UNIVERSIDADE DE ÉVORA

DEPARTAMENTO DE BIOLOGIA

Environmental factors influencing the settlement of *Diplodus* spp. juveniles in temperate marine rocky shores

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Orientação:
Prof. Doutor Frederico de Oliveira Almada
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Mestrado em Biologia da Conservação

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Dissertação

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Since this is a very personal part of this work, I hope you'll excuse me for taking the liberty of expressing myself in my native language! =)

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Abstract

Long-term monitoring programs can reveal trends emerging from yearly fluctuations that are difficult to evaluate in short-term studies. A 10-year study focused on four *Diplodus* species whose juveniles regularly use coastal reefs as nursery areas was performed in a recently classified Marine Protected Area (MPA). Differences in microhabitat use and a photographic guide for juvenile identification is provided. Environmental variables such as sea surface temperatures, air temperature, wave period and wind speed explain 53,4% of the interannual variation in juvenile abundances. A decreasing trend observed in juveniles from 2012-2017 was mirrored by adult fish landings in adjacent fishing ports, highlighting the importance of monitoring programs within MPA's for the management of species with commercial interest. Furthermore, the biogeographic transition between warm and cold temperate habitats along the Portuguese coast, enables the identification of sudden settlement pulses of warm water species in years with milder temperatures.

Fatores ambientais que podem influenciar o recrutamento de juvenis de *Diplodus* spp. em recife temperado

Resumo

Os programas de monitorização a longo prazo são importantes por permitirem destrinçar pequenas variações interanuais, impossíveis de verificar em estudos de curta duração. Este estudo de 10 anos realizou-se numa zona marinha rochosa normalmente usada como berçário por algumas espécies de *Diplodus* recente classificada como Área Marinha Protegida (AMP). Variáveis ambientais como a temperatura da água do mar (SST) e do ar, período da onda e velocidade do vento explicam 53,4% da variação interanual das suas abundâncias. De 2012 a 2017 as suas abundâncias têm sofrido um decréscimo acentuado, também observado nos desembarques da pesca comercial destas espécies em lota. Esta relação mostra a importância dos trabalhos de monitorização em AMP para a gestão de recursos pesqueiros. A localização biogeográfica da costa portuguesa, na transição entre águas temperadas quentes e frias, permite observar pulsos de recrutamento de espécies de águas mais quentes interrompidos por ciclos de águas mais frias.

1 Literature overview

1.1 Climate change and global warming

It is consensual amongst the scientific community that our world is on the fringe of significant climate changes (Cook et al., 2013). It is also accepted that we are going through an era marked by global warming like it has never been seen before (Hansen et al., 2006; Potter et al., 2017) and that its major causes originate in, or are closely related to human activities (Lineman et al., 2015). Greenhouse effects are mostly caused by increasing emissions of carbon dioxide within earth's atmosphere (Solomon et al., 2009) as well as the uncontrolled use of CFC's until the mid-90's (Ramanathan & Feng, 2009). Adding to these effects, and as their direct consequence, sea water temperatures are raising, leading to the melting of the polar icecaps, ice sheets and glaciers (Carson & Harrison, 2008; NASA Global Climate Change, 2018). This leads to sea level rise (NASA Earth Observatory; Peltier, 1998; Buis, 2017) which could drastically affect most of the world´s coastal areas causing tremendous ecological and economic impacts contributing to the collapse of commercial fisheries (Jackson et al., 2001). Sea currents could also be disrupted, affecting Earth's atmospheric weather (e.g. high and low pressures that can cause severe storms) and, ultimately, affect the Earth´s rotation (Peltier, 1998).

1.2 A transition area between marine cold and warm water species

To shed some light on local and regional shifts of fish abundances along the Portuguese coast it is important to understand the weather and oceanographic macro-phenomenon in the northeastern Atlantic. The Portuguese continental shelf has been considered a transition zone between "cold and warm waters" as it is bathed by southern warm superficial currents and cold northern waters and also superficial upwelling (Almada et al., 2013). One of the factors that influence these particular weather conditions is the Azores High (also known as the Azores anticyclone) directly related with the North Atlantic Oscillation - NAO (Taylor & Stephens, 1998; NCEI, 2018). The NAO can be measured as an index that shifts its position between positive and negative values influencing winter conditions across the North Atlantic

Region, hence influencing the Portuguese coast. According to its values, different weather conditions can be felt. This means that, in a year of high positive NAO index the Portuguese winters will be sunny, dry and cold, with prominent northern winds. Waters that are usually transported from the south and southwest do not reach the shoreline, leading to low Sea Surface Temperatures (SST), with upwelling events making nearshore waters even cooler. On the other hand, if the NAO index reaches negative values, winters will be extremely rainy, as the storm track is pushed further south, and the warmer southwest winds become prominent. These factors combined bring higher SST values, with warmer nearshore water temperatures. (Hurrell, 1995; Henriques et al., 2007; NCEI, 2018; NOAA National Weather Service Climate Prediction Center, 2018) (figure 1).

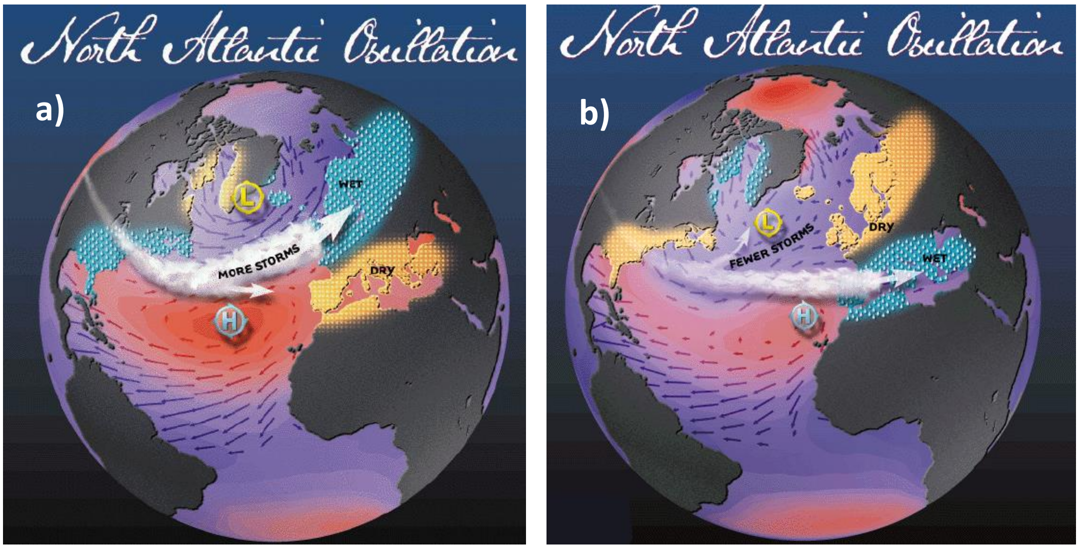

Figure 1 - NAO – North Atlantic Oscillation. a) Positive NAO; b) Negative NAO (https://www.ldeo.columbia.edu/res/pi/NAO/).

Another factor that influences these conditions are the warm superficial waters coming from the Gulf Stream that branches in the North Atlantic Drift and then, part of it deflects southwards on the British Isles resulting in the Portugal Current (PC) (Pérez et al., 2001; Arístegui et al., 2004, 2009) (figure 2).

Coming from the south and moving northwards along the Portuguese coast line, we have the Portugal Coastal Countercurrent (PCCC) during Winter, Autumn and Spring. During Summer the PCCC weakens considerably (almost disappearing) and is replaced by the Portugal Coastal

Under Current (PCUC) running at a depth between 100 and 200 metres (figure 2). These are low oxygen waters but with a very high nutrient content (Pérez et al., 2001; Arístegui et al., 2004).

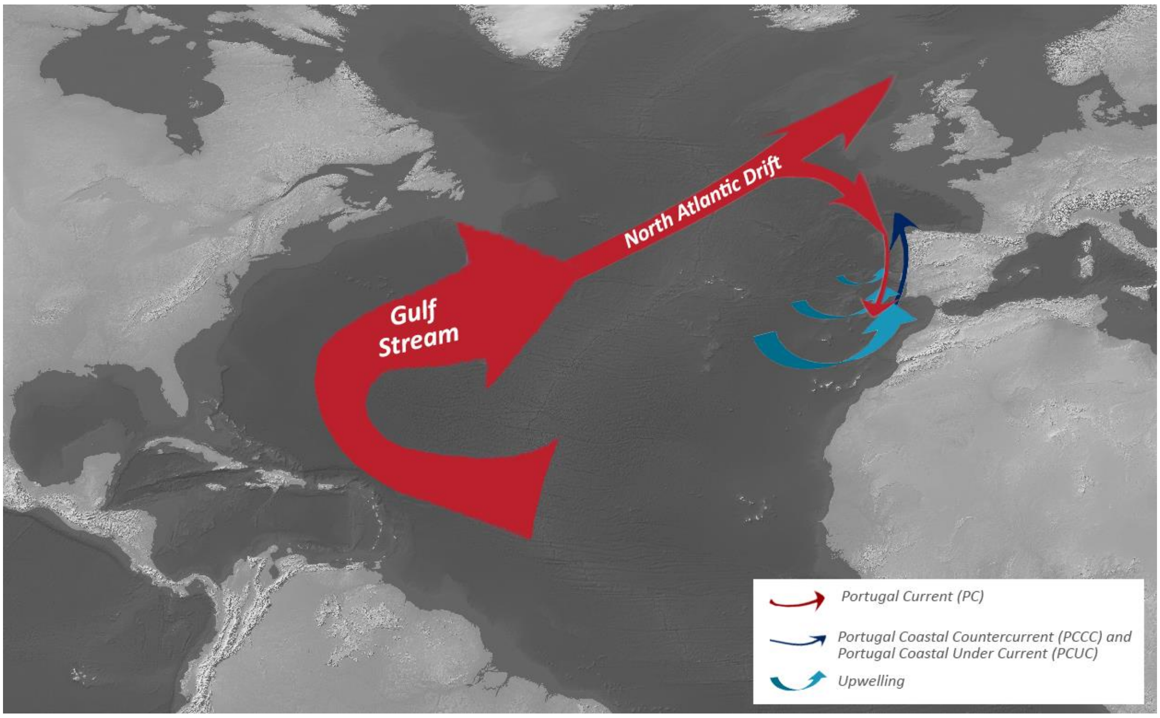

Figure 2 - Simple schematics of the currents influencing the Portuguese coast.

Since the position of the Azores High influences the circulation of the currents that reach the Portuguese coast, this means that the limits of warmer and colder waters will also shift their position across the coastline. As this happens, we can also expect a shifting in the fish communities that occur in these areas especially considering the warm water ichthyofauna (Henriques et al., 2007) and the early life stages that are presumably more sensible to environmental shifts (Pörtner & Peck, 2010).

1.3 The importance of long-term vs. short-term studies

Nowadays, due to the ever-growing world population, it is of crucial importance to understand how species and ecosystems intertwine and relate so that political decisions can be grounded on solid environmental and biological data.

Long biological data series, gathered over a period of several years allow us to identify small ecological shifts that could not be observed (or could even be misleading) in short-term studies of one, two or even three years (Hughes et al., 2017). We can find several examples in the literature, mostly from the Northwest Atlantic: North Sea (*e.g.* Barceló, Ciannelli, Olsen, Johannessen, & Knutsen, 2016; van der Veer et al., 2015) and the British Isles (*e.g.* Hawkins et al., 2009; Heath et al., 2012), that corroborate the previous statement. These reviews with a wider time frame are less common across the Southwest Atlantic Coast (but see Grilo et al., 2009; Horta E Costa et al., 2014). Long-term studies in this biogeographic region are of extreme importance because of its dynamic frontier between warm and cold water species (Almada et al., 2013; Horta E Costa et al., 2014). The lack of long-lasting monitoring studies within this region could be a consequence of the shortage of funds attributed to research and science in these countries compared to more developed ones (e.g. England, Germany or Norway). These studies, when applied to marine ecology, usually include scuba-diving sampling techniques, which are expensive, time consuming and require skilled manpower. They are also dependent on weather and sea conditions which, in this region, limits the diving season to a few months per year. As an alternative and, simultaneously, a way to keep costs low, regular intertidal monitoring within the area of interest focused on juveniles that depend on these habitats during the settlement phase could answer important questions. Although this should be regarded as a complement and not an alternative to scuba-diving monitoring, it enables us to answer similar questions. In addition, depending on the fish species to be monitored - since several species recruit to the near shore - the species status and stocks could be accessed using this methodology (Vinagre et al., 2010; Dias et al., 2016) during a longer period of time. This is the case of commercial species, such as the white seabream, *Diplodus sargus*, that are known to recruit to the rocky intertidal in their early life stages before moving into their subtidal adult habitats (Vinagre et al., 2010; Dias et al., 2016).

Which sampling technique is more effective or applicable depends mostly on the target species, local hydrodynamics and topography. Two different examples can be given from two Marine Protected Areas (MPA) that have been monitored for the past 10 years. One of them is Parque Marinho Luiz Saldanha (PMLS) located near Sesimbra, adjacent to the Sado estuary. This MPA sets on an area of coast (52,8 km^2 (Zupan & Klaui, 2018)) surrounded by high cliffs that descend almost vertically into the sea. This coast is protected from the predominant northeast winds and enables almost perfect diving conditions (Beldade et al., 2006). On the other hand, because of the high vertical cliffs, there is no extensive rocky platforms or access from land to intertidal monitoring at Arrábida. The other example is Área Marinha Protegida das Avencas (AMPA), located at Parede, near Cascais close to the Tagus River estuary. Similarly, to the previous MPA, this shoreline is also facing south but, in contrast, presents extensive horizontal rocky platforms. This short stretch of protected rocky area (0,6 km^2 (Zupan & Klaui, 2018)) is less protected from the predominant northeastern winds and is under the influence of the Tagus River runoff, making underwater visibility and overall diving conditions very poor. On the other hand, because of its long rocky platforms, it is easy to access the intertidal from land. These are two examples of long-term studies that take into account local conditions to optimize monitoring techniques in two close (50 km apart) Marine Protected Areas.

Summing-up, long-term studies across the coast line can be of extreme importance to manage commercial fish stocks, to detect non-indigenous species (NIS) and to monitor climate change.

1.4 Nursery areas, anthropogenic pressure and Marine Protected Areas (MPAs)

Estuaries and reef systems play an important role as nursery grounds for many marine species (Caselle & Warner, 1996; Adams et al., 2006; Dias et al., 2016). These habitats are a source of fish and other marine organisms that replenish the surrounding areas (Swearer et al., 1999). These can be some of the most productive systems in the marine ecosystems, hence the importance to protect them. Along the Portuguese coast, intertidal rocky reefs are nursery grounds for *Diplodus* species and, in the west central coast, the Parede-Avencas has been recently classified has a Marine Protected Area (MPA). Although the term is frequently under discussion, an MPA, essentially, is a marine area where human activities are more strictly regulated than the in surrounding waters (Horta e Costa et al., 2017). Benefiting from this protection status there is an overall advantage for marine organisms and a spill over effect to adjacent areas, which may also benefit local populations that depend on those marine resources. This happens when the organisms inside a marine reserve overflow to the adjacent fishing grounds, replenishing them (Buxton et al., 2014). There can be several levels of protection in an MPA depending on the activities permitted within it. Specific areas can be classified as Total Protection Areas (TPAs), where no human activities are permitted, or Partially Protected Areas (PPAs), where some human activities are permitted. Even within the PPAs, several degrees of protection can be found. Another issue that concerns MPA management is the effective size of the reserve. What is better, a large MPA or several small MPAs spaced between them and within the array of the larval dispersal range for the marine organisms we are trying to protect. This has been a controversial issue over the past decades. Studies show that limiting and reducing human activities in an MPA increases its resilience to the introduction of non-indigenous species (NIS) (Ardura et al., 2016) along with the increase fish biomass (Abecasis et al., 2014; Horta e Costa et al., 2016; Zupan et al., 2018). These works highlight the importance of the MPAs dimension and their levels of protection. The study performed by Ardura et al. (2016) supports that small to medium size MPAs are more resistant to NIS introduction than larger ones (stretches of coast smaller than 60km), especially if there are several of them spaced within a small distance of each other. As regulation and protection level goes hand-in-hand, it is best to keep them as restricted as possible (Zupan et al., 2018). This raises a different problem, especially if these MPAs are located close to metropolitan areas (e.g. a beach to where people usually convey to in large numbers during summer). In these cases, it is of extreme importance to make a great effort to inform and educate the local populations about the purposes of an MPA and what advantages they could bring later. Although we still have a long way to go, coastal communities are getting more aware of the importance of their local ecosystems, helping in the implementation and protection of new MPAs and actively trying to prevent their ecological degradation.

1.5 Study Area characterization

1.5.1 The characteristics of this small area of Portuguese Coast

As previously stated above, the Portuguese coast (figure 3c) is located in a very special and particular site where northern temperate cold and southern warm waters converge. In addition to its biogeographic location the study area corresponds to a small patch of shore with a very peculiar topography, encompassing long horizontal rocky platforms with a very gentle slope. Reefs are interspersed with small patches of sand along the coastline. Across the rocky platforms we can find several tide pools and natural inshore-offshore channels carved on the reef platforms. Our study area comprises two of these channels (figure 3a) that run along the intertidal platform and become isolated from the surrounding waters at low tide, entrapping the demersal and pelagic fauna that was present during high tide. These channels can attain a length of more than 100 metres and allow both observation and capture of all individuals that are unable to escape into the subtidal.

