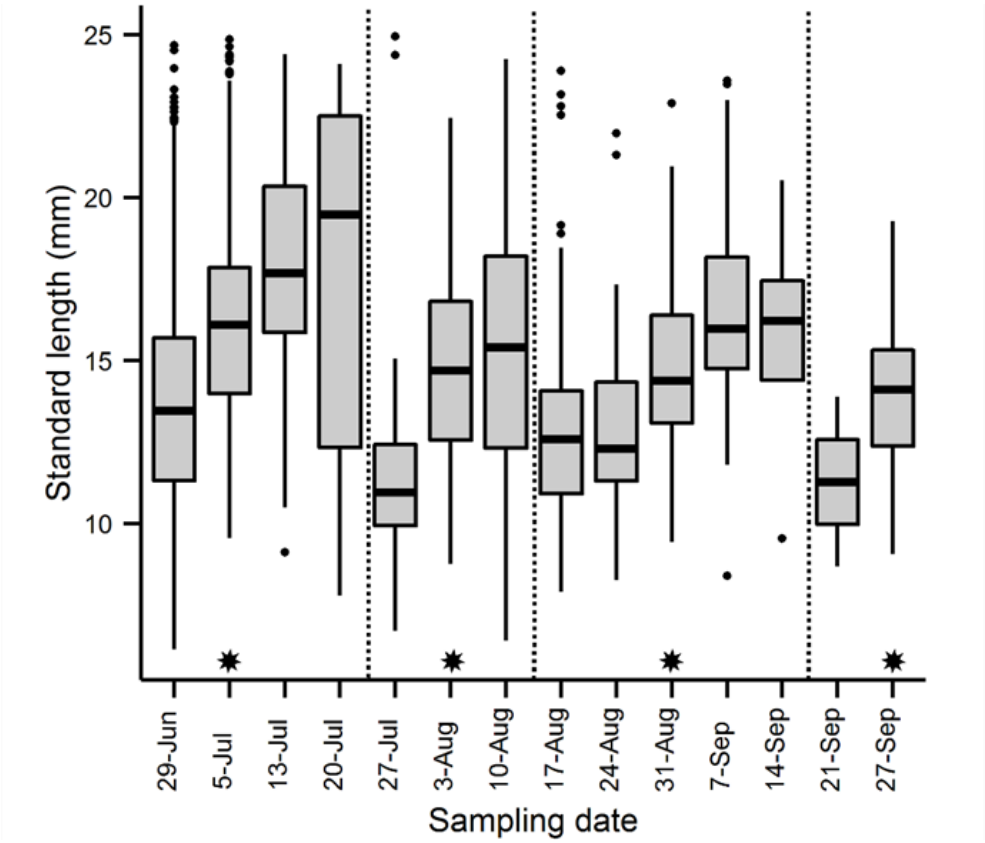

Figure 4.2.3. Length distribution of weekly sampled *T. trachurus* recruits. Stars indicate weeks of peak recruitment and dashed lines separate recruitment cohorts.

Estimated back-calculated hatching dates of recruits occurred from May 10 until September 3, with a decrease in hatching frequency over time (Figure 4.2.2 B). Three clear hatching cohorts (peaks) were detected, with a fourth one that was less distinct and had few individuals. Overall, SL of recruits decreased from the first to the fourth hatching cohort, with fish hatched in the first cohort recruiting at significantly larger sizes than fish from the third cohort (X^2=23.85; df=3; p<0.0001; n=2126; posthoc: 1st vs. 3rd cohort: X^2= 5.94; p< 0.001; Figure 4.2.4). Fish belonging to different recruitment groups could have hatched within the same hatching cohort and, inversely fish from the same recruitment group could belong to different hatching cohorts (Figure 4.2.5). This indicates variability in the pelagic larval duration (PLD) of this species.

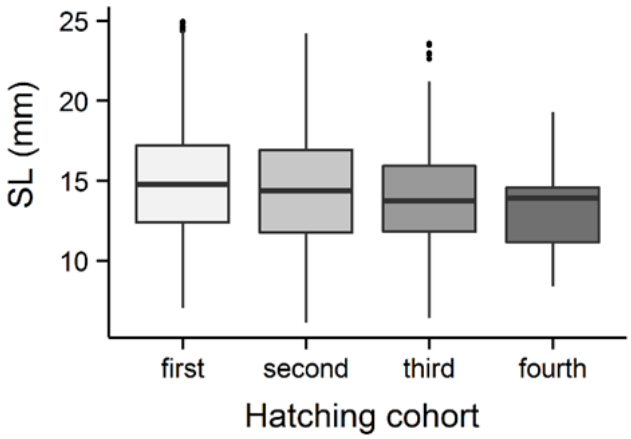

Figure 4.2.4. Length distribution of Trachurus trachurus recruits for each hatching cohort.

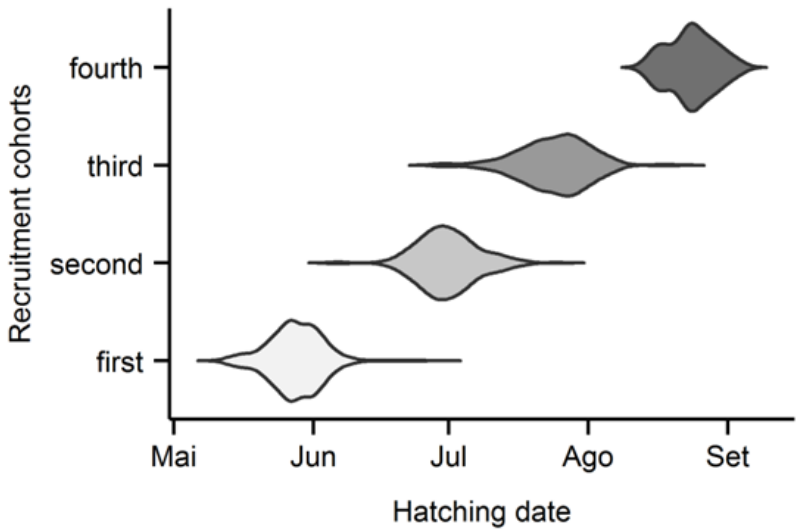

Figure 4.2.5. Hatching period of recruitment cohorts; areas are scale-independent.

The recruitment time series encompassed 4 new and 3 full moon events, while during the estimated hatching period 5 full and 5 new moon events occurred (Figure 4.2.2 A, B). For each lunar cycle, there were two tidal cycles, with spring tides occurring on the same day or one day after full and new moon and the tidal range extending from 1 m to 3.5 m. Until the beginning of June, the upwelling index was around or below 0, indicating a period of respectively no upwelling or downwelling (Figure 4.2.2C). From the beginning of June onwards, the upwelling index steadily increased. SST increased until the beginning of June from around 17 to 20ºC, then decreased to 16ºC by the end of July and increased again,

stabilizing around 19ºC until the end of the sampling period (Figure 4.2.2D). A similar temporal pattern was observed for the surface currents in the marine park, as from the start of June currents changed from less stable conditions including days with northward currents, to almost exclusively south and south-eastward currents (Figure 4.2.2F).

After a stepwise forward and backward selection process, the GAM model containing the factor moon phase, and variables upwelling (UP_INDEX) and U current component (U) as smoothing functions was the one with the lowest AIC (523.1) and explained the largest part of the deviance (71.7%; Table 4.2.2; formula 1). No strong residual pattern was observed and no auto-correlation was detected (Figure S4.2.3 A, B). Adding factor SITE to the model increased the AIC and the factor was not significant.

$$REC_i = \alpha_i + \beta_i \times MOON_i + f(UP\_INDEX_i) + f(U_i) + \varepsilon_i \quad (1)$$

with REC_i being the number of recruits at time i, $\alpha_i + \beta_i$ are the regression parameters, f() stands for the smoothing functions and ε_i is the residual error vector.

The moon phase had a strong influence on the recruitment of Atlantic horse mackerel, with higher numbers of recruits collected after the third quarter and new moon phases (Figure 4.2.2A, 4.2.6A). Results of the smoothed variables indicated that recruit abundance decreased with decreasing east-west current component (U) and was higher at positive values (Figure 4.2.7 A). Recruitment decreased with increasing upwelling index (Figure 4.2.7 B).

Table 4.2.2. Model results of the generalized additive model of the recruitment data and of the generalized additive mixed model of the hatching data with and without random effect. Following information is provided for each model: explained deviance (Dev. expl.), adjusted r^2 (r^2 adj.) and Akaike Information Criterion (AIC). For each predictor factor moon phase (fMOON), up-welling index (UP_INDEX), U-current component (U) and SST are listed: degrees of freedom (df) or effective degrees of freedom (edf) for smoothed variables, Chi-square statistics ($\chi 2$) for GAM and F-statistics for GAMM models (F), and significance value (p).

Model type	Dev. expl.	r^2 adj.	AIC	log Lik. (df)	Predictors	df / edf	χ^2 / F	p
GAM	71.7	0.46	523.08	248.41 (13.13)	fMOON	3.00	19.6	<0.001
					UP_INDEX	3.41	41.4	<0.001
					U	3.99	34.4	<0.001
GAMM	-	0.75	295.21	-138.6 (9)	fMOON	3.00	13.3	<0.001
					UP_INDEX	1.00	168.0	<0.001
					SST	3.75	12.8	<0.001
GAMM	-	0.40	284.14	-132.07 (10)	fMOON	3.00	17.9	<0.001
with					UP_INDEX	1	13.5	<0.001
random effect					SST	6.41	7.0	<0.001

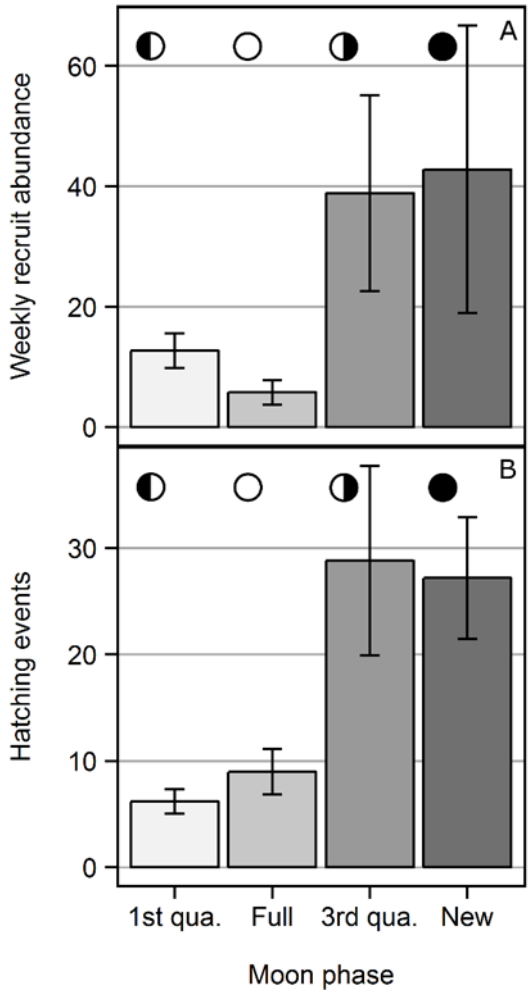

Figure 4.2.6. Mean recruit abundance (A) and mean hatching intensity (B) at each moon phase; error bars indicate standard error.

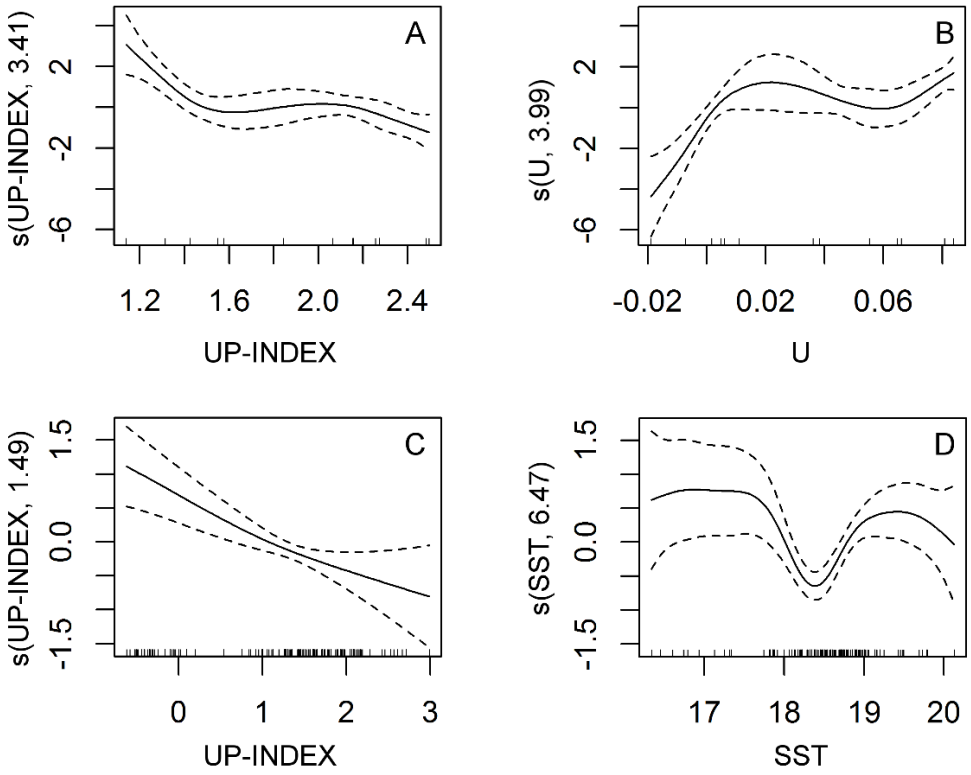

Figure 4.2.7. Estimated smoothing curves and 95% confidence bands (dashed line), illustrating the influence of U current component (A) and up-welling (B) on recruit abundance and up-welling (C) and SST (D) on hatching intensity.

The optimal combination of environmental variables for the model of the hatching patterns contained the factors moon phase and hatching cohort, the variables upwelling index (UP_INDEX) and SST with a smoothing function (Table 4.2.2; formula 2). Including the factor cohort as a random effect lead to a much better fit than the model without this dependence structure (AIC=284.1 vs. AIC=292.4; likelihood ratio test: $p<0.005$; Table 4.2.2) and eliminated the residual autocorrelation (Figure S4.2.4 D vs. B). No clear patterns were observed when plotting model residuals against fitted values and against environmental variables.

$$HATCH_i = \alpha + \beta_i \times MOON_i + \beta_i \times f(UP\_INDEX_i) + f(SST_i) + \alpha_i + \varepsilon_i \quad (2)$$

,where $\alpha + \beta_i$ are the regression estimates, f indicates a smooth function, α_i stands for the random intercept of the random factor cohort and ε_i is the residual error vector.

Certainly more fish hatched during third quarter and new moon periods (Figure 4.2.2B, 4.2.6B). The hatching abundance was negatively related to the upwelling index (Figure 4.2.7 C). A non-linear relationship was found between SST and hatching (Figure 4.2.7 D.

4.2.5 Discussion

This study recorded for the first time abundant recruitment of post-larval and early juvenile Atlantic horse mackerel to the nearshore (~100 m offshore), suggesting that this species is using shallow water rocky reefs as nursery areas. This result is innovative, given that high larvae and egg abundances were usually detected above the shelf edge (Farinha and Borges, 1994, Farinha and Lopes, 1996, Fives *et al.*, 2001, Álvarez *et al.*, 2012) and only few studies have occasionally registered the occurrence of newly hatched larvae in the nearshore (Sabatés *et al.*, 2003, Borges *et al.*, 2007a, Borges *et al.*, 2007b), without focusing specifically on recruitment patterns of this species. Our results indicate that Atlantic horse mackerel utilises the nearshore during their early growth period and clearly associates with floating structures. This association has been observed for other carangid species (e.g. Masuda and Tsukamoto, 2000, Dempster and Kingsford, 2004, Masuda *et al.*, 2008). Possible reasons for this behaviour include finding conspecifics, not dispersing of the school at night, predator avoidance, predatory behaviour and migration (e.g. Fréon and Dagorn, 2000, Castro *et al.*, 2002, Masuda *et al.*, 2008). A thigmotactic behaviour of Atlantic horse mackerel was observed in the Mediterranean by Deudero *et al.*, 1999 and Massutí *et al.*, 1999, who studied the functioning of fish aggregating devices (FADs). In contrast to our study, FADs were installed further offshore (4-7 nautical miles) and had only sampled Atlantic horse mackerel larger than 36 mm (with average fork length = 64 mm). This might indicate that post-larval and early juvenile stages can use the nearshore as a nursery area before moving to deeper waters, their main adult habitat (Borges and Gordo, 1991, Lloris and Moreno, 1995, Sousa *et al.*, 2005).

We show that *T. trachurus* have a clear depth preference, as 99% aggregated in surface collectors. This goes along with previous studies on vertical distribution which recorded eggs and larvae predominantly at the surface (Southward and Barrett, 1983, Coombs *et al.*, 1996, Borges *et al.*, 2007a). Our results also indicate that Atlantic horse mackerel forms schools during the larval and postlarval phase, consisting of different size and age individuals which had likely hatched on different days. These schools are assumed to be distributed patchily as the number of recruits varied largely among SMURFs without any clear pattern, and no significant relationship between sampling site and recruitment intensity.

The highest abundance of recruits was recorded in the first two weeks of the sampling period with the number of recruits decreasing strongly afterwards, but staying somewhat stable over the rest of the sampling period. One could hypothesise that this decrease is associated with the end of the spawning season, but in our region it extends to the summer and autumn (Barraca, 1964, Borges *et al.*, 1977, Arruda, 1984, Borges and Gordo, 1991). Therefore, variability in oceanographic conditions might be a better explanation for these temporal changes in recruitment.

Despite differences in abundance, recruitment peaks followed a clear cyclic pattern with a roughly monthly periodicity. A strong relation between recruitment patterns and the lunar phase was found. Most fish recruited during the waning (third quarter) and new moon periods. For many fish species, a lunar periodicity in recruitment has been established mostly in tropical (e.g. Robertson *et al.*, 1988, Sponaugle and Cowen, 1996, Lozano and Zapata, 2003), but also in temperate reef systems (e.g. Kingsford, 1992, Tricklebank *et al.*, 1992, Aalbers, 2008), although the type of moon phase is species specific. Different ecological hypotheses have been proposed to explain these lunar settlement patterns, such as the association of the lunar phase with specific tidal currents (e.g. Sponaugle and Cowen, 1996, 1997), a synchronization with periodic spawning (Robertson *et al.*, 1990, Meekan *et al.*, 1993, Sponaugle and Cowen, 1994), or predation risk avoidance related to nocturnal light conditions (Robertson *et al.*, 1988, Sponaugle and Cowen, 1994). If the dependence of recruitment on the lunar phase would be associated with tidal amplitude, one would expect recruitment to happen on a semilunar basis (Sponaugle and Cowen, 1994). However, at our sites, recruitment was maximal during new moon, but minimal during full moon.

A synchronization of recruitment with spawning might be plausible, since hatching was also synchronized with the lunar phase and spawning occurring just two to three days earlier according to the water temperature during this period (Pipe and Walker, 1987, Cunha *et al.*, 2008). Nonetheless, a certain level of synchronization of spawning and recruitment might not be the only cause for a lunar dependent recruitment, since the PLD of recruits was very variable and many were younger or older than 30 days when reaching the SMURF (Sponaugle and Cowen, 1994).

The avoidance of predation risk could also be of significance, especially from the third quarter phase onwards, when both moon illumination and the time when the moon is visible over the horizon progressively decrease, until a minimum is reached during new moon. But the effect of potentially important predators has not been studied. Another explanation for lunar recruitment could be a relation with the reef soundscapes. Maximal sound intensity created by biological processes occurs during new moon in both tropical (Staaterman *et al.*, 2014) and temperate reefs (Radford *et al.*, 2008), which might increase the ability of fish to detect and to orient to reefs. This would be an interesting topic for future research.

The back-calculated information from otoliths gave a picture on the hatching pattern of the Atlantic horse mackerel based on fish who survived the larval phase (recruits to SMURFS). This pattern followed a clear lunar cycle with distinct hatching cohorts. This species is a batch spawner, i.e. females spawn their eggs in several batches during the spawning season. A lunar spawning pattern around new and full moon has also been described for the carangid species *Trachurus declivis* (Jordan, 1994), *Caranx melampygus*, *C. ignobilis* (Johannes, 1978), and only around full moon for *C. mate* and *Carangoides fulvoguttatus* (Johannes, 1978). Nevertheless, all of these species, except of *T. declivis*, are carangids with a tropical and nearshore distribution. A lunar spawning periodicity might benefit the synchronization with an optimal arrival period to the nearshore (Johannes, 1978, Robertson *et al.*, 1990), which would support our assumption of a coupling between spawning and recruitment of Atlantic horse mackerel. Furthermore, spawning around a specific moon phase might facilitate the aggregation of fish and increase the fertilization success. Spawning aggregations have been recorded for other tropical nearshore carangid species (Johannes, 1978, Whaylen *et al.*, 2004, Graham and Castellanos, 2005).

