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Settlement patterns of mussel recruits at different temporal scales in the Peniche-Berlengas area (Southwest Portugal).

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Dissertation for Master's degree in Marine Biology

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Declaração de autoria do trabalho:

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

Institutions

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"Façam o favor de ser felizes..!"

Raul Solnado

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Resumo

As Áreas Marinhas Protegidas são ferramentas de gestão e conservação, que estendem a proteção das espécies-alvo a todo o ecossistema dessa área, aumentando assim a preservação do habitat e da biodiversidade. No entanto, as políticas de proteção nem sempre se traduzem num aumento da população de uma determinada espécie. Este é o caso do mexilhão azul (*Mytilus galloprovincialis*) na Reserva Natural das Berlengas (Portugal), onde não se observou o aumento da população adulta com a política de proibição implementada em 1981. Os fatores que regulam a incorporação de novos indivíduos na população (recrutamento) são difíceis de isolar devido às interações complexas que atuam em diferentes escalas espaciais e temporais. O estudo dos padrões de assentamento espacial e temporal é um método indireto largamente aceite para inferir processos que ocorrem antes do assentamento. O efeito da Reserva Natural das Berlengas na dinâmica da população do mexilhão azul, *M. galloprovincialis*, foi descrito dentro da mesma e a diferentes distâncias da área protegida, a fim de comparar os efeitos da distância à reserva e os diferentes mecanismos de chegada de larvas e possíveis repercussões da reserva nas áreas adjacentes. Foram recolhidas séries temporais de assentamento em duas frequências diferentes, mensalmente e a cada dois dias. As amostras mensais foram utilizadas para descrever os efeitos sazonais na chegada de larvas, enquanto os efeitos das características hidrodinâmicas de maior frequência foram estudados através de uma amostragem mais intensa (de 2 em 2 dias) durante o pico da época reprodutiva (primavera). As diferenças encontradas nos dados de temperatura e concentração de Chl-*a* encontrados entre o arquipélago das Berlengas e principalmente as localizações mais a norte da costa perto de Peniche, junto com os resultados de assentamento de juvenis de *M. galloprovincialis* podem indicar a presença de uma frente de *upwelling* entre as ilhas e a costa continental, o que poderia explicar a falta de chegada de larvas às ilhas durante as épocas favoráveis de *upwelling*. Desta forma, a Reserva Natural das Berlengas tem um papel de reservatório em vez de fonte de larvas para outros locais.

Palavras-chave: Assentamento; Recrutamento; *Mytilus galloprovincialis*; Áreas Marinhas Protegidas; Reserva Natural das Berlengas.

Abstract

Marine Protected Areas are management and conservation tools, which extend the protection of target species to the entire ecosystem enclosed in that area, enhancing habitat preservation and biodiversity. Nonetheless, protection policies are not always translated in an increase on certain species population size. This is the case of the blue mussel (*Mytilus galloprovincialis*) at the Berlengas Natural Reserve (Portugal), where adult population size has not increased with the non-take policy implemented in 1981. Factors regulating the incorporation of new individuals to the population (recruitment) are hard to isolate due to the complex interactions acting at different spatial and temporal scales. The study of spatial and temporal settlement patterns is a widely accepted indirect method for inferring pre-settlement processes because integrates different aspects involved in recruitment. The effect of Berlengas Natural Reserve on population's dynamics of the blue mussel, *M. galloprovincialis*, will be described within the reserve and at different distances from the protected area, in order to compare the effects of distance to the reserve and the different larval delivery mechanisms and possible spill-over effects from the reserve to the adjacent areas. Settlement time series at two different frequencies (monthly and daily) were collected. Monthly samples were used to describe seasonal effects on larval delivery and high frequency hydrodynamic features effects were studied through a more intense sampling (every two days) during the peak of the reproductive season (spring). The differences in temperature data and concentration of Chl-*a* found between the Berlengas archipelago and especially the northern locations of the mainland coast near Peniche, along with the results of *M. galloprovincialis* juvenile settlement may indicate the presence of an upwelling front between the islands and mainland coast, which could explain the lack of larval delivery to the islands during favourable upwelling seasons. Thus, the Berlengas Natural Reserve plays a role as sink instead as a source of larvae to other locations.

Key-words: Settlement; Recruitment; *Mytilus galloprovincialis*; Marine Protected Areas; Berlengas Natural Reserve.

Theme Choice

The importance of establishing marine protected areas is mostly related to biodiversity conservation, fisheries management and also as tools for researchers (Edgar *et al.* 2007). Marine protected areas have a specific goal to each different ecosystem, but their function is usually related to the enhancement of certain habitats or the abundance of particular target species (Halpern 2003). The influence of marine protected areas on ecological functioning of marine ecosystems can have a great variety of responses from one region to another. Depending on the complexity of processes occurring within each area, the reactions of species are very difficult to predict (Halpern 2003). MPAs can indirectly favour unprotected locations, acting as an enhanced source of adults, juvenile and larvae for sink-adjacent areas (Cudney-Bueno *et al.* 2009) and in some cases the expected enhancement on protected population is not directly observed (Cole *et al.* 2011).

About the efficacy of MPAs, it has been determined that location, size and spacing of MPAs are crucial factors on MPAs design which are going to condition their efficiency, and should be selected according to habitat requirements and meta-population dynamics of key species. Understanding of those dynamics require the development of models that include spatial components, especially regarding to the dispersal phase of planktonic larvae (Willis *et al.* 2003, Sale *et al.* 2005). In this context, it is of great interest the conducting of a study about the actual influence of marine protected areas on population dynamics of relevant species. *Mytilus galloprovincialis* is a complex life cycle species frequently employed as a model due to several reasons: it is very common to found in the Iberian Peninsula, and a bit all over the world, so results can be easily extrapolated; mussels are engineering species creating habitat for others and increasing diversity; mussel fishery and aquaculture are relevant both in Portugal and worldwide (FAO 2010). In the particular case of *M. galloprovincialis* populations in the Berlengas Natural Reserve, adult populations have not significantly increased since the non-take regulation has been established. Protection policies are expected to have a direct effect on adult mortality and indirectly on population size but the lack of this direct relationship suggest the presence of other limiting factors which might be related to earlier stages. Hence, I focused this thesis on the study of differences on larval supply and post-settlement processes (growth) of mussels within the Berlengas Natural Reserve and at different distances from the actual protected area, which may help to understand the meta-population dynamic of the species in the area and evaluate the effectiveness of the actual design of the marine protected area for the conservation of this species.

Literature review

Marine Protected Areas

Marine Protected Areas (MPAs) are spatially delimited areas of the marine environment that are managed at least in part, for conservation of biodiversity and fishery management (Edgar *et al.* 2007). In spite of each reserve having its own objectives, types of regulation and management and thereby species are not equally protected, MPAs' potential as tools for fishery management and conservation has been widely recognized and their advocates support their benefits as protection against overexploitation, biodiversity's conservation and habitat's protection (National Research Council 2001). Until now, only a small percentage of the ocean is protected as marine reserve, banning all types of fishing, however the total number of reserves is already above 200 around the world (Spalding *et al.* 2008).