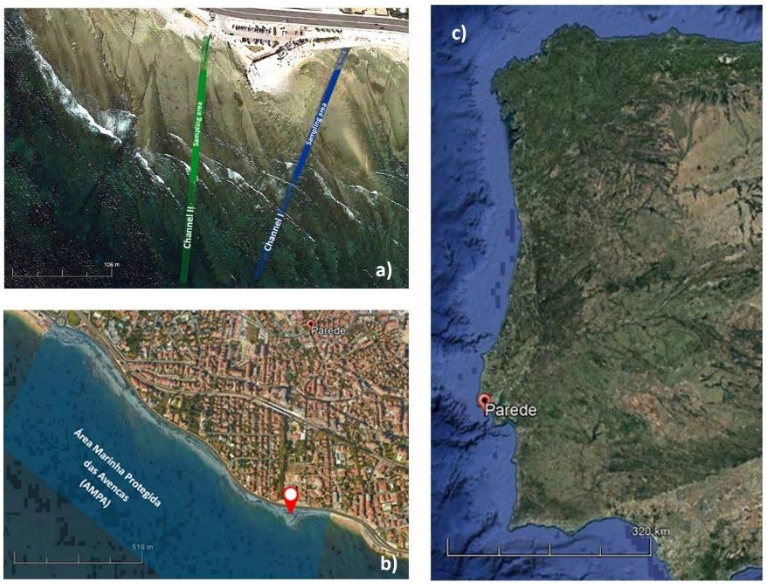

Figure 3 - Sampling stations designated Channel I and Channel II; b) Overview of the study area mapping the recently classified marine protected area (AMPA); c) Study area located in the western coast of Continental Portugal.

Moreover, the intertidal non-resident ichthyofauna, including several *Diplodus* species, occur in large numbers in this MPA (figure 3b), which is simultaneously the most recent and smallest along the Portuguese coast. These conditions make it the perfect grounds to implement a long-term monitoring station.

As stated above it is also of major importance to protect this area as it is located in a region that is heavily populated, with its pressure increasing seasonally during spring and summer, simultaneously with the settlement of several juveniles in these nursery grounds.

1.6 Species description

White seabream - *Diplodus sargus* (Linnaeus, 1758) (figure 3a)

Distribution: Subtropical species (48°N - 36°S, 18°W - 42°E) that occurs in the Eastern Atlantic, from the Bay of Biscay to Cape Verde and the Canary Islands including the Mediterranean and Black Sea, also from Angola to South Africa (Froese & Pauly, 2018).

Sexual maturity: Females may reach sexual maturity during the second year of life with approximately 16,7cm total length (TL) (in the Azores) (Morato et al., 2003). In the Azores, they spawn from March to June (with SST between 15º e 17ºC) (Morato et al., 2003). In south Continental Portugal aquaculture facilities they spawn from mid-December until early June (Pousão-Ferreira et al., 2005).

Eggs: Pelagic eggs are found during Spring in the Mediterranean and later the larvae can be found offshore, more than 1 mile from the coast line (Macpherson & Raventos, 2006). In captivity, all eggs hatch between 64 and 66 hours (Divanach et al., 1982).

PLD: Pelagic larval duration ranges from 14 to 17 days in the Mediterranean (Di Franco et al., 2011).

Figure 4 – a) *Diplodus sargus*; b) *Diplodus vulgaris*; c) *Diplodus cervinus*; d) *Diplodus puntazzo*.

Settlement: In the Mediterranean, new settlers range from 0.9 to 1.4 cm standard length (SL) (mean 1.11 + 0.01 cm) (Franco & Guidetti, 2011). In Continental Portugal west coast, settlers occur in inshore reefs from March/May until October/November (Dias et al., 2016). In the Azores, settlement usually occurs from late May to July, with juveniles remaining in their nursery grounds for approximately 2.5 months. Settlers leave the nursery grounds to join shoals of juveniles from late July until September (Morato et al., 2003).

Habitat: In the Mediterranean, settlers show a preference for a substrate consisting primarily of pebbles, but also recruited to substrates of sand or gravel and small or medium blocks (García-Rubies & Macpherson, 1995). La Mesa et al. (2011) describes a preference for rocky reef habitats but also sand and *Posidonia oceanica* banks. In the Atlantic, adults prefer rocky bottom habitats, and their highest habitat suitability occurs [over rock] between the depths of 5 and 10 m and when the distance to rock is less than 120 m. (Central Portugal) (Abecasis et al., 2014).

Activity: Individuals are more active during the day than at night (with diurnal feeding habits) and shelter at night inside crevices and holes (Mediterranean) (Di Lorenzo et al., 2016).

Common Two-Banded Seabream – *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) (figure 3b)

Distribution: This subtropical species (50°N - 40°S, 26°W - 36°E) occurs in the Mediterranean Sea, southwestern Black Sea; eastern Atlantic from the Canary Islands and Madeira north to Brittany (France) and in Angola (Froese & Pauly, 2018).

Sexual maturity: In southern Portugal male and female size at first maturity is approximately 17,2cm TL (Gonçalves et al., 2003). Spawning starts in the Autumn (September) ending in the Spring (April)(Gonçalves et al., 2003). In captivity spawning occurs from mid-December until early April (south Portugal) (Pousão-Ferreira et al., 2005).

Eggs: In the Mediterranean pelagic eggs can be found during winter (Macpherson & Raventos, 2006). Larvae can be found offshore, more than 1 mile from the coast line (Macpherson & Raventos, 2006).

PLD: According to Galarza et al., (2009) their PLD varies from 29 to 58 days. In the Mediterranean and lasts for a mean time of 41 days (Macpherson & Raventos, 2006).

Settlement: According to Bussotti & Guidetti, 2011, these fish start settling during spring in the Adriatic Sea. In the Mediterranean they have 2 settlement peaks in November and March/April. They show a preference for a substrates consisting primarily of pebbles, but also recruit to substrates of sand or gravel and small or medium blocks (Mediterranean) (García-Rubies & Macpherson, 1995).

Habitat: In the Mediterranean there is a slight preference for rocky reef habitats but these fish are also found over sand and *P. oceanica* banks (where food is abundant) (Abecasis et al., 2009; La Mesa et al., 2011). According to Sala & Ballesteros (1997) this species can be found after a depth of 3m.

Activity: In the south of Portugal, smaller fish (<12cm Total Length) show high values of residency staying in the same area for a month or more and having a small home range of less than 1Km (Abecasis et al., 2009).

Zebra Seabream – *Diplodus cervinus* (Lowe, 1838) (figure 3c)

Distribution: Compared to previous ones this subtropical species (47°N - 35°S, 19°W - 36°E) shows more affinity to temperate warm waters. It occurs in the eastern Atlantic from the Bay of Biscay and the Mediterranean to South Africa including Madeira and the Canary islands. It is absent from Cape Verde, off Senegal and the Gulf of Guinea (Froese & Pauly, 2018).

Sexual maturity: In captivity, spawning occurs from mid-March until mid-April in southern Continental Portugal (Pousão-Ferreira et al., 2005)

Eggs: In the Mediterranean pelagic eggs can be found during spring with larvae occurring offshore (Macpherson & Raventos, 2006).

PLD: Pelagic larval duration takes approximately 17 days in the Mediterranean (Raventós & Macpherson, 2001).

Settlement: Young settlers can be found in inshore areas from July until September in the Mediterranean (García-Rubies & Macpherson, 1995).

Habitat: In the Mediterranean settlers show preference for substrates consisting primarily of pebbles, but also recruit to substrates of sand or gravel and small or medium blocks (García-Rubies & Macpherson, 1995; Henriques et al., 1999).

Activity: This species is commonly found in the central Portuguese coast and usually occurs isolated or in groups with no more than 5 fish (Henriques et al., 1999).

Sharpsnout Seabream – *Diplodus puntazzo* (Walbaum, 1792) (figure 3d)

Distribution: This subtropical species (42°N - 28°S, 26°W - 42°E) has more affinities to temperate warm waters compared to *D. sargus* and *D. vulgaris*. It occurs in the eastern Atlantic from the Bay of Biscay (rare) to Sierra Leone, the Canary Islands, and Cape Verde, including the Mediterranean from the Black Sea to the Strait of Gibraltar. It is also found off South Africa (Bauchot & Hureau, 1986; Froese & Pauly, 2018).

Sexual maturity: In captivity spawns from late October until mid-January in southern Portugal (Pousão-Ferreira et al., 2005).

Eggs: In the Mediterranean pelagic eggs can be found during autumn with larvae occurring offshore (Macpherson & Raventos, 2006).

PLD: In the Mediterranean their PLD lasts on average 33 days (Macpherson & Raventos, 2006).

Settlement: In the Mediterranean, settlement occurs during October and November. Settlers show a preference for substrate consisting primarily of pebbles, but also recruit to substrates of sand or gravel and small or medium blocks (García-Rubies & Macpherson, 1995).

Habitat: In the Mediterranean there is a clear preference for rocky reef habitats (La Mesa et al., 2011).

Activity: In Arrábida, central Portuguese coast, adults and juveniles of this species are usually observed in small groups of no more than 5 fish (Henriques et al., 1999).

1.7 Main goals of this work

1.7.1 Shifting environmental conditions and their effects on *Diplodus* spp. young settlers

An overview of the changing abundances of four *Diplodus* species (*D. sargus*, *D. vulgaris*, *D. cervinus* and *D. puntazzo*) together with shifting environmental conditions is provided over a ten-year period. The analysis aims to unravel a set of environmental variables that affect the settlement effectiveness of these commercial species.

1.7.2 Settlement period/reproduction season duration and seasonal peaks for four *Diplodus* species

This work will evaluate the monthly and seasonal variation in the settlement (and consequently the reproduction season) of four *Diplodus* species, providing important data for the management of these species. Yearly variations of these parameters highlight the importance of long-term studies to define adult no-take seasons. Additionally, since these fish settle in intertidal reefs in regions that are under heavy human pressure, it is also important to define protection periods for the settlement grounds of the juveniles, especially in MPA's with an extensive intertidal rocky area.

1.7.3 Evaluate if local data obtained for juveniles is mirrored by adult fish landings in nearby fishing ports

Monitoring juvenile fish can provide information on future abundances of local/regional adult populations. On the opposite perspective, decreasing trends on fish landings of a particular set of species may anticipate a decreasing trend in juvenile populations. This positive feedback cycle is most concerning in species whose geographical dispersion is limited to a short pelagic egg and larval phases.

1.7.4 Provide a small photographic identification guide to discriminate young juveniles belonging to closely related species (in Appendix I)

In the beginning of this work it was harder than previously anticipated to distinguish between *Diplodus* juveniles belonging to different species. While the adults are easily identified some species of this genus start with vertical stripes along the flank and loose this pattern later in the juvenile phase (Bauchot & Hureau, 1986). For that reason, a small photographic guide is provided, and genetic tests were implemented in order to guarantee that the taxonomic identifications of these small settlers are correct.

2. Manuscript: Warm enough? Environmental factors influencing the settlement of *Diplodus* spp. juveniles in Atlantic temperate rocky shores

2.1 Abstract

Long-term biological data series on marine species are useful to reveal trends emerging from yearly fluctuations that are difficult to evaluate in regular short-term studies. They can also clarify the importance of different environmental factors in fish reproduction and settlement by the comparison of results from different years. Furthermore, ecological driven effects should become more evident when monitoring larvae and juvenile fish stages compared to adults that are presumably more robust. This study focused on four sparid species belonging to the genus *Diplodus* (*D. sargus*, *D. vulgaris*, *D. cervinus* and *D. puntazzo*) with significant commercial interest and whose early life stages regularly use rocky coastal areas. Regular intertidal surveys starting in 2009 at the central west coast of Portugal, show extreme interannual fluctuations in early settlers abundances, especially *D. sargus*, with a concerning decreasing trend between 2012 and 2017. According to this study, the main environmental variables that can explain 52% of the variation are: water temperature, wave period, air temperature and wind speed. Warmer years seem to contribute with small surges of *D. cervinus* and *D. puntazzo* settlers that are typically found in southern latitudes, highlighting the dynamic biogeographic frontier along the Portuguese coast.

2.2 Introduction

Fishing has been an important human activity representing a good source of protein and nourishment. However, this activity has taken such proportions that we are now on the verge of rupture, fishing beyond the possibilities that marine ecosystems have to replenish their populations (Thompson et al., 2002). It is therefore crucial to provide strong background knowledge of each species and communities to the management agencies of commercial fish stocks.

Some of the most common species along the Portuguese coast are *Diplodus sargus* (Linnaeus, 1758) (White Seabream), *D. vulgaris* (Geoffroy Saint-Hilaire, 1817) (Common Two-Banded Seabream), *D. cervinus* (Lowe, 1838) (Zebra Seabream) and *D. puntazzo* (Walbaum, 1792) (Sharpsnout Seabream) (Gonçalves et al., 2003; Pousão-Ferreira et al., 2005; FAO (Food & Agriculture Organisation), 2012). These four species are closely related but present small differences in their biological cycles and ecological niche preferences (Sala & Ballesteros, 1997). Most data is available for the Mediterranean and, for example, *D. vulgaris* spawns during winter, *D. sargus* and *D. cervinus* during Spring and *D. puntazzo* during Autumn (Macpherson & Raventos, 2006). This could imply that their preferential temperatures, defining reproduction or pelagic larvae duration (PLD), are also different (Morato et al., 2003; Franco & Guidetti, 2011). In the Mediterranean the settlement period of new *D. sargus* recruits starts in May, while *D. vulgaris* starts in November, *D. cervinus* in July and *D. puntazzo* in October (García-Rubies & Macpherson, 1995). According to Morato et al. (2003), in the Azores, *D. sargus* first settlers also arrive in late May. On the other hand, Pajuelo et al. (2003) in a study performed at the Canary Islands, found different settlement periods, starting in October for *D. cervinus*. Vinagre et al. (2010) in a study performed along river estuaries in the Portuguese coast also reported latitudinal preferences, probably related with the northern distribution limits of these species and their water temperature preferences along with other environmental variables. They found higher abundances of *D. sargus* and *D. vulgaris* in the northern estuaries and higher abundances of *D. puntazzo* in southern estuaries. With global warming affecting our oceans and marine resources, and the risk of tropicalization of the world marine temperate systems, it is crucial to monitor and follow these biogeographical transition zones (Perry et al., 2005; Cook et al., 2013; Horta E Costa et al., 2014) in which the Portuguese coast is included.

Adult seabreams also show differences in microhabitat niche preferences. According to Sala & Ballesteros (1997), *D. sargus* is very abundant in the surf zone, unlike *D. vulgaris,* whose densities increase significantly with depth and were not present at shallower sampled areas. *D. puntazzo*, although more abundant in deeper waters, did not show significant differences in densities between depth zones (Sala & Ballesteros, 1997). The same authors also show distinct algal feeding habits and different feeding rates in the different zones. Although with partial overlapping ecological requirements, there is evidence to support a robust stratification of their ecological niches. This segregation probably helps to reduce interspecific competition which is an expected consequence of their close phylogenetic relationships (Summerer et al., 2001). As closely related species they share similarities such as being omnivorous (Sala & Ballesteros, 1997) and showing preference for rocky substrates over soft sandy bottoms (Morato et al., 2003; La Mesa et al., 2011; Abecasis et al., 2014).

Seabreams are regularly captured in coastal and artisanal fisheries. Some species are not easy to discriminate and therefore they are frequently reported under the generic name “seabreams” ("sargos" in Portugal). Given their financial importance, it is vital to better understand their ecological interactions and roles in coastal ecosystems.

Considering the port landings from the genus *Diplodus* alone in 2016, reported captures resulted in a total of 776 t of fish, representing 0,8% of the total fish landings weight, and an income of 3,3 million €, expressing 2,3% of the total income in Continental Portuguese fish landings (INE, 2017). However, we suspect that being the target of artisanal fisheries and recreational anglers the real captures and incomes are probably largely underestimated (although we cannot provide a specific value). If the previous assumption is true it is crucial to gather additional biological information on these species, if possible, maintaining a continuous sampling over a broad period of time. Emphasizing the importance of long-term studies (Hawkins et al., 2009; Heath et al., 2012; Van Der Veer et al., 2015; Barceló et al., 2016), such a monitoring program would allow us to perceive dramatic or even small fluctuations in seabream communities throughout the years. The extension of their interannual settlement phase, their yearly abundance variation and even their yearly growth rates can be tracked while sampling juveniles in intertidal rocky areas (Duarte-Coelho et al., 2018). Some of these variations are related with environmental factors that will influence their maturation period, their reproduction timings, their pelagic larval dispersal period (PLD) and their settlement waves (Gonçalves & Erzini, 2000; Gonçalves et al., 2003; Morato et al., 2003; Abecasis et al., 2009; Franco & Guidetti, 2011).

Temperate marine rocky shores are of extreme importance to small fish settlers, working as important nursery areas for a number of species (Harmelin-Vivien et al., 1995; Caselle & Warner, 1996; Swearer et al., 1999; Adams et al., 2006; Dias et al., 2016). So, understanding during which developmental phases these species are dependent on such areas can contribute for a better management of commercial fish stocks. Understanding which environmental variables could be influencing their survival and growth rates and predict the outcome of the next reproductive season would be crucial, especially considering the rate of depletion of most marine resources. Providing a methodological framework and tools to replicate this monitoring program in different areas along the Portuguese coast would be a valuable contribution to discriminate local from regional effects in the future.

2.3 Materials and methods

2.3.1 Study area

This study was carried out from March 2009 to May 2018 along the intertidal rocky shore of Parede/Avencas, on the west coast of continental Portugal (38°41'08''N, 9°21'20''W) (figure 4c). This shoreline is oriented in a south-southwestern direction in the northern margin near the entrance of the Tagus river. Sampling was performed in two natural channels which become isolated from subtidal adjacent areas during low tide, forming a long stretch of enclosed sea water (figure 4a)). The two channels have markedly different habitats, with the first one (channel I) being essentially composed of plain rocky platforms and sandy bottoms and the second (channel II) being a more complex habitat with rocky bottom and large rocks and boulders. The length of the sampling areas in each channel was 110m.