In addition to this lunar pattern, upwelling was negatively correlated to recruitment and hatching. This supports the study of Santos *et al.* (2001), which revealed a negative correlation between upwelling during the winter spawning season and the overall yearly recruitment to the fishery in Portugal for the Atlantic horse mackerel. In our study, when summer upwelling intensified, hatching decreased rapidly, which can also indicate that less individuals survived the larval pelagic phase. The second and third hatching peaks occurred during small relaxations of upwelling intensity. Although, the upwelling index was found to be highly positively correlated with CHLa concentration, the negative correlation of hatching and recruitment with upwelling found could be driven by upwelling generated

turbulence and offshore advection (Fiúza *et al.*, 1982, Rossi *et al.*, 2013) in spite of an increase of phyto- and zooplankton production (Cunha, 1993, Moita, 2003).

Intensified offshore advection of surface water induced by upwelling (offshore Ekman transport; Relvas *et al.*, 2007) can transport larval fish offshore (Rodríguez *et al.*, 1999, 2004) and potentially increase larval mortality due to dispersal to unfavourable areas (Bakun and Parrish, 1980, Parrish *et al.*, 1983). This could partially explain the decrease of recruits aggregating at SMURFs, as the majority of fish that settled after the first peak had hatched during upwelling conditions when offshore advection and turbulence might have transported many larvae to areas away from shore. Indeed, in our study stronger south and south-eastward currents occurred when upwelling started.

Earlier studies on other pelagic fish, suggested that they have developed a reproductive strategy which involves spawning before intensive upwelling periods. Such a strategy might benefit egg and larval retention, but also larval and juvenile growth at a later stage (Cushing, 1974, Lasker, 1978, Cury and Roy, 1989, Roy *et al.*, 1989, Bakun, 1996, Cubillos *et al.*, 2001). Future studies should explore this hypothesis for Atlantic horse mackerel.

Concerning the other environmental factors used in the model analysis, SST was significantly related to hatching, and although its enhancement of the explained variance was minimal, it improved the residual pattern of the model. In addition to the lunar phase and upwelling, the third significant variable of the recruitment model was the U-current component (eastward). Recruitment increased with an increase of the U-component from negative to positive values, which means a change in current direction to the east, thus towards the Portuguese coast. Naturally, the U-component was highly collinear to the V-current component (northward) and the significant relationship found applies to the surface current in general. The available data are restricted to the regional scale water circulation and lacks resolution to analyse small scale processes such as nearshore and tidal currents, which likely are important for fish recruitment (e.g. Findlay and Allen, 2002, Sim-Smith *et al.*, 2013).

Fish collected in weeks following each of the four observed recruitment peaks were larger and older than fish that settled during peaks. This could be due to several reasons: i) these fish belonged to the previous recruitment pulse of fish arriving to the nearshore, and kept

growing locally; ii) they have spent more time offshore until arriving to the nearshore for any unknown reason, delaying recruitment; or iii) all fish might have arrived to the nearshore at the same time, but took longer to associate with the SMURFs. In general, when comparing hatching cohorts, the size of recruits decreased over the sampling period. Additionally, fish of the first hatching cohort had a higher growth rate than fish of the following cohorts (unpublished data). We hypothesise that environmental conditions prevailing during larval growth might have caused these differences.

The first cohort developed during a period of downwelling conditions and at the start of upwelling, whereas fish of the later cohorts grew only during increasing upwelling conditions with increasing Chla concentration (Cunha, 1993). On average, the temperature was higher during the larval growth of the first cohort than for the other cohorts and this might have led to a faster larval growth and to a larger size at recruitment of the first cohort (McCormick and Molony, 1995, Takahashi and Watanabe, 2004, Sponaugle *et al.*, 2006). Meekan *et al.* (2003) observed that larval growth was higher during absence of upwelling, when water temperatures were higher and prey abundance lower, concluding that temperature was more important for larval growth than prey abundance. This supports our results as fish of the second and third hatching cohorts were smaller and had a lower growth rate (unpublished data). The large majority of fish from the first hatching cohort recruited to our SMURFs during the first and highest recruitment peak. Similar results were found in other species with faster larval growth and higher rate of survival (Houde, 1987, Hare and Cowen, 1997, Wilson and Meekan, 2002, Takahashi *et al.*, 2012) associated to higher recruit abundance (Shima and Findlay, 2002, Watanabe *et al.*, 2003, Jenkins and King, 2006, Robert *et al.*, 2007).

In summary, although the exact mechanisms are still unknown, a clear relationship between the lunar cycle and both spawning and recruitment of Atlantic horse mackerel to nearshore environments was shown. Also, a relationship between upwelling and recruitment was found. These can be major mechanisms driving population fluctuations and hence key information for management of such an important commercial species.

This study indicates for the first time that nearshore areas might be important nursery grounds for the growth of postlarval and juvenile Atlantic horse mackerel. Several questions remain unanswered and are the basis for future research. Whether such nearshore aggregations are occasional and weather dependent or consistent; if different nearshore

habitats or coastline features are more favourable than others for recruits; and what is the influence of local hydrodynamics on these patterns are all open questions. Nevertheless, our results suggest that coastal marine protected areas might benefit this highly important commercial species by providing shelter to its vulnerable early stages.

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4.2.8 Supporting information

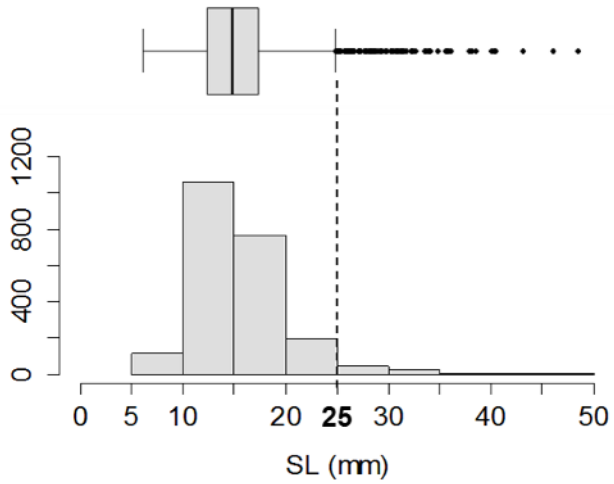

Figure S4.2.1. Standard length distribution of Atlantic horse mackerel collected with SMURFs.

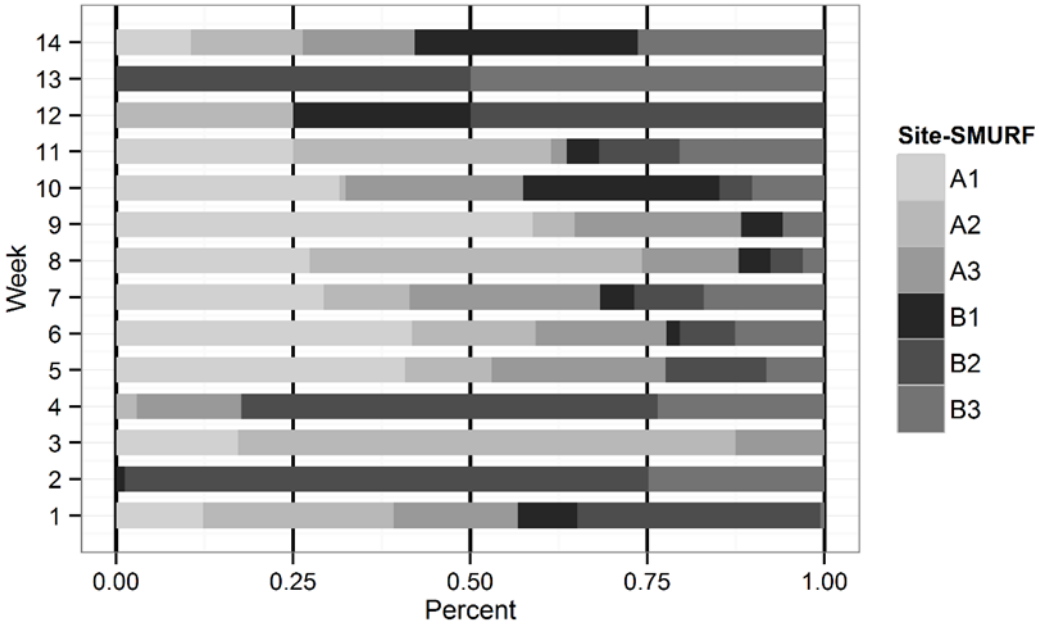

Figure S4.2.2. Spatial distribution in percentage of weekly settlers to collectors 1, 2 and 3 in site A and B.

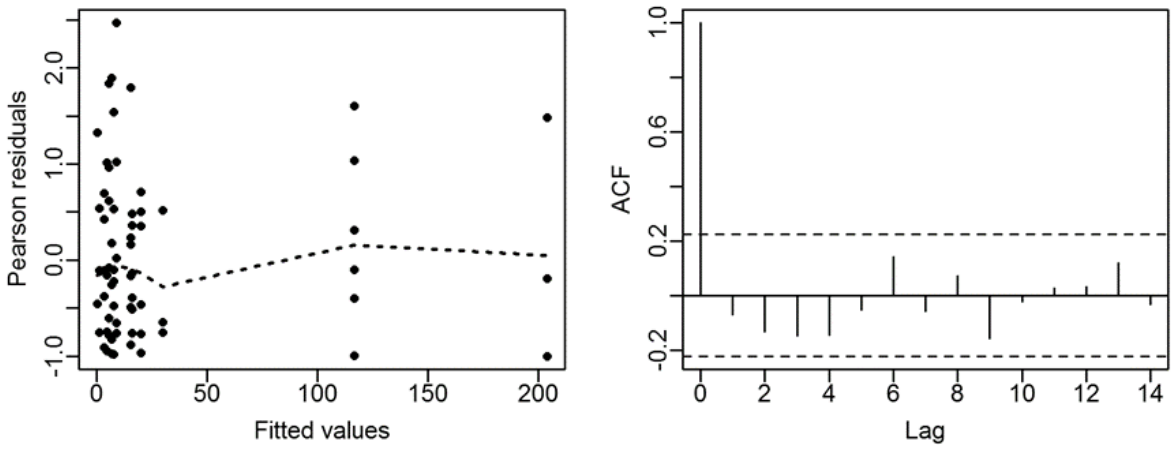

Figure S4.2.3. **Recruitment model validation**. Scatterplots of residuals (method: Pearson) vs. fitted values (A) and autocorrelation plot of residuals (B) from the best recruitment model.

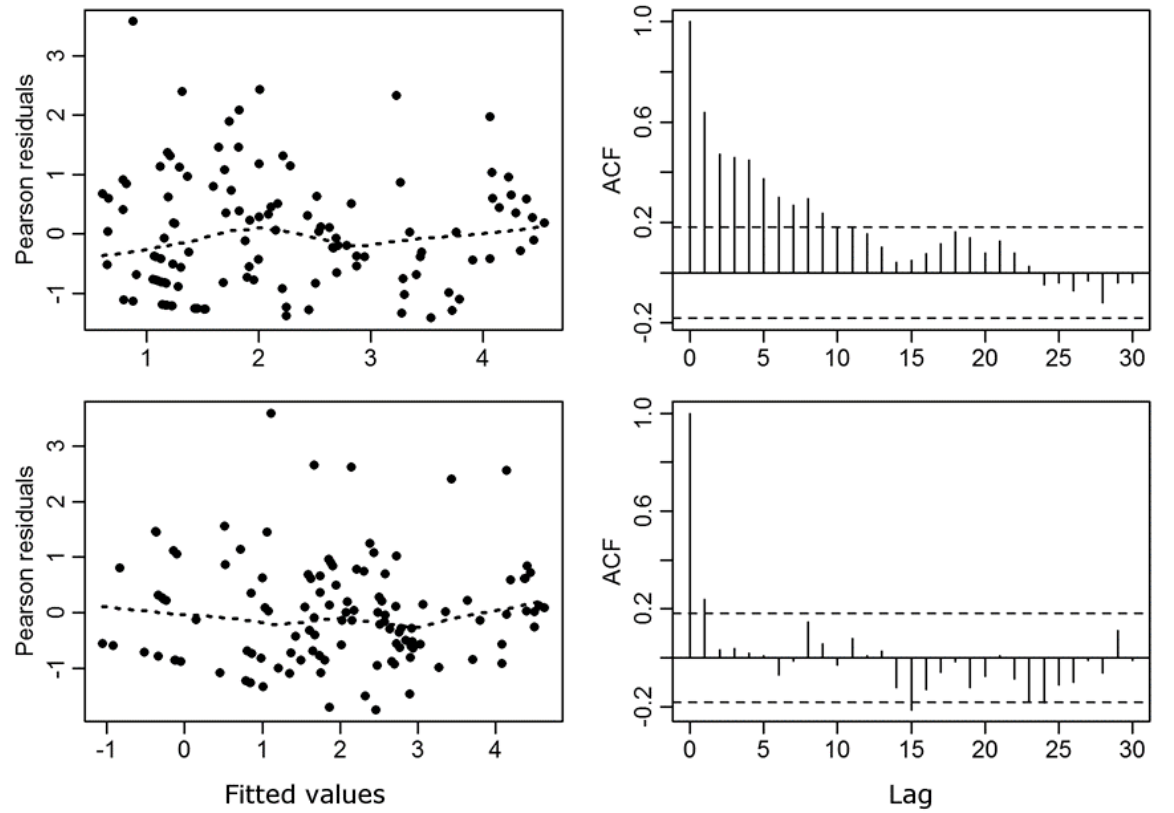

Figure S4.2.4. **Hatching model validation**. Scatterplot of residuals (method: Pearson) vs. fitted values and autocorrelation plot of residuals from the hatching model excluding a random intercept (A and B, respectively) and including a random intercept (C and D, respectively).

Chapter 5.1

Characterization of 15 polymorphic microsatellite loci in the temperate reef fish *Lepadogaster lepadogaster*, developed using 454-sequencing

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5.1.1 Abstract

The clingfish, *Lepadogaster lepadogaster* is a reef fish species, abundant in temperate nearshore rocky reefs of the Eastern Atlantic and central and Eastern Mediterranean. To study genetic variability and population connectivity of this species, we developed fifteen polymorphic microsatellite markers. These were tested in one population and all but one, showed no departure from Hardy-Weinberg equilibrium. Average overall observed heterozygosity was 0.66 and allelic richness was 8.9. Two primer pairs revealed possible linkage disequilibrium. These markers open perspectives for population genetic studies of this species to unravel connectivity and population biology, vital information for future conservation studies.

Keywords: Temperate reefs, intertidal, larval retention, *Lepadogaster*, microsatellites

5.1.2 Text

Understanding factors affecting recruitment is central to conservation biology and marine ecology (Almany *et al.*, 2007). Marine populations have traditionally been considered as open, with larval pelagic phases ensuring connectivity, depending on current patterns and on pelagic phase duration. Recent studies have challenged this view, by demonstrating larval retention and local recruitment (e.g. Jones *et al*., 2005, Almany *et al*., 2007) and showing that larval behaviour can modulate dispersal (see Leis 2006 and Leis *et al*., 2011). This has pronounced consequences for local populations' dynamics, and must be considered when managing reef fish populations in marine protected areas (Quetglas, 2000), particularly when aimed at conservation of nursery areas.

Clingfishes (Gobiesocidae) are distributed worldwide in tropical and temperate seas (Briggs, 1955) among which *Lepadogaster lepadogaster* is particularly abundant, distributed from northwest Spain (Galicia) to northwest Africa, the Canary and Madeira Islands and in the Mediterranean. It has recently been separated from the sympatric species *Lepadogaster purpurea*, based on genetic and morphological differences (Henriques *et al*., 2002). Both species occur on intertidal and subtidal boulder fields but differ in breeding season and microhabitat preferences (Henriques *et al*., 2002). Behavioural (Gonçalves *et al*., 1998) and ecological (Henriques *et al*., 2002) studies are scarce. Pelagic larval duration is

short (Beldade *et al*., 2007, Tojeira *et al*., 2012) and the larvae have an inshore distribution (Borges *et al*., 2007) and can be found in different stages of development close to the adult´s reefs (R. Borges unpublished results). Juveniles and adults have a ventral sucking disk which allows them to explore microtopography and to resist strong water currents prevalent in their habitats. Larval swimming capabilities increase until settlement, when the larvae stop swimming and, having developed the sucking disk, remain at the bottom (Faria and Gonçalves 2010). Clingfish larvae might thus remain nearshore during their entire pelagic stage.

To assess genetic variability and the realized larval dispersal of *Lepadogaster lepadogaster*, we have developed and characterized polymorphic microsatellite loci for this species. Whole genomic DNA was isolated (CTAB method, Doyle and Doyle, 1990) from three individuals of different populations of *L. lepadogaster,* from ethanol preserved muscle tissue. Next generation '454' sequencing (Duke University), performed with a 1/8 genome coverage, produced 129,849 individual sequences, ranging from 40 to 984 bp with an average fragment size of 350 bp, of which 7.9% contained microsatellites. Raw sequences were searched for di- to hexa-nucleotide microsatellites, of 4 repeats minimum, using MSATCOMMANDER (Faircloth, 2008). Primers were designed with PRIMER3 (Rozen and Skaletsky, 2000), and 25 arbitrary primer pairs were tested for polymorphism using 15 individuals from several locations sampled. An M13-tail (TGTAAAACGACGGCCAGT) was added at the 5' end of all forward primers to enable fluorescent-dye labeling (Schuelke, 2000).

Of all the primer pairs tested, 15 were polymorphic (Table 5.1.1). Sequences have been deposited in GenBank (accession no. JX448551 through JX448565). Amplification reactions in 10 µL contained 10 ng of genomic DNA, 1x Qiagen HotStart *Taq* buffer, 200 µM of dNTP's, 0.04 µM of forward primer, 0.16 µM of reverse primer and fluorescently-labeled M13 primer, and 0.5 U of HotStart *Taq* polymerase (Qiagen).

PCR were conducted in Perkin-Elmer GeneAmp7200 (Waltham, MA, USA) with the following program: 15 min at 95° C, 30 cycles composed of 30 s of denaturation at 95º C, 45 s at the annealing temperature (Table 5.1.1) and 45 s elongation at 72° C, followed by an additional 8 cycles composed by 30 s of denaturation at 95º C, 45 s at 53ºC and 45 s elongation at 72° C, after the cycles a final 30 min elongation step at 72º C was performed. PCR products were amplified with M13 primers end-labelled with different fluorescent dyes,

FAM, ATT550 or HEX in order to multiplex. Fragments were separated on an ABI3130XL automated sequencer (Applied Biosystems, Foster City, CA, USA) with Rox350 size standard. Alleles were scored using Peak Scanner 1.0 (Applied Biosystems).

Variability of these markers was tested on 40 individuals from Southern Portugal (near Sagres). The number of alleles per locus (*n*), and the observed (H_O) and expected (H_E) heterozygosities (Table 5.1.1) were calculated with GENETIX 4.05 (Belkhir *et al*., 1996-2004).

Most loci were highly polymorphic, with 2 (Lep2) to 24 (Lep24) alleles; H_O ranged from 0.36 to 0.82 and H_E from 0.33 to 0.93. No significant heterozygote deficiency was observed, except for Lep24. High frequency of null alleles is likely at this locus, as confirmed by further analysis using MICRO-CHECKER (van Oosterhout *et al*., 2004). We tested for linkage disequilibrium between all pairs of loci according to the Black & Krafsur (1985) procedure, the significance of the results were tested with 10000 permutations, at the 5 % level, two pairs were significant, Lep7- Lep14 (p=0.014), and Lep11-Lep20 (p=0.016).

These microsatellite loci should be useful for assessing genetic structure at different geographical scales. Due to their high variability they should also be useful for fine-scale genetics and recruit assignment in Marine Protected Areas (such as the Arrábida Natural Park, Portugal), where *L. lepadogaster* is a model to understand larval retention and the consequent role of the Marine Park in fostering recruitment and spill over effects of great importance in conservation biology.

Table 5.1.1. Characterization of the polymorphic microsatellite loci identified in *Lepadogaster lepadogaster* (Genbank accession numbers JX448551 to JX448565).