These marine reserves have shown some positive results as generating an increase in biomass and density of populations and species diversity by limiting the exploitation of marine resources in those areas (Alcala and Russ 1990, Polunin and Roberts 1993). Other studies of marine reserves effects demonstrated that species in different trophic levels are likely to have different reactions (Palumbi 2004). However, aside from the effects inside MPAs there are also consequences to the neighbourhood. Cudney-Bueno *et al.* (2009) found that MPAs affect fish stocks in adjacent areas, through larval dispersal and migrations of juveniles or adults, these protected areas can act as an enhanced source of adults, juvenile and larvae for sink-adjacent areas. These results brought important implications for the establishment and management of MPAs, thereby oceanographic features, bathymetry, hydrography, and the retention and transport of individuals into or out of MPAs can be critical factors in MPAs design (National Research Council 2001). Also, understanding population dynamics of target species and their connectivity patterns between MPA's and adjacent areas is crucial information for an effective design of a network of marine protected areas (Botsford *et al.* 2003, Shanks *et al.* 2003, Gerber *et al.* 2005).

The Berlengas Natural Reserve (BNR) is located in Central-West Portugal, about 6 miles off Cabo Carvoeiro, Peniche, and it is one of the 30 areas which are officially under protection in the country. The BNR was declared a protected area in 1981 and later in 1998 it was enlarged, incorporating now the entire Berlengas's archipelago (Berlenga Island, Estela and Farilhões islets). The BNR has now 9541 hectares overall, of which 99 hectares are land area and 9442 hectares are marine area. The reserve was created with the purpose of preserve the rich fauna and flora of the area from overexploitation ensuring a sustainable development. Inside the BNR, it is not allowed commercial fishing to vessels not-registered at the Peniche Port Authority, trawl fishing, gill nets, trap fishing or shellfish collecting (Queiroga *et al.* 2009). Despite the restrictive policy on bivalve harvesting, the adult mussel population is not increasing their biomass on the rocky intertidal of the Berlengas Natural Reserve (ICNF pers. Com.).

Mytilus galloprovincialis, as a model species.

The blue mussel *Mytilus galloprovincialis* is a bivalve belonging to the Mytilidae family. It is also known as Mediterranean mussel for being native of Mediterranean Sea, and is usually found in the temperate intertidal rocky shores, establishing dense populations (Hockey and van Erkom Schurink 1992, Branch and Steffani 2004). *M. galloprovincialis* is a complex life cycle species with a planktonic larval phase and a sessile or sedentary adult (Figure 1). *M. galloprovincialis* is a gonochoric species, releasing millions of gametes for external fertilization at each reproductive event (Cáceres-Martinez and Figueras 1998). Although this species is capable of reproducing all year long, they usually show two major peaks in spring and autumn depending on environmental conditions (Ferrán *et al.* 1990, Ferrán 1991, Villalba 1995).

The planktonic larval stage has an estimate duration ranging from 14 days (Satuito *et al.* 1994) to 6 weeks (Chicharo and Chicharo 2000), depending mostly on the temperature and food concentration (Bayne 1965, Widdows 1991, Lutz and Kennish 1992) showing a considerable developmental plasticity under the influence of ambient conditions (Bayne 1965). This variability on pelagic larval duration can also affect their dispersal capacity (Picker and Griffiths 2011).

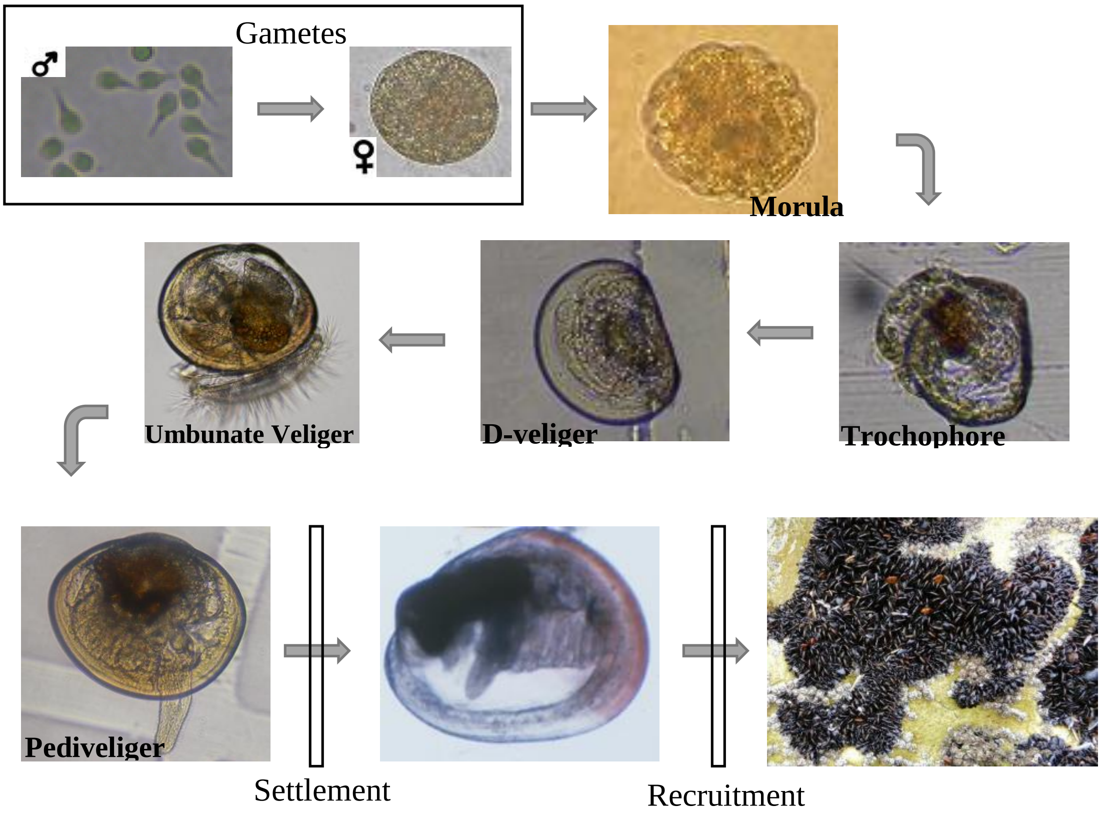


Figure 1 - *Mytilus galloprovincialis* life cycle. Photographs by Cristina Rodríguez.

The recognition of the high economic value of this species have warned scientists of the importance of conduct studies to research the factors controlling recruitment, either the regulating processes before settlement (dispersal and larval supply), the actual process of settlement (substrate choice and availability) and the ones affecting post-settlement (growth and mortality) (Connell 1985, Underwood and Fairweather 1989) (Figure 2).

During the pelagic larval stage, multiple physical and biological processes determine the balance between mortality, dispersal and retention within parental habitats and hence larval supply to the coast (Pineda *et al.* 2009). Settlement plays a strong role in mussels' population dynamics as the process linking larval and benthic stages (Connell 1985), and it's not just dependent on larval supply but also in the features of coastal ocean and nearshore hydrodynamics (Von der Meden 2009), as in the characteristics of the available substrate (Pulfrich 1996). Early settlers suffer extremely high mortalities, and because of that, the recruitment i.e. the number of new individuals incorporated to the population, is usually evaluated on function of the survivals after mortality post-settlement stabilizes some weeks later (Connell 1985). After settlement, the regulating processes are a combination of predation, competition for space and disturbance by biotic and abiotic events (Pineda 2000, Noda 2004, Navarrete *et al.* 2005). In order to comprehend the functioning of marine benthic systems is fundamental to relate all these factors influencing population dynamics (Pineda *et al.* 2009).