The study area, along with the adjacent surroundings, has recently been classified as a Marine Protected Area (MPA)(RCM-64-2016), being currently designated Área Marinha Protegida das Avencas (AMPA)(figure 4b).

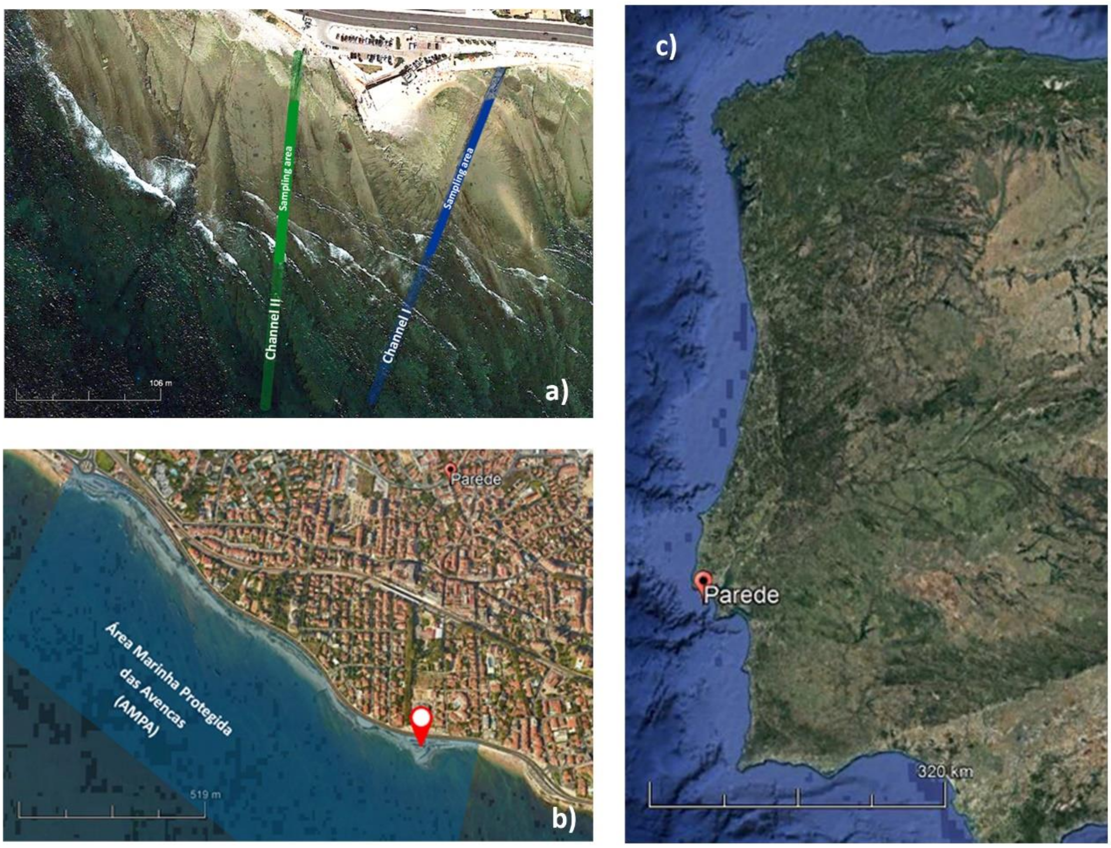

Figure 5- a) Sampling stations designated Channel I and Channel II; b) Overview of the study area mapping the recently classified marine protected area (AMPA); c) Study area located in the western coast of Continental Portugal.

2.3.2 Field sampling procedures

The intertidal non-resident ichthyofauna was sampled using non-destructive methods during low tide in each full and new moon periods. Fish were caught with small hand nets (mesh size <1mm for the smaller cohorts and larger hand nets mesh size between 0,3 and 1 cm for larger individuals). During field surveys, species abundances were estimated and at least 10 individuals were randomly sampled. Two additional individuals were captured to include the smallest and largest individuals sighted in the sampling protocol. The objective of this second non-random sampling was to obtain a size range (maximum and minimum) each fifteen days. The smaller individuals were useful to identify the length of the recruitment phase and the largest were used to estimate the size of transition from the intertidal nursery grounds to the subtidal adult habitats. Captured fish were identified and total, along with standard length, was measured to the nearest millimetre. For small juveniles (usually < 1cm) whose taxonomic status could not be immediately confirmed by their morphological characteristics, captures also included the collection of a small fin clip that was immediately preserved in ethanol for posterior genetic analysis. Sampled individuals were then released in the site where they were previously captured. During the 10 year sampling period a total of 235 field surveys were performed.

Environmental variables such as water temperature, sea and weather conditions were registered for each sampling session.

2.3.3 Data acquisition on environmental factors and fish landings

NAO index values were obtained from the NOAA site (http://www.cpc.ncep.noaa.gov/products/precip/CWlink/pna/norm.nao.monthly.b5001.current.ascii). Environmental data: wind speed (K), wave height (m), wave period (s), air temperature (°C), were obtained from WindGuru (https://old.windguru.cz/pt/). Since biological sampling was performed each 15 days a mean of the values gathered for environmental variables was calculated for the 15 days prior to each sampling. Tidal range values were obtained from Instituto Hidrográfico (http://www.hidrografico.pt/previsao-mares.php).

Water temperature was acquired during each sampling session and the predator abundances (*Dicentrarchus punctatus* in this case) were gathered from our data.

Fish landings were provided by DGRM (Direção-Geral de Recursos Naturais, Segurança e Serviços Marítimos).

2.3.4 Data and statistical analysis

Biological and environmental data were analysed with Primer-E (version 6.1.6). A draftsman plot was drawn to disentangle possible correlated variables. A logarithmic transformation was implemented to environmental variables to scatter their values and a square root transformation was applied to biological data as a way of giving less importance to the more abundant species and taking the less common species into account. All values were then normalized and a resemblance matrix was made using Euclidean distance for environmental data and Bray-Curtis similarity for biological data.

2.3.4.1 Biological data

An MDS (Non-metric Multi-Dimensional Scaling) analysis was performed followed by a PCO (Principal Coordinates Ordination) to understand how much variation can be explained by each axis (PCO1 and PCO2). However, this last test does not show which variables are contributing the most so, to clarify this issue, I tested the data with a PERMANOVA (Permutational MANOVA).

2.3.4.2 Environmental data

A CAP (Canonical Analysis of Principal coordinates), a PCO and a PCA (Principal Component Analysis) were performed. Since all results were similar, I opted to show only the PCO, because graphically this is the test that provides more information. In line with the analysis of biological data, to clarify which variables are more important to explain the results, I tested the data with a PERMANOVA.

2.3.4.3 Environmental vs biological data analysis

A RELATE analysis (Testing matched resemblance matrices) was implemented to test matched biological and environmental resemblance matrices, using a Spearman rank correlation coefficient.

To identify which environmental variables are more important to explain this model a BEST (Biota and/or Environment matching) test was performed. This test calculates the best correlations highlighting the ones that are more influent over the variation of biological data. To check how much variation these variables are allowing the model to explain I performed a DistLM (Distance based linear models). This model describes the patterns in the biological data using the environmental variables.

2.4 Results

2.4.1 Population structure and abundances

The overall results obtained over the last 10 years for the four *Diplodus* species show a clear decreasing trend in the number of seabream juveniles present in the study area (figure 5). There was a high peak of recruitment in 2011 and a sharp decline since then. Considering that *D. sargus* is the most abundant species, this trend mostly explains a decline in the number of juveniles from this species.

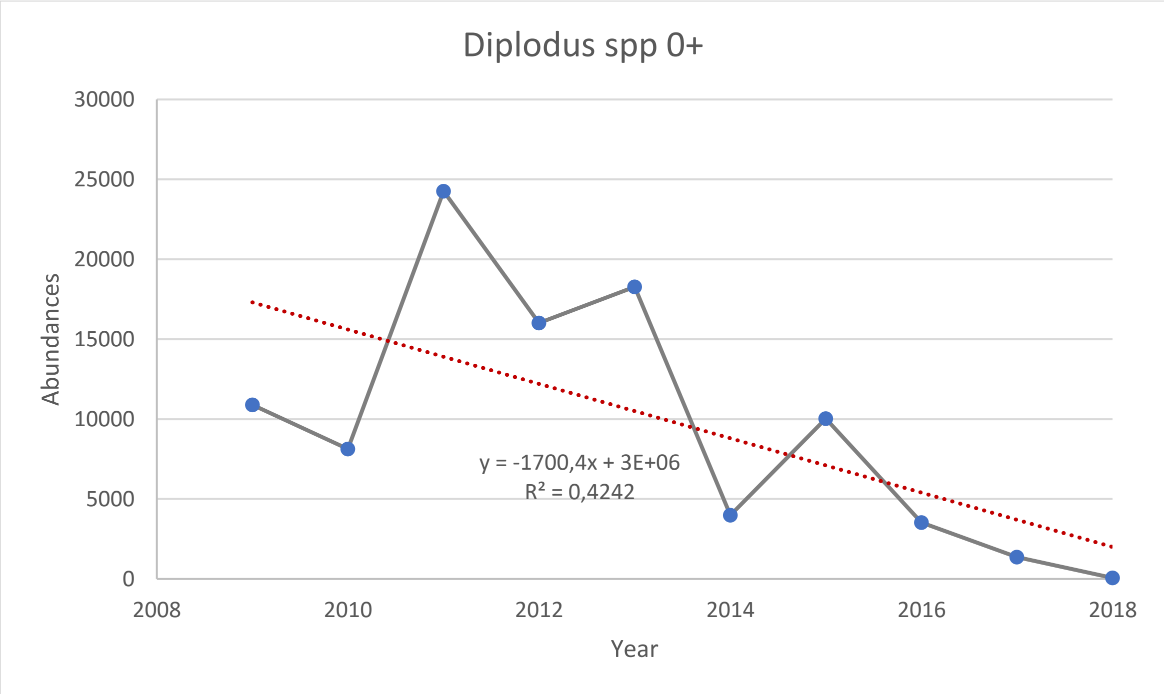

Figure 6 –Overall abundances of the four *Diplodus* species along a 10 year period. This data refers only to the recruits (0^+ cohort) of each particular year within both channels sampled in the study area.

In contrast with the regular predominance of juvenile *D. sargus*, in the spring of 2009 there was a peak in *D. vulgaris* abundances and a decrease of *D. sargus* (figure 6).

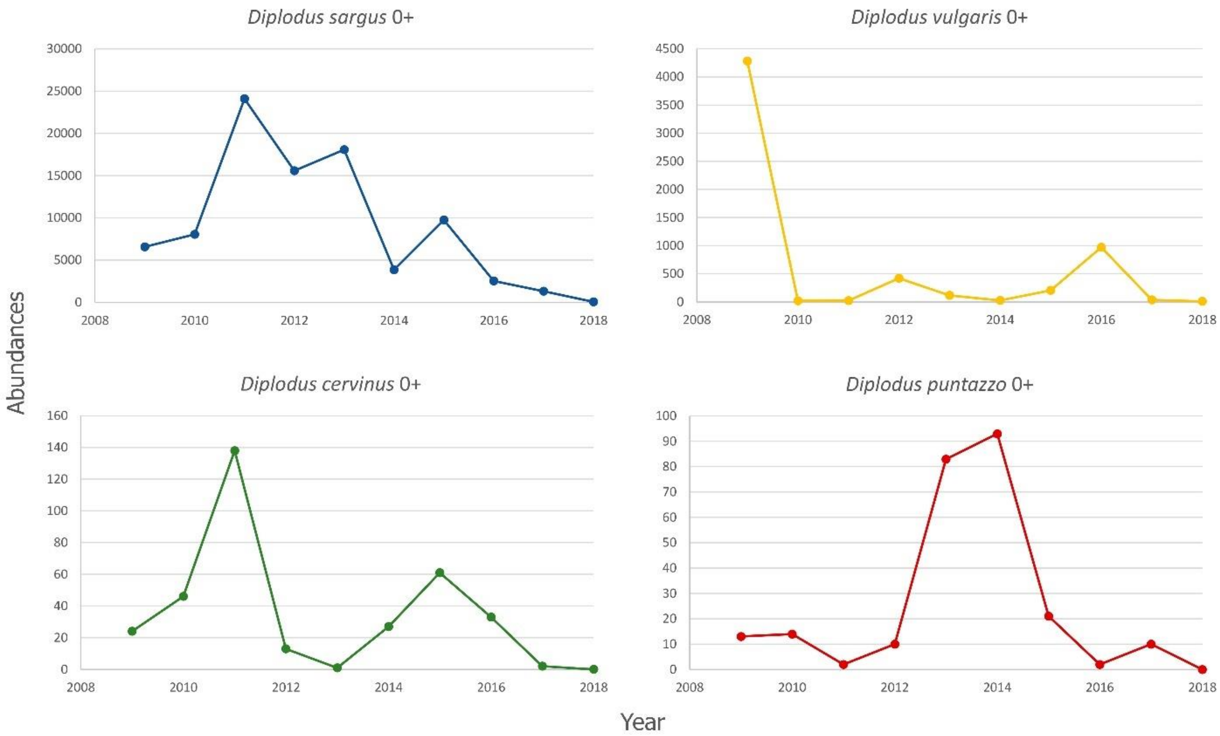

Figure 7 – Total abundances of each species per year along the 10 year sampling period. Data includes exclusively the recruits of the year (0⁺ cohort) within both channels sampled in the study area. Note: the abundance scales are different due to the highly dissimilar abundances of each *Diplodus* species in the study area.

Examining the overall abundances for each year and for each species it becomes evident that *D. sargus* is the most abundant and the most affected species by the decreasing trend starting in 2012 until 2017. Abundances declined from the tens of thousands to approximately one thousand.

D. vulgaris, shows a different trend. Being the second most abundant species in our study area it presents an unusual peak in 2009, followed by a sharp decline in the following years. A slight recovery occurred in 2012 and 2016, which could be taking advantage of fewer numbers of *D.sargus*, moving into their niche.

The remaining two species, *D. cervinus* and *D. puntazzo*, are much less abundant in the study area. *D. cervinus* follows a trend that closely follow the tendencies of *D. sargus* over the years, except in 2013 when they reached their lowest value. Regarding *D. puntazzo*, they usually occur in very low numbers, except for 2013 and 2014 when they reached their higher abundances over the past 10 years. Interestingly this peak in the abundances of *D. puntazzo* roughly corresponds to a decrease in *D. cervinus* abundances.

Interannual variability can be further evaluated by an analysis of the monthly distribution of each species abundances (figure 7). Looking at the younger cohort of these species (0^+ juveniles of the year), we can get information on their recruitment and also reproduction period within the area throughout the year (figure 8). This analysis highlights the sequential occupation of these intertidal rocky platforms by the juveniles of each species. The first one to settle is *D. vulgaris*, making its first appearance in March with a peak of recruitment in (early) May, being observed until October. The second species is *D. sargus*, present from April until November and having their recruitment peak in (late) May. The third wave of recruitment is performed by *D. cervinus*, starting their recruitment in June with a peak in July. One interesting remark about this species is that they spend the whole year within this nursery area, unlike all the others, that abandon it after a few months moving into deeper waters or visiting the same area but during high tide. Chronologically, the last species to settle is *D. puntazzo*. First juveniles occur in late August, having a peak in November and stay within these nursery grounds until April of the following year.

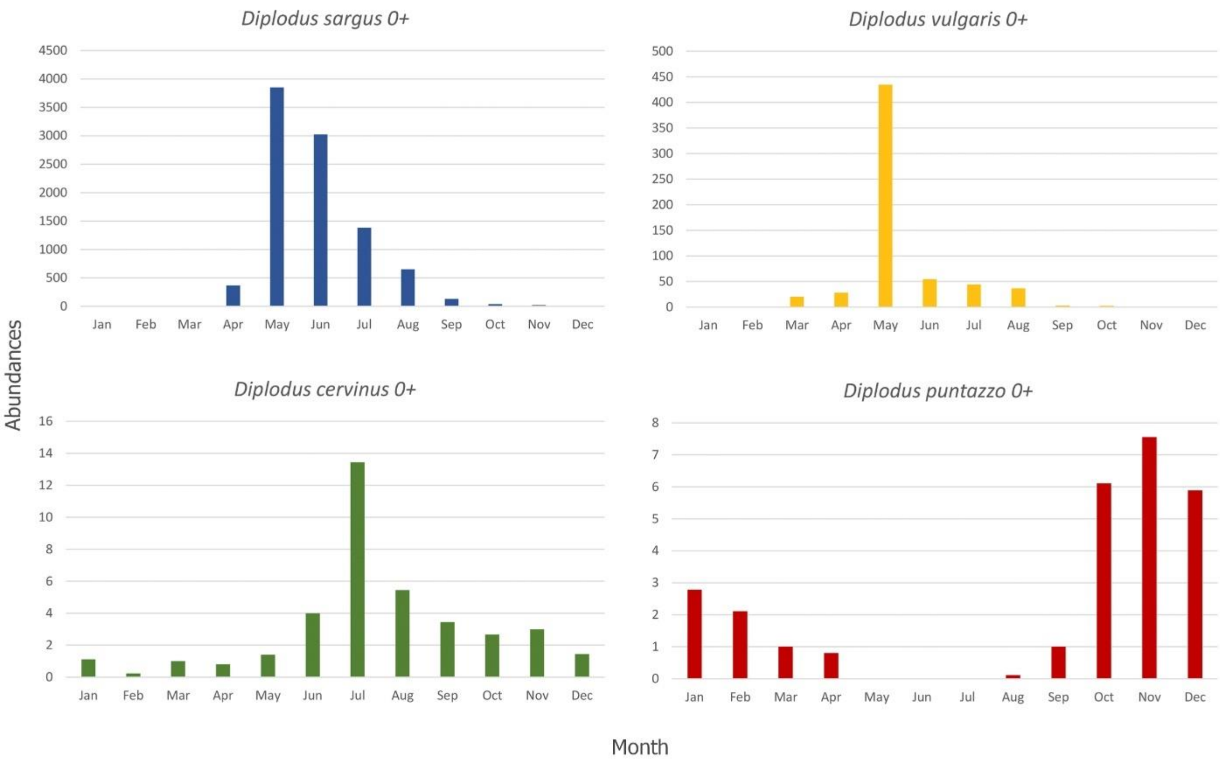

Figure 8 – Mean abundances per month of each *Diplodus* species along the 10 year survey in the study site. Note: the abundance scales are different due to the highly dissimilar abundances of each *Diplodus* species in the study area.