Locus	Repeat motif	Primer sequence (5'-3')	Ta (°C)	Allele size range (bp)	n	HE	HO
Lep2	(GATT)4	F: ACCATTGGCTATGATTTGGCG	58	172-204	2	0.3	0.4
		R: CCGTCCCTGGACCTAAACG					
Lep3	(CCCAGC)5	F: ACCCGTAGCCAAAGCCAG	50	241-269	7	0.7	0.7
		R: CATGGCTTCATCGGGAAGTG					
Lep4	(GGA)7	F: TAGCGACAGGTGCGTCAG	56	212-224	5	0.7	0.8
		R: CCCAGACTCAGAGGTGCAG					
Lep6	(GA)3(GCA)9	F: TCCGAGGCACAGTGAATGC	48	275-311	4	0.5	0.4
		R: CGAGGCGCCATTATTCCATC					
Lep7	(CA)17	F: CCCTATTTGGCTGCTGGAC	56	147-167	5	0.6	0.6
		R: GGTGGAGTGGTTTGACAGG					
Lep9	(GT)5TT(GT)8	F: CCAGCTTTACCGATGTGGC	52	176-203	8	0.8	0.8
		R: GCCTCCCTTCTGTAAGCCC					
Lep11	(GT)12	F: ACTGACTGCAGGCGTTG	52	191-195	6	0.6	0.6
		R: AGAAATGACAGCTTCAGATAATCCC					
Lep13	(GT)14TT(GT)3	F: CTCGCAATACCCCATAATGAC	54	180-195	11	0.8	0.7
		R: ACCCTGCACCCACAGATAAG					
Lep14	(GT)21	F: TAGTGAGGCCTGGTCTTGG	54	211-286	17	0.7	0.8
		R: GTGCTCCTCATGCCAACC					
Lep15	(GGGTTA)5	F: GGCTGGCACATGTTACTCG	52	185-289	7	0.5	0.4
		R: CCCTACCACATCCCACGG					
Lep17	(GGAT)8	F: CCAGTGGTTTGCTAATGTCCC	52	256-296	10	0.8	0.8
		R: TCAGCCAATCAGCGTTCAC					
Lep20	(CTTT)6(CT)3	F: CCATCGAAGGCATGCACAG	52	239-267	8	0.7	0.8
		R: CACAAGGAGTCAAACTGTTCCG					
Lep21	(CAT)6…(GA)4(GT)11	F: GCATATGTTCCCTGGAAGCTG	52	252-283	12	0.7	0.7
		R: AAATTGGGCCAGAACCTGC					
Lep23	(GATT)8	F: GGACGATAAACGGACAGCG	56	211-251	8	0.8	0.7
		R: CCTGTGTCTCGATCATATGCG					
Lep24	(CA)22	F: GGGAGTCGTTCTGCACCTAC	50	163-235	24	0.9	0.8
		R: TGGCACGTGTGTCGCATC					

The following details are reported: name, motif, primer sequence and annealing temperature (Ta ºC). Also descriptive statistics are presented, based on one population analysed, number of alleles *n*, and expected and observed heterozygosities, H_E and H_O.

5.1.3 Acknowledgments

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Chapter 5.2

High inter-annual variability in connectivity and genetic pool of a temperate clingfish, matches dispersal model predictions

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Photograph on previous page was taken by Diana Rodrigues.

5.2.1 Abstract

Adults of most marine benthic and demersal fish are site-attached, with the dispersal of their larval stages ensuring connectivity among populations. Here, we used an interdisciplinary approach to study population connectivity and dispersal of the shore clingfish *Lepadogaster lepadogaster* along the south west Iberian Peninsula in 2011 and 2012. We predicted high levels of self-recruitment and distinct populations, as larvae of this intertidal rocky reef species have a short pelagic duration (11-18 days) and all its developmental stages have previously been found near adult habitats. Genetic analysis based on microsatellites and a biophysical dispersal model countered our prediction. Adult sub-populations separated by up to 300 km of coastline displayed no genetic differentiation, revealing a single connected population with larvae potentially dispersing long distances over hundreds of km. Despite this, parentage analysis performed on recruits from one focal site within the Arrábida Marine Park (Portugal), revealed 2.5 7.7% of self-recruitment in 2011 and 2012, respectively, suggesting that both long- and short-distance dispersal play an important role in the replenishment of these populations. Population differentiation and patterns of dispersal, which were highly variable between years, could be linked to the variability inherent in local oceanographic processes. Overall, our measures of connectivity based on genetic and oceanographic data highlight the relevance of long-distance dispersal in determining the degree of connectivity, even in species with short pelagic larval durations.

Keywords: Lepadogaster, Gobiesocidae, marine dispersal, population connectivity, parentage, biophysical model, oceanography

5.2.2 Introduction

Adults of most marine benthic and demersal fish are site-attached, with the dispersal of their larval stages ensuring connectivity (e.g. Keough and Swearer, 2007). Due to the difficulties in tracking small larvae and because early connectivity studies focused on the larvae of temperate species with poor swimming capabilities (Blaxter, 1986, Miller *et al.*, 1988), initial research considered fish larvae as passive particles vulnerable to large current systems (Roberts, 1997). Such studies revealed the existence of long-distance dispersal, resulting in highly connected open populations (e.g. Williams *et al.*, 1984, Siegel *et al.*, 2003). In contrast, a growing number of studies have recently shown significant levels of

self-recruitment and reduced dispersal distances in many tropical (e.g. Taylor and Hellberg, 2003, Jones *et al.*, 2005, Gerlach *et al.*, 2007) and a few temperate fish species (e.g. Galarza *et al.*, 2009, Swearer and Shima, 2010). These studies, combined with findings of persistent nearshore larval distributions of coastal species (Borges *et al.*, 2007a, Borges *et al.*, 2007b) and increasing evidence of active larval behavior (Kingsford *et al.*, 2002, Fisher, 2005, Paris *et al.*, 2013), show that larval dispersal can be restricted and populations can be more demographically closed over ecological time scales than previously assumed.

Although ocean currents are a major factor affecting larval transport (e.g. Jenkins *et al.*, 1999, Simpson *et al.*, 2014), local conditions may favor reduced dispersal distances and larval retention (Largier, 2003, White *et al.*, 2010). Some fish larvae significantly interact with currents by migrating vertically, changing their swimming speed and direction to actively find settlement habitats (e.g. Kingsford *et al.*, 2002, Leis and Carson-Ewart, 2003, Paris *et al.*, 2013). The degree of dispersal and connectivity among populations is essential to our understanding of the persistence and resilience of disturbed populations as such, it is critical that we understand the processes that shape connectivity patterns (Eckert, 2003, Botsford *et al.*, 2009).

Many methods have been developed to overcome the knowledge deficit in marine fish larval connectivity. Indirect approaches include genetic connectivity studies (e.g. Hedgecock, 1994, Selkoe *et al.*, 2010), microchemistry of otoliths as geochemical markers (e.g. Swearer *et al.*, 1999, Fontes *et al.*, 2009) and biophysical models, which are used to reconstruct dispersal tracks, population connectivity and to identify potential sink and source populations (e.g. Cowen *et al.*, 2006, Assis *et al.*, 2015). Direct approaches include mark-recapture methods with stable barium isotopes or tetracycline immersion (e.g. Jones *et al.*, 2005, Thorrold *et al.*, 2006), assignment tests (Manel *et al.*, 2005) and parentage analysis with highly polymorphic genetic markers (Jones and Ardren, 2003, Saenz-Agudelo *et al.*, 2012).

In this study, we tested the prediction of high population structure and low connectivity among populations of a temperate clingfish, *Lepadogaster lepadogaster*. Our prediction was based on a combination of biological, ecological and oceanographic features applicable to this species. These include, a short pelagic larval duration (PLD; Raventós and Macpherson, 2001, Beldade *et al.*, 2007, Tojeira *et al.*, 2012), the occurrence of all larval developmental

stages in the nearshore environment of some study sites (Beldade *et al.*, 2006a, Borges *et al.*, 2007b), the hatching of well-developed larvae compared to pelagic species (Tojeira *et al.*, 2012) and oceanographic data suggesting retention at some of the study sites (Relvas *et al.*, 2007). Based on microsatellites, we estimated population structure and connectivity through F-Statistics and multivariate and assignment methods. We then ran simulations of oceanographic particle displacement to infer connectivity.

5.2.3 Material and Methods

5.2.3.1 Study Species

The shore clingfish *Lepadogaster lepadogaster*, Bonnaterre (1788) a Gobiesocidae, is a small cryptobenthic temperate reef fish, strictly restricted to smooth boulders and stones with little biological cover in the intertidal zone of rocky coasts (Henriques *et al.*, 2002, Beldade *et al.*, 2006b). It occurs from the British Isles to North-west Africa, including the Mediterranean and Black Sea (Hofrichter, 1995, Henriques *et al.*, 2002). Egg clutches attached to rocks can contain 60 to 265 eggs (Hofrichter, 1995). Males aerate and rub the eggs until hatching (Tojeira *et al.*, 2012). *L. lepadogaster* has an embryonic development of 16 days and hatches well-developed larvae with an open mouth and anus, fully pigmented eyes and with lengths of 5.2-5.3 mm (Tojeira *et al.*, 2012). The PLD of *L. lepadogaster* lasts 11-18 days (Beldade *et al.*, 2007, Tojeira *et al.*, 2012); this PLD is comparably short in relation to other temperate rocky reef species (Raventós and Macpherson, 2001).

5.2.3.2 Study area and sampling

The Arrábida Marine Park (AMP), located in central western Portugal, was a preferred location for this study since previous work on larval fish assemblages in this area described a very near-shore and near-reef distribution for several rocky reef fish species, indicating the potential for larval retention close to the adult habitat (Beldade *et al.*, 2006a, Borges *et al.*, 2007a, Borges *et al.*, 2007b). The AMP can be regarded as a continental island since it is separated from the nearest rocky reef systems by 40 km (to the north) and 60 km (to the south) of sandy coastline. Additionally, a review on the oceanography of the Western Iberian system described this region as being retentive with reduced offshore advection (Relvas *et al.*, 2007). In 2012, adult fish were sampled at a focal site within the AMP (ARR) and in areas to the north and south. Northerly sites included rocky coasts close to Lisbon (LIS),

Peniche (PEN) and São Martinho do Porto (MAR), while southerly sites included Sines (SIN), Almograve (ALM) and Barranco (BAR) (Figure 5.2.1, Table 5.2.1). Additionally, adults were collected in ARR and LIS in 2011 and recruits were collected from ARR in 2011 and 2012. In total, the sampling area encompassed 390 km of Portuguese coastline and sites were separated by an average of 65 km.

Adults and juveniles were collected at low tide during spring tides under boulders and stones in large boulder fields and in tide pools between rocky platforms. Adult fish were sampled before the recruitment season (February-June), while recruits were collected in autumn after recruitment (August-September). In order to roughly estimate population size at each site, the density of *L. lepadogaster* at ARR was determined via a field survey of all suitable boulder habitat, and extrapolated to the other sites. ARR was chosen for the field survey because of its suitable habitat and high fish density.

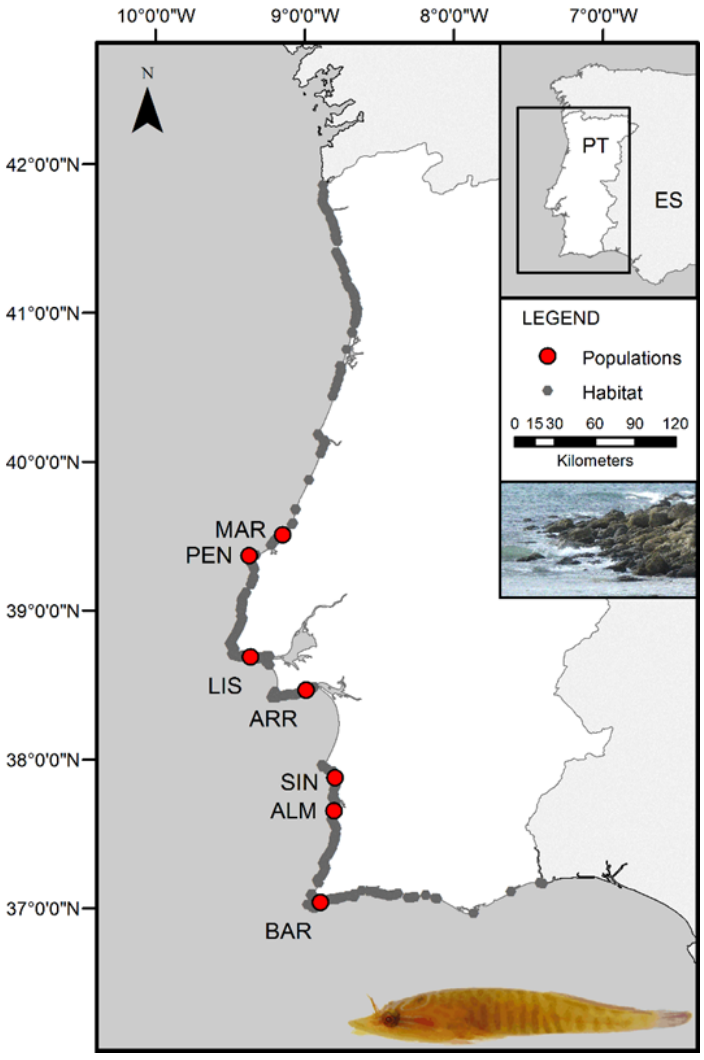

Figure 5.2.1. Study area. Displayed are sampling sites (red points with site labels as in Table 5.2.1) and possible habitat sites of *L. lepadogaster* in grey, selected by the presence of boulders in intertidal rocky reefs. Images on the right side show an adult fish (photo by P. Coelho) and a typical habitat site. Country maps were provided by the Portuguese Institute of Hydrography.

5.2.3.3 Ethics statement

For this study approximately 0.3 cm^2 tissue of the dorsal fin was clipped from adult *L. lepadogaster* and stored in 96% ethanol. Juvenile fish were euthanized at sampling locations and stored in 96% ethanol. *L. lepadogaster* is not a species of conservation concern (IUCN, 2014: Least Concern) and thus no specific permits were required except for sampling at ARR. This permit was issued by the Portuguese Nature Conservation Institute (ICNF). We followed the guidelines of the Association for the Study of Animal Behaviour (ASAB) (ASAB guidelines, 2012), as no specific protocol was required in the ICNF permission. Adult fish were released directly back to the same location where they were collected. Several fish were followed after this procedure in aquaria and no mortality due to handling was observed and fin regeneration occurred within weeks.

Table 5.2.1. Summary statistics of sampled adult and recruit populations from 2012 and 2011.

Pop_ID	Site	Adults/ Recruits	Year	N	% P	Mean SL ± SD	*A*	A_{rich}	H_E	H_O	F_{IS}
Mar_A_12	MAR	adults	2012	39	21	54.9±6.4	9.17	8.23	0.8	0.7	0.05*
Pen_A_12	PEN	adults	2012	39	16	54.2±5.2	9.58	8.25	0.7	0.7	0.08***
Lis_A_12	LIS	adults	2012	40	55	47.4±5.8	8.42	7.47	0.7	0.6	0.13***
Arr_A_12	ARR	adults	2012	40	14	52.8±4.1	9	8.14	0.8	0.6	0.14***
Sin_A_12	SIN	adults	2012	40	9.3	45.3±6.0	9.92	8.58	0.8	0.7	0.06**
Alm_A_12	ALM	adults	2012	24	43	45.3±6.0	7.83	7.83	0.8	0.7	0.08**
Bar_A_12	BAR	adults	2012	36	7	37.0±5.6	9.75	8.85	0.8	0.7	0.08***
Lis_A_11	LIS	adults	2011	30	41	56.1±4.5	10.3	10	0.8	0.7	0.09***
Arr_A_11	ARR	adults	2011	29	10	51.3±7.0	8.92	8.55	0.8	0.7	0.13***

Legend: Population label (Pop_ID), Site abbreviation as in Figure 5.2.1, Number of individuals sampled (N), estimated proportion of population sampled (% P), mean standard length ± standard error (Mean SL ± SD), mean number of alleles across loci (A), mean allelic richness (A_{rich}), observed (H_O) and expected (H_E) heterozygosities and heterozygote deficiency (F_{IS}). SiFgnificance levels are indicated (*p<0.05, **p<0.01, ***p<0.001).

Genomic DNA from fin- (adults) and muscle tissue (juveniles) was extracted as in (Henriques *et al.*, 2002), excluding the phenol/chloroform purification step. Fifteen microsatellite loci were amplified and analyzed, as described in Teixeira *et al.* (2013; chapter 5.1), but with higher primer specific annealing temperatures for most loci. Due to low polymorphism and some amplification inconsistency, only 12 of the initial 15 loci were used for data analysis.

5.2.3.4 Data Analysis

Genetic diversity

The mean number of alleles across loci (A), expected (H_E) and observed (H_O) heterozygosity, and heterozygote deficiency (F_{IS}) were determined with GENETIX, 4.05 (Belkhir K. *et al.*, 1996). Standardized allelic richness was estimated in R 3.1 (R-Core-Team, 2009) using the R package standARich vl1.00 (Alberto, 2006). Correction of p-values for multiple comparisons was performed using the false discovery rate in QVALUE (Storey and Tibshirani, 2003).

Population Structure and Connectivity

GENETIX was used to estimate F_{ST}, pairwise population differentiation, for each locus and over all loci. The probability of the F statistic being greater than zero was calculated with a permutation approach (10,000 replicates). Additionally, significant differentiation was confirmed by 95% confidence intervals (CI) above zero (Palsboll *et al.*, 2007), calculated using the R package "diversity". The software FreeNA (Chapuis and Estoup, 2007) was used to detect null alleles and to compare global and pairwise F_{ST} between our raw microsatellite data and a corrected version after applying the excluding null alleles (ENA) correction method with 1,000 bootstrap repetitions. Furthermore, D values, a differentiation index that is not affected by the number of alleles per locus (Jost, 2008, Keenan *et al.*, 2013), were estimated (averaged over loci) with the R package "DEMEtics" (Gerlach *et al.*, 2010). Here, p-values were obtained by 1,000 bootstrap resampling. Correction of p-values for multiple comparisons were performed using the false discovery rate in QVALUE (Storey and Tibshirani, 2003).

Isolation-by-distance (IBD) analyses were conducted for both pairwise F_{ST} and Jost's D-values independently in R by using the mantel.randtest function in the R package ade4 and 10,000 permutations. For these analyses, shoreline distance was calculated with the R-package "gdistance" (Van Etten, 2014).

The software STRUCTURE version 2.1 (Pritchard *et al.*, 2000) was used to assign individual genomes to a number of genetic populations estimated to minimize Hardy-Weinberg disequilibrium. Analysis was conditioned with an initial burn-in of 50,000 cycles followed by 100,000 additional cycles using an admixture ancestry model with correlated allele frequencies. Runs for each K (1 to 8) were repeated 20 times. Both Pritchard's (Pritchard *et*

al., 2000) L(K) criterion and the delta K criterion of Evanno *et al.* (2005) were applied using STRUCTURE HARVESTER (Earl and von Holdt, 2011). The software CLUMPP (version 1.1.2) was used to align the 20 replicate cluster analyses for the chosen K, by using the Greedy algorithm (Jakobsson and Rosenberg, 2007). Results of CLUMPP were then visualized with the software DISTRUCT (version 1.1; Rosenberg, 2003).

Furthermore, a Discriminant Analysis of Principal Components (DAPC), a multivariate method to resolve population structure, was used. A DAPC can assign individual genotypes to predefined groups without being dependent on assumptions of Hardy-Weinberg equilibrium or linkage disequilibrium, while being as sensitive as STRUCTURE (Jombart *et al.*, 2010). The dapc function within the R package adegenet performs a Principal Component Analysis (PCA) using the dudi.pca function from the ade4 package and a linear discriminant analysis (DA) with the lda function from the MASS package (Jombart *et al.*, 2010). For our data set 53 PCs, comprising 87% of the genetic information, were retained (Figure A in S5.2.3 supporting information).

Population and parentage assignment

To assign or exclude recruits to/from the 7 sampled populations, we used a partial Bayesian classification method (Rannala and Mountain, 1997) in GENECLASS 2 (Piry *et al.*, 2004) together with a Monte Carlo re-sampling method (Paetkau *et al.*, 2004). All adult fish (N=317) from the sampled populations were used as reference and 10,000 genotypes were simulated from these reference populations for the Monte Carlo algorithm. A recruit was assigned to a population if its probability of assignment was >0.05 for only one population. A recruit was determined as immigrant if the probabilities of assignment were below 0.05 for all populations (Waser and Hadfield, 2011).