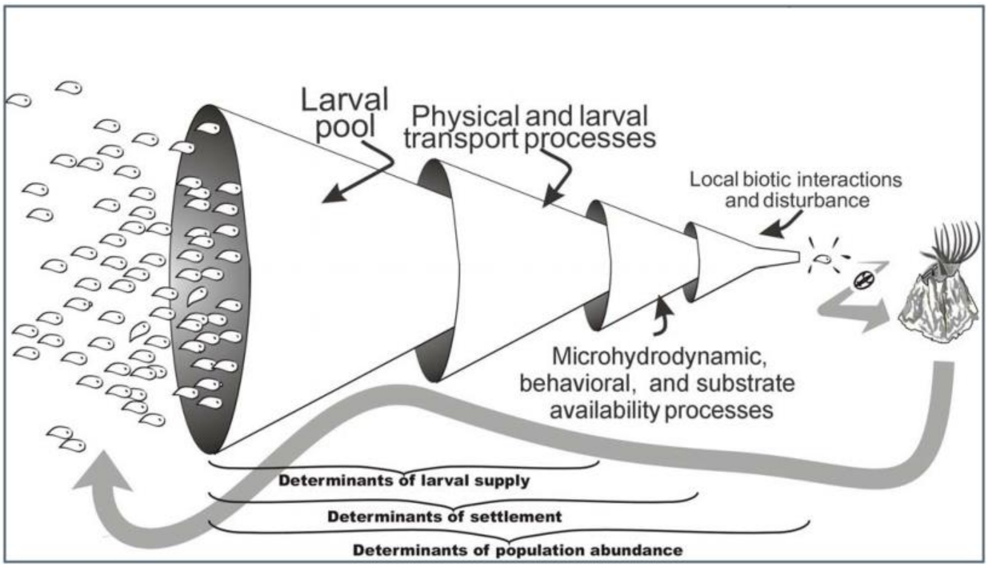


Figure 2 - Processes influencing dynamics population of coastal species with planktonic larval stages. Adapted from Pineda (2000).

Survival during larval development and large-scale offshore oceanographic processes are usually considered main factors determining larval supply (Pineda *et al.* 2009). It is assumed, because of larval limited swimming capacity, that they can be transported by oceanic currents over a long time and distance (Scheltema 1986, Caley *et al.* 1996), which made scientist suppose that coastal species with a planktonic larval phase are demographically open and highly "connected" through larval transport (Roughgarden *et al.* 1985, Sale 1991).

Among the physical processes affecting larval transport, winds and frontal structures are some of the most relevant (Queiroga *et al.* 2007). Shelf-winds inducing upwelling have been studied recurrently because of the high productivity associated and the physical mechanisms involved in along and cross-shore transport (Roughgarden *et al.* 1988, Wing *et al.* 1995, Morgan *et al.* 2009c). Due to limited larval swimming capacity, upwelling systems have traditionally been considered as dispersive ecosystems, where larvae are passively transported in the surface layer (Roughgarden *et al.* 1988). The wind-driven upwelling moves the Ekman layer (surface layer) offshore (northerly winds in the Northern hemisphere), and carries larvae away from settlement sites (Alexander and Roughgarden 1996, Connolly and Roughgarden 1999, Connolly *et al.* 2001). Coastal upwelling events are also closely related with food availability, once it brings up water rich in nutrients which together with sun light in the photic zone promotes phytoplankton development, creating better conditions to larvae survival (Fraga *et al.* 1988, Rico-Villa *et al.* 2009). Larvae might be accumulated at the upwelling front and when the wind relax or reverse, causing downwelling, larvae accumulated would be carried back to the shore with the surface layer (southerly winds in the northern hemisphere) (Farrell *et al.* 1991).

Although it has been widely accepted that coastal marine populations were highly "connected" (Roughgarden *et al.* 1985, Sale 1991), recent technological advances combined with a recognition of the importance of larval behaviour have led to a paradigm shift, in which is proposed that self-recruitment is much more common than previously suggested (Swearer *et al.* 2002, Levin 2006). Self-recruitment is defined as the larval retention within their native habitats (Sponaugle *et al.* 2002). Upwelling areas have been suggested as retentive environments for some species (Shanks and Brink 2005, Morgan *et al.* 2009c, Shanks and Shearman 2009). Metaxas (2001) reviewed how larval behaviour influence dispersal since they are capable of vertical migration, limiting its transport by ocean currents due to the unequal strength and direction along the water column. The water density (Sameoto and Metaxas 2008), phytoplankton concentration (Raby *et al.* 1994), temperature (O'Connor *et al.* 2007) and small variations in water chemistry (Atema and Cowan 1986) are some of the factors influencing larval behaviour. According to Raby *et al.* (1994), bivalve veliger larvae vertical distribution is positively correlated with chlorophyll *a* concentration, in a stratified water column. Temperature, besides affecting larval distribution, is a regulator of planktonic stage duration by altering metabolism and growth rate of individuals (O'Connor *et al.* 2007).

Fronts are also relevant structures involved both in larval retention/accumulation and cross-shore transport (Reviewed by Queiroga *et al.*

(2007). Fronts are natural boundaries between two masses of water with different physical characteristics, which can act as actual barriers for larvae, accumulating them and also influencing their transport. Accumulation arises from convergence of surface waters at the front and this convergence movement can play an important role in cross-shore transport. Nonetheless, accumulation and transport in fronts is also dependent on larval swimming capabilities and their vertical position in the water column. Commonly studied examples are the upwelling fronts. An upwelling front forms at a certain distance from shore where the cold salty and nutrient-rich upwelled water meet the less dense, nutrient-poor surface oceanic water (Shanks *et al.* 2000). When upwelling favourable winds relaxes or reverses, the less dense oceanic surface layer moves back to the coast and in certain conditions the upwelling front might stay intact and move onshore transporting the accumulated material.

For understanding the importance of larval transport and concentration processes within the frontal structures, two components must be related. In one hand we have the existence of downwelling events at one or at the two sides of the front (Queiroga *et al.* 2007). In Figure 3 is represented an upwelling front with downwelling at the two sides of the front (Shanks *et al.* 2000).

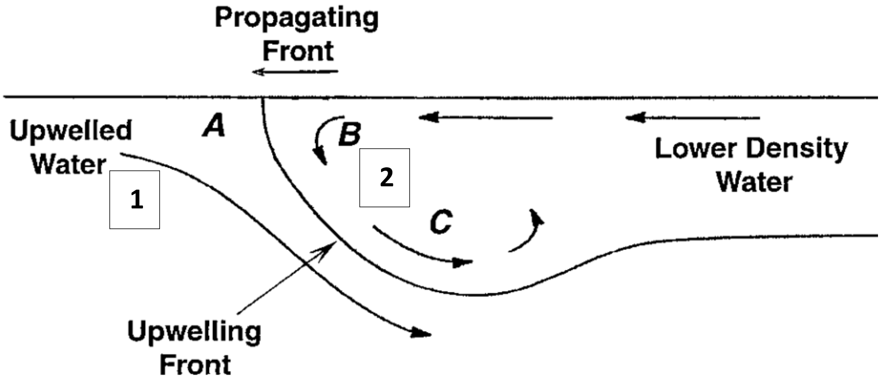


Figure 3 – Illustration of the water movements within an upwelling front propagating shoreward. A, B and C are larval concentration points depending on their initial position and migration capacities. Strong swimmers larvae with near-surface preferences can overcome the flow and become concentrated in A or B depending on their origin. In the other hand, if the larvae have weak swimming capabilities or slow behavioural response, they will be carried down with the flow before going up again and concentrate at C. Adapted from (Shanks *et al.* 2000).