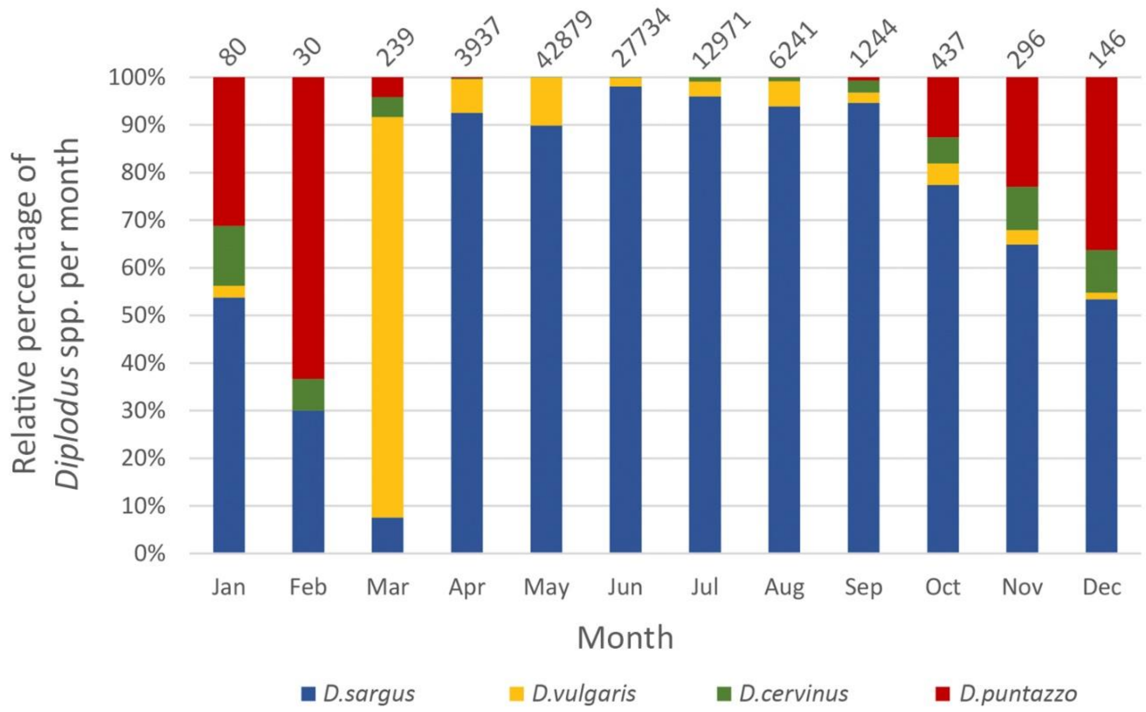

Figure 9 – Relative percentages of monthly occupation rates along the 10 year survey in the study site. Values on the top of each bar represent the total number of individuals present in that particular month.

In figure 8 it is possible to compare monthly percentage of occupancy per species showing the high contribution of juvenile *D. sargus* along the year. In fact, *D. sargus* contributes with more than 90% of the total abundances over the past 10 years.

Considering *D. sargus* alone and analysing figure 9 it becomes evident that the first settlers and peaks of settlement vary from year to year. The start of their settlement period varies between April and May but, the peak of abundances may occur from May to July depending on the year. With this in mind, it is important to emphasize that the duration of their settlement period also changes over the years between 5 months in 2014 and 8 months in 2011. Abbreviating, there is a high variability within the onset of the settlement period along with the peak abundances throughout the year and permanence in the nursery areas.

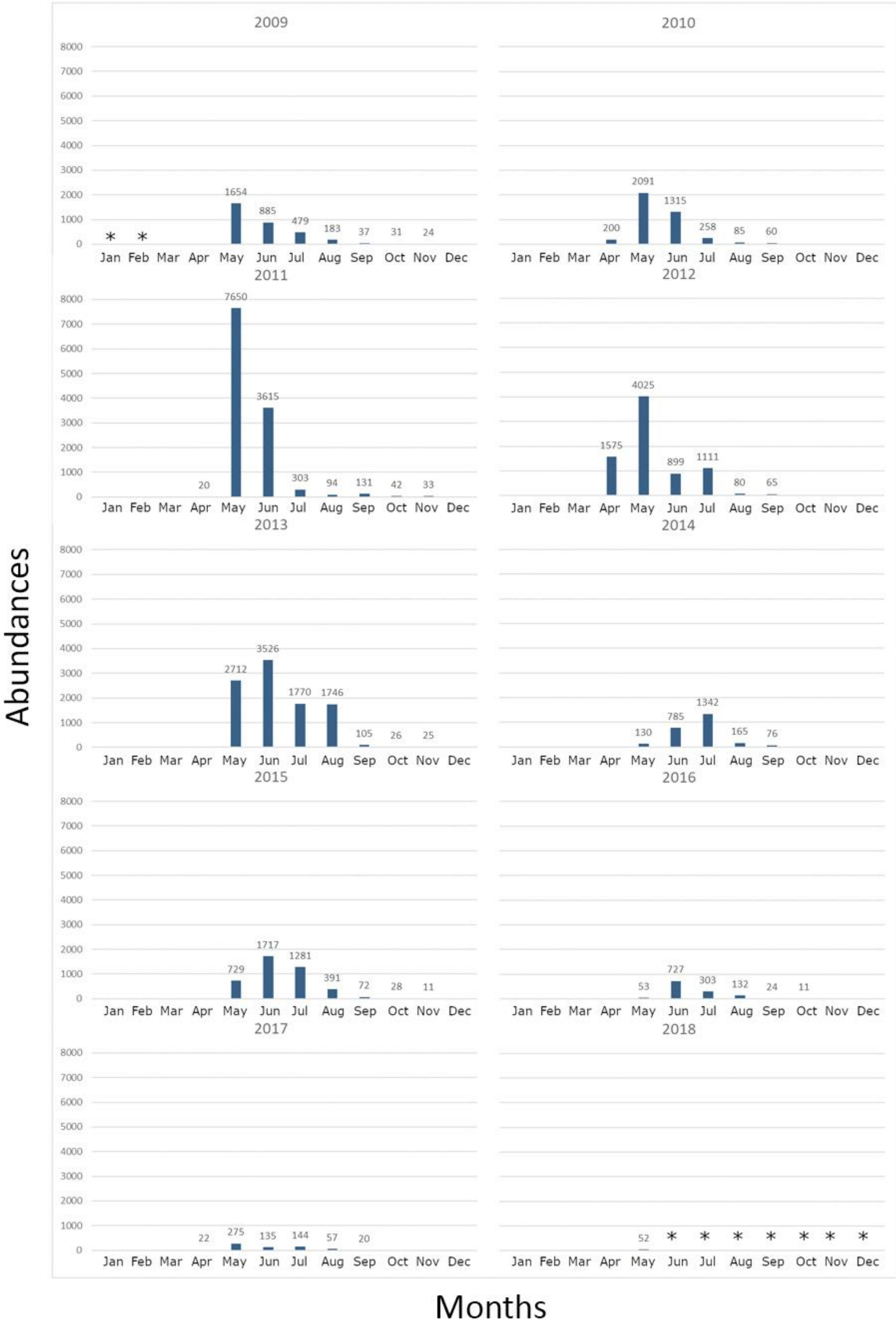

Figure 10 – Mean abundances of *D. sargus (the most representative species in the study area)* per month along the 10 year survey in the study site (when more than 10 individuals were sighted) (* shows months that were not sampled).

2.4.2 Fish landings in nearby fishing ports

Seabreams are commercial species and an important economic resource for coastal and artisanal fisheries. Landings in ports from regions that are adjacent to our study site (from north to south – Nazaré, Peniche, Ericeira, Cascais, Sesimbra and Setúbal) were evaluated for the last 9 years (figure 10). Since available data shows that the number of vessels, fishing permits and certified fishermen did not change significantly (see figure 24 Appendix I) over the last years we will assume that fish landings represent the available adult population in the region (i.e. abundances *Diplodus* species). Catch per unit of effort (CPUE) was not calculated because data on operation time for the fishing gears that usually catch *Diplodus* species were not available. Another drawback of this analysis is that we cannot discriminate the relative contribution of each species to fish landings (although we know that the most common species reported in fish landings on Portuguese ports are also *D. sargus*).

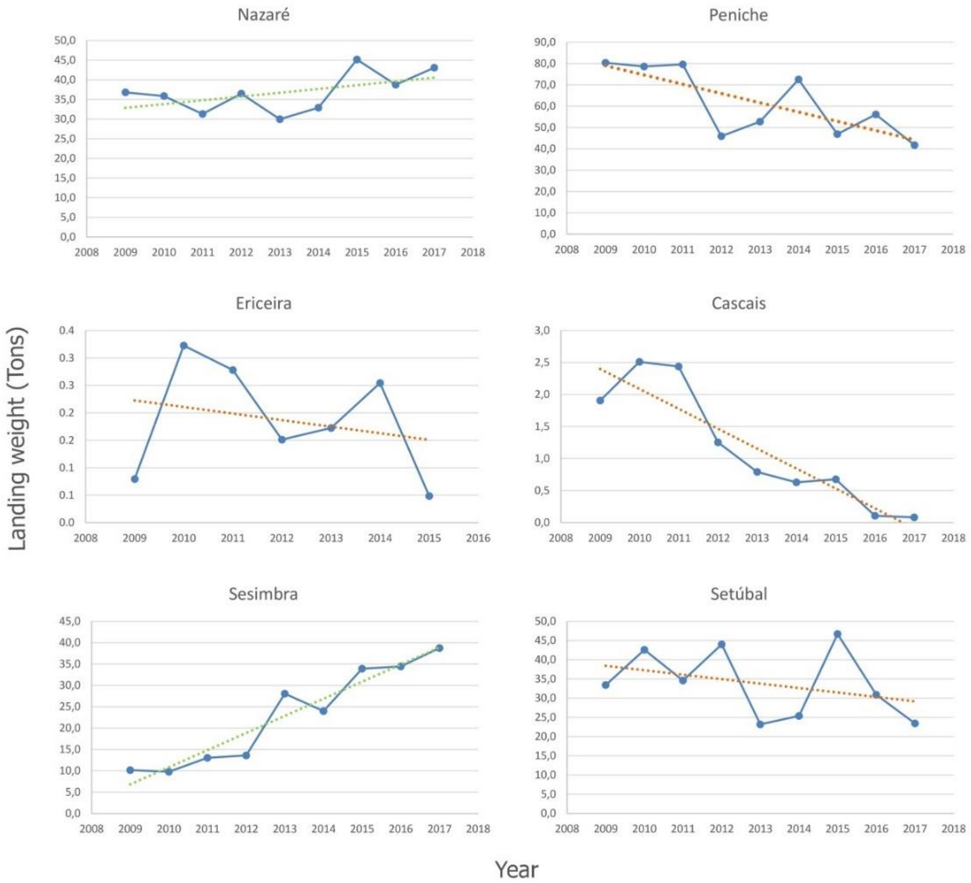

Figure 11 – Total seabream (*D. sargus*, *D. vulgaris*, *D. cervinus*, *D. puntazzo*) landings in the ports surrounding the survey area in the west coast of Continental Portugal. Negative trendlines are

showed in red and positive trendline is showed in green (Nazaré: y = 0,9628x - 1901,5 R^2 = 0,2706; Peniche: y = -4,3472x + 8812,5 R^2 = 0,553; Ericeira: y = -0,1196x + 242,5 R^2 = 0,0632; Cascais: y = -0,311x + 627,22 R^2 = 0,8352; Sesimbra: y = 4,0081x - 8045,4 R^2 = 0,9164; Setúbal: y = -1,1568x + 2362,4, R^2 = 0,124).

Taking these limitations into consideration it is remarkable to note that at Cascais, the port that is adjacent to our study area, we also find a sharp decline in seabream landings from 2011 to 2012 which continues until 2017. Although this is particularly evident in Cascais, other nearby fishing ports (Peniche, Ericeira and Setúbal) also show an overall decreasing trend.

The exceptions are Sesimbra and Nazaré which show an increasing trend of seabream landings. From these last two fishing ports only Sesimbra is a large port showing a steady increase of seabream landings over the last 10 years. Although there may be many valid hypothesis to explain these contrasting results it is worth to emphasizing that Sesimbra fishing port is located in the vicinity of a large MPA, the Parque Marinho Prof. Luiz Saldanha, implemented in 1998 (DR23/98-14 Oct.) where spillover effects of several commercial species, including *D. sargus*, were already demonstrated (Costa et al., 2013; Abecasis et al., 2015).

2.4.3 Environmental and biological data analysis

2.4.3.1 Analysis of the environmental data

An nMDS analysis of the environmental variables comparing different years is shown in figure 11. Although 2014 and 2016 appear to be overlapped there are no clear clusters, meaning that there is a high variability in the conditions occurring each year. However, the year 2012 is somehow isolated from all remaining years.

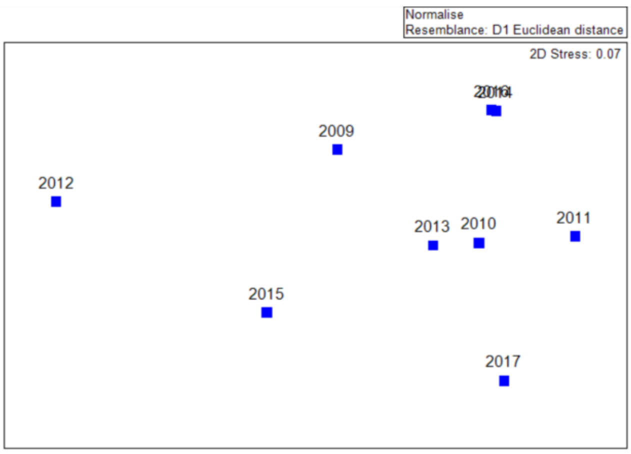

Figure 12 - nMDS analysis of the environmental variables along the 10 year survey in the study site.

A complementary analysis of the environmental data with a PCA showed that the first Principal Coordinate (PC) explains 47,8% of the variation and the second PC increases the cumulative percentage to 63,1%.

To understand the contribution of each environmental variable to the distribution of each month/year sampling period a PCO was plotted representing the overall seasonal variation (figure 12). As expected, the seasonality of environmental variables is quite evident. In addition, the cumulative percentage of explanation for both axis is 63,13% (similar to the PCA results reported above). Although much variability remains unexplained by this multivariate model it is, nevertheless, robust enough to be compared with the biological data reported in this work.

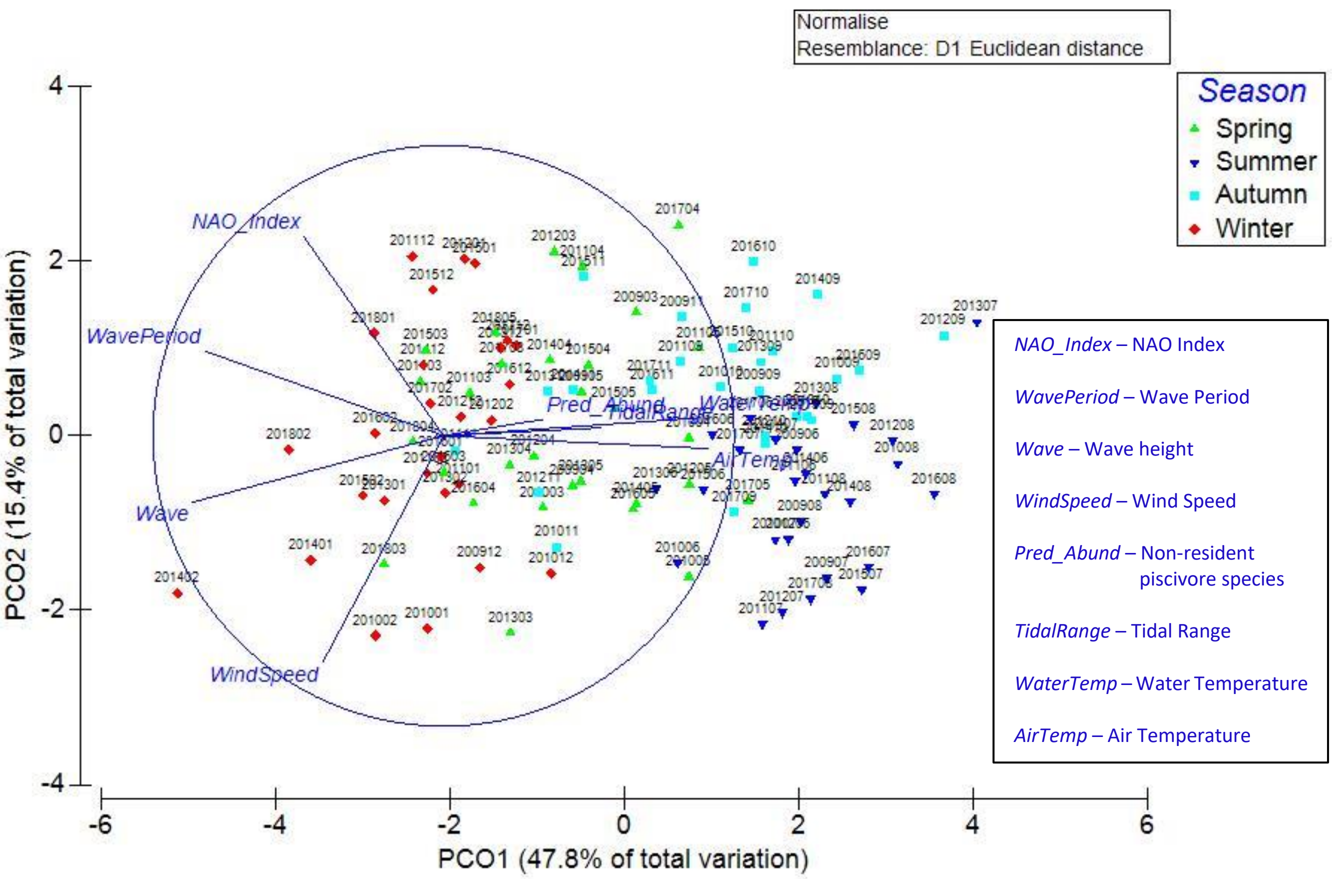

Figure 13 – PCO plotted for overall environmental data throughout our sampling period using seasonal variation as an ordination factor.