To further infer the possible origin of the parents and offspring, we used the maximum likelihood approach implemented in COLONY v.2.0 (Wang, 2004, 2010), which assigns parentage from individual multilocus genotypes. Recruits (sampled at ARR in 2011, 2012) were used as offspring, while all adult populations were used as candidate male and female parents. The rate of allele dropout used was 0.05 and the rate of other errors was 0.01 (Wang, 2004). A full-likelihood method was used. We assumed polygamous mating systems for both males and females since in previous aquaria experiments it was observed that females spawn several times during the spawning season with different males and males have eggs from

different females. The analysis was run four times and only relationships with a probability higher than 0.95 were considered. Both methods were used to estimate self-recruitment in ARR, the proportion of local recruits that come from local parents (Burgess *et al.*, 2014) and to infer dispersal trajectories along sampled populations.

Dispersal model

Estimates of population connectivity of *L. lepadogaster* were inferred by developing individual-based Lagrangian Numerical Simulations (LNS; as reviewed by Cowen *et al.*, 2006, Fossette *et al.*, 2012) using early life history parameters that are known for this species. The experiments were performed following the framework implemented by Assis et al. (2015), utilizing daily data derived from the Hybrid Coordinate Ocean Model (HYCOM), a high-resolution hind cast of three-dimensional velocity fields forced by wind stress, wind speed, precipitation and heat flux (Chassignet *et al.*, 2007). This model is able to resolve oceanic filaments, fronts, meandering currents and eddies, some key processes needed to accurately simulate dispersion of drifting larvae (Chassignet *et al.*, 2007, Lett *et al.*, 2008).

Individual particles simulated drifting larvae of *L. lepadogaster* by incorporating a maximum PLD of 18 days (Beldade *et al.*, 2007) and a set of state variables such as location (longitude and latitude), age (day), developing stage (competent or non-competent for settlement) and status (dead or alive; dead are considered particles that have not found habitat for settlement after 18 days). Suitable habitats (Henriques *et al.*, 2002) along the SW Iberian Peninsula were quantified using a Geographic Information System (GIS) and gridded to match 0.05° (~9 km^2) spatial resolution. Particles were released from each cell every 6 hours during the spawning season of *L. lepadogaster* (from March to August; Henriques *et al.*, 2002 and pers. observation) and allowed to drift for the maximum PLD. The geographical position of each particle was determined every hour (24 steps per day) using bilinear interpolation of velocity fields, while integrating in the path equation a 4th Order Runge-Kutta adaptive time-step (e.g. Lett *et al.*, 2008).

Contrasting transport experiments (passive vs. vertical migration) were performed based on the early life history of this species. In the passive transport experiments, particles used the surface (0 meters) or the bottom (10 meters) layers independently. In the vertical transport experiments, particles were allowed to move from the surface to the bottom layers on the 10^{th} day of PLD, which is when the larvae acquire competence for settlement (Faria and

Gonçalves, 2010, Tojeira *et al.*, 2012). Additional parameters such as mortality rate and population sizes were not included, since this information is not available for *L. lepadogaster*. This model was used to estimate potential distances and patterns of dispersal and not to quantify larval dispersal.

Simulations were run independently per year, with data for the period 2002 to 2012. The resulting aggregated trajectories allowed the production of connectivity matrices between all pairs of cells. Paired probabilities were inferred by determining the number of steps in the path of a single particle, released from cell i, that achieved cell j, divided by the overall simulated time steps (18 days PLD * 24 steps a day). The annual matrices resulting from the different experiments were averaged to account for inter-annual variability. This allowed the determination of the maximum and average distance travelled by particles connecting pairs of cells. The mean proportion of retention and effective settlement were also determined and differences between experiment types were tested using a Kruskal-Wallis Rank Sum test followed by a post-hoc pairwise test for multiple comparisons of mean rank sums (Nemenyi-test). Lagrangian dispersal simulations were developed in R (R-Core-Team, 2009) using the packages: abind, calibrate, doParallel, igraph, ncdf4, parallel and raster.

5.2.4 Results

5.2.4.1 Genetic diversity

In total, 317 adult and 79 juvenile *L. lepadogaster* with a mean standard length of 51.2±8.8 mm and 18.2±4.1 mm, respectively, were successfully genotyped for 12 polymorphic microsatellites. Percentage of population analyzed ranged from 5.8 to 54.9% (Table 5.2.1). The number of alleles per locus ranged from 9 (loci LP3, 4, and 15) to 41 (locus LP24). Allelic diversity was very similar between populations as the mean number of alleles across loci varied from 7.8 to 10.3 (Table 5.2.1). Allelic diversity of recruits was similar to adult populations. Mean allelic richness (A_{rich}), standardized to 24 individuals as the minimum common sample size, ranged from 7.5 (LIS_A12, n= 40) to 10.0 (LIS_A11, n=29). Unbiased expected (H_E) and observed heterozygosity (H_O) ranged from 0.72 to 0.81 and 0.56 to 0.74, respectively. All populations had low but significant F_{IS} values, indicating a departure from Hardy-Weinberg equilibrium (HWE; heterozygote deficit) (Table 5.2.1 and Table S5.2.1 in supporting information).

We used the software FreeNA to test for null alleles in our data and their influence on our results. The null allele frequency per locus and population ranged from 0 to 0.027 (Table S5.2.2 A in supporting information). Global F_{ST} for all loci and pairwise F_{ST} were very similar, when estimated from raw allele frequencies or from allele frequencies corrected for null alleles by using the ENA correction of Chapuis and Estoup (2007) (Tables S5.2.2 B and C in supporting information). This suggests that null alleles have very little influence on our analysis; therefore all further tests were performed with uncorrected allele frequencies.

5.2.4.2 Population Structure and Connectivity

Global F_{ST} and Jost's D value were 0.0227 and 0.0762, respectively. Pairwise F_{ST} and Jost's D values showed very similar significant differences between populations (Table 5.2.2). Fewer F_{ST} values were significant, when considering 95% confidence intervals (Table 5.2.2). F_{ST} and D values ranged from -0.003 to 0.067 and -0.001 to 0.189, respectively. D values are less affected by diversity and sample size and were in better concordance with other analyses. According to D-values, in 2012 the most southern population (BAR) was significantly different ($p<0.01$) from all other populations and SIN was different to all populations north of ARR. In general, there was an increasing differentiation with distance, which coincided with the significant result of the isolation by distance analysis with pairwise F_{ST} and D values ($p= 0.0087$ and $p = 0.0015$, respectively; S4 in supporting information). Adult individuals from ARR and LIS in 2011, were significantly different ($p<0.01$) from each other and were also significantly different from both the same populations sampled one year later and from all other populations sampled in 2012. The ARR recruits from 2012 were significantly different ($p<0.01$) to the most northern population (MAR) and also to SIN and BAR in the south. The 2011 recruit population at ARR was significantly different from all other populations except for the 2011 adults sampled at the same site. D-values were higher for year to year comparisons than site to site, as the D-values of LIS and ARR between 2011 and 2012 were 0.152 and 0.123, respectively and so clearly higher than the D value of MAR and BAR that are separated by 700 km of coastline (0.112, highest D-value among 2012 populations).

Table 5.2.2. Genetic difference among adult, recruit populations and years.

	Mar A12	Pen A12	Lis A12	Arr A12	Sin A12	Alm A12	Bar A12	Lis A11	Arr A11	Arr R12	Arr R11
Mar_A12	--	0.001	-0.003	0.002	0.006*	0.007	0.030***	0.037***	0.048***	0.006*	0.023***
Pen_A12	0.024	--	0	0.006*	0.009**	0.008*	0.033***	0.038***	0.047***	0.003	0.025***
Lis_A12	-0.001	0.015	--	0.004	0.006*	0.003	0.029***	0.039***	0.046***	0.003	0.019***
Arr_A12	0.012	0.027	0.02	--	0.004	-0.002	0.023***	0.038***	0.051***	0.005	0.023***
Sin_A12	0.043**	0.035**	0.024*	0.024	--	0.009*	0.025***	0.045***	0.053***	0.012**	0.024***
Alm_A12	0.043**	0.052**	0.022	0.003	0.034*	--	0.032***	0.035***	0.046***	0.004	0.027***
Bar_A12	0.112**	0.109**	0.086**	0.074**	0.080**	0.081**	--	0.055***	0.067***	0.036***	0.041***
Lis_A11	0.139**	0.150**	0.152**	0.153**	0.175**	0.146**	0.219**	--	0.022***	0.046***	0.037***
Arr_A11	0.138**	0.121**	0.119**	0.123**	0.128**	0.140**	0.189**	0.099**	--	0.051***	0.011**
Arr_R12	0.038*	0.02	0.02	0.01	0.038*	0.02	0.106**	0.177**	0.131**	--	0.023***
Arr_R11	0.072**	0.070**	0.057**	0.064**	0.064**	0.080**	0.117**	0.138**	0.03	0.055**	--

Pairwise F_{ST} (above diagonal) and D-values (below diagonal); significance levels are indicated for permutation tests (*p<0.05, **p<0.01, ***p<0.001) and underlined F_{ST} values are significant also when considering confidence intervals. Population labels are explained in Table 5.2.1.

The STRUCTURE analysis revealed 3 clusters using L(K) and ΔK methods. All studied populations contained these three genetic populations in different proportions (Figure 5.2.2). The composition was very similar among adult populations in 2012, except for the most southern population (BAR). There was a clear difference between populations from 2011 and 2012. The 2012 recruit population matched better with the 2012 adult populations, and the 2011 recruit population matched better with the 2011 adult populations (Figure 5.2.2).

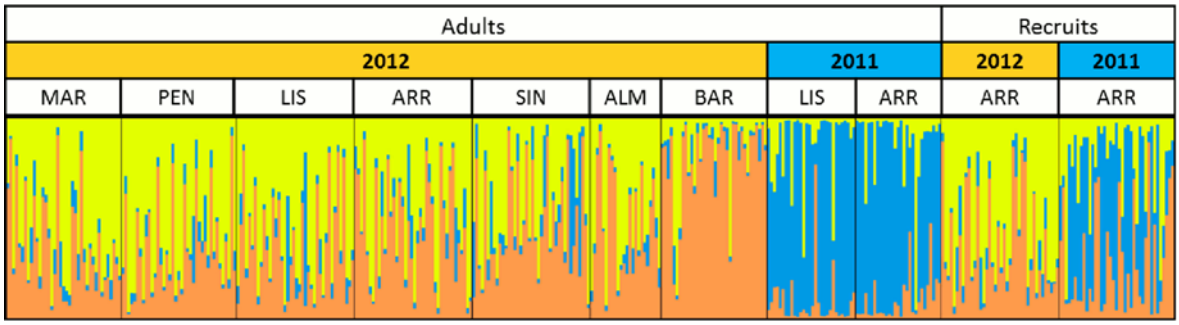

Figure 5.2.2. Averaged bar plot of STRUCTURE Bayesian clustering with K=3. Vertical bars displayed for each individual show the estimated membership proportions in the three clusters colored in yellow, blue and orange. Populations are separated by black vertical lines and described by site abbreviations as in Figure 5.2.1, sampling year, adult and recruit populations.

A similar pattern could be described from the DAPC analysis, whereby 2011 and 2012 populations are differentiated along the first discriminant function that comprised most of the genetic variation (Figure 5.2.3). This temporal distinction was even more apparent when densities were plotted only along the first discriminant function (Figure 5.2.3, top-right). At the second discriminant function (represented by the y-axis), the most southern location (BAR) stood out from the other 2012 populations, and, to a lesser extent, LIS adults were separated from ARR adults in 2011. The two recruit populations were separated along the first discriminant function. Overall, 61% and 81% of adults were correctly assigned to their geographical population in 2012 and 2011, respectively (Figure B in S5.2.3 supporting information), when the recruit populations were excluded. When included, 47% and 62% of adult fish were correctly assigned. Both the STRUCTURE and the DAPC analyses showed that there is more difference between adult populations in 2011 and 2012 for the same sites than among spatially separated populations from the same year.

5.2.4.3 Assignment test and parentage analysis

The assignment test detected only very few significant assignments, supporting low genetic structure among these populations. From the 2012 recruits collected at ARR, one was assigned to the population of BAR and one immigrant was not assigned to any of the analyzed populations. In 2011, one recruit was assigned to LIS and another one to ARR.

The parentage analysis identified 6 and 8 parent-offspring pairs in 2011 and 2012, respectively. In 2011 parents originated from ARR (3), SIN (2) and ALM (1) and in 2012 from LIS (5), ARR (1) and ALM (2). This means that over these two years, 5% of recruits came back to their natal population (ARR) and, 13% had a parent from either the next northern or the two next southern populations sampled. Recruits that self-recruited to ARR in 2011 (n=3) were identified to be the offspring of just one adult fish with a 100% probability. Also, 4 out of the 5 recruits originating from LIS in 2012 shared a parent.

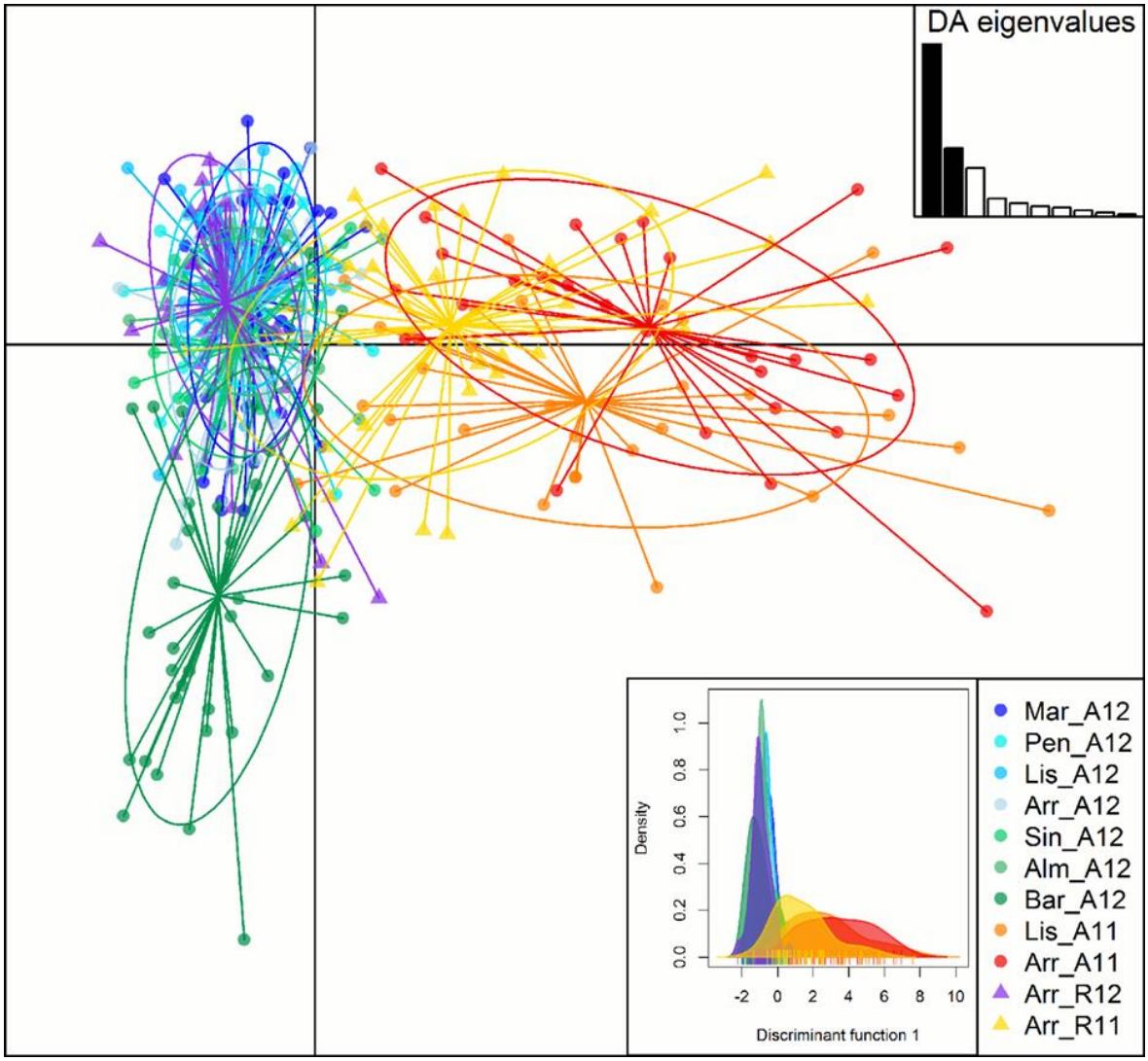

Figure 5.2.3. Scatterplot of the DAPC. Individual adult genotypes are represented in dots and recruit genotypes in triangles. Populations are distinguished by colors and 95% inertia ellipses. Barplot of DA eigenvalues (bottom right) displays the proportion of genetic information comprised in each consecutive discriminant function. X and Y axis of the scatterplot describe the first and second discriminant function (explaining 81.8% and 31.6% of genetic variance, respectively). Left from the legend is a density plot of the genetic information from only the first discriminant function.

5.2.4.4 Dispersal model

The numerical simulations using HYCOM velocity fields for an entire 11-year period allowed us to track the fate of 7,920 particles released from 93 distinct coastal cells (736,560 particles in total). Trajectories were obtained for particles that had successfully settled to a site within 18 days of PLD and these resulted in two types of connectivity matrices: 1) on a yearly basis including 2011, 2012 and 2010 and 2) averaged for an 11-year period. Simulations ran over 11 years revealed that particles spending their entire pelagic phase in a

deeper water layer (10 m) had a significantly higher probability of retention compared to particles staying at the surface, or migrating vertically from the surface to the bottom layer at the 10th day of PLD, when the larvae acquired competence for settlement (Faria and Gonçalves, 2010) (Figure 5.2.4a; Kruskal-Wallis and Nemenyi test, $p<0.001$). Also, particles that stayed at the bottom layer (10 m) had a significantly higher probability of effective settlement compared to the other two particle conditions (Figure 5.2.4b; Kruskal-Wallis and Nemenyi test, $p<0.001$). This means that by staying near the bottom, more particles are locally retained and are also able to find suitable habitat for settlement during their PLD. Particles that stayed at the surface at the beginning or for the whole pelagic phase had a higher probability of being advected offshore and were thus excluded from the model. Both the probability of retention and effective settlement were not significantly different between surface and migration particles (Kruskal-Wallis and Nemenyi test, $p>0.05$).

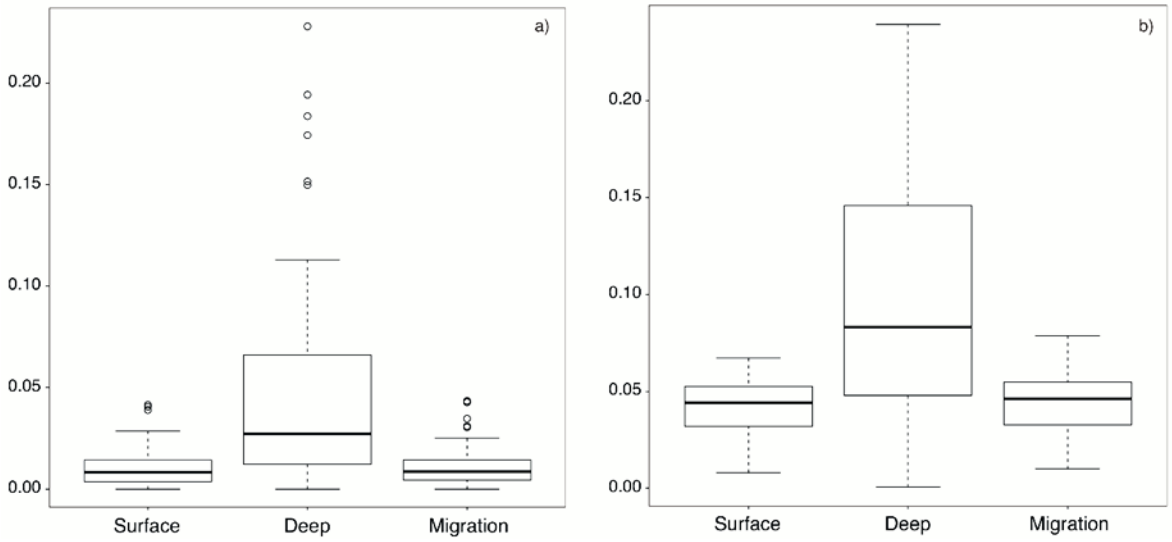

Figure 5.2.4. Comparison of particle type. Probability of (a) retention and (b) effective settlement according to particle type (surface, deep and migration).

Particles in deeper waters had an average dispersal capacity of 89.8±70.1 km compared to 105.9±77.0 km and 104.5±75.8 km, for surface particles and vertically migrating particles, respectively. For all three particle types, probability of connectivity declined exponentially with increasing coastline distance, with deep particles having the fastest decline (Figure A in S5.2.5 supporting information). The longest distance undertaken by a larva that remained near the bottom was 378.0 km. However, such dispersal events were very rare, as seen in the connectivity matrices (Figure 5.2.5 A, B, C and Table A in S5.2.5).