The downwelling movement in the anterior edge (1) of the front is caused by the convergence of the upwelled water and the lower density advanced front. In the posterior edge (2), the movement of the lower density water is faster than the overall front movement, resulting in downward movements. The other component is the swimming capacities of larvae in order to keep the depth and not be carried downwards with the flow as the upwelling front moves shoreward.

At the end of planktonic stage, when *M. galloprovincialis* reach a shell length of approximately 0,25 mm (pediveliger larvae), mussel larvae have the capacity to attach in hard substrates using their byssus) a group of strong filaments secreted by mussel's foot) (Hammond and Griffiths 2004). This process, named settlement, consists of larvae contacting and connecting with substrate and also a metamorphosis of individuals (Connell 1985). According to Bayne (1965), settlement takes place in two steps, larvae attach primarily in filamentous structures (eg: algae) and secondarily in a hard substrate. Larval settlement is highly variable in space and time, and this variability have been explained according to several biotic and abiotic factors related not just to larval supply but also to the settlement process itself and the substrate choice (Peteiro *et al.* 2007). Biotic factors can be assumed to be the presence of individuals of the same species (Tumanda *et al.* 1997), presence of algae covering substrate (Hunt and Scheibling 1996, O'Connor *et al.* 2006), or particular biofilms (Bao *et al.* 2007). On the other hand there are abiotic factors influencing settlement magnitude including temperature (Pineda 1991, Garland *et al.* 2002), substrate physic-chemical properties (Pulfrich 1996, Alfaro *et al.* 2006), and also daylight and orientation (Bayne 1965).

Transition to the benthos is a critical moment on the mussel's life cycle and the mortality of early settlers is often vast. Post-settlement mortality processes and post-larvae migrations difficult the assessment of recruitment and increase the temporal and spatial variability of the process (Peteiro 2009). Post-settlement mortality rates can be very high (Hunt and Scheibling 1997), the success of metamorphosis and development of juveniles is conditioned by larvae's physiological condition (Phillips 2002, 2004). Post-settlement mortality may also increase due to environmental conditions such as temperature and salinity, food scarcity or even due to hydrography of habitat and physical disturbance (Hunt and Scheibling 1997, Guichard *et al.* 2003, Alfaro *et al.* 2006). In addition to predation

(Rilov and Schiel 2006, Peteiro *et al.* 2007), intra-specific competition is also an important regulator process of recruitment (Hunt and Scheibling 1997). According to Steffani and Branch (2003) better recruitment and growth rates are achieved at sites exposed to wave action due to greater food supply. Similarly, opportunities for settlement may decrease with the strength of wave action (Branch and Steffani 2004).

Quantitative studies of larval dispersal and connectivity jointly with the study of transitional processes to the benthos are fundamental, since they can lead to a better understanding of several issues about population dynamics, processes of local extinction and recolonization, spread of invasive species, species response to climate change and even marine reserves design (Becker *et al.* 2007, Domingues *et al.* 2012).

The main goal of this thesis is to understand early-life processes involved in the blue mussel *Mytilus galloprovincialis* meta-population dynamics and connectivity around the Berlengas Natural Reserve. Larval supply, larval delivery mechanisms and post-settlement processes were compared between a series of locations inside and outside of the reserve's limits using settlement time series and cohort analysis.

Objectives

The main objective of this thesis is to understand the mussel population dynamics in the Berlengas Natural Reserve and adjacent areas, focusing on the larval supply and transitional processes from the planktonic stage to the benthos. Larval supply was evaluated by comparing settlement time series at two different frequencies (monthly and every two days) inside and outside the reserve's limits. We also used those time-series to compare larval delivery mechanisms between locations, and General Additive Models (GAMs) were built to test environmental effects on mussel settlement, in some representative stations. As a proxy of differences in post-settlement success between locations, population structure has been compared by cohort analyses. The data set obtained from this work will also be used in the parameterization and validation of an Individual Coupled Physical Biological Models (ICPBM), which is being developed under the same project LarvalSources.

Methodology

Surveys and sampling sites

The characterization of spatial and temporal settlement patterns is commonly used as an indirect method to understand pre-settlement processes. Thereby, in order to deduce larval dispersal patterns and possible connectivity between populations is required a combined study of settlement patterns and local oceanography (Dudas *et al.* 2009).

For this study, one Marine Protected Area in Western Iberia Upwelling Ecosystem was chosen: Berlengas Natural Reserve. One other reserve is being studied similarly, *Parque Natural da Arrábida,* located near Setúbal, although the field work of this reserve couldn't be included in this thesis. The Berlengas Natural Reserve, off Peniche, is located in the Eastern North Atlantic Upwelling Region, characterized by strong and frequent coastal upwelling events during spring and summer months (Wooster *et al.* 1976, Fraga *et al.* 1988, Queiroga *et al.* 2007, Alvarez *et al.* 2008).

The chosen sampling stations, represented in Figure 4, are distributed inside the marine reserves and in the neighborhood at different distances. Inside Berlengas Natural Reserve two sampling sites had been selected: Forte and Buzinas, chosen by the easy access on foot and by boat. Outside the reserve we selected five other sampling sites: Foz do Arelho (Prainha), Baleal, Casa (Portinho de Areia), Praia da Consolação and Praia de Porto-Dinheiro.

Settlement time series were collected at two different frequencies, monthly and every two days. Monthly samples were performed at the lower tide of each month between December 2013 and July 2014 with the purpose of describing seasonal effects on larval supply. We have no samples from January 2014 due to the storms, which prevented the fulfilment of the schedule. In May 2014, the expected month to represent the peak of mussel's reproductive season in spring, every other day samplings were carried out in order to evaluate the effects of high frequency hydrodynamic features.