A Season/Year PERMANOVA analysis was made in order to find which periods are significantly different and if there is an interaction between them.

The results show that there are differences between years (P<0.001) and also, as expected, between seasons (P<0.001). PERMANOVA results also show that there is no interaction between season and year (P=0.211).

2.4.3.2 Analysis of biological data

The nMDS plot representing *Diplodus* spp. abundances show similar results during winter and autumn regardless of the year under analysis (figure 13). In contrast, spring values and, to a lesser extent, summer values are scattered in this multidimensional analysis. In other words, biological data varied greatly among years mostly during spring season. It is interesting to point the dissimilarities of spring values from 2009 and 2016.

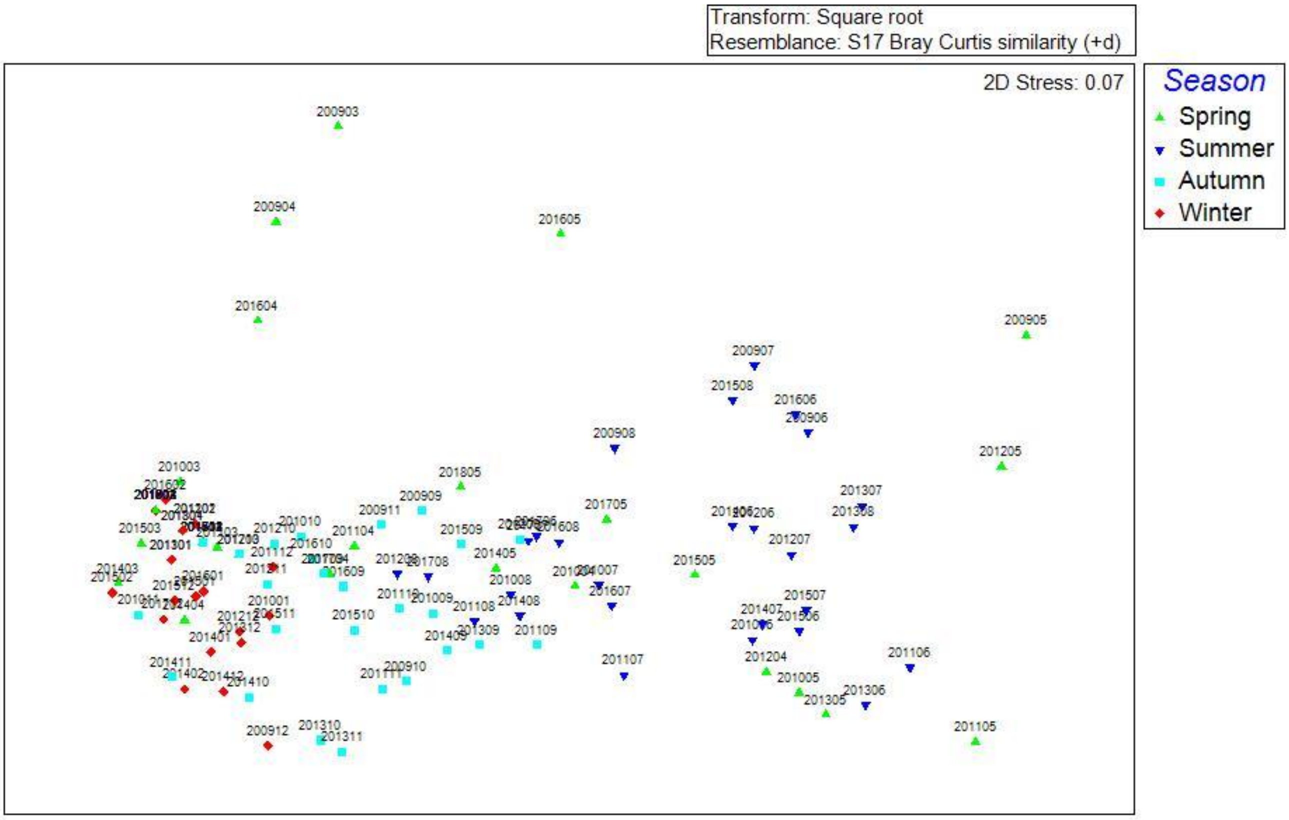

Figure 14 - nMDS analysis of the *Diplodus* spp. seasonal abundances from 2009 to 2018.

Environmental variables were overlapped on a PCO analysis of juvenile *Diplodus* spp. abundances for a preliminary evaluation of the relationships between both environmental and biological data (figure 14).

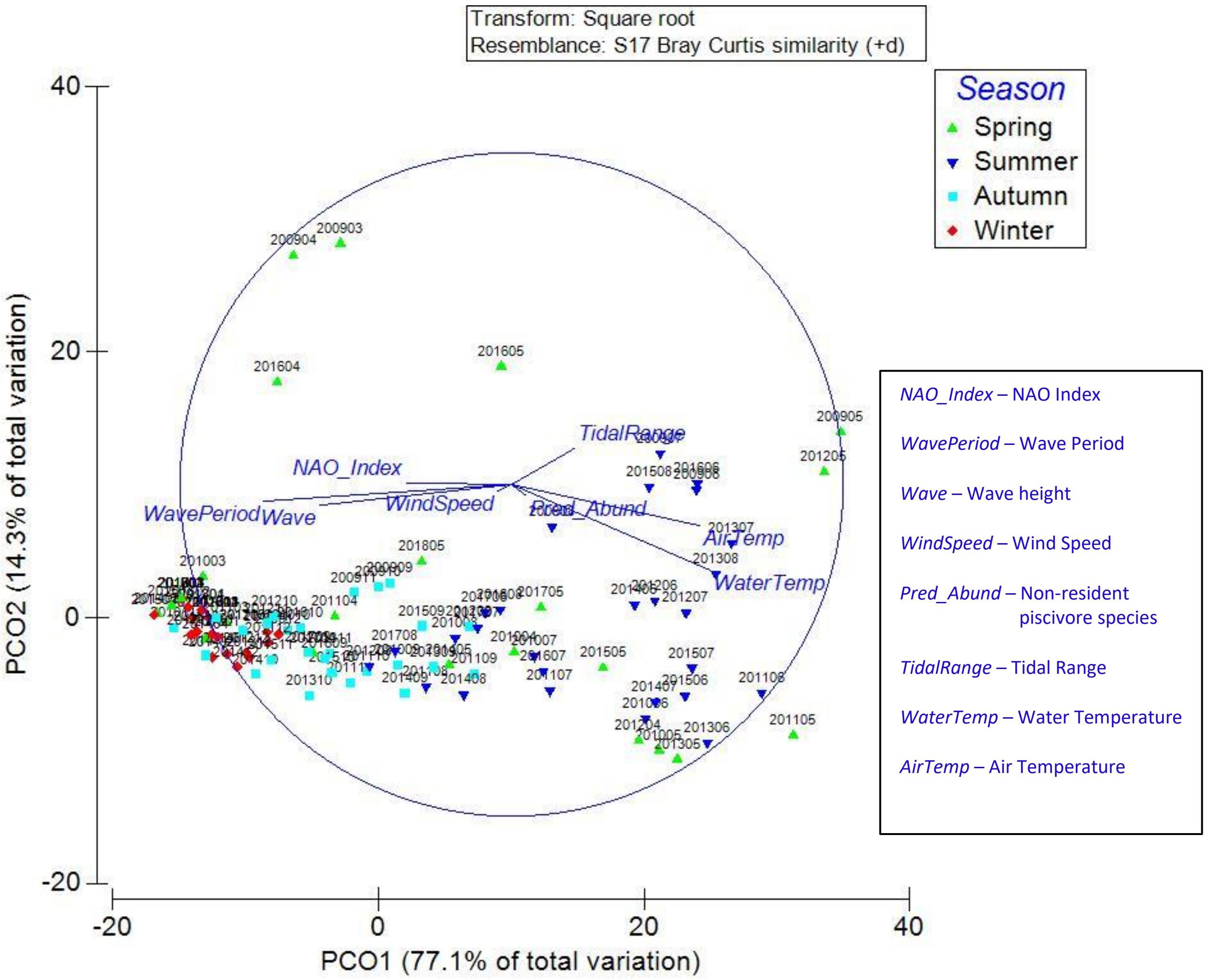

Figure 15 – PCO analysis of the *Diplodus* spp. abundances plotted against environmental data from 2009 to 2018 using seasonal abundances as an ordination factor.

A cumulative percentage of 91,4% for PCO1 and PCO2 strengthens the conclusion that biological data can effectively be represented by reducing its dimensionality. Similarly, to what was described before, winter values are clustered close together and spring values are widely scattered representing the high variability in the abundances during spring throughout the different years. A PERMANOVA analysis was made to evaluate the influence of different environmental factors in the distribution of *Diplodus* spp. abundances.

Results for biological data were similar to the ones obtained with the environmental data. Significant differences were found between years (P<0.01) and, as expected, between seasons (P<0,001), but no effect of year versus season was detected (P=0.61).

2.4.3.3 Analysis of environmental vs biological data

Interactions between environmental and biological data were tested with RELATE (Testing matched resemblance matrices). RELATE tests how well the relationships in the biological resemblance matrix match up to the ones in the environmental matrix using a Spearman rank correlation coefficient. This test gave us a result of Rho= 0,235 (P=0,001). The significance level is given in percentage meaning that this correlation is significant. However, the Rho value is extremely low (with good results presenting Rho~1), meaning that the correlation pattern between environmental and biological data are not similar.

An alternative approach is to find which environmental variables are more important to explain the model. BEST (Biota and/or Environment matching) analysis returns the best sets of correlations between the biological and each individual environmental variable. The best model that results from this test includes the following variables: 1) water temperature; 2) wave period. A correlation coefficient of Rho= 0,46 (P=0,001) (with 1000 permutations) point to these two variables as the most important ones to explain the variability in the juvenile fish abundance data.

However, the objective is to evaluate how much of the variation in the biological data these environmental variables are explaining. With this objective in mind a DistLM (Distance based linear models) test was performed. This model describes the patterns of variation in fish abundances using the environmental variables to reach the best result. To understand if there is a correlation between the biota and each of the environmental variables it is important to analyse the values from the marginal tests. The best solution from this test using the AICc criteria (Akaike information criterion), that gives the maximum value of the likelihood function to the model, was a group of four environmental variables that includes: 1) water temperature; 2) wave period; 3) air temperature and 4) wind speed.

Altogether these four environmental variables explain 53,4% (R^2) of the variation in *Diplodus* spp. abundances over the period 2009-2018. The results of this model are represented in figure 15.

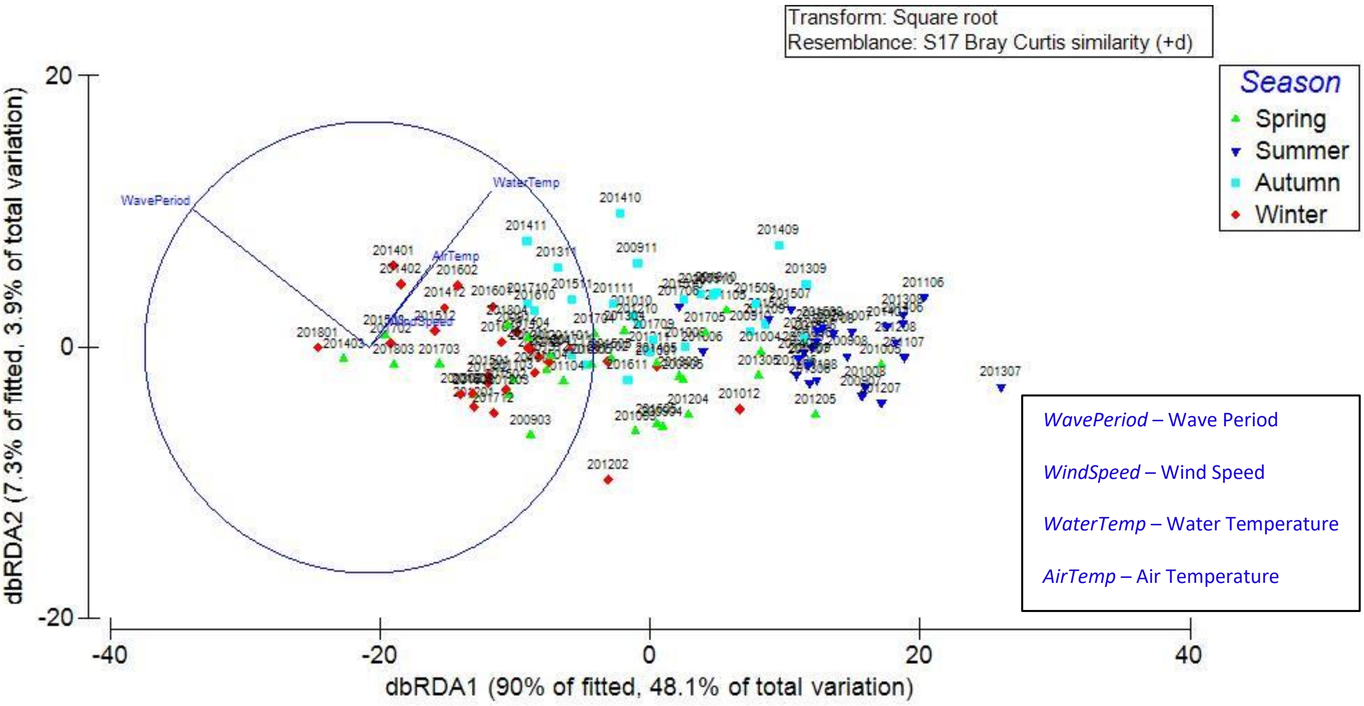

Figure 16 - Representation of a multivariate analysis relating *Diplodus* spp. abundances and the four most important environmental variables selected by the DistLM model.

2.4.4 Relevant environmental data

Reducing the dimensionality of the environmental variables without significantly losing the explanatory power of the model is crucial to interpret results and to test predictive hypothesis in the future. In the following sections the environmental variables selected by the models described above are presented in detail. Although the NAO index was not one of the variables chosen by those models I consider important to present this data, due to its influence over a vast array of oceanographic and climatologic processes. Additionally, it is important to clarify that the NAO index tested before corresponds to its values along the entire year. The data presented below addresses only winter and spring NAO which are expected to affect directly the reproduction success and the settlement of juveniles in their nursery areas, respectively.

2.4.4.1 The North Atlantic Oscillation – NAO

The graphic bellow (figure 16) displays the NAO index values over the past 67 years. During this large temporal window there are specific periods with distinctive values that are worth to mention. For example, 2012 and 2016 presented the highest winter NAO values over the past 67 years. Since 2005 we had experienced some of the lowest spring NAO values, only comparable in the late fifties, and in 2017 we find one of the lowest values from the past fifty years.

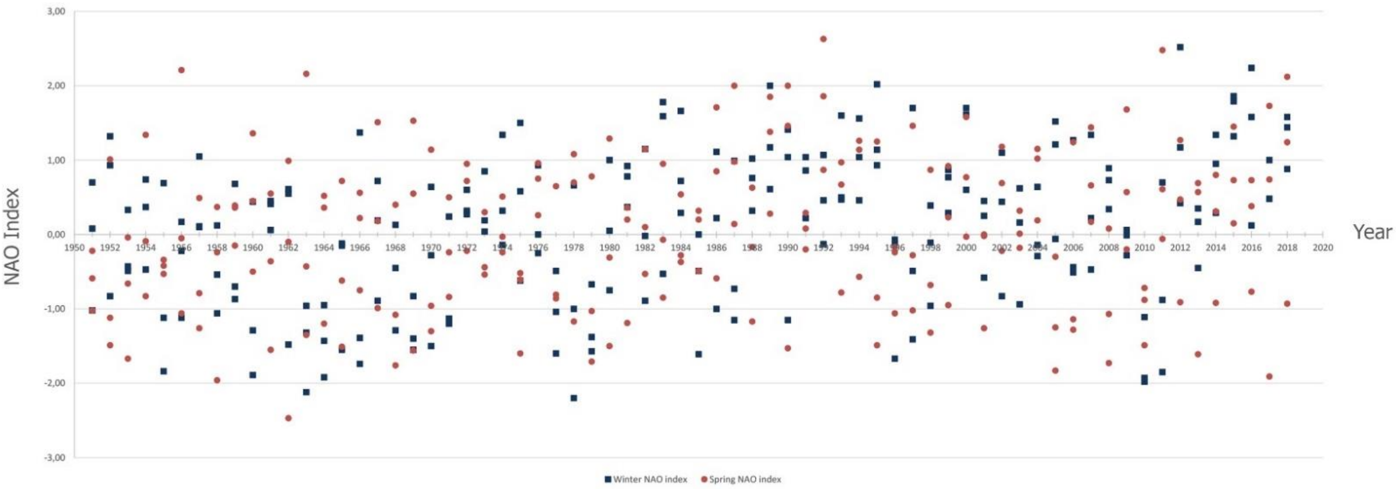

Figure 17 – Winter (blue squares) and Spring (red dots) NAO index values since 1955.