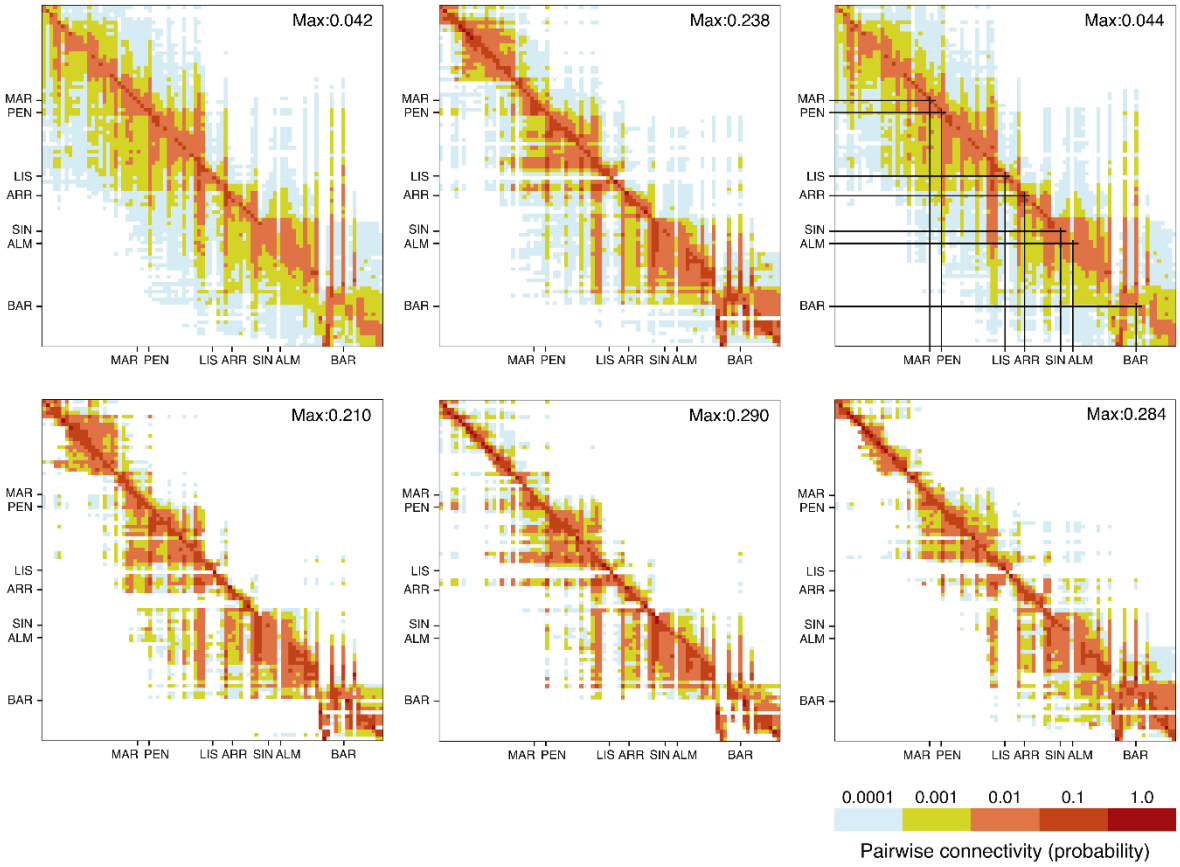

Figure 5.2.5. Connectivity matrices for the SW Iberian coast. Displayed in color are probabilities of particle released in site i (y-axis) that settle to site j (x-axis); averaged over simulations ran from 2002 to 2012 for particle type surface (a), deep (b) and migration (c) and yearly averaged for particle deep for 2010 (d), 2011 (e) and 2012 (f). The diagonal represents self-recruitment, area left from the diagonal shows northward dispersal and right from the diagonal southward dispersal. Value in the top right corner indicates the connection made with the highest probability. Axis labels indicate genetic sample sites. First scale color (blue) reaches from a probability of 0 to 0.001 and the last (red) from 0.1 to 1.0.

Connectivity matrices averaged over simulations ran from 2002 to 2012 (Figure 5.2.5; A, B, C) also showed higher probabilities of retention (more orange and red cells along diagonal) and shorter dispersal distances (narrower spread around diagonal) for particles staying in deeper waters. In general, these matrices indicated a higher probability of effective dispersal northwards rather than southwards. Furthermore, the 11-year connectivity matrices showed that all sites sampled for genetic analysis were connected by larval dispersal despite low probabilities of connectivity.

Yearly connectivity matrices for 2010, 2011 and 2012 for particles staying in deeper water, a behavior expected to occur in our study species, showed less spread along the diagonal, indicating reduced long-distance connectivity, when analyzing each year separately (Figure 5.2.5; D, E, F). However, inter-annual differences in dispersal patterns were

apparent, with more particles being advected northwards (left from the diagonal) in 2010 and 2011, whereas in 2012 more southward dispersal (right from the diagonal) occurred. Connectivity matrices show that around the most southern location (BAR) less dispersal occurred northwards, suggesting a connectivity barrier around Cape São Vicente.

When results of the dispersal model were summarized for the whole area of the AMP the probability of particles settling to the AMP was 99 and 68 times higher, in 2010 and 2011 respectively, when originating from southern sites than for particles released from northern sites (Figure B in S5.2.5). However, in 2012 this difference was only 10 times higher for southern particles. Particles that were released from the AMP in 2010 and 2011 had 32 and 28 times higher probability, respectively, to settle to northern sites than to southern sites. In all three years the probability of particles being retained in the AMP was 11 to 15 times higher than for particles arriving from southern sites.

5.2.5 Discussion

This study revealed high genetic connectivity among *Lepadogaster lepadogaster* populations throughout the study area, contradicting our hypothesis of high larval retention in natal areas. However, connectivity decreased with coastline distance and could be explained by oceanographic effects. The large temporal variability was an unpredicted finding; population differentiation and patterns of dispersal were highly variable between years and this could be explained by inter-annual variation in current patterns. These results highlight the important role of oceanographic conditions in mediating recruitment patterns despite biological traits which suggested a high level of local recruitment. Larval depth distribution also seems to be an important factor influencing connectivity, as modelling results showed that remaining deeper significantly restricted dispersal distance.

5.2.5.1 Spatial scale

In this study, significant heterozygote deficits, in comparison with the expected heterozygosity under the HWE, were found for all populations. However, it is not expected that a departure from HWE will affect our analysis, indeed previous studies have used these methods to study population differentiation, despite HW limitations (Assis *et al.*, 2013, Silva *et al.*, 2014). The heterozygote deficiency found here can be caused by several factors such

as a Wahlund effect, inbreeding or null alleles. Sampling more than one genetic population and treating it as one can result in a Wahlund effect. For our data little spatial sub-structuring was found within populations. However, temporal variation in the annual pool of recruits could result in distinct genetic populations co-occurring and in consequently significant F_{IS}.

High inbreeding levels are not expected to occur since *L. lepadogaster* can reach high densities and exhibit polygamy. Additionally, the high population genetic diversity and low spatial population structuring found in our study does not support the occurrence of inbreeding leading to a heterozygote deficit.

Another reason for significant F_{IS} could be the occurrence of null alleles in our data. Estimated null allele frequencies were below 0.2, except for two combinations of locus x population ($p<0.27$; Table A in S5.2.2 supporting information), indicating that null alleles were uncommon to rare (Dakin and Avise, 2004). Null alleles did not affect our genetic analyses of population differentiation, since pairwise F_{ST} for both the original and corrected datasets were similar. Also the DAPC analysis, that does not require populations to be in HWE (Jombart *et al.*, 2010), showed a similar differentiation as the STRUCTURE analysis. Regarding the parentage analysis, although null alleles could result in an underestimation of the average exclusion probability at a locus, this effect has been shown to be negligible at frequencies below 0.2 (Dakin and Avise, 2004, Wang, 2010). Moreover, since null alleles can lead to a false exclusion of parentage (Dakin and Avise, 2004, Wang, 2010), we can expect an underestimation of parent offspring pairs rather than an overestimation in our study.

Using 12 highly polymorphic genetic markers we inferred that populations of *L. lepadogaster* were genetically similar along 300 km of coastline and consequently highly connected. Seven out of eight populations from 2012 were placed in one single genetic cluster and genetic diversity was very similar among the 2012 adult populations. Our biophysical model for the SW Iberian coastline supported these results by confirming that all sites sampled for genetics can be connected by dispersal and that it is possible for *L. lepadogaster* larvae to disperse long distances over hundreds of kilometers.
Despite this high connectivity, the dispersal model revealed higher probabilities for larval retention and exponentially declining probabilities of dispersal with distance. This is in agreement with the significant isolation-by-distance result obtained. The parentage analysis,

a direct method used to estimate connectivity, identified 14 parent-offspring pairs that showed either self-recruitment to ARR or larval dispersal from the nearest sampled populations. From these parent-offspring pairs, 50% of recruits in 2011 and 13% in 2012 self-recruited to ARR.

Both dispersal and retention play an important role in the replenishment of *L. lepadogaster* populations, as shown by our results from both indirect and direct measurements of connectivity, which revealed not only high connectivity and possible long-distance dispersal, but also significant levels of self-recruitment and higher probabilities for short-distance dispersal. These results also support the hypothesis that local recruitment is not incompatible with long-distance dispersal and that they may often occur together in marine species (e.g. Christie *et al.*, 2010, Underwood *et al.*, 2012).

This study further demonstrates that it can be misleading to make assumptions on the degree of population connectivity based solely on a few known early life history traits, such as egg type and PLD. This supports previous studies that showed either higher than expected connectivity (e.g. Galarza *et al.*, 2009) or, on the contrary, small scale genetic differentiation where genetic homogeneity was expected (e.g. Horne *et al.*, 2011). Indeed, some genetic studies have found no relation with either egg type (planktonic vs. demersal) and/or PLD for several reef fish species (e.g. Purcell *et al.*, 2006, Weersing and Toonen, 2009). Although PLD might be a weak predictor of the degree of gene flow in marine systems, PLD estimates might also be very limited, failing to consider their variability within and among populations (e.g. Wellington and Victor, 1992, Bay *et al.*, 2006).

Larvae of *L. lepadogaster* have a low critical swimming speed and it is hypothesized that strong swimming abilities are not needed since they can be retained nearshore on the benthic layer where currents are expected to be weaker due to bottom friction (Faria and Gonçalves, 2010). However, nearshore currents in the study site can be much faster than the registered critical swimming speed (unpublished data). Generally, hydrodynamics in the nearshore area are complex due to the effects of wind, tides, buoyancy, waves and nearshore currents predominantly flowing parallel to shore can be shifted to nearshore and offshore water exchanges (reviewed in Gawarkiewicz *et al.*, 2007). Thus specific coastal hydrodynamic conditions could likely allow a significant number of *L. lepadogaster* larvae to disperse and eventually reach other populations, contributing to a flattening of genetic differences among

them. This assumption meets the 2nd functional dispersal group described in Shanks (2009) for which longer than "normal" dispersal distances were estimated from genetics, although species of this group have a short PLD, and whose larvae stay close to the bottom to avoid advection. To sustain genetic homogeneity among populations, only a few individuals per generation are needed to be exchanged (Slatkin, 1993), whereas to maintain ecologically viable populations through time a supply of self-recruitment is often necessary (Jones *et al.*, 2005).

The parentage analysis revealed that three and four recruits were assigned to the same parent, respectively. This might indicate that *Lepadogaster* larvae that belonged to the same egg clutch and likely hatched at the same time disperse together in a larval pool and likely settle to the same area. Evidence of such cohesive dispersal has been found before for coral reef fishes (Ben-Tzvi *et al.*, 2012, Bernardi *et al.*, 2012).

5.2.5.2 Temporal scale

All analyses in this study revealed strong temporal genetic divergence, with adult gene pools having significantly different compositions between years. This annual difference was even larger than the spatial genetic difference between the two most distant sites (MAR and BAR), which were separated by 700 km of coastline. Moreover, the spatial genetic divergence was also different between years. Although in 2012 all populations (excluding the most southern one: BAR) had a very similar genetic composition, in 2011 the nearby populations of LIS and ARR were more differentiated from each other.

Few genetic studies on reef fish include temporal comparisons and those that do, mostly showed no inter-annual differences (Gerlach *et al.*, 2007, Jones *et al.*, 2010, Félix-Hackradt *et al.*, 2013), except for (Hogan *et al.*, 2010, 2011). Our results do not suggest that these differences are caused by either a bottle neck or sweepstake effect (i.e. only a subset of the adult population successfully contributing to subsequent recruitment) since the allele diversity of the recruits was similar to that of the adults (Hedgecock, 1994, Beldade *et al.*, 2012). Also, we do not attribute this inter-annual difference to the effect of small sample sizes as the estimated proportion of the population sampled at LIS was very large (41.2% and 54.9% in 2011 and 2012, respectively).

We propose two possible hypotheses to explain this strong inter-annual difference in the adult gene pools: i) oceanographic transport; and ii) density dependent recruitment influenced by storms. The first could be due to differences in coastal currents during the spawning season of *L. lepadogaster*, which could lead to larval pools arriving to adult habitats from different source populations in different years (Hedgecock, 1994, Selkoe *et al.*, 2006, Siegel *et al.*, 2008). For example, in 2011 parents other than those originating from ARR were identified by parentage analysis to belong to the populations of SIN and ALM, whereas in 2012 most parents were from LIS. These inter-annual differences match significant differences in the genetic composition of recruits sampled in ARR between 2011 and 2012. This result was consistent in all population genetic analysis and also in the dispersal model, which showed differences in the origin of recruits settling to ARR between those two years.

During late spring and summer months, in addition to the predominant upwelling derived southward currents, inner-shelf northward counter-currents transporting warm water can occur from the South of Portugal until AMP (Relvas *et al.*, 2007). These could lead to northward transport of larvae and could explain the assignment of recruits sampled in ARR as originating from SIN and ALM and even to the most southern population BAR (assignment test). Connectivity matrices retrieved from our dispersal model showed that successful settlers would mostly arrive from the next southern population, but inter-annual differences in dispersal patterns were observed when comparing 2010, 2011 and 2012 connectivity matrices individually. In 2010 and 2011 more particles were advected northwards, whereas in 2012 more southward dispersal occurred compared to both previous years. When focusing just on the probability of larvae settling to ARR, the observed year-to-year differences suggest that variable current patterns could cause the variation of genetic composition in recruit populations. Similarly, two successive studies of (Hogan *et al.*, 2010), Hogan *et al.* (2011) found that genetic structure and connectivity among adult populations of a Caribbean reef fish changed over time due to yearly differences in local retention and dispersal patterns. Inter-annual differences in local retention and self-recruitment were also reported for a coral reef fish in the Red Sea by Nanninga *et al.* (2015).

The second hypothesis to explain the observed temporal differences could be an effect of storms combined with density dependent recruitment success. *L. lepadogaster* occurs in discrete patchy habitats (Gonçalves *et al.*, 1998) in which populations are expected to be

relatively stable over time as older fish might occupy most of the restricted space, while recruits might have a low probability of successful settlement because of density dependence. This stability, however, could collapse if a significant number of old fish die and habitat is made available for new recruits to settle and influence the population. The LIS adult individuals sampled in 2012 were significantly smaller than in 2011, suggesting that the population in 2012 consisted of younger fish. But, no differences in size were observed for the ARR adult populations. Mortality of a large proportion of the adult population could be due to the end of the natural life span or also due to winter storm events including big waves resulting in strong habitat disturbance. Indeed, at the end of October and beginning of November 2011, extreme storms occurred and wave heights of 4.5 to 5.5 m were obtained from a numerical model for the area near LIS and ARR (WANA model, Puerto del Estado Spain ; Figure S5.2.6). A storm event causing high mortality of *L. lepadogaster* fish could thus be an explanation for the decrease of allelic diversity in the adult populations from 2011 to 2012. This decline was much higher in Lisbon, which is more exposed to swells, compared to the protected reef in ARR.

Several studies have found a strong relation between population connectivity and oceanographic features, such as coastal and ocean circulations, eddies and shifts of predominant currents (e.g. White *et al.*, 2010, Schunter *et al.*, 2011). The simulations performed here for SW Iberia described oceanic advection with detail, allowing further inferences for dispersal and retention levels beyond populations sampled for genetics. Results of the dispersal model were consistent with the genetic results (White *et al.*, 2010, Schunter *et al.*, 2011). However, a limitation of most biophysical models lies in representing correctly nearshore hydrodynamics (Leis *et al.*, 2011). Future studies on reef fish ecology directed at analyzing fine-scale current conditions in the reef and looking at alongshore currents nearshore are needed to help clarify the above hypothesis.

Furthermore, in a future study a higher number of adult fish should be sampled, since it might enhance the number of parent-offspring pairs revealed by the parentage-analysis (Saenz-Agudelo *et al.*, 2009) as well as the accuracy of the allele frequencies in the populations. Compared to the parentage-analysis, the assignment test detected only very few dispersal trajectories, thus it might lack power to assign recruits correctly when used in a high gene flow scenario despite having highly polymorphic loci (~10; e.g. Saenz-Agudelo *et al.*, 2009, Christie *et al.*, 2010, Lowe and Allendorf, 2010).

Overall, the genetic population structure, supported by the dispersal model, indicate significant gene flow due to long-distance larval dispersal that would lead to a homogenization of allele frequencies among sites over an evolutionary time scale. However, the occurrence of temporal genetic divergence in adult and recruit populations suggests that *L. lepadogaster* populations might be strongly affected by short-term events such as storms and that populations are highly influenced by the larval pool composition recruiting to the adult habitats. These larval pools would need to be a mixture of fish originating from a variety of source populations to allow for a decrease in the spatial genetic structure within years. But larval pool compositions might differ inter-annually, likely due to stochastic processes such as highly variable alongshore currents, to establish adult populations in the proximate year that differ in their gene pool compared to the previous year.

The *L. lepadogaster* population of ARR is not as closed as expected; not only does it depend on the supply of recruits from other populations but it can also provide other sites with larvae, thus functioning simultaneously as a source and sink. A long-term analysis of adults and recruits from all populations over several years would be ideal to follow the temporal dynamics of these source-sink patterns, something that cannot be revealed in studies focused on a single sampling event. The results of this study show that these temporal scales are of great importance to understand connectivity patterns in coastal reef fish species, thereby providing important information for the design of a network of MPAs.

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5.2.8 Supporting information

S5.2.1 Summary statistics

Table S5.2.1. Summary statistics for adult and recruit samples of *Lepadogaster lepadogaster* collected along the Portuguese west coast. Shown are expected and observed heterozygosities (HE and HO), heterozygote deficiency (FIS) and p-values of FIS for each population and loci.