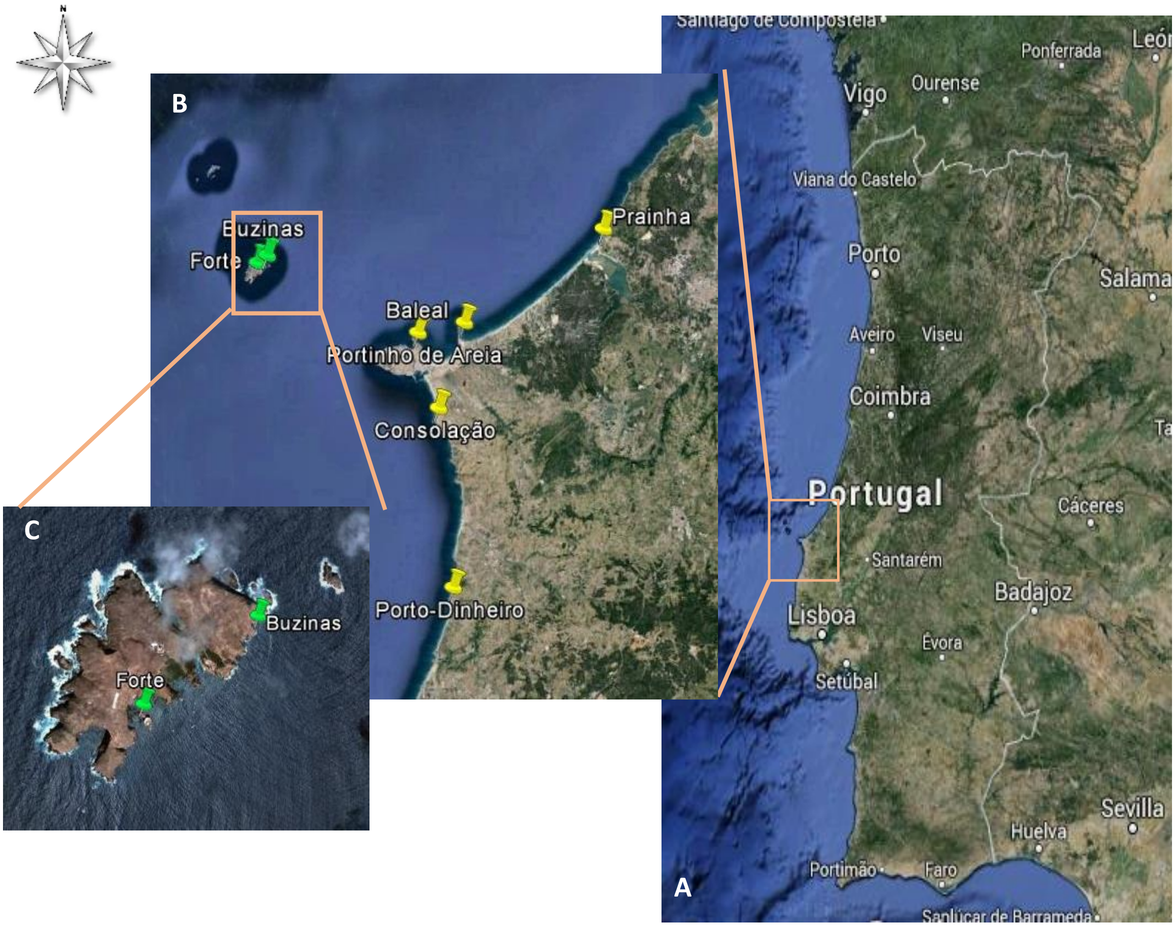


Figure 4 - Sampling sites in area of Peniche. (C) Berlengas Natural Reserve: Buzinas and Forte; (B) Neighbouring area of the reserve: Foz do Arelho (Prainha), Baleal, Casa (Portinho de Areia), Consolação e Porto-Dinheiro.

To evaluate mussel settlement along the year, three squares of 6x6 cm of natural substrate at each sampling site were randomly scraped every month (Figure 5A), while in May three artificial collectors (scouring pads with 6x6 cm) (Figure 5B) were deployed only in two locations: one inside the reserve (Buzinas) and another outside (Baleal) and replaced every other day. The souring pads collectors were chosen for being more successful than other types as the rope collectors, according to Ramirez and Cácerez-Martínez (1999). Each collection samplings took between 2 and 3 days, depending on tidal levels, meaning that between the first and last collecting of each month there were no more than 3 days.

Samples were placed in individual plastic bags and kept frozen until their processing at the laboratory. For all samples of artificial and natural substrates were separated all the individuals under a stereo microscope, subsequently counted and measured with *uEye Cockpit Software* (Version 4.02.0000) and *ImageJ Software*.

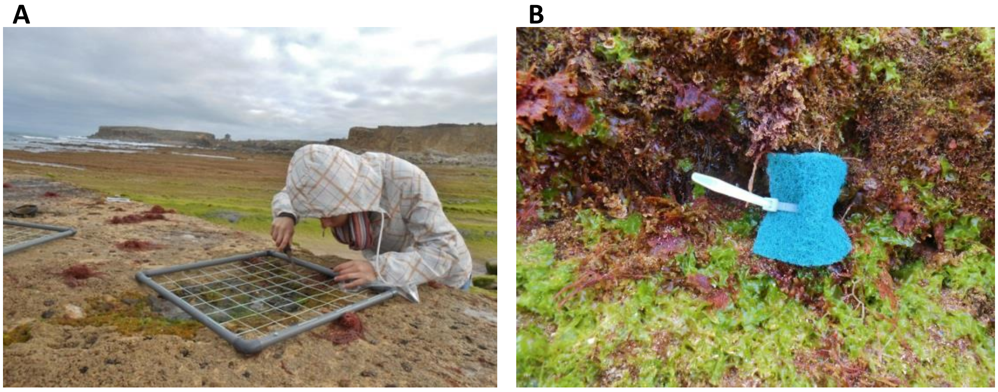


Figure 5 – Collecting natural substrate samples (A) and artificial collectors (scouring pads) (B).

Data analysis

The values of concentration of Chl-*a* were obtained from MODIS data (Feldman and McClain 2006), and the sea surface temperature and salinity values were extracted from the HYCOM model (Bleck 2002), whose resolution don't allow differentiation between Forte and Buzinas, so therefore we only have one value for Berlengas. The upwelling index was calculated based on wind velocity data from CliM@UA®. All the environmental parameters, Chl-*a* concentration, sea surface temperature and salinity, were measured near each sampling location (Figure 6), except for upwelling index which was calculated for one representative point of the study area.

Warmer temperatures detected in the Berlengas Islands together with the lower values of Chl-*a* concentration might be indicating the presence of an upwelling front between the island and mainland. To check its existence we have created a new variable, *DifTemp*, using the difference between Berlengas temperature and each mainland locations. Positive differences indicates that the temperature is warmer near the Island, and negative values indicates warmer temperatures near the mainland coast, and consequently, means the proximity of the upwelling front to the island. Fronts don't usually show an immediate response to upwelling favourable winds, so we did a cross-correlation to identify what would be the delay between the upwelling favourable winds and the formation of the front. Cross-correlations using all locations together showed a maximum at lag-5. When doing it by locations, we observed different delays between locations, but maximum correlation was always between lag-3 (Baleal, Casa and Consolação) and lag-5 (Porto Dinheiro and Foz do Arelho). We also created other two new variables, average of UI for the previous 5 and 10 days, *UI5d* and *UI10d*, to evaluate the influence of the persistence of upwelling or downwelling events into the analyses.

Thereafter the relationship between the *DifTemp* and *UI5d* and *UI10d* was checked as well as their interaction between locations using a General Additive Model (GAM). The interaction between UI and location was significant for every location, highlighting the influence of the topography on the formation of this front and its influence in the results.