Focusing more carefully on the years related to the biological survey reported in this work some interesting patterns emerge. Figure 17 clearly shows that in 2012 there was an inversion between the spring and winter NAO index values. This is remarkable because the onset of a multiyear declining trend in the abundances of *Diplodus* spp. also started in 2012.

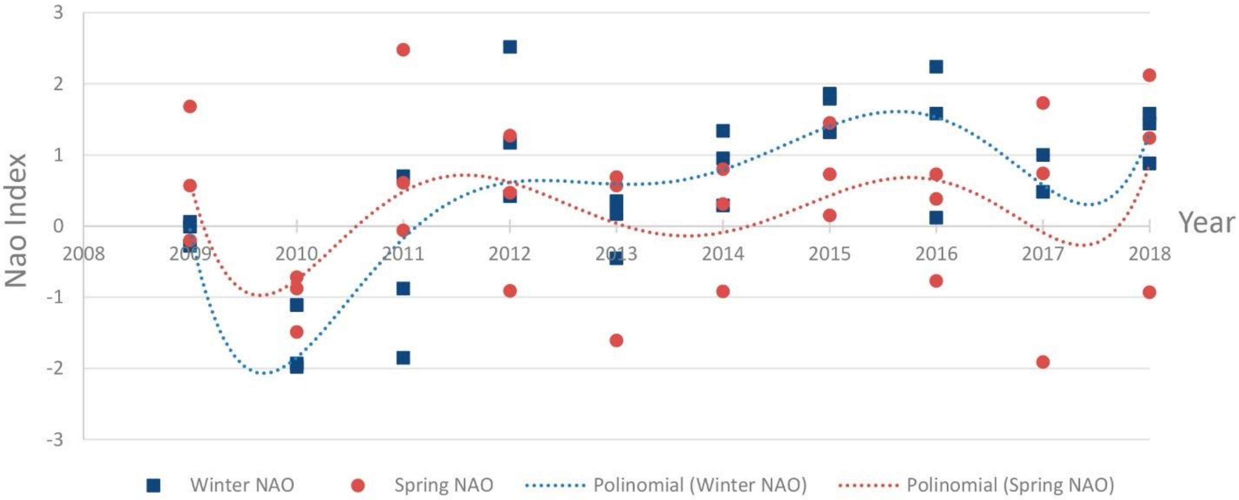

Figure 18 – NAO index polynomial trends for Winter (blue squares) and Spring (red dots) from 2009-2018. Winter and Spring seasons are represented by the mean values of each month.

2.4.4.2 Water Temperature (SST)

While working with marine species, especially considering coastal species that use the intertidal as nursery areas, it is important to consider environmental variables such as sea surface temperatures (SST). The SST in the study area (figure 18) show that in 2012 all temperature values seem to be quite low, with the lowest reported winter temperatures (13ºC).

On the other hand, the highest summer and autumn temperatures were registered in 2014. This was also the year with the highest variation amplitude between winter/spring and summer/autumn temperatures.

In 2016 an unusual phenomenon occurred with the winter temperatures (mean 14.7ºC) reaching higher values than the spring temperatures (mean 13.2ºC). In fact, focusing on spring temperatures there is an overall decline from 2010 (mean 15.9ºC) until 2016 (mean 13.2ºC).

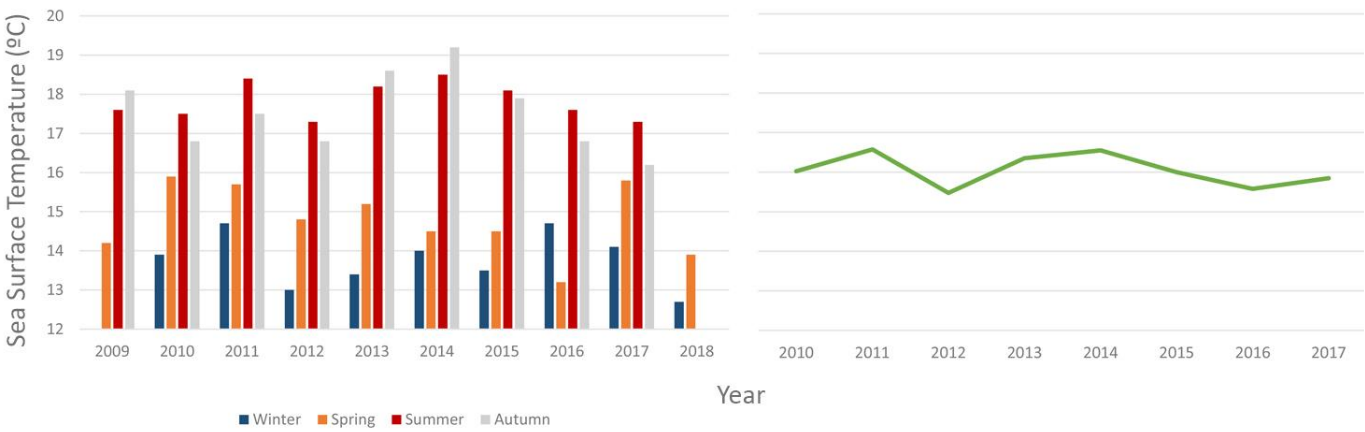

Figure 19 – Seasonal mean sea surface temperatures (SST) registered from 2009 to 2018. The green line shows the yearly variation of mean SST.

2.4.4.3 Air Temperature

Air temperature is an environmental variable that is directly related with the water temperature, especially considering superficial water in intertidal areas. The mean values for each season and each year are presented in figure 19. Since the beginning of this survey, 2012 was the year with the lowest yearly mean temperature (16.2ºC). Comparing each season, autumn temperatures present higher variations, with a maximum amplitude ranging from 17.7ºC to 20.5ºC.

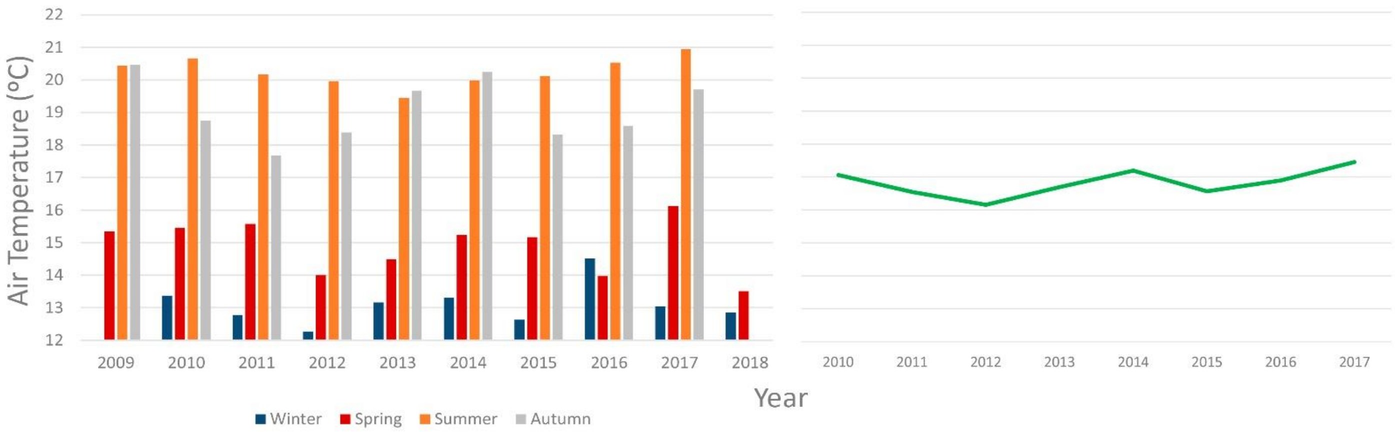

Figure 20 – Mean seasonal and yearly air temperature registered from 2009-2018. The green line shows the yearly variation of mean air temperature.

2.4.4.4 Wave Period

Yearly and seasonal variation in the wave period estimated for the study area is shown in figure 20. Once again, 2012 shows the lowest yearly mean values for this variable (10.0 s) that steadily increase until 2014. The highest values are reported in winter, with an interannual peak in 2014 (13.5 s). The lowest values are reported in summer, with its lowest value in 2012 (8.3 s).

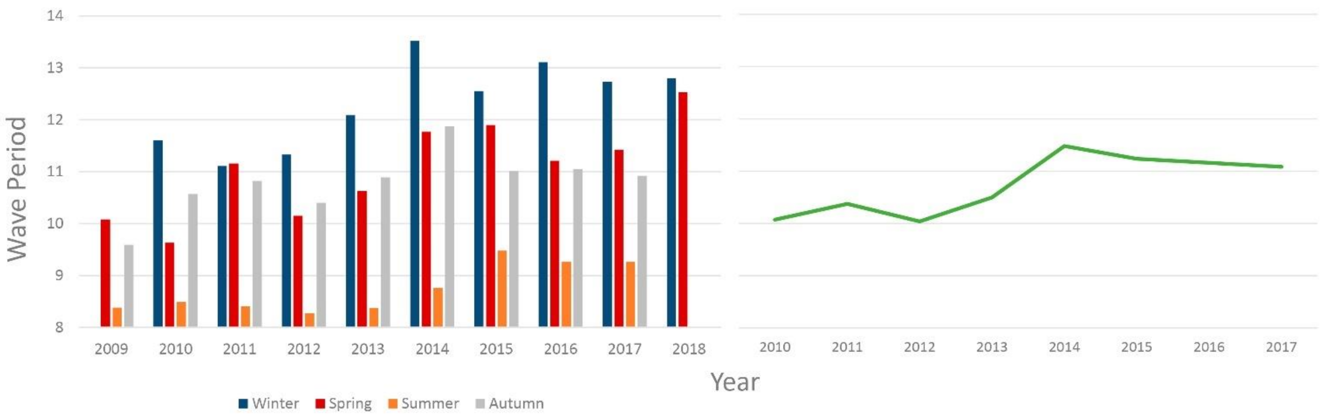

Figure 21 - Mean seasonal and yearly values for wave period (in seconds) registered from 2009-2018. The green line shows the yearly variation of mean wave period values.

2.4.4.5 Wind Speed

Wind speed is an environmental variable that can strongly affect surface sea conditions. Data presented in figure 21 shows mean values for wind speed estimated for each season of each year. The general trend is similar to the one described before for other environmental variables with the lowest values reported in 2012 (8.9 kn). The highest values registered along a period of 10 years peaked during the winter of 2014 (12 kn).

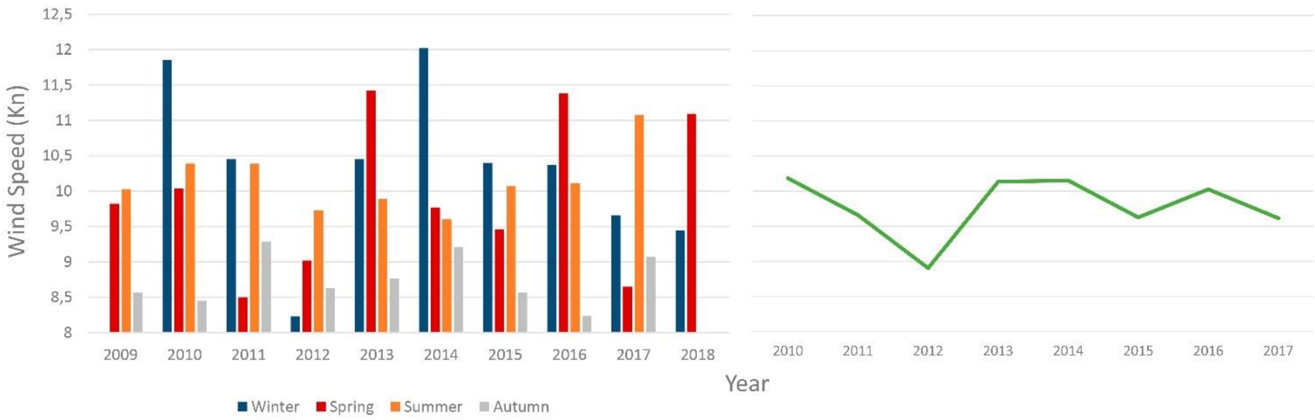

Figure 22 - Mean seasonal and yearly values for wind speed (in knots) registered from 2009-2018. The green line shows the yearly variation of mean wind speed values.

2.4.4.6 Non-resident piscivore species (Dicentrarchus punctatus)

An unusual phenomenon that is important to report was observed in 2016 with an explosive increase of *Dicentrarchus punctatus* juveniles in our study area, which could not be explained so far. This is a voracious piscivore species that prey on the *Diplodus spp*. and other small coastal organisms. Their abundances over these 10 years were regularly low but in 2015 their numbers start increasing to the hundreds and in 2016 they peaked with more than 18000 individuals reported during summer surveys (figure 22).

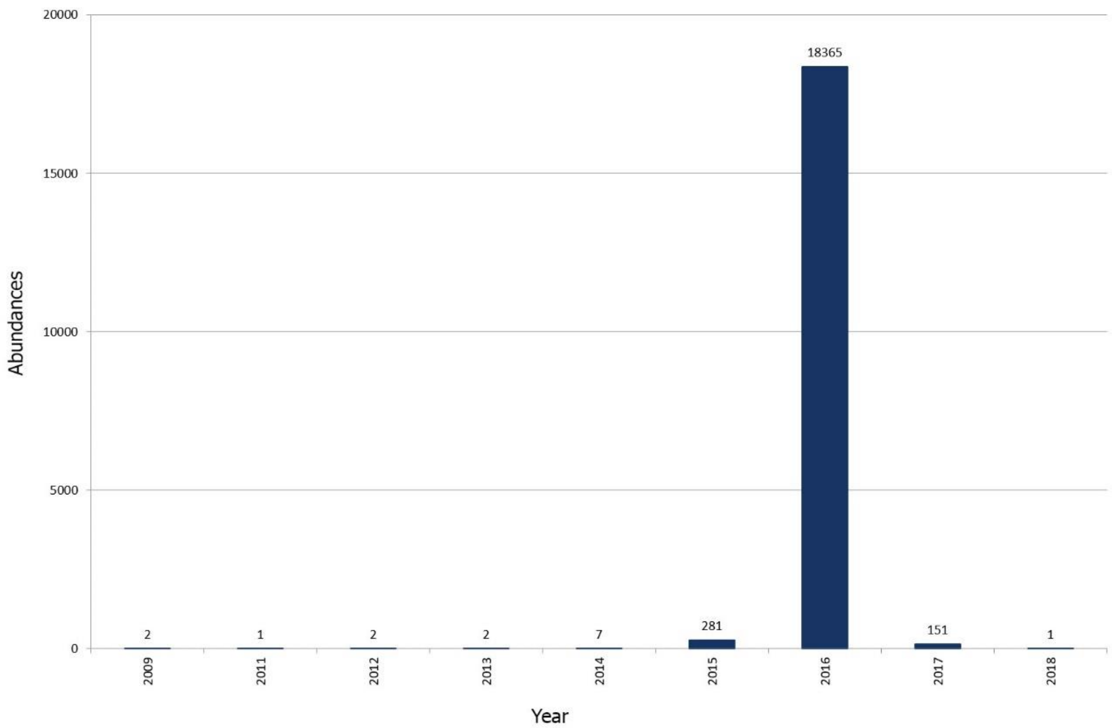

Figure 23 – Overall abundances of *Dicentrarchus punctatus* registered from 2009-2018.

This event had a huge impact on *Diplodus* spp. abundances. These predators arrived during summer, in July, and rapidly increased their numbers with a maximum observed in August. They rapidly consumed the young settlers of several fish species, including *D. sargus* and *D. vulgaris*, initiating a sharp decline until November when they were no longer observed in the study area. Therefore, it is expected that the scarce abundances of *Diplodus* spp. found in 2016 are mostly explained by the explosive increase of these predator species and not by other environmental variables.

2.5 Discussion

2.5.1 *Diplodus* spp. response to local environmental conditions

Long-term studies provide interesting results over short-term ones as they pinpoint periods of rapid ichthyofaunal changes interspersed with long periods of apparent stability (Henriques et al., 2007). Differences in species abundances also become evident when analysing fish landings over the years (Gamito et al., 2013). According to Teixeira et al. (2014) these trends can be potentially affected by climate change and can be related to environmental variables, such as SST (Gamito et al. 2015) or wind speed (Klein et al., 2018).

During 2012 there was an inversion between the winter and spring NAO indexes apparently affecting multiple environmental parameters. Environmental variables such as: SST, wind speed and direction, rain fall, river runoff, upwelling index and chlorophyll concentrations can explain, to a limited extent, the abundances of fish populations (Henriques et al., 2007; Vinagre et al., 2009; Gamito et al., 2013; Bento et al., 2016; Klein et al., 2018).