	Lp3	Lp4	Lp9	Lp11	Lp13	Lp14	Lp15	Lp17	Lp20	Lp21	Lp23	Lp24
MAR_A_12												
N	38	39	39	39	39	39	39	39	39	39	38	39
H_E	0.773	0.686	0.753	0.757	0.688	0.917	0.519	0.817	0.812	0.678	0.641	0.941
H_O	0.684	0.744	0.821	0.769	0.410	0.872	0.564	0.949	0.692	0.615	0.711	0.744
F_{IS}	0.117	-0.085	-0.091	-0.017	0.407	0.050	-0.089	-0.164	0.149	0.093	-0.111	0.212
p-value (F_{IS})	0.057	0.151	0.105	0.364	0.000	0.085	0.136	0.003	0.014	0.107	0.078	0.000
PEN_A_12												
N	39	39	39	39	39	39	39	39	39	39	39	39
H_E	0.753	0.719	0.785	0.710	0.652	0.880	0.526	0.800	0.801	0.776	0.541	0.953
H_O	0.744	0.769	0.718	0.769	0.410	0.718	0.487	0.795	0.744	0.641	0.513	0.846
F_{IS}	0.013	-0.070	0.086	-0.085	0.374	0.186	0.075	0.006	0.072	0.175	0.052	0.113
p-value (F_{IS})	0.347	0.181	0.110	0.137	0.000	0.000	0.185	0.377	0.119	0.010	0.239	0.002
LIS_A_12												
N	40	40	40	40	40	39	40	40	40	40	40	39
H_E	0.752	0.722	0.765	0.714	0.682	0.873	0.456	0.783	0.785	0.658	0.550	0.933
H_O	0.750	0.600	0.575	0.725	0.375	0.821	0.450	0.725	0.725	0.525	0.500	0.769
F_{IS}	0.003	0.171	0.250	-0.016	0.454	0.061	0.013	0.075	0.078	0.204	0.091	0.177
p-value (F_{IS})	0.401	0.027	0.001	0.361	0.000	0.088	0.350	0.125	0.115	0.006	0.137	0.000
ARR_A_12												
N	40	40	38	40	40	39	40	40	39	40	39	39
H_E	0.768	0.709	0.780	0.738	0.661	0.859	0.440	0.837	0.816	0.722	0.685	0.937
H_O	0.750	0.650	0.737	0.675	0.425	0.692	0.450	0.825	0.615	0.575	0.641	0.692
F_{IS}	0.023	0.084	0.056	0.086	0.360	0.196	-0.023	0.015	0.248	0.206	0.065	0.264
p-value (F_{IS})	0.309	0.157	0.183	0.111	0.000	0.000	0.321	0.319	0.000	0.005	0.199	0.000
SIN_A_12												
N	40	40	39	40	40	40	39	40	40	40	40	40
H_E	0.772	0.697	0.835	0.769	0.647	0.832	0.367	0.798	0.852	0.730	0.703	0.941
H_O	0.750	0.675	0.897	0.775	0.525	0.825	0.385	0.725	0.825	0.475	0.675	0.850
F_{IS}	0.029	0.031	-0.076	-0.008	0.191	0.009	-0.050	0.092	0.032	0.352	0.041	0.097
p-value (F_{IS})	0.277	0.299	0.087	0.394	0.023	0.323	0.255	0.088	0.226	0.000	0.263	0.005
ALM_A_12												
N	24	24	24	24	24	24	24	24	24	24	24	24
H_E	0.796	0.717	0.786	0.606	0.747	0.818	0.473	0.833	0.794	0.812	0.642	0.941
H_O	0.667	0.792	0.833	0.708	0.500	0.833	0.375	0.958	0.708	0.333	0.708	0.833
F_{IS}	0.166	-0.106	-0.062	-0.174	0.335	-0.019	0.211	-0.154	0.110	0.595	-0.106	0.116
p-value (F_{IS})	0.034	0.140	0.204	0.046	0.001	0.292	0.062	0.011	0.092	0.000	0.139	0.007
BAR_A_12												
N	35	36	35	36	35	36	36	35	29	33	36	36
H_E	0.740	0.708	0.791	0.701	0.677	0.871	0.471	0.836	0.750	0.728	0.757	0.914
H_O	0.714	0.750	0.829	0.611	0.400	0.722	0.417	0.800	0.793	0.606	0.722	0.833
F_{IS}	0.035	-0.060	-0.049	0.129	0.413	0.173	0.117	0.044	-0.059	0.170	0.046	0.090
p-value (F_{IS})	0.268	0.129	0.218	0.051	0.000	0.001	0.099	0.191	0.165	0.020	0.214	0.024
LIS_A_11												
N	30	30	30	29	28	27	30	29	30	30	30	30
H_E	0.725	0.783	0.862	0.807	0.848	0.934	0.666	0.868	0.815	0.840	0.630	0.970
H_O	0.700	0.700	0.833	0.724	0.607	0.815	0.667	0.793	0.867	0.700	0.533	0.900
F_{IS}	0.035	0.107	0.034	0.104	0.288	0.130	-0.001	0.087	-0.064	0.169	0.156	0.073
p-value (F_{IS})	0.266	0.090	0.219	0.082	0.000	0.005	0.418	0.073	0.147	0.009	0.063	0.011
ARR_A_11												
N	29	29	29	29	28	23	29	29	29	29	29	29
H_E	0.790	0.707	0.768	0.710	0.785	0.862	0.641	0.837	0.803	0.742	0.575	0.955
H_O	0.793	0.655	0.724	0.655	0.464	0.652	0.690	0.793	0.690	0.655	0.586	0.621
F_{IS}	-0.004	0.075	0.058	0.078	0.413	0.247	-0.077	0.053	0.143	0.119	-0.019	0.354
p-value (F_{IS})	0.413	0.195	0.204	0.162	0.000	0.000	0.168	0.183	0.030	0.058	0.355	0.000
ARR_R_12												
N	40	40	39	40	37	40	40	39	39	40	40	40
H_E	0.798	0.722	0.792	0.685	0.672	0.846	0.480	0.819	0.828	0.763	0.369	0.952
H_O	0.850	0.650	0.641	0.600	0.378	0.700	0.425	0.641	0.590	0.500	0.350	0.425
F_{IS}	-0.066	0.101	0.193	0.126	0.441	0.174	0.116	0.219	0.291	0.348	0.052	0.557
p-value (F_{IS})	0.136	0.103	0.005	0.063	0.000	0.001	0.123	0.002	0.000	0.000	0.186	0.000
ARR_R_11												
N	39	39	39	39	33	32	38	39	38	38	39	38
H_E	0.773	0.686	0.793	0.722	0.787	0.780	0.651	0.764	0.811	0.528	0.550	0.963
H_O	0.615	0.539	0.718	0.487	0.788	0.719	0.711	0.718	0.605	0.447	0.359	0.737
F_{IS}	0.206	0.218	0.096	0.328	-0.001	0.079	-0.092	0.061	0.256	0.155	0.350	0.237
p-value (F_{IS})	0.006	0.013	0.080	0.000	0.422	0.096	0.144	0.162	0.000	0.038	0.001	0.000

S5.2.2 FreeNA results - checking for null alleles

Table S5.2.2A. Estimated null allele frequency for sampled populations (first row) at each locus (first column).

Pop / Loci	Mar_A12	Pen_A12	Lis_A12	Arr_A12	Sin_A12	Alm_A12	Bar_A12	Lis_A11	Arr_A11	Arr_R12	Arr_R11
Lp3	0.031	0.000	0.001	0.000	0.000	0.048	0.000	0.010	0.000	0.000	0.086
Lp4	0.000	0.000	0.051	0.028	0.000	0.000	0.000	0.073	0.006	0.050	0.077
Lp9	0.000	0.024	0.093	0.034	0.000	0.007	0.000	0.000	0.057	0.080	0.055
Lp11	0.000	0.023	0.000	0.043	0.000	0.000	0.031	0.036	0.039	0.063	0.141
Lp13	0.155	0.140	0.176	0.127	0.072	0.122	0.169	0.121	0.162	0.176	0.022
Lp14	0.036	0.089	0.035	0.075	0.000	0.000	0.089	0.051	0.091	0.076	0.000
Lp15	0.000	0.005	0.000	0.000	0.000	0.063	0.000	0.033	0.000	0.076	0.047
Lp17	0.003	0.000	0.006	0.018	0.020	0.000	0.023	0.041	0.008	0.099	0.017
Lp20	0.073	0.008	0.025	0.095	0.000	0.029	0.000	0.000	0.075	0.121	0.100
Lp21	0.061	0.080	0.073	0.098	0.153	**0.264**	0.058	0.058	0.028	0.158	0.028
Lp23	0.000	0.009	0.044	0.023	0.010	0.000	0.000	0.067	0.000	0.014	0.123
Lp24	0.092	0.054	0.070	0.122	0.047	0.042	0.037	0.025	0.169	**0.268**	0.111

Legend
0.2< x
0.1 < x < 0.2
0.05 < x< 0.1

Table S5.2.2B. Comparison of global F_{ST} per locus derived from raw microsatellite data and after applying the excluding null alleles (ENA) correction method from (Chapuis and Estoup, 2007).

Locus	F_{ST} w/o ENA	F_{ST} w ENA
Lp3	-0.003	-0.002
Lp4	0.001	0.001
Lp9	0.007	0.007
Lp11	0.052	0.05
Lp13	0.057	0.041
Lp14	0.011	0.012
Lp15	0.099	0.099
Lp17	0.005	0.005
Lp20	0.007	0.008
Lp21	0.033	0.034
Lp23	0.026	0.025
Lp24	0.003	0.005
All loci	0.023	0.022

Table S5.2.2C. Comparison of pairwise F_{ST} derived from raw microsatellite data and after applying the excluding null alleles (ENA) correction method from (Chapuis and Estoup, 2007).

without ENA

	Mar_A12	Pen_A12	Lis_A12	Arr_A12	Sin_A12	Alm_A12	Bar_A12	Lis_A11	Arr_A11	Arr_R12
Pen_A12	0.001									
Lis_A12	-0.003	0								
Arr_A12	0.002	0.006	0.004							
Sin_A12	0.006	0.009	0.006	0.004						
Alm_A12	0.007	0.008	0.003	-0.002	0.009					
Bar_A12	0.03	0.033	0.029	0.023	0.025	0.032				
Lis_A11	0.037	0.037	0.039	0.038	0.045	0.035	0.055			
Arr_A11	0.049	0.047	0.046	0.052	0.053	0.047	0.068	0.022		
Arr_R12	0.006	0.003	0.003	0.005	0.012	0.004	0.037	0.046	0.052	
Arr_R11	0.023	0.024	0.018	0.023	0.024	0.027	0.041	0.037	0.011	0.022

with ENA

	Mar_A12	Pen_A12	Lis_A12	Arr_A12	Sin_A12	Alm_A12	Bar_A12	Lis_A11	Arr_A11	Arr_R12
Pen_A12	0.002									
Lis_A12	-0.003	-0.001								
Arr_A12	0.002	0.006	0.004							
Sin_A12	0.007	0.009	0.006	0.003						
Alm_A12	0.009	0.008	0.005	0	0.009					
Bar_A12	0.03	0.033	0.029	0.022	0.025	0.033				
Lis_A11	0.032	0.032	0.032	0.032	0.039	0.03	0.049			
Arr_A11	0.043	0.042	0.04	0.048	0.05	0.042	0.063	0.021		
Arr_R12	0.008	0.005	0.005	0.008	0.013	0.006	0.039	0.039	0.047	
Arr_R11	0.022	0.024	0.018	0.022	0.024	0.028	0.039	0.034	0.013	0.024

S5.2.3 Discriminant analysis of Principal Components (DAPC)

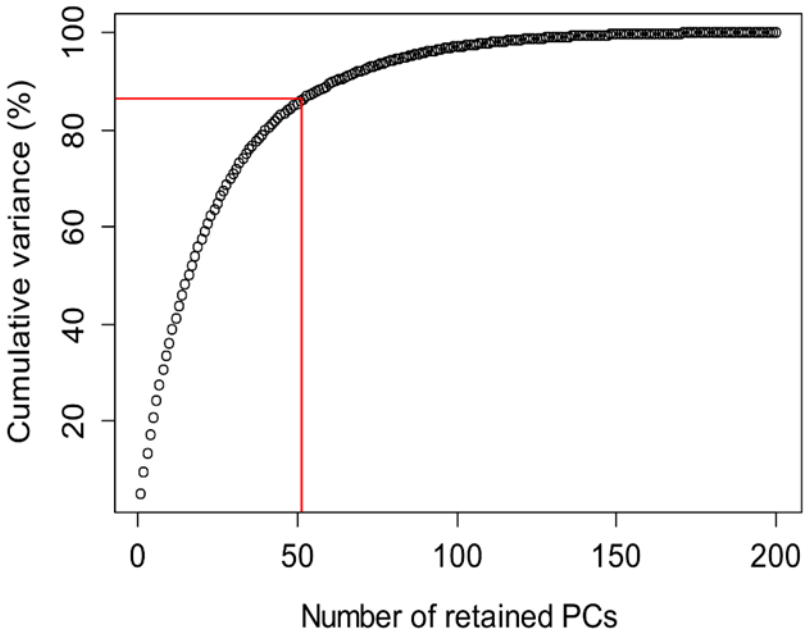

Figure S5.2.3A. Principal Component Analysis of the DAPC; red line indicates number of PCs retained (53) for the DAPC and the genetic information comprised by this number.

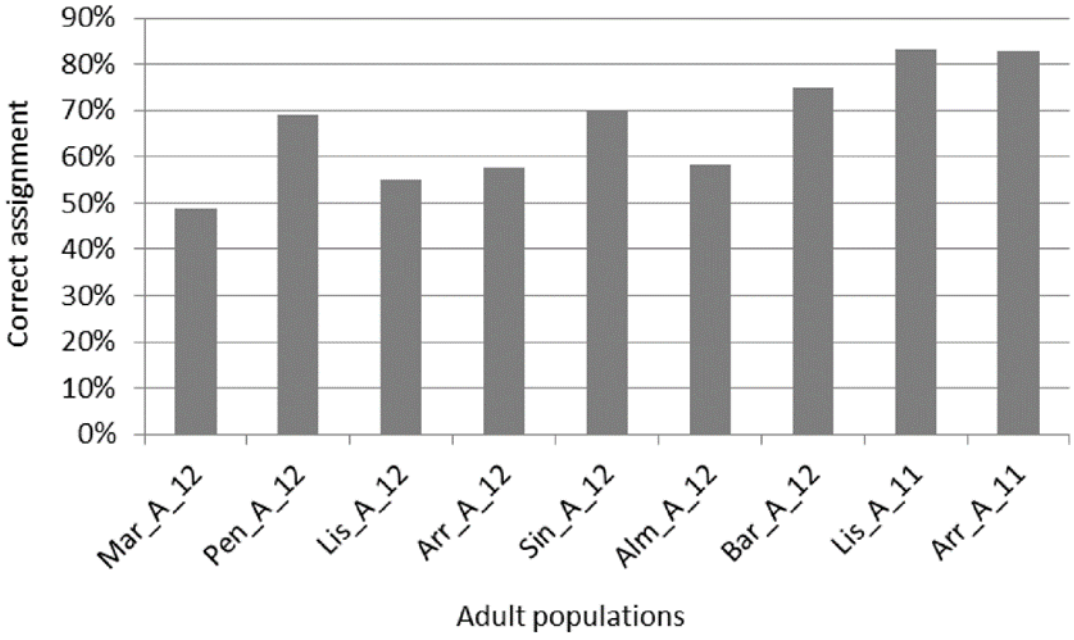

Figure S5.2.3B. Correct assignment of adult fish to their geographical population in 2012 and 2011, with recruits being excluded from the DAPC.

S5.2.4 Isolation by distance analysis

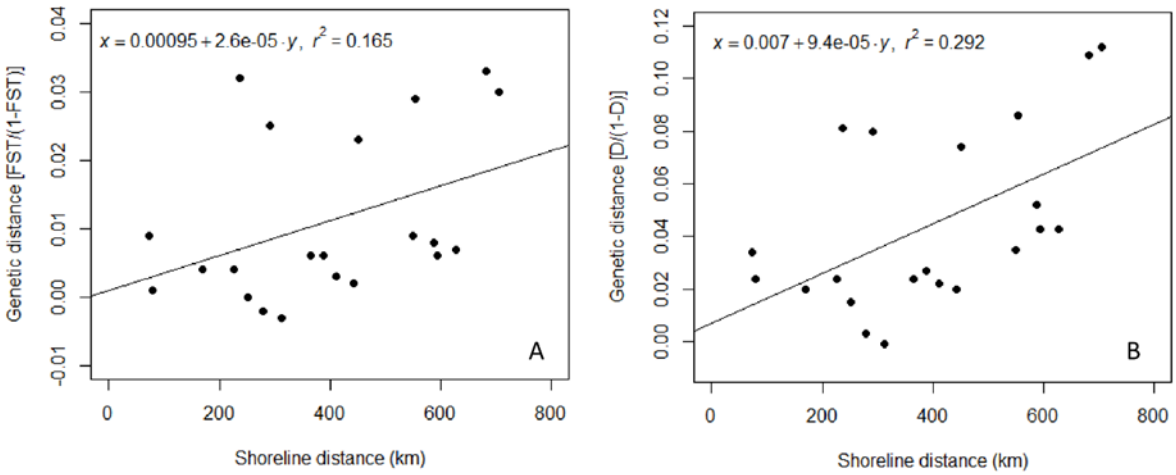

Figure S5.2.4. Linear regression analysis of shoreline distance with genetic distance, estimated from pairwise F_{ST} (A) and Jost's D values (B).

S5.2.5 Dispersal Model

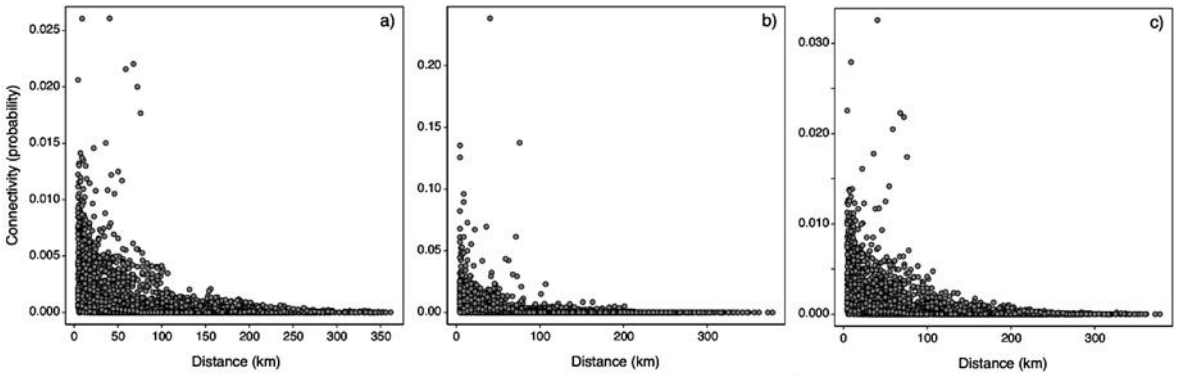

Figure S5.2.5A. Coastline distance related to the probability of connectivity averaged over simulations ran from 2002 – 2012 per particle type surface (a), deep (b) and migration (c); notice different scales of y-axis.

Table S5.2.5A. Paired probabilities of connectivity among genetic sample sites for particles of type deep, migration and surface, averaged over simulations ran from 2002 - 2012 and yearly averages for 2010, 2011 and 2012. Shaded rows indicate retention probabilities.