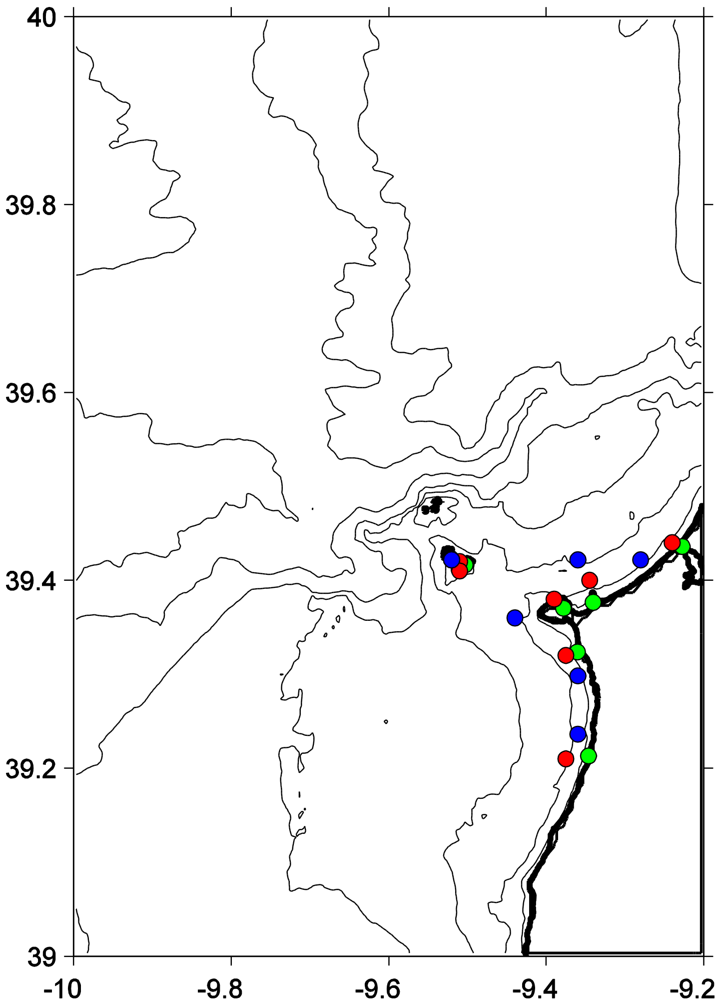


Figure 6 – Position of the collection points of environmental data (Chl-*a* – green; temperature and salinity – blue), in relation to the sampling sites (red).

Settlement abundance data was divided in two groups. Once mussel settlement occurs when the individuals have between 0.220 mm and 0.400 mm, the first group included the individuals with less than 0.600 mm, "early settlers" (the ones recently settled, with less than 15 days), and the second group the individuals between 0.600 mm and 2 mm, "plantigrades" (until approximately 2 months old) (Bayne 1976). This way, the smaller individuals could be a more representative way to estimate larval delivery, once the larger group integrates also a bigger amount of different types of mortality and migration.

Settlement synchrony among stations was assessed through cross-correlation of settlement time-series. Delays in settlement between stations would indicate different larval delivery mechanisms between them. Delivery mechanisms were evaluated through Generalized Additive Models (GAMs) which were built to test environmental effects on mussel settlement.

GAMs supplement general linear models (GLMs) by allowing for the exploration of non-linear functional relationships between dependent and explanatory variables. GAM models fit predictor variables independently by smooth functions rather than by assumed linear or quadratic relationships, and still allow combining parametric and non-parametric relationship for different variables. These analyses and their applicability to ecological modelling has been explored in several books (Hastie and Tibshirani 1990, Ruppert *et al.* 2003, Wood 2006, Zuur *et al.* 2007, Zuur *et al.* 2009).

In order to observe from a different point of view, the data was plotted with the scaled abundance by dividing the individual's number of each replicate by the maximum of individuals of the same month, between every locations. Thereby settlement is represented between 0 and 1, and can be easily compared between locations.

Size-frequency distributions of the settlers were used to identify cohorts, with FISAT II Software (Version 1.2.2). The cohort analysis and its evolution through time was used as a proxy for post-settlement growth rate. ANCOVA was used to assess the differences in growth rate between sampling sites.

All the statistical analyses was performed in R Software, Version 3.0.2. The variables used to build the GAMs will be the same employed by the ICPBM (Individual Coupled Physical Biological Models) that LarvalSources project is developing to predict connectivity matrices for mussel populations. Settlement time series and GAMs results will be implemented in the parameterization and validation of the ICPBM, since its development implies a rigorous validation process which requires high quality data-sets to compare with the model predictions (Hannah 2007, Peliz *et al.* 2007).

Results

Environmental parameters

The environmental variables monitored in this study have shown seasonal patterns, as much as we could see during the sampling period. The sea surface temperature had relatively the same evolution during the sampling months in every sampling sites, showing a typical increase in temperatures from winter to spring with mean minimum temperatures around 13.35 ºC in February and mean maximum temperatures of 17.32 ºC in June (Figure 7).

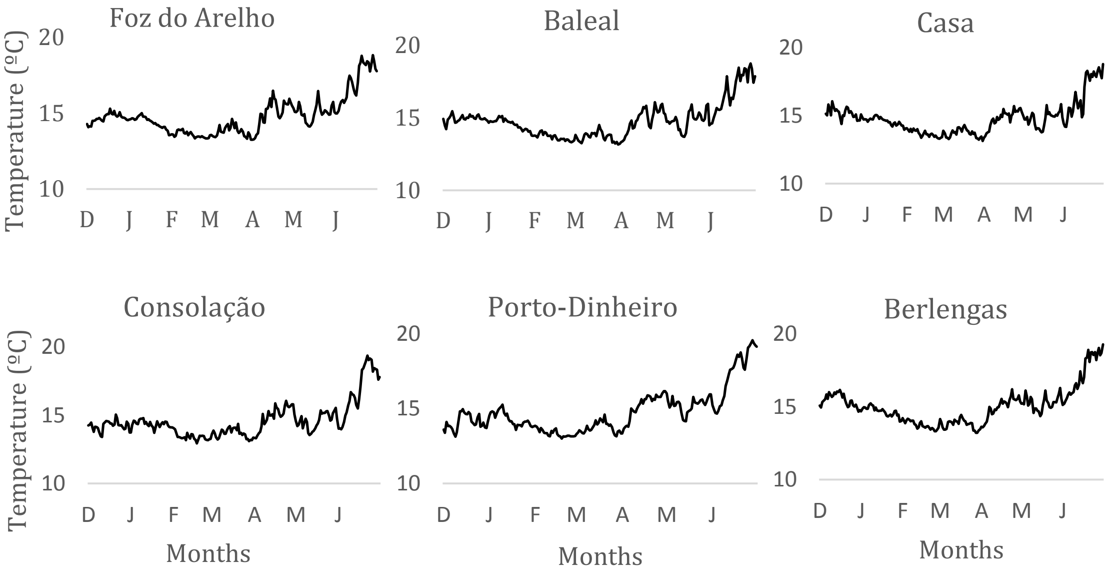


Figure 7 - Evolution of temperature during the sampling months in the different sampling locations.