Considering *Diplodus* spp. abundances, there has been a drastic decrease in the number of new settlers from these fish populations since 2012 (Duarte-Coelho et al., 2018). Fish landings in nearby fishing ports have also been diminishing dramatically for the same period (DGRM Cascais 2.4t in 2011 to 1.3t in 2012).

Considering the environmental variables available for this study the best biological model (DistLM) explains 52% of the variability in our sampling area using SST, wave period, air temperature and the wind speed altogether. Although this model reflects only about half the variability in our data, when compared to other studies using similar environmental variables (e.g. Teixeira et al., 2015 and Klein et al., 2018), they are well within their range values and both stress the importance of testing a large array of environmental variables.

In the Mediterranean it was already known that *D. vulgaris*, *D. sargus*, *D. cervinus* and *D. puntazzo* reproduction and settlement occur in sequential periods along the year (Vigliola et al., 1998). First settlers of *D. vulgaris* and *D. sargus* occur during spring, *D. cervinus* during late spring/early summer and *D. puntazzo* in late summer/early autumn (García-Rubies & Macpherson, 1995). Being similar congeneric species, two hypotheses could explain the sequential onset of their reproductive and settlement periods: 1) water temperature and other environmental preferences that are met in different periods along the year and; 2) habitat/food competition, which would favor a settlement displacement in nursery areas. All four species present subtropical affinities, although with different northern distribution limits (Froese & Pauly, 2018). This may show their inherent capability to withstand different (warmer or cooler) water temperatures.

D. vulgaris northern limit is 50ºN, followed by *D. sargus* northern limit at 48ºN, *D. cervinus* reaches 47ºN and *D. puntazzo* is only reported until 42ºN (Froese & Pauly, 2018). *D. vulgaris* is the species with the distribution range that extends further north being expected that they can endure colder water temperatures. As expected this was the species whose juveniles were reported to settle earlier in spring. On the opposite side, *D. puntazzo*, is the species with a southernmost distribution range (its northern limit is the Portuguese coast) and therefore it is expected to have preference for warmer water conditions. This was also the species whose juveniles only settle in late summer or even during autumn.

The second hypothesis reported above considers interspecific competition. As shown by Sala & Ballesteros (1997), at least three of these species (*D. sargus*, *D. vulgaris* and *D. puntazzo*) may have habitat overlapping at younger ages when juveniles are still on their nursery grounds. Although *D. sargus* shows a clear preference for the surge zone and *D.vulgaris* is more likely to be found in deeper waters, these two habitats overlap in shallow waters with a gentle slope (Sala & Ballesteros, 1997). Our study also showed evidence of that when looking at the abundance fluctuations of *D. sargus* and *D. vulgaris* settlers during the years of 2009, 2012 and 2016. During these years there was a decline in *D. sargus* numbers and an increase of *D. vulgaris* abundances within our sampling area. It was as if they were occupying the vacant space that was left open.

In 2014 both species experienced a drastic decline in their numbers. This was one of the warmest years with the highest yearly SST mean which may favor southernmost species with a preference for warmer waters. Although not suitable for these two *Diplodus* species, conditions were perfect for *D. puntazzo* that reached its higher abundances over a 10 year period. This warming pattern is even more evident when comparing summer and autumn, respectively, the reproduction and settlement seasons for this particular species.

Regarding *D.cervinus*, these species peak abundances were reached in 2011 which was also a year with one of the highest SST values amongst the sampling years. The difference between

2011 and 2014 (the other warm year) is that in the later, the high temperatures that contributed to this high yearly mean were felt during autumn and in 2011 they were felt during summer. This may show the importance of high SST values felt during summer for the reproductive success of *D. cervinus*.

On a larger framework our results also show that the genus *Diplodus* represents an excellent model to track environmental changes over an extensive period of time.
Since the settlers of *D. sargus* alone make up more than 90% of the total abundances, we will now give particular focus to this species. *D.sargus* is an extremely resilient species regarding temperature (Vinagre et al., 2009), wide distribution range (Froese & Pauly, 2018) and opportunistic feeding habits (Sala & Ballesteros, 1997). Even though they can endure such a wide array of environmental conditions (Vinagre et al., 2009) their interannual abundances vary greatly in our study area. Could their reproductive success be limited to a small scope of intermediate SST values with a sharp population contraction whenever these conditions are not met? Looking at how the settlement period of this species contracts and expands over the years could provide additional insights. However, during the warm 2014, the settlement period was extremely short, lasting only 5 months, and in the other warm year 2011, this species had the longest settlement period, lasting for 8 months. The months when they reach peak abundances in recruitment are also very variable (May through July) over the years, where no direct relationship between these fluctuations and monthly water temperature patterns seem to stand out. The drop in 2016 abundances can be explained by the unprecedent number of piscivore species (*Dicentrarchus punctatus*) present in the area during that same year.
For the reasons described above we can conclude that more complex relations are at work. Most of these patterns are only noticeable when long lasting observations take place, reinforcing once again the importance of long-term studies for monitoring rocky coastal areas.

2.6 Conclusions and future perspectives

It will be interesting to complement our data with more environmental variables such as the rain fall, the Tagus river runoff, the upwelling index and variation in chlorophyll concentrations (Dias et al., 2016; Klein et al., 2018) to evaluate if we are able to improve our model.

It will also be interesting to track how *D. puntazzo* reproduction and settling cycles react to a warming ocean era (Carson & Harrison, 2008) especially in a frontier zone of warm and cold temperate waters such as the Portuguese coast. In 2006, Vinagre et al. (2010) either failed to detect it or *D. puntazzo* had not reached as far north as we find it today. Is its northern limit moving poleward with warming sea conditions or are we facing a cycle that passed undetected due to the duration of the sampling period?

It would be of high importance to obtain similar data in different regions along the Portuguese coast. A number of monitoring stations at different latitudes would provide additional data on the environmental preferences and ecological relationships between these four *Diplodus* species. Such information would be of extreme importance for Portuguese seabream fishing stock management.

2.7 Acknowledgments

We would like to thank DGRM for historical data on fish landings and WindGuru for historical environmental data. Câmara Municipal de Cascais e EMAC/Cascais Ambiente for sponsoring the project Monitorização da AMP Avencas (from February 2017 until August 2017). Oceanário de Lisboa for engaging and supporting Project Rebreathe, financing part of this long-term monitoring program. And MARE-ISPA for the use of their equipment and working space.

3. Discussion and general conclusions

3.1 Main conclusions

At first when we started this study, we thought that we could predict what would happen to the adult populations of *Diplodus* spp. by following the recruits of these species. Now, looking back at our results and comparing them to fish landing data from the surrounding areas, we suspect that fish landings may also anticipate the abundances of juveniles in their local nursery grounds. Although additional data is needed to clarify this issue there is an underlying assumption that needs to be addressed: it is possible that the recruitment of this species to their nursery grounds is more dependent on the local adult population than what it was assumed before. If this is true and local fish landings are dependent on local juvenile recruitment and vice-versa, then egg and larval dispersion may reveal to be much less effective than what was previously assumed. This has direct conservation implications because replenishment of local populations from adjacent areas may reveal to be slower, leaving population recovery mostly dependent on occasional years of (local) successful reproduction and recruitment.

The sharp decline of local adult fish landings and *Diplodus* spp. recruits in their nursery grounds during 2012, shows that there must have been an array of biophysical variables that compromised local populations.

These long-term studies can also allow us to keep track of climate changes, especially in our study area where we find a transitional zone of cold and warm temperate species. It could enable us to highlight interannual trends, such as the one reported in this work, and even "predict" what will happen to these local communities in the years to come enabling us to take management actions accordingly. As these fluctuations in marine communities can have economic impacts at local and regional scales and the species studied in this work are of high commercial importance for local and artisanal fisheries, it is important to understand the combined effects of environmental pressures and fisheries. In addition, we also presented data on a sudden increase of a population of piscivore predators (*D. punctatus*) that would have passed unnoticed if our sampling protocol was only targeting *Diplodus* species. These results underline the importance and the interaction between environmental, human and ecological impacts on local *Diplodus* spp. populations calling the attention for the complexity associated to the management of marine resources.

With continuous monitoring programs at the community level, we can add information that could become useful at larger geographic scales. To undercut the pitfalls that result from this complex, and to a large extent unpredictable, interactions, it is crucial to protect significant patches of habitat that could later replenish the surrounding communities and fishing areas. A group of small MPA's that could act as breeding and nursery grounds for these species with the expected spillover effect to adjacent areas would represent a major step forward for the preservation of inshore communities along the Portuguese coast. These MPA's could also translate into a network of monitoring stations along the Portuguese coast.

4. Appendix I

4.1 Preliminary analysis

4.1.1 The commercial importance of the genus *Diplodus*

Information regarding *Diplodus* fish landings in the surrounding ports and within continental Portugal is presented below to provide an overview of their importance for coastal fisheries. Seabreams are an important commercial asset for Portuguese costal fisheries with fish landings fluctuating between 724 to 866 tons in the last 9 years (Figure 23a) which represents a mean revenue of 3,3 million euros per year (Figure 23b).

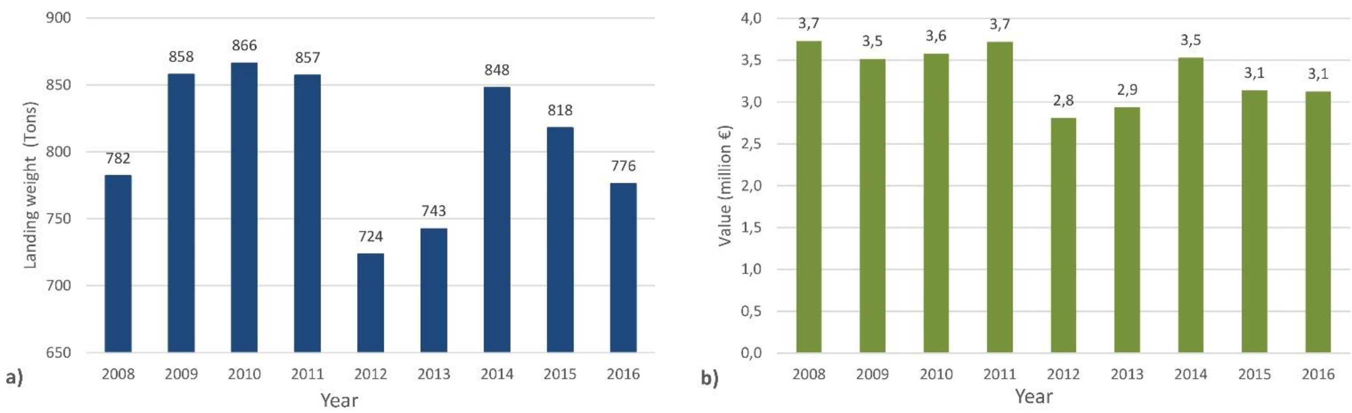

Figure 24 – a) Total landing weight (tons) of *Diplodus* spp. (data includes the four species presented in this study, unidentified *Diplodus* sp. and also occasional reports on *Diplodus bellottii*) in continental Portugal per year (DGRM); b) Income in millions of euros of the total landings of *Diplodus* spp. in continental Portugal per year (DGRM).

In Continental Portugal seabream landings do not follow any stable trend over the past 10 years. Instead, data shows a fluctuating pattern with the lowest value observed in 2012 and landings dropping since 2014 (figure 23a). It is worth to note that this decrease in the landings of seabreams could be a consequence of a variation in CPUE. Although we were not able to calculate this index, no significant changes were observed in the number of vessels, number of fisherman or fishing permits throughout the last 9 years (figure 24).

Figure 25 – Interanual variation of different variables related with fishing effort: Blue – number of listed fisherman per year; Red – number of registered vessels per year; Grey – number of fishing permits issued per year (INE, 2017).

Given these results in seabream fisheries it is important to highlight that in Portugal the minimum legal size for capture is 15 cm total length (TL) (Abecasis et al., 2009). This is strikingly incongruent with the fact that some of the most common species (e.g. *Diplodus sargus*) only fully mature with a mean size of 17 cm (TL) (Gonçalves et al., 2003; Morato et al., 2003). This fact alone paves the way for unsustainable overfishing/resource depletion and represents a singular example of the need to bring scientific data into the current legislation.

4.1.2 Genetic barcoding - DNA sampling and taxonomic identification

Field work

To confirm the identification of the small *Diplodus* juveniles (<1.5 cm) found in the study area, a small subsample of 60 *Diplodus* spp. individuals were collected to perform genetic validation tests of the specimens captured. The 60 samples collected during field work over a 10 year period varied from the entire fish, for morphological characterization when smaller individuals, to fin clips of larger ones. Fish were previously anaesthetized with MS222 and tissue samples were immediately labelled and preserved in ethanol following the recommendations of the ethics committee at ISPA.

Laboratory work

Total genomic DNA was extracted from all samples with REDExtract-N-Amp Kit (Sigma-Aldrich) following the manufacturer's instructions. A fragment of 16S rDNA was amplified with the following primers, 16SFor (5'- AAGCCTCGCCTGTTTACCAA -3') and 16SRev (5' – CTGAACTCAGATCACGTAGG – 3'), as described in Almada *et al* (2005), in a total volume of 20µl (10µl of REDExtract-N-Amp + 0,8µl of 16SFor + 0,8µl of 16SRev + 4µl of DNA + 4,4µl of ultra-pure water). The amplification process was conducted as follows: one cycle of 4 min at 94° C, 30 cycles of [1 min at 94°C,1 min at 55°C and 1 min at 72°C], and one last cycle of 10 min at 72°C. The PCR was performed in a VWR Doppio and the PCR products were purified and sequenced at Stab Vida.

DNA analysis

Once the chromatograms arrived from StabVida they were analysed and edited using CodonCode Aligner (Codon Code Corporation). The edited sequence was then inserted in the Nucleotide Blast section of the NCBI site (https://blast.ncbi.nlm.nih.gov/Blast.cgi) in an extensive search of highly similar sequences. The results were carefully analysed and the taxonomic identifications confirmed. The genetic barcoding results corroborated the field identifications.

4.1.3 Recruiting patterns and moon cycle

It is commonly assumed that the moon cycle can influence the activity patterns and life cycles of marine organisms. This relationship has been related, among others, to the avoidance of predators by larvae and juvenile fish (Sponaugle & Cowen, 1994). Taking this information into account and hypothesizing that juveniles use intertidal areas as nursery areas to escape larger predators in subtidal areas, the onset of the settlement period and the peaks of settlement for each species where analysed for each year. A clear preference for new moon periods was expected in order to avoid being visually detected by their predators.

Considering the arrival of the first recruits, *D.sargus* first settlers show preference for the new moon phase in six out of ten years (table 1). Apparently, the remaining species from the same genus show no preference between these two moon periods. However, it is important to note that *D. sargus* are the most representative species within the sampling area. As a consequence, the absence of such pattern for other *Diplodus* species could result from the lower number of individuals sampled in these intertidal areas.

Table 1 - First yearly occurrence of young settlers per month and per moon cycle for each species studied. An asterisk shows a year with no individuals present from the 0⁺ cohort. Grey shades are the months that still were not sampled at the time of this work.

	D.sargus		***D.vulgaris***		***D.cervinus***		***D.puntazzo***	
Year	Month	Moon	Month	Moon	Month	Moon	Month	Moon
2009	5	Full Moon	3	New Moon	3	New Moon	10	New Moon
2010	4	New Moon	3	New Moon	4	Full Moon	9	New Moon
2011	4	New Moon	4	New Moon	5	New Moon	11	New Moon
2012	3	New Moon	5	Full Moon	6	New Moon	11	Full Moon
2013	5	New Moon	6	Full Moon	10	New Moon	8	Full Moon
2014	5	New Moon	5	New Moon	7	New Moon	9	New Moon
2015	5	Full Moon	5	Full Moon	6	Full Moon	10	New Moon
2016	5	Full Moon	4	Full Moon	6	New Moon	*	*
2017	4	New Moon	5	Full Moon	6	Full Moon	12	Full Moon
2018	5	Full Moon	5	Full Moon				
Mode	**5**	**New Moon**	**5**	**Full Moon**	**6**	**New Moon**	**9-11**	**New Moon**

In addition, supporting the results reported by other authors, different *Diplodus* species arrive to intertidal areas in different months, following a sequence that starts with *D. vulgaris* in early spring, *D. sargus* in late spring, *D. cervinus* in the summer and *D. puntazzo* in the autumn (García-Rubies & Macpherson, 1995; Morato et al., 2003; Dias et al., 2016).

In 2016 the usage of the study area by juveniles of different species was affected by a major change in the local fish community. This change is important to explain the lack of data on *D. puntazzo*, the species whose juveniles settle later along the year and was previously addressed in this work.

Yearly abundance peaks of the four species studied also show no pattern that can be assigned to new or full moon periods (table 2). In other words, the abundance peaks of young juveniles on intertidal rocky areas do not follow any fluctuation pattern that can be related to the moon cycle. This probably occurs because these fish remain in these intertidal habitats until they are large enough to adventure into subtidal areas.

Table 2 – Yearly maximum abundances per month and per moon cycle for each species studied. An asterisk shows a year with no individuals present from the 0^+ cohort. Grey shades are the months that still were not sampled at the time of this work.