		Deep				Migration				Surface			
FROM	TO	AVG	2010	2011	2012	AVG	2010	2011	2012	AVG	2010	2011	2012
MAR	MAR	0.0415	0.0380	0.0815	0.0505	0.0125	0.0142	0.0116	0.0194	0.0127	0.0122	0.0098	0.0176
PEN	MAR	0.0129	0.0048	0.0207	0.0110	0.0028	0.0018	0.0021	0.0008	0.0018	0.0020	0.0024	0.0008
LIS	MAR	0.0003	0.0004	0.0000	0.0000	0.0005	0.0000	0.0009	0.0000	0.0003	0.0000	0.0001	0.0000
ARR	MAR	0.0011	0.0003	0.0010	0.0000	0.0001	0.0000	0.0001	0.0000	0.0001	0.0000	0.0001	0.0000
SIN	MAR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ALM	MAR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
BAR	MAR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MAR	PEN	0.0006	0.0004	0.0001	0.0007	0.0022	0.0017	0.0015	0.0015	0.0026	0.0020	0.0010	0.0021
PEN	PEN	0.0144	0.0140	0.0167	0.0139	0.0133	0.0118	0.0097	0.0159	0.0145	0.0120	0.0095	0.0159
LIS	PEN	0.0003	0.0004	0.0000	0.0000	0.0003	0.0008	0.0006	0.0002	0.0003	0.0006	0.0001	0.0000
ARR	PEN	0.0017	0.0030	0.0012	0.0005	0.0004	0.0009	0.0003	0.0000	0.0003	0.0006	0.0003	0.0000
SIN	PEN	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000	0.0003	0.0000	0.0000
ALM	PEN	0.0001	0.0013	0.0000	0.0000	0.0001	0.0006	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000
BAR	PEN	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MAR	LIS	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PEN	LIS	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
LIS	LIS	0.0804	0.0670	0.1366	0.0919	0.0108	0.0085	0.0066	0.0070	0.0105	0.0076	0.0075	0.0075
ARR	LIS	0.0033	0.0018	0.0087	0.0019	0.0010	0.0006	0.0003	0.0000	0.0010	0.0006	0.0002	0.0001
SIN	LIS	0.0000	0.0000	0.0000	0.0000	0.0001	0.0004	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000
ALM	LIS	0.0001	0.0000	0.0000	0.0000	0.0001	0.0003	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000
BAR	LIS	0.0001	0.0001	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MAR	ARR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PEN	ARR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0001	0.0000	0.0000	0.0003
LIS	ARR	0.0003	0.0001	0.0008	0.0000	0.0016	0.0012	0.0014	0.0015	0.0021	0.0012	0.0009	0.0048
ARR	ARR	0.0213	0.0157	0.0205	0.0297	0.0208	0.0158	0.0176	0.0211	0.0209	0.0131	0.0150	0.0189
SIN	ARR	0.0037	0.0051	0.0024	0.0008	0.0050	0.0056	0.0061	0.0062	0.0034	0.0012	0.0031	0.0045
ALM	ARR	0.0113	0.0040	0.0078	0.0135	0.0050	0.0056	0.0056	0.0026	0.0033	0.0026	0.0020	0.0022
BAR	ARR	0.0069	0.0103	0.0080	0.0009	0.0006	0.0010	0.0003	0.0000	0.0004	0.0001	0.0006	0.0000
MAR	SIN	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PEN	SIN	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
LIS	SIN	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ARR	SIN	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
SIN	SIN	0.0623	0.0460	0.1308	0.0315	0.0182	0.0133	0.0225	0.0182	0.0162	0.0126	0.0230	0.0170
ALM	SIN	0.0297	0.0200	0.0753	0.0081	0.0072	0.0054	0.0040	0.0061	0.0061	0.0058	0.0040	0.0049
BAR	SIN	0.0015	0.0007	0.0005	0.0059	0.0002	0.0001	0.0000	0.0000	0.0001	0.0003	0.0000	0.0000
MAR	ALM	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PEN	ALM	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
LIS	ALM	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
ARR	ALM	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000	0.0001
SIN	ALM	0.0018	0.0011	0.0019	0.0021	0.0026	0.0040	0.0011	0.0029	0.0023	0.0033	0.0012	0.0028
ALM	ALM	0.0113	0.0090	0.0153	0.0124	0.0061	0.0075	0.0060	0.0054	0.0058	0.0065	0.0074	0.0060
BAR	ALM	0.0023	0.0014	0.0041	0.0023	0.0003	0.0001	0.0007	0.0001	0.0001	0.0002	0.0001	0.0000
MAR	BAR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PEN	BAR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
LIS	BAR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0001	0.0000	0.0000	0.0000
ARR	BAR	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0005	0.0001	0.0000	0.0008
SIN	BAR	0.0000	0.0000	0.0000	0.0000	0.0002	0.0000	0.0000	0.0002	0.0028	0.0010	0.0003	0.0078
ALM	BAR	0.0000	0.0000	0.0000	0.0000	0.0007	0.0002	0.0001	0.0008	0.0034	0.0023	0.0009	0.0071
BAR	BAR	0.0119	0.0175	0.0123	0.0147	0.0044	0.0021	0.0061	0.0069	0.0030	0.0027	0.0049	0.0009

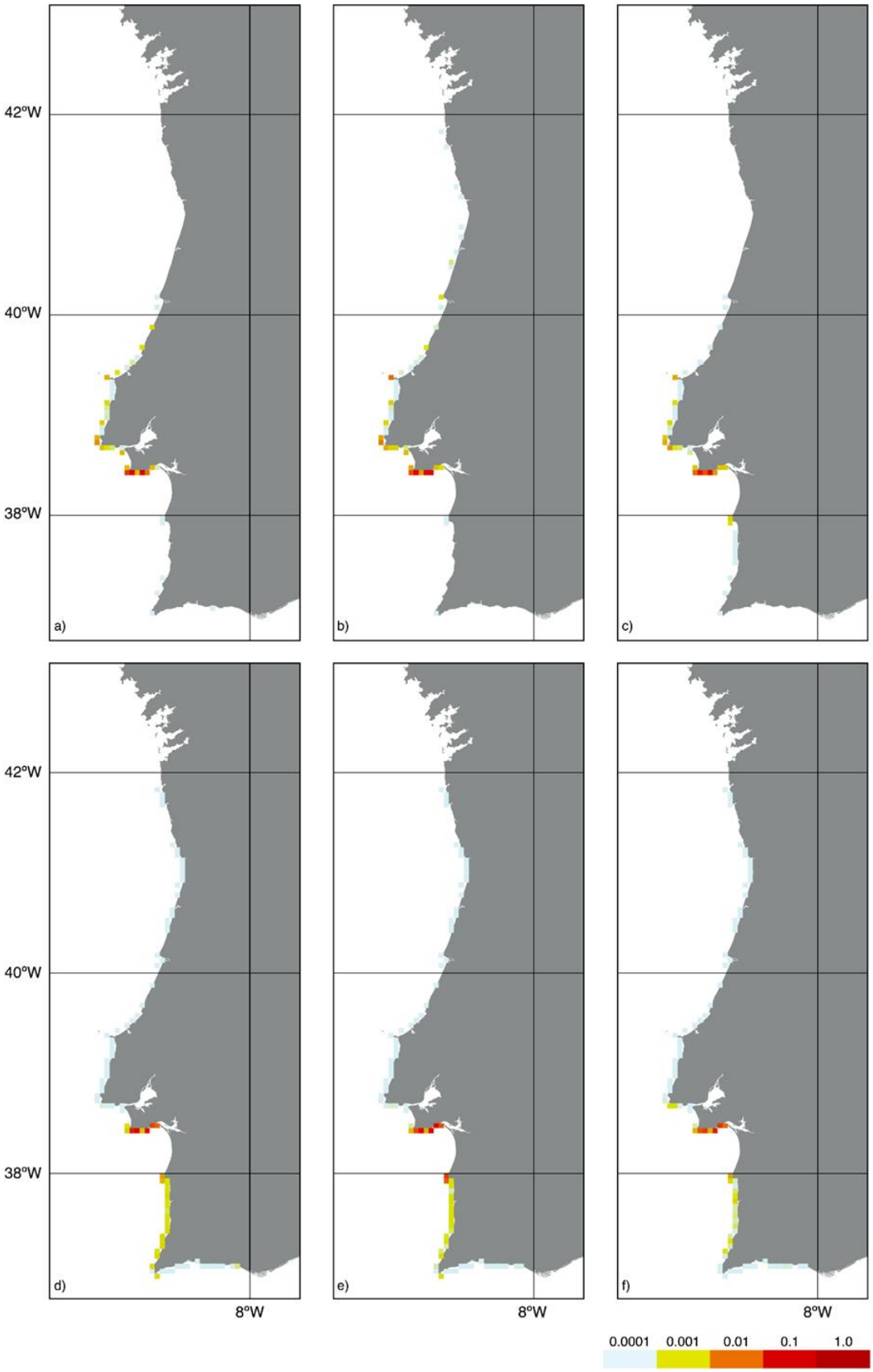

Figure S5.2.5B. Connectivity map with probabilities of where particles settle after being released from the Arrábida Marine Park (framed) in 2010 (a), 2011 (b) and 2012 (c) and probabilities of the origin of particles that settled into the marine park in 2010 (d), 2011 (e) and 2012 (f).

S5.2.6 Wave and Wind patterns in winter 2011/12

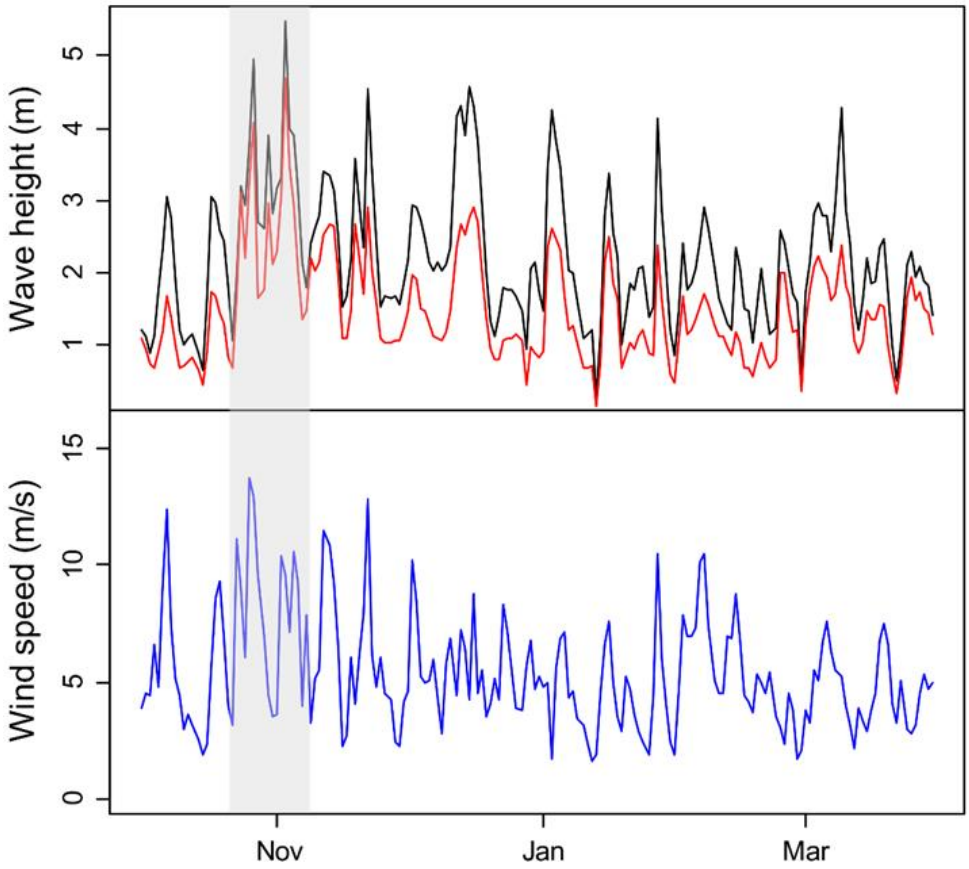

Figure S5.2.6. Pattern of wave height for the area of Lisbon (black line) and Arrábida (red line) and wind speed of Lisbon during winter 2011/12, retrieved from an oceanographic model (Puerto del Estado Spain)(Puerto del Estado Spain)(Puerto del Estado Spain); shaded area indicates a period when very strong storms were registered in Portugal.

Chapter 6
General Discussion

6. General Discussion

In this thesis several aspects of the early life stages of fish occurring near rocky reefs, were analysed and a deeper understanding of the temperate nearshore environment was gained.

6.1 Thesis overview

After assessing the most adequate collection method (Chapter 2, Figure 6.1), recruitment patterns of a temperate nearshore fish assemblage were studied at various spatial and temporal scales, were analysed in relation to oceanographic processes (Chapters 3 and 4), and early life history traits (ELHT) were also studied (Chapter 4.1). Secondly, population connectivity and larval dispersal of a common reef fish were investigated by using a genetic and biophysical modelling approach (Chapter 5, Figure 6.1). Key findings are described and discussed below.

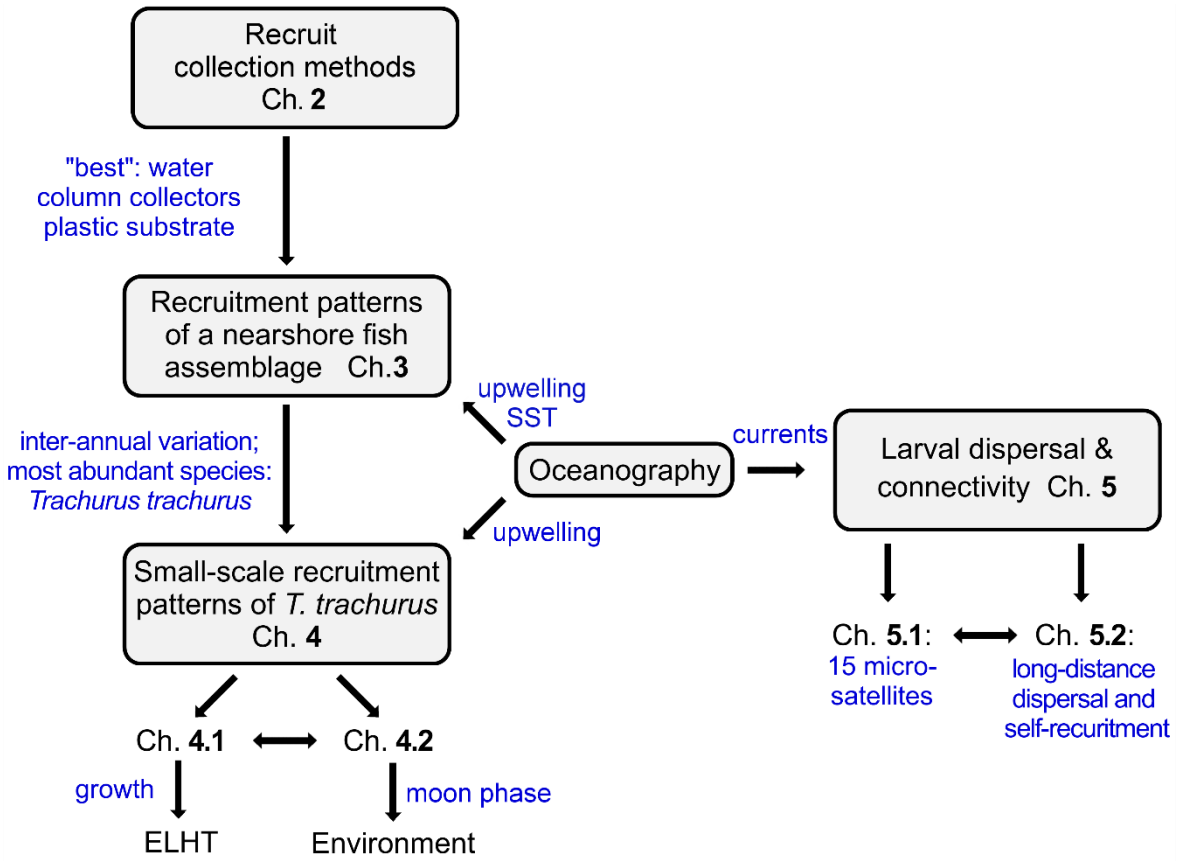

Fig. 6.1 Scheme of the thesis structure with main findings in blue.

Standardized collectors filled with plastic substrate (SMURFs) installed in the water column (as in Ammann, 2004, Caselle *et al.*, 2010a), stood out from artificial substrate collectors deployed at the sea floor (Vallès *et al.*, 2006), mainly due to easier handling and replicability (Chapter 2). In contrast to previous studies that used SMURFs, our study registered the recruitment of a pelagic fish species, the Atlantic horse mackerel (*Trachurus trachurus*). And this enabled the first investigation of early life stages of this highly important commercial species (Chapters 2-4), showing for the first time the adequacy of these standardized collectors to sample recruits of pelagic fishes as well.

Apart from this species, all the other fish collected were cryptobenthic and benthopelagic, most from species commonly found in the Arrábida Marine Park (AMP) (Gonçalves *et al.*, 2003, Beldade *et al.*, 2006b). Yet, three species that were abundant in this study were rare or variable in previous adult fish surveys (Gonçalves *et al.*, 2003, Horta e Costa, unpublished data, Beldade *et al.*, 2006b). Curiously, the cryptobenthic species *Apletodon pellegrini* and *A. incognitus* of the family Gobiesocidae (clingfish) have not been recorded in the AMP before (Gonçalves *et al.*, 2002). Both species are very alike to the common *A. dentatus*, suggesting that in previous underwater surveys these three species were not distinguishable. A second explanation could be that these two clingfish have expanded their distribution range and started to occur in the AMP after the last cryptobenthic fish survey in 2003 (Beldade *et al.*, 2006b). *A. pellegrini* has been recorded to occur in the eastern Atlantic from Madeira to South Africa (Hofrichter, 1995, Hofrichter and Patzner, 1997) and *A. incognitus* in the Mediterranean Sea and Azores Islands (Hofrichter and Patzner, 1997, Bilecenoglu and Kaya, 2006).

Thus, the occurrence of these species at the AMP could reflect a northward extension of their distribution, supporting the suggested tropicalization hypothesis of the AMP fish assemblage proposed by Horta e Costa *et al.* (2014). In that study, a predictive model developed from a 12 year time series of the fish assemblage structure and a set of most related local oceanographic variables, found that over the last 50 years species with tropical affinities have increased in frequency compared to cold-temperate species at AMP. And this trend coincided with an increase in mean winter SST (Horta e Costa *et al.*, 2014).

On the other hand, species reported as common in the area and that had been frequently sampled in previous larval surveys (Beldade *et al.*, 2006a, Borges *et al.*, 2007), were not

recorded in our recruit sampling. This could reflect high larval mortality until settlement. But the total absence of some species common in the area might be due to (i) a high selectivity of SMURFs because of the species specific habitat choice at settlement and (ii) a too long distance between collectors and the reef (~100 m) for species that might retain near the reef during their larval development. Studying the natural settlement habitat and testing SMURFs at different distances to the reef might provide a better understanding of the selectivity of these collectors.

Nevertheless, some species were collected regularly in this study, showing the practicality of these standardized collectors for simultaneously monitoring recruitment at different locations (Chapters 2 and 3). Recruitment patterns at various temporal and spatial scales were detected. These patterns were related to different biotic and abiotic factors depending on the scale, as it has been found in previous studies. Here, recruitment varied weekly within a recruitment season (Chapters 2 and 4) and also between the three years studied (Chapter 3). The inter-annual variation in average recruitment per collector was very high for all taxa, with differences among years (excluding sampling periods of zero catch) spanning 1 to 2 orders of magnitude for reef fish species and even 3 orders of magnitude for the pelagic-neritic species Atlantic horse mackerel (Chapter 3). Such variation has been observed also in other reef fish recruitment studies (McFarland *et al.*, 1985, Sponaugle and Pinkard, 2004, Vallès *et al.*, 2008). The temporal patterns found were different between taxa, which agrees with previous studies that investigated several species (Chapter 3; Sponaugle *et al.*, 2012b, Félix-Hackradt *et al.*, 2013).

Regarding the effect of biotic factors on recruitment, we found that higher larval growth was related to higher recruitment intensity (Chapter 4), assuming that higher larval growth lead to higher survival of larvae. This concurs with the "growth-mortality hypothesis" (Houde, 1987, Anderson, 1988) that looks at the relationship of larval condition and recruitment success and which has been supported before by many recruitment studies (e.g. Hare and Cowen, 1997, Searcy and Sponaugle, 2001, Vigliola and Meekan, 2002, Johnson, 2008, Samhouri *et al.*, 2009, Johnson *et al.*, 2012).

Larval growth was higher during absent and weak upwelling, when water temperature was higher and prey abundance lower (Chla used as a proxy; Chapter 4). This suggests that temperature was more important for larval growth than prey abundance. Very similar

findings were revealed for a reef fish species by Meekan *et al.* (2003); and there are other studies that have found as well an impact of oceanographic factors on larval conditions (McCormick and Molony, 1995, Meekan *et al.*, 2003, Takahashi and Watanabe, 2004, Sponaugle *et al.*, 2006). Larval growth and size can also be affected by biotic factors such as maternal and paternal conditions (Green and McCormick, 2005, Raventos and Planes, 2008).

In this thesis, the relationships found between temporal recruitment patterns and environmental variables were species-specific and dependent on the temporal scale studied (Chapters 3 and 4). At a small scale (weekly; Chapter 4) *T. trachurus* recruitment correlated with the lunar cycle, similarly to what has been described for tropical reef fish (Robertson, 1992, Lozano and Zapata, 2003, Ben-David and Kritzer, 2005). A recruitment peak during new moon as found here (Chapter 4), has been reported by other authors (Doherty, 1991, Lozano and Zapata, 2003). An explanation often assumed for this pattern is the avoidance of predation risk related to nocturnal light conditions (Robertson *et al.*, 1988, Sponaugle and Cowen, 1994). Furthermore, a lunar synchronization of recruitment of Atlantic horse mackerel to the nearshore at the time of metamorphosis could be essential for schooling, a behaviour commonly observed near reefs (pers. observation).

At a small scale (weekly, Chapter 4) and at a larger scale (inter-annual, Chapter 3) it was found that upwelling can have a great influence on recruitment. In temperate coastal environments, the influence of upwelling and/or downwelling processes on the ecology of fish and invertebrates has been deeply studied, in particular on factors such as adapted spawning time, larval survival, and taxon-specific larval transport mechanisms (Bakun, 1996, Santos *et al.*, 2001, Queiroga *et al.*, 2005, Mace and Morgan, 2006). Regarding reef fish recruitment, Caselle *et al.* (2010a) found, in a long-term study using SMURFS, that regional upwelling in summer months correlated positively to settlement of rock fish species. Moreover, other variables associated to the upwelling process such as water current and SST also were correlated with recruitment patterns, indicating that recruitment is affected by several abiotic factors rather than just by the upwelling index alone (Chapter 3 and 4; Caselle *et al.*, 2010a).

Upwelling comes along with a decrease of coastal temperature, increase in offshore advection and a higher production of phyto-and zooplankton (Cunha, 1993, Moita, 2003,

Relvas *et al.*, 2007). Therefore, upwelling can impact recruitment negatively due to higher offshore transport and colder SST, or positively by providing higher food availability. These differences can help explaining why the effect of upwelling on recruitment was different between taxa (Chapter 3). A positive effect of upwelling was found for most species, since highest mean abundances occurred in the year of highest upwelling intensity. However recruitment of the most abundant reef fish species recruiting to SMURFs, *S. hepatus*, was affected negatively by upwelling (Chapter 3).