A Generalized Additive Model (GAM), Table 1, was performed to identify temperature seasonality and variability inter-locations using the day of the year and location as explanatory variables. Both variables demonstrated a significant relationship, explaining 84.9% of the variability observed (GAM, Table 1, Figure 8). Regarding to location, the baseline applied to perform it was Baleal. The temperature in Baleal was similar to every locations ($p>0.05$) except Consolação and Berlengas ($p<0.001$). Consolação on average was colder than the rest of sampling sites, while Berlengas was regularly warmer than the rest of the locations. The seasonal pattern (Figure 8) shows lower temperatures during the winter and a typical increase in temperature during the spring-summer, although the lack of data between July and November.

Table 1 - Structure of the General Additive Model selected to describe Temperature. S.E.: standard error; e.d.f.: estimated degrees of freedom.

Parametric coefficients	**Estimate**	**Std. Error**	**t**	**Pr(>|t|)**
Intercept (Baleal)	14.786	0.034	429.037	$<2\times10^{-16}$ ***
Casa	-0.014	0.048	-0.278	0.781
Consolação	-0.189	0.048	-3.868	1.2×10^{-4} ***
Berlengas	0.260	0.048	5.332	1.15×10^{-7} ***
Foz do Arelho	0.034	0.048	0.705	0.481
Porto Dinheiro	-0.01	0.048	-0.207	0.836

Approximate significance of smooth terms	**edf**	**F**	**p-value**
s(YearDay)	8.958	780.5	$<2\times10^{-16}$ ***

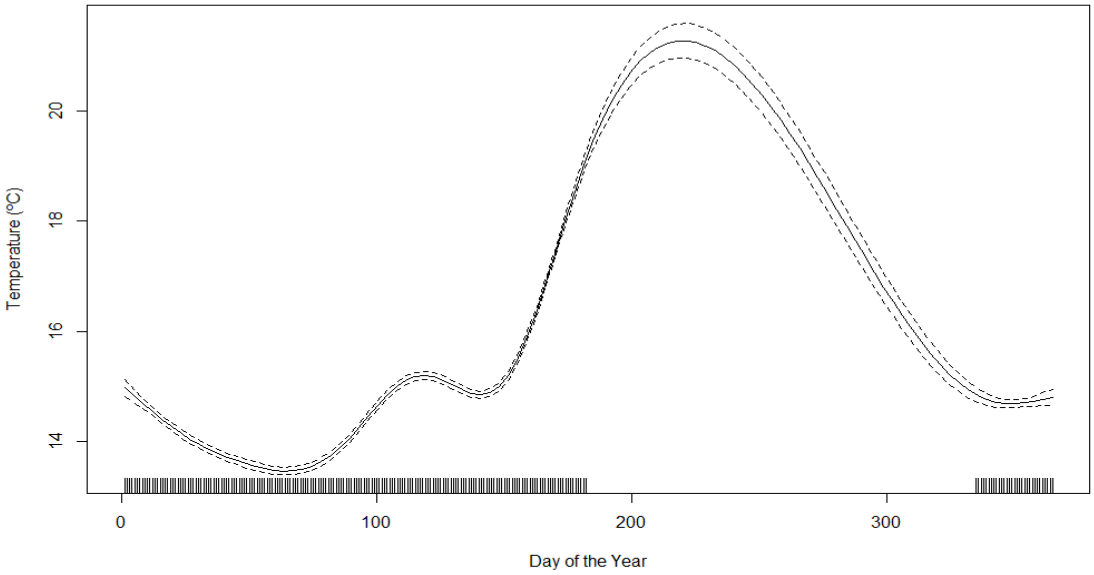


Figure 8 - Result of the Generalized Additive Models (GAM), showing the partial effect of Day of the Year on Temperature. Dotted lines indicate 95% confidence intervals, and tick marks along the X-axis below each curve represent effect values where observations occurred.

The evolution of salinity along the months was similar in the 6 locations (Figure 9). There was almost no variation, with mean minimum values of salinity around 35.83‰ in May and mean maximum values of approximately 36.14‰ in December.

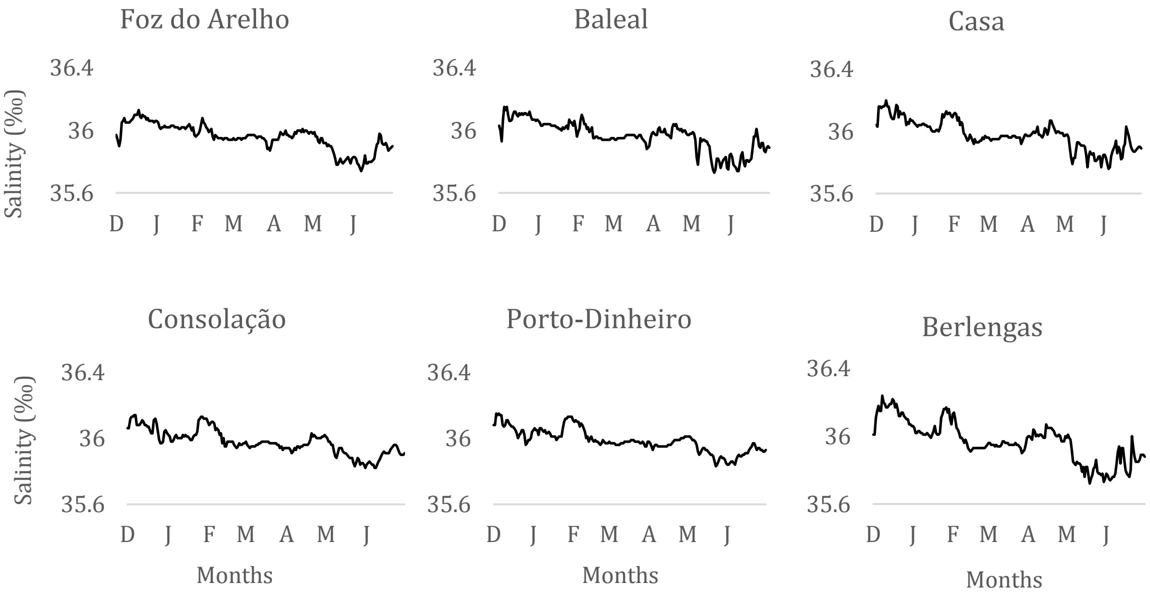


Figure 9 - Evolution of salinity during the sampling months in the different sampling locations.

A GAM, Table 2, was performed to identify salinity seasonality and variability between locations using the day of the year and location as explanatory variables. Both variables demonstrated a significant relationship, explaining 77.9% of the variability observed. The baseline applied to perform it was Baleal, regarding to location. The salinity in Baleal, Berlengas and Foz do Arelho was similar ($p>0.05$) and Casa, Consolação and Porto Dinheiro had on average higher values of salinity than Baleal ($p<0.01$). The seasonal pattern (Figure 10) shows a typical decrease in salinity during winter-spring and predicts an increase during the summer, although we have no data between July and November.

Table 2 – Structure of the General Additive Model selected to describe Salinity. S.E.: standard error; e.d.f.: estimated degrees of freedom.

Parametric coefficients	Estimate	Std. Error	t	Pr(>|t|)
Intercept (Baleal)	35.963	0.003	12174.577	$<2x10^{-16}$ ***
Casa	0.017	0.004	4.012	$6.37x10^{-5}$ ***
Consolação	0.013	0.004	3.034	0.00246 **
Berlengas	0.007	0.004	1.686	0.09209 .
Foz do Arelho	-0.007	0.004	-1.742	0.08176 .
Porto Dinheiro	0.022	0.004	5.327	$1.18x10^{-7}$ ***

Approximate significance of smooth terms	edf	F	p-value
s(YearDay)	8.913	468.7	$<2x10^{-16}$ ***

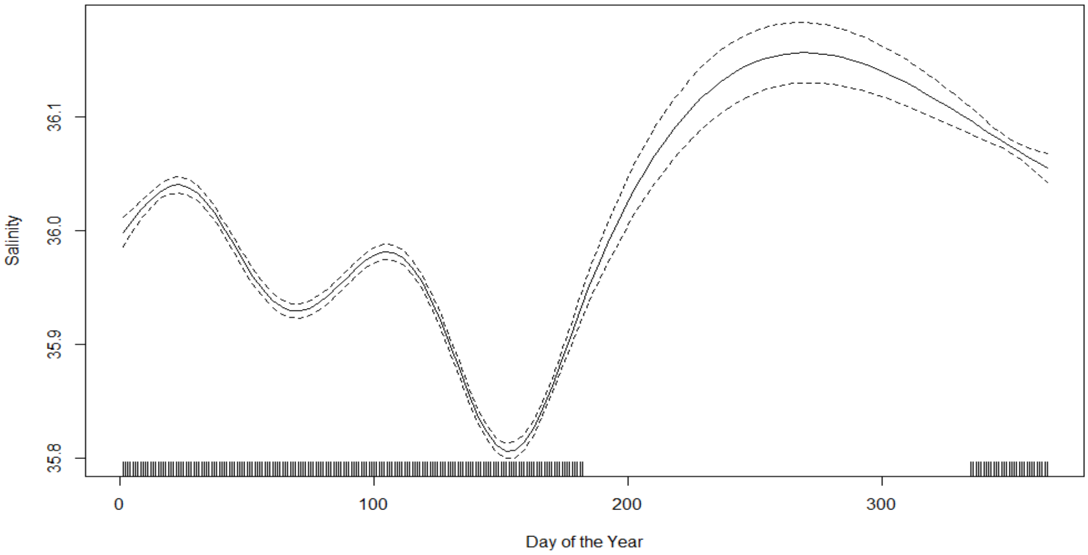


Figure 10 - Result of the Generalized Additive Models (GAM), showing the partial effect of Day of the Year on Salinity. Dotted lines indicate 95% confidence intervals, and tick marks along the X-axis below each curve represent effect values where observations occurred.

The concentration of Chl-*a* (Figure 11) had some differences between the sampling sites. The peaks of concentration occurred simultaneously in every locations, in the end of March and in May, however with very different scales. In the Berlengas Natural Reserve, the major peak in the end of March only reached 4.56 mg/m^3, and stayed below 1mg/m^3 the rest of the months. In Baleal and Casa the peaks were higher, with 10.67 mg/m^3 and 9.13 mg/m^3 respectively in end of March, and 7.65 mg/m^3 and 4.26 mg/m^3 respectively in May. In Foz do Arelho and Porto-Dinheiro, the higher values were 17.42 mg/m^3, and 14.57 mg/m^3 correspondingly, in early spring and 14.47 mg/m^3 and 15.21 mg/m^3 in May. Consolação has the higher concentrations of Chl-*a* with a first peak in March of 13.06 mg/m^3 and a major one in May with 42.37 mg/m^3.

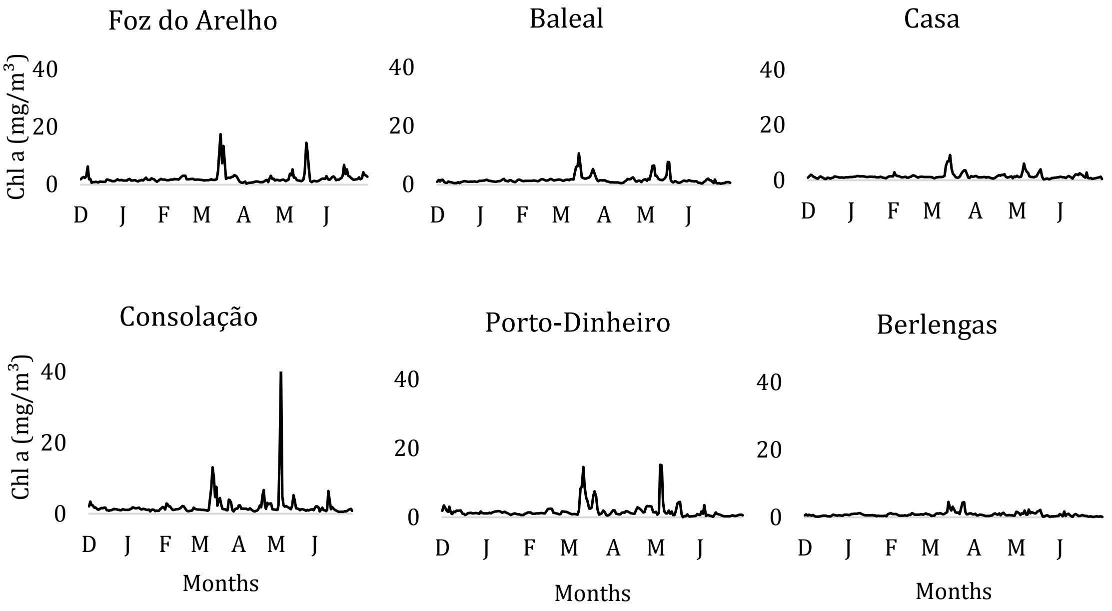


Figure 11 - Evolution of Chl-*a* concentration (mg/m^3) during the sampling months in the different sampling locations.

The concentration of Chl-*a* did not show such a relevant seasonal pattern. The GAM, only explains 10% of variability using the Day of the Year and Location as explanatory variables, but we can still detect the typical spring maximum, spring transition (Figure 12). Regarding to locations, Baleal was applied as the baseline (Table 3). The concentration of Chl-*a* in Baleal was similar to Casa and Porto Dinheiro ($p>0.05$). Consolação and Foz do Arelho have shown higher values of Chl-*a* ($p<0.5$), and both locations in the Berlengas Island (Buzinas and Forte) showed significantly lower values ($p<0.001$).

Table 3 - Structure of the General Additive Model selected to describe the Concentration of Chl-*a*. S.E.: standard error; e.d.f.: estimated degrees of freedom.

Parametric coefficients	**Estimate**	**Std. Error**	**t**	**Pr(>|t|)**
Intercept (Baleal)	1.570	0.124	12.631	$<2\times10^{-16}$ ***
Buzinas	-0.722	0.176	-4.105	4.27×10^{-5} ***
Casa	-0.101	0.176	-0.575	0.566
Consolação	0.426	0.176	2.424	0.015 *
Forte	-0.759	0.176	-4.316	1.7×10^{-5} ***
Foz do Arelho	0.607	0.176	3.451	5.74×10^{-4} ***
Porto Dinheiro	0.144	0.176	0.815	0.415

Approximate significance of smooth terms	**edf**	**F**	**p-value**
s(YearDay)	3.541	17.71	1.52×10^{-13} ***