	D.sargus		***D.vulgaris***		***D.cervinus***		***D.puntazzo***	
Year	Month	Moon	Month	Moon	Month	Moon	Month	Moon
2009	5	Full Moon	5	New Moon	12	Full Moon	10	New Moon
2010	5	Full Moon	8	New Moon	8	New Moon	11	Full Moon
2011	5	Full Moon	6	New Moon	7	Full Moon	11	New Moon
2012	5	New Moon	5	Full Moon	7	New Moon	12	Full Moon
2013	5	Full Moon	7	Full Moon	10	New Moon	10	Full Moon
2014	7	New Moon	6	Full Moon	11	New Moon	12	Full Moon
2015	7	Full Moon	8	Full Moon	7	Full Moon	11	Full Moon
2016	6	New Moon	5	Full Moon	7	New Moon	*	*
2017	5	New Moon	5	New Moon	6	Full Moon	12	Full Moon
2018	5	Full Moon	5	Full Moon				
Mode		**Full Moon**	**5**	**Full Moon**	**7**	**New Moon**	**10-11**	**Full Moon**

To evaluate if there are differences in the overall abundances of *D. sargus*, the most representative species in these intertidal areas, consecutive samplings in new and full moon periods were compared for the entire duration of this study. No fluctuating pattern was detected (figure 25) resulting in the conclusion that although *D. sargus* juveniles preferentially settle during new moon periods in the spring, they remain in their settlement grounds until they are large enough to move into subtidal areas in the summer.

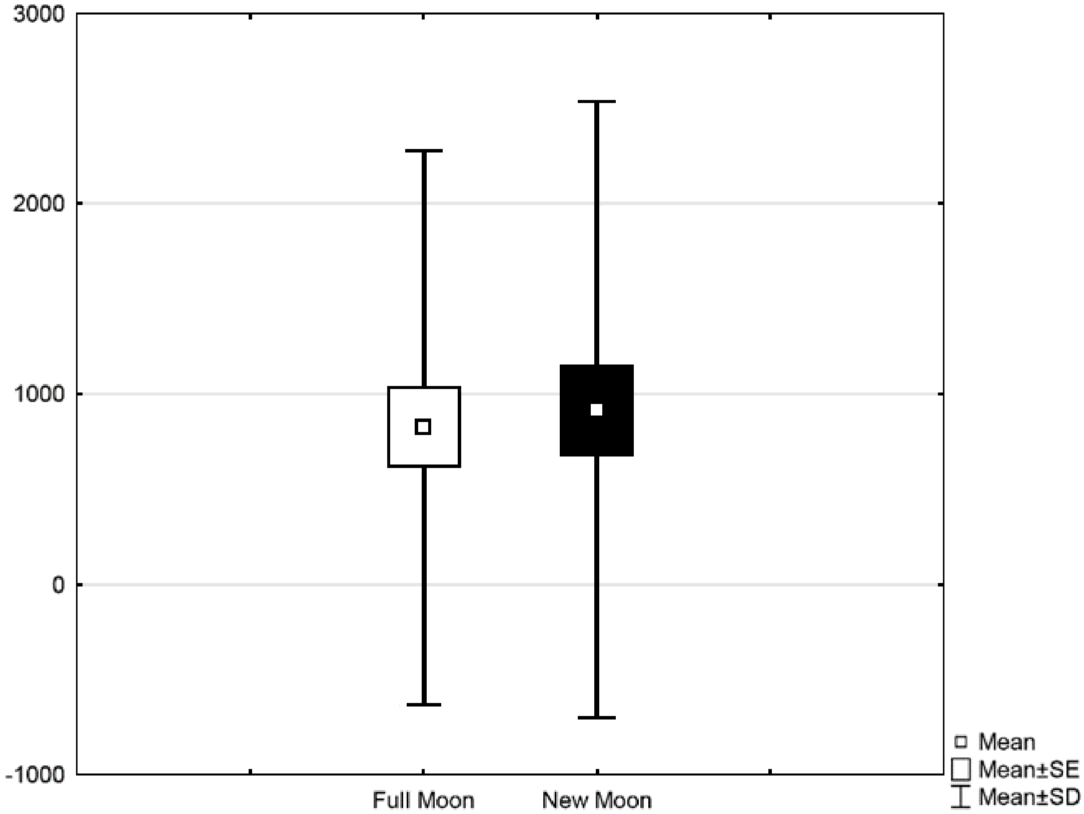

Figure 26 - Comparison of the monthly abundances of juvenile *D. sargus* along the entire study registered in full moon and new moon periods (Wilcoxon Matched Pair test analysis Z=0,36 and p=0,72) (Statistica Version 13.3 – TIBCO Software Inc.).

4.1.4 Occupation patterns of intertidal areas by juvenile *Diplodus* spp.

Day vs. night sampling

The use of space can vary widely along the day and along the year. To determine the occupation pattern of *Diplodus* spp., samplings were performed during day and night from March 2009 until July 2010. To evaluate the importance of intertidal rocky areas along a period of 24h, abundances registered during the day were compared with the abundances registered during the night in the same date. The results are shown in figure 26.

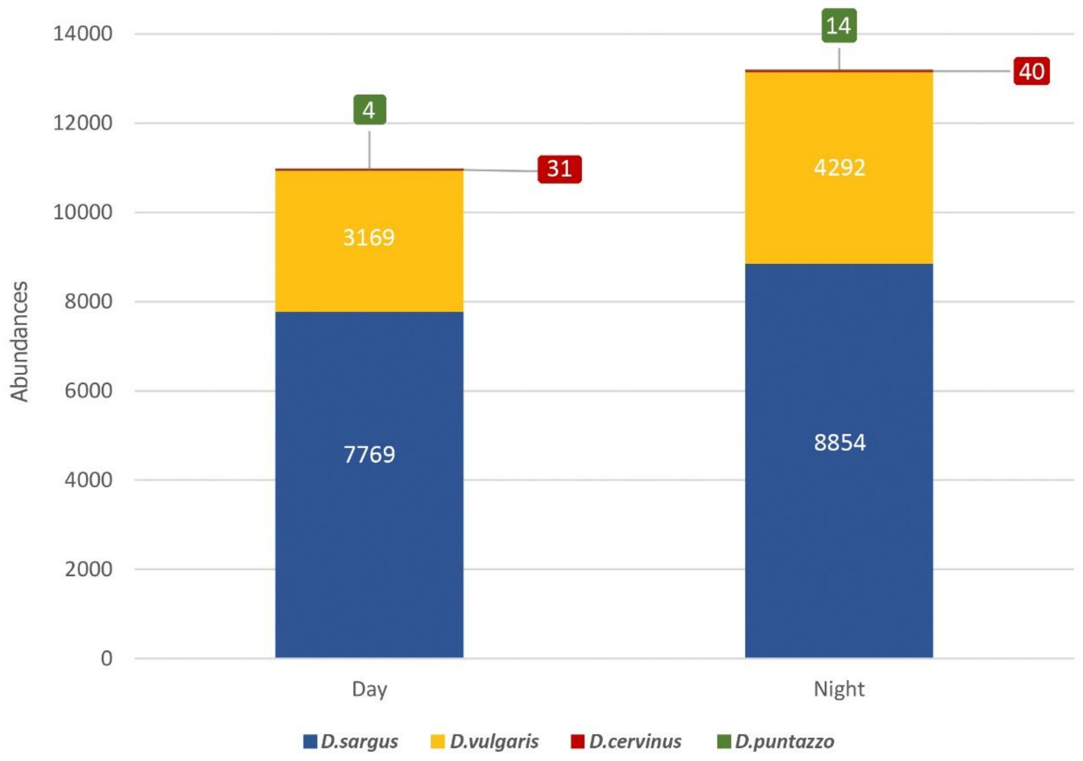

Figure 27- Total abundances of *D. sargus, D. vulgaris, D. cervinus and D. puntazzo* during day and night along the sampling period.

All four species show slightly higher abundances during the night, although no significant statistical differences were detected (table 3).

Table 3 – Results of the Wilcoxon Matched Pairs Test comparing the abundances registered in the same day for each *Diplodus* species from March 2009 to july 2010 *(Statistica Version 13.3 – TIBCO Software Inc.)*

	Z	p-value
D.sargus Day vs Night	0,62	0,538
D.vulgaris Day vs Night	0,49	0,626
D.cervinus Day vs Night	0,15	0,878
D.puntazzo Day vs Night	1,28	0,201

Non-significant differences in day and night abundances could be due to factors like:

- It is more difficult to sample juvenile fish during the day due to the reflection of light on the water surface along with their cryptic behaviour and morphology making the identification and quantification of young settlers much harder;
- During the day these fish are more active and can, therefore, easily evade the hand nets while sampling;
- The sampling area is a marine protected area (MPA) but also a public beach with a very high anthropogenic pressure especially during the summer, affecting the intertidal areas that are sampled during the day.

These factors and the absence of differences between the abundances registered in both periods lead us to concentrate exclusively on night sampling.

Channel I vs. channel II sampling

Field surveys were performed in two distinct channels that cross the intertidal platform in an inshore/offshore direction. Channel I topography, with rock platform and sandy substrate, is less complex than channel II, with rocks and boulders providing shelter to larger individuals belonging to a diversified array of species.

Channel I abundances were consistently higher during the entire sampling period (figure 27), probably because potential predators of juvenile *Diplodus* spp. could be detected at greater distances due to its less complex topography.

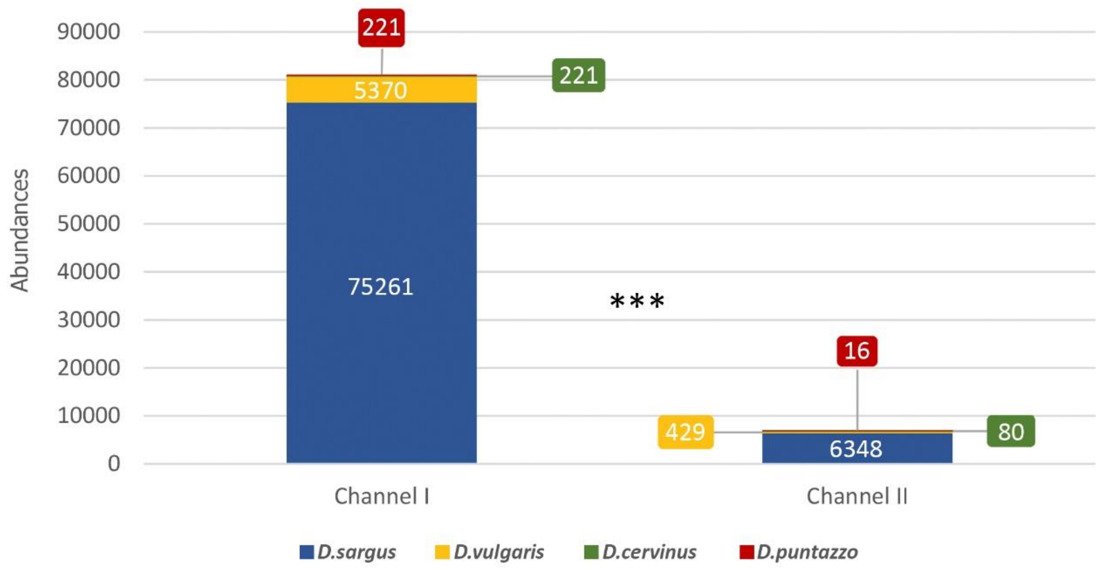

Figure 28 – Total abundances of *D.sargus, D.vulgaris, D.cervinus and D.puntazzo* in Channel I (less complex topography) and Channel II (more complex topography) in night surveys along the entire sampling period. * show that there are significant differences between them.**

These differences were analysed with a non-parametric matched pairs Wilcoxon test and the results were highly significant for the four *Diplodus* species analysed here (table 4).

Table 4 - Results of the Wilcoxon Matched Pairs Test applied to *Diplodus* species abundances in the two channels that were samples in this study

	Z	p-value
D.sargus Channel I vs Channel II	7,22	0,000 ***
D.vulgaris Channel I vs Channel II	3,72	0,000 ***
D.cervinus Channel I vs Channel II	4,61	0,000 ***
D.puntazzo Channel I vs Channel II	5,47	0,000 ***

Although it is shown in literature that these juveniles settle on rocky shores and rocky substrates (La Mesa et al., 2011; Abecasis et al., 2014) no differences in the juvenile microhabitat where previously compared in the Atlantic (but see Bussotti & Guidetti, 2011, for the Adriatic sea). Although one could assume that small fish prefer the apparent security of a complex rocky bottom, it is also important to understand that these demersal fish aggregate in large shoals and other benthic cryptic piscivorous species (e.g. Gobiidae, Blenniidae and Cottidae) may take more advantage of this habitat complexity to predate on them.

5. Appendix II

5.1 Limitations of this work

5.1.1 The problems of using SST on the intertidal area

When using SST there are some limitations that one should be aware of. Some of them will be listed below:

- When taking measurements in the field the water temperature can vary greatly in the surrounding area of just a few tens of metres because of the water column depth. Also, the data gathering is widely dispersed in time, as they are done only when the field surveys are performed, in our case, only every 15 days.

- The same problem happens when working with satellite data, as the value for each cell will be the mean of the temperatures within it. This becomes an even bigger issue when working with nearshore values, as most of the times the cell you need is located partly in land and over the sea, the mean will consider the higher values found in land and give erroneous values for the sea surface temperature in that area. Another problem is that with the evolution of the satellite sensors technology, results from 10 years ago are much less precise and difficult to compare with the values gathered from the new satellites.

- The best way to get accurate SST values is to have a few data loggers dispersed around your study area as they can be set to record values with the time interval you want/need. Although, despite the fact that these are quite expensive, it is also very laborious and time consuming to retrieve the data.

5.1.2 Values of the environmental variables come from a model

Some of the values we have for the environmental variables (the ones from WindGuru) are estimated from different sources and are based on computer models not representing real life values. In the future we intend to use real values measured within or near the study area from more accurate sources.

5.1.3 Why the use of an intertidal type of monitoring instead of underwater visual census

As previously stated in chapter 1.3 the area we are monitoring is moderately protected from the predominant northern winds and is still strongly affected by the Tagus runoff. These two factors combined make diving conditions very poor during most of the year. Visibility is very poor, there are strong underwater currents, and because of the long rocky platforms, it is difficult to approach by boat. On the other hand, having long rocky platforms makes it perfect for an intertidal type of monitoring. This is a cheaper method, easy to perform with no extra skills required (like scuba diving) and easy to assess as fish become confined to a stretch of 110m of shallow water. This makes it a perfect monitoring station for a long-term study. Although, the best way to perform this monitoring work would be to have the intertidal samplings along with the underwater diving census, due to the reasons given before this could not be achieved.

5.1.4 Some difficulties of working with the genus *Diplodus*

5.1.4.1 Morphological similarities between juveniles of different species

For the untrained eye most of the recently settled and small individuals from different *Diplodus* species look alike and can be easily misidentified. In figure 28 we show individuals of the four species monitored in this survey that already show their characteristic colour pattern. However, some individuals can present very similar patterns. In the beginning of this monitoring program, it was sometimes hard to distinguish *D. sargus* from *D. vulgaris* since they usually overlap their settlement period. *D. cervinus* and *D. puntazzo* are also almost identical at a younger age, but their recruitment season does not overlap making their identification easier. However, this was not clear during the first sampling years, and therefore taxonomic identifications of several individuals were confirmed using DNA markers for genetic barcoding. A photographic record of all samples was also registered.

This genetic confirmation is still being used for taxonomic identifications whenever the morphological identifications are not clear during field surveys.

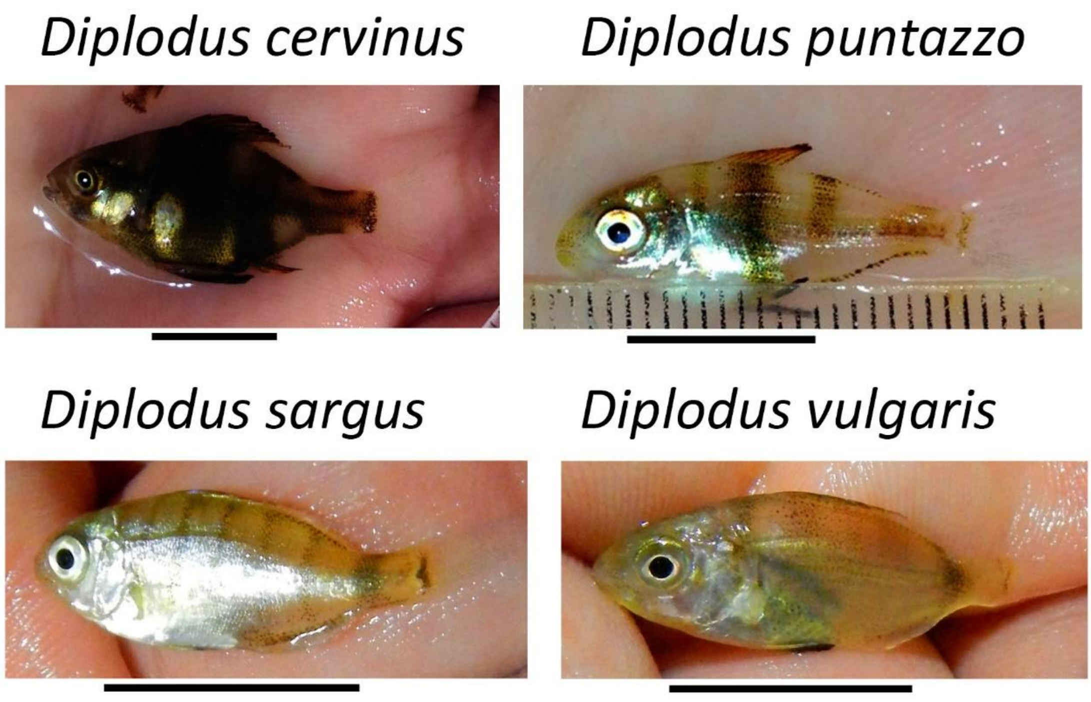

Figure 29 - Individuals of each *Diplodus* species studied in this monitoring program. All juveniles are recent settlers belonging to the 0^+ cohort. Scale bar corresponds to 1cm.

6. References

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