Indeed, species specific relationships between abiotic variables (lunar- and semi-lunar cycle; wind, water temperature, turbulence, upwelling/downwelling) and recruitment were also found in previous studies (Thorrold *et al.*, 1994, Lozano and Zapata, 2003, Raventos and Macpherson, 2005, Lemberget *et al.*, 2009, Caselle *et al.*, 2010b). Species may differ in their relationship with abiotic factors due to differences in life history traits and in orientation and swimming abilities.

The analysis of fine scale recruitment patterns in 2011, revealed that recruitment of Atlantic horse mackerel was as well negatively affected by upwelling. However, the annual comparison of recruitment in chapter 3 suggests that Atlantic horse mackerel requires medium upwelling conditions for successful recruitment, since here recruitment was low in the year of lowest upwelling intensity. This can indicate that the relationship between upwelling and larval survival follows the optimal environmental window concept (a dome-shaped curve) that was established for small pelagic fish (clupeoids) in wind-driven upwelling systems (Cury and Roy, 1989, Roy *et al.*, 1992). In general, based on previous findings, this theory defends that recruitment success is highest when upwelling and/or wind intensity is moderate. But specific relationships dependent on the species and the area considered have been found (Cury and Roy, 1989, Roy *et al.*, 1992, Shin *et al.*, 1998).

Recruitment patterns in the AMP are affected by abiotic variables and this influences the composition on the adult's reef fish assemblage (Henriques *et al.*, 2007, Horta e Costa *et al.*, 2014). However, a two year survey in the AMP by Beldade *et al.* (2006b) revealed that the adult cryptobenthic assemblage was stable over time (11 species out of 29, 38%, were recorded in this thesis) and these authors suggested that the cryptobenthic assemblage is shaped rather by post-settlement processes. Indeed, mortality during settlement and post-settlement phase has been found to be very high (Caselle, 1999, Almany, 2004, e.g. Doherty

et al., 2004, Planes *et al.*, 2009) and hence the effect of recruitment on the adult population might be density-dependent (Jones, 1990). However, post-settlement processes have not been studied yet in this system.

In this thesis, at a small spatial scale of around 13 km, recruitment varied more at a fine scale between collectors within sites than between sampling areas (Chapter 3). The fine scale variation found herein is unlikely due to microhabitat structure, as collectors were around 100 m offshore from the nearest rocky reef. A more plausible reason might be a patchy distribution of pre-settling fish (larval patch hypothesis; Cowen, 1985, Doherty and Williams, 1988) and small-scale hydrodynamics (Breitburg *et al.*, 1995). Other studies that used artificial substrates to monitor recruitment also found little variation over 10s of km (Vallès *et al.*, 2008, Caselle *et al.*, 2010a). However, when studying recruitment in the natural habitat, spatial variation was found to occur at a small scale related to habitat features (e.g. Tolimieri, 1995, Sponaugle *et al.*, 2012b, Félix-Hackradt *et al.*, 2014), whereas oceanographic and stochastic processes and topography affecting the larval influx were believed to cause larger scale spatial patterns (e.g. Caselle and Warner, 1996, Vigliola *et al.*, 1998, Hamilton *et al.*, 2006, Fontes *et al.*, 2009).

The correlations between oceanographic processes and recruitment intensity were probably due to the effect on larval survival and transport to the reef at the end of the pelagic phase. But environmental processes also majorly influence the dispersal of early fish larvae, and in this way affect the genetic structure and connectivity of populations (Chapter 5; White *et al.*, 2010, Schunter *et al.*, 2011). For example, this thesis revealed that population differentiation and patterns of larval dispersal of *Lepadogaster lepadogaster* were highly variable between years and this could be explained by inter-annual variation in alongshore current patterns.

Correlations between population structure and oceanographic processes have been found before regarding coastal and ocean circulation, eddies and shifts of predominant currents (White *et al.*, 2010, Schunter *et al.*, 2011). Inter-annual variation in alongshore currents could have been the reason why the origin of *L. lepadogaster* recruits settling to the AMP was different between years (Chapter 5.2). Similar results were found in other studies (Hedgecock, 1994, Selkoe *et al.*, 2006, Siegel *et al.*, 2008). Nanninga *et al.* (2015) studied a coral reef fish species and also observed inter-annual differences in local retention and dispersal patterns of fish larvae, which may lead to high inter-annual variation in genetic

structure and connectivity among adult populations (Chapter 5; Hogan *et al.*, 2010, 2011). These findings confirm that recruitment not only has an important effect on the size and demography of fish populations through variation in recruit abundance, but also on the structure of populations through the variable origin of recruits (Chapter 5; Doherty and Williams, 1988, Roughgarden *et al.*, 1988, Jones, 1990, Cowen and Sponaugle, 2009).

The species studied regarding population connectivity and larval dispersal (*L. lepadogaster*) did not settle to recruitment collectors (Chapter 3), despite its common occurrence in the study area and the observed occurrence of recruits in the intertidal during the studied periods. Furthermore, this species exhibits a short pelagic larval duration (PLD) of 11-18 days (Raventós and Macpherson, 2001, Beldade *et al.*, 2007, Tojeira *et al.*, 2012), well-developed larvae at hatching (Tojeira *et al.*, 2012), and all larval developmental stages have been collected in the nearshore environment (Beldade *et al.*, 2006a, Borges *et al.*, 2007). All these characteristics together with the general acceptance that distance of larval dispersal is positively correlated with PLD (Shanks *et al.*, 2003), lead to the strong assumption that *L. lepadogaster* larvae may retain near the natal reef and that populations should be weakly connected.

However, the interdisciplinary approach in chapter five revealed high connectivity among populations and the possibility of long-distance larval dispersal. Nevertheless, significant levels of self-recruitment and higher probabilities for short-distance dispersal were also recorded (Chapter 5). Hence, local recruitment is not incompatible with long-distance dispersal and both can play an important role in the replenishment of a population (Chapter 5; Christie *et al.*, 2010, Underwood *et al.*, 2012). Furthermore these results reinforce that PLD known for a species is not always a good proxy for larval dispersal (Shulman and Bermingham, 1995, Purcell *et al.*, 2006, Galarza *et al.*, 2009, Horne *et al.*, 2011, Riginos *et al.*, 2011, Riginos *et al.*, 2014) and that other factors such as larval behaviour and local oceanography need to be considered.

In summary, the major findings of the thesis are:

- standardized recruit collectors such as water column SMURF used in this study are an effective method to monitor fish recruitment of some species in the nearshore of rocky reef environments;

- high temporal variation of recruitment was detected and found to be related to environmental processes;
- the temporal recruitment patterns and the link with environmental variables were species-specific and distinct depending on the temporal scale;
- long distance dispersal and high connectivity were found for *L. lepadogaster* despite its short PLD and local larval development in the nearshore;
- self-recruitment and long distance dispersal occurred simultaneously for the same species.

6.2 Implications to conservation management

In this thesis settlement (reef fish), nearshore recruitment (Atlantic horse mackerel) and larval dispersal (clingfish), exhibited clear temporal patterns, contributing to the overall recruitment patterns of fish populations. This information is very important for the design of fishery and conservation strategies.

Due to the influence of recruitment on the dynamics and persistence of populations, understanding processes and patterns of recruitment has been a long-standing goal in marine ecology, fishery science and conservation. Studying the relationship between patterns of recruitment and oceanographic and climatic processes is essential to estimate the effects of current and future environmental changes (e.g. climate change) and to support a more sustainable fishery management. Moreover, the conservation and management of important fishing resources, biodiversity and habitats seek to know how the persistence of populations can be maintained and in this regard the investigation of recruitment patterns and processes assumes great importance, in particular to the design and management of Marine Protected Areas (MPA); hence, the results provided by this study can deliver useful information for the management of the Arrábida Marine Park.

The Inter-annual and within seasonal patterns of recruitment found in this thesis, correlated with the oceanographic processes described in chapters 3 and 4. For example, upwelling and its consequences may highly influence the recruitment success of pelagic and also reef fish species in the AMP and likely as well along the total coastline of the Iberian Upwelling System. These findings, together with outcomes of previous studies on the adult fish

assemblage (Henriques *et al.*, 2007, Horta e Costa *et al.*, 2014), demonstrate that oceanographic conditions of different spatio-temporal scales affect the dynamic and structure of fish populations in the AMP and need to be considered in conservation management.

Additionally, the interaction between recruitment and regional oceanographic processes indicated that coastal populations receive recruits from other areas and thus that fish populations of the AMP are to some degree open and connected to other populations along the Portuguese coast, even when considering a highly site attached species with expected high potential of retention. Both self-recruitment and recruits of *L. lepadogaster* originating from northern or southern populations were registered in the AMP (Chapter 5). Thus the AMP does not only receive recruits from other populations but it can also provide other sites with larvae, thus functioning simultaneously as a source and sink. This is critical information for conservation management and for the design of a network of MPAs. Indeed, there is no study yet that has analysed connectivity of fish populations among the existing Portuguese MPAs (Berlengas MPA; Arrábida Marine Park, Sudoeste Alentejano and Costa Vicentina).

Moreover coastal areas might not just benefit its coastal species but also pelagic-neritic species that use the nearshore as a nursery ground (Chapter 4) by protecting their early growth after recruitment. For example, the AMP might serve as a nursery ground and as an area for forming schools of the common Atlantic horse mackerel (Chapter 4), one of the most important fish resources in Portugal. The results herein provide novel knowledge on the early life stage and nearshore recruitment patterns of this species that may translate into valuable information for the management of this species and shows the potential conservation effect that coastal MPAs can have.

6.3 Future Research

Ideally, a regular monitoring of recruitment patterns with collectors, of larval dispersal and population connectivity should be continued in order to better understand the spatio-temporal patterns found. In particular, analyses made to test relationships with oceanographic processes will gain accuracy with longer data time series covering a larger

range of oceanographic and climatic conditions. Throughout the thesis several ideas and new research questions arose that could be addressed in future research, namely:

i) Recruitment patterns

For a deeper understanding of the relationship between small scale recruitment patterns and oceanographic processes, a weekly or even finer sampling scheme is recommended in order to analyse the effect of fine scale environmental patterns such as the lunar cycle and tide-related processes (e.g. tidal bores; Findlay and Allen, 2002). Concerning this, on-site measurements, temperature data loggers and current profilers will be necessary to study in-time local scale oceanographic processes. Moreover, a permanent monitoring of these interactions should be considered to gain a deeper understanding of the effect of long term environmental changes in response to global warming, which potentially include changes in upwelling (Miranda *et al.*, 2012, Pires *et al.*, 2013, Bakun *et al.*, 2015).

Regarding Atlantic horse mackerel, a monitoring of recruitment over a longer time period and for several years could show if this carangid species has developed a reproductive strategy in response to upwelling conditions as it has been found for other pelagic species in upwelling areas (Cury and Roy, 1989, Roy *et al.*, 1989, Bakun, 1996, Cubillos *et al.*, 2001).

In order to understand the influence of larval condition on recruitment success in relation with environmental factors, it would be highly interesting to study ELHT as well for other species (e.g. *S. hepatus*) sampled in this study. Furthermore, it would be necessary to estimate the loss of settlers due to post-settlement processes, by for example monitoring the abundance of settlers to SMURFS and of recruits in the natural reef (visual census) (Macpherson and Raventos, 2005, Félix-Hackradt *et al.*, 2013) or by investigating predation with cage experiments (Steele and Forrester, 2002, Vallès *et al.*, 2006).

In general, of great interest would be a study that combines different sampling methods, such as light-traps, recruit collectors and visual census, in order to collect several ontogenetic stages from larvae to adult fish. Such a sampling design could allow for a correlation analysis between larval supply, recruitment and adult fish abundance and by this making conclusions on the degree to which local reef fish populations are influenced by local larval production, recruitment or by post-settlement processes (Caselle *et al.*, 2003, Félix-Hackradt *et al.*, 2013). In addition, combining such a sampling design to follow individual cohorts and with

the respective otolith microstructure analysis on the different ontogenetic stages, can further allow studying the relationship of ELHT and recruitment success (i.e. selective mortality; D'Alessandro *et al.*, 2013, Murphy *et al.*, 2014).

ii) Larval dispersal and population connectivity

Despite the fact that knowledge on population connectivity is elemental to successfully protect fish populations, very little is known about fish larval dispersal and population connectivity along temperate coastlines such as the Portuguese one (Chapter 5, F. Solomon unpublished data). Therefore, it is important to address these research topics in future studies, which could target several species with different ELHTs and include the following aspects (outcomes from Chapter 5): (i) sampling over several years to further investigate temporal patterns, to understand their variability and to exclude the chance of observing just a single atypical event; (ii) analysing adults and recruits from all studied populations to describe the overall source-sink network; (iii) combining direct and indirect approaches (e.g. parentage-analysis and/or otolith microchemistry with genetic population structure and/or bio-physical dispersal model) with the observation of oceanographic processes. This would reveal further critical information for fishery and conservation management. For example a network of marine protected areas (MPA) intends to increase ecological and economic benefits, while decreasing negative social, economic and cultural effects of a single large no-take area (Almany *et al*., 2009, Gaines *et al*., 2010, Grorud-Colvert *et al*., 2014). For the design and establishment of multiple interconnected MPAs it is fundamental to have prior information on population connectivity, the movement and dispersal of larvae, juvenile and adult fish (Christie *et al*., 2010b, Grorud-Colvert *et al*., 2014, Green *et al.*, 2015).

iii) Coastal and nearshore oceanography

Understanding oceanographic processes occurring in the coastal and nearshore environment is very important since most coastal fish start and end their pelagic phase in this environment (Cowen and Sponaugle, 2009). Still, there is very little knowledge on the oceanography of this area. The nearshore environment is often shallow, and a weakening of currents is expected because of bottom friction, but processes in this area are highly complex by the effects of wind, tides, buoyancy, and waves and nearshore currents predominantly flowing parallel to the coastline can be shifted to nearshore and offshore water exchanges (reviewed in Gawarkiewicz *et al.*, 2007). Observations and analysis of small-scale local processes in the nearshore can allow identifying features favouring larval retention, ranging from eddies,

upwelling “shadows”, or reduced flow near headlands (Cowen and Castro, 1994, Hamilton *et al.*, 2006, Mace and Morgan, 2006, Pineda *et al.*, 2007). Furthermore, this information needs to be integrated into bio-physical models to improve the still very limited representation of the coastal and nearshore area and to increase the resolution and accuracy of bio-physical models (e.g. Sponaugle *et al.*, 2012a).

iv). Larval capabilities

In addition to oceanographic processes, larval behaviour, especially swimming, sensory, and orienting abilities of larvae, can greatly influence larval dispersal and recruitment. The observations made in this study on the Atlantic horse mackerel suggest that larvae can migrate from the continental shelf to the nearshore and that highest peaks of recruitment occur around new moon. From this, several questions arose: Can larvae of this and other studied species orient and swim actively towards the coast? What are the relevant senses that allow orientation towards the reef: smell, solar and celestial cues or sound? The latter has been found to be higher during new moon (Radford *et al.*, 2008, Staaterman *et al.*, 2014), so could this also be a reason why more recruits were collected around new moon?

Swimming abilities of *L. lepadogaster* increase with larval development (Faria and Gonçalves, 2010), but do not exceed local currents (unpublished data) and larvae can potentially disperse over long distances (Chapter 5.2). Nevertheless, in larval fish surveys all developmental stages of *L. lepadogaster* were found nearshore and close to the rocky reef (Beldade *et al.*, 2006a, Borges *et al.*, 2007) and a parentage analysis revealed self-recruitment (Chapter 5.2). Thus, behavioural abilities other than swimming might be more relevant for interacting with the nearshore environment and that remain to be investigated.

Investigating larval behaviour can provide relevant information for the understanding of recruitment patterns and of larval dispersal and/or retention near reefs and self-recruitment. For example, important research fields would be the study of the natural settlement habitat, ontogenetic habitat transitions and the overall ontogenetic development of swimming, sensory, and orientation abilities. The integration of such behaviours in bio-physical models will be of major importance to obtain more realistic estimates of dispersal (Leis, 2007). Laboratory and field equipment are advancing in this research field (Piercy *et al.*, 2014, Irisson *et al.*, 2015) and already on-going research on species of the AMP should be extended (Tojeira *et al.*, 2012, Silva *et al.*, 2014).

In this thesis we showed that recruitment of a nearshore fish assemblage varied strongly within a summer recruitment season and between three consecutive years. This inter-annual variation matched with patterns of oceanographic processes and parameters. At a species level we found that recruitment and as well hatching of the Atlantic horse mackerel, was affected by the lunar cycle and coastal upwelling. Furthermore, dispersal patterns of the clingfish *L. lepadogaster* also matched with variation in ocean currents. Overall, this study demonstrates, that a multidisciplinary study approach, including further research fields mentioned above, is necessary to improve our understanding of the highly complex, interactive process of recruitment.

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Appendix

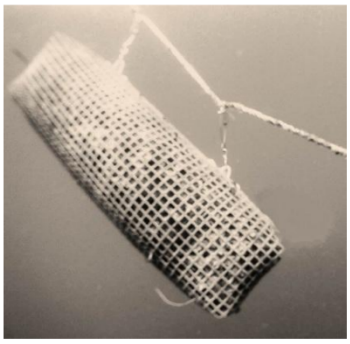
Appendix 1

Flyer explaining our SMURF study to fishermen and divers

Photograph on previous page was taken by Elisabeth van Beveren.

Estudo de juvenis de peixes através de SMURFs

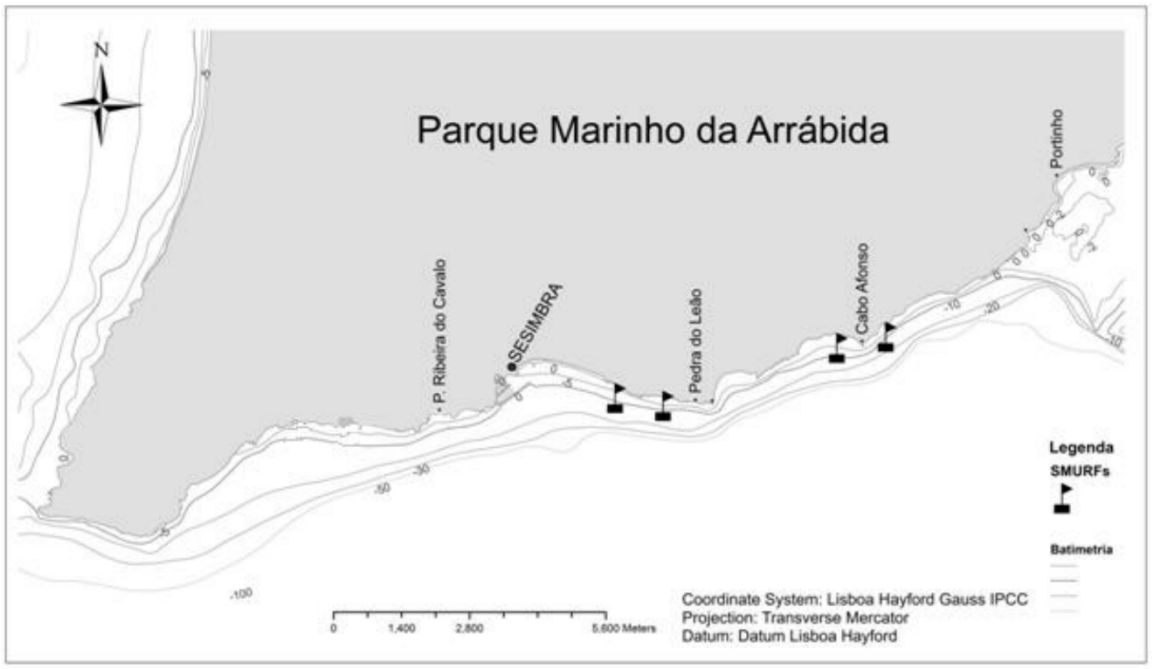
Caros Navegadores, Pescadores e Mergulhadores



Durante a Primavera e o Verão voltará a decorrer no Parque Marinho Luiz Saldanha o estudo no qual irão ser utilizadas estas estruturas, a que chamamos SMURFs, que podem servir de abrigo para os peixes juvenis.

O objetivo é conhecer melhor quais os peixes que vêm de outros locais ou que nascem neste local e que usam estas águas para crescerem até serem adultos.
Os locais onde estão colocados os SMURFs estarão identificados com bóias brancas.

E estes serão os locais onde serão colocados:



Pedimos a vossa colaboração para não perturbar este estudo, pois poderemos assim contribuir com novas informações importantes sobre as espécies deste Parque Marinho!

Obrigado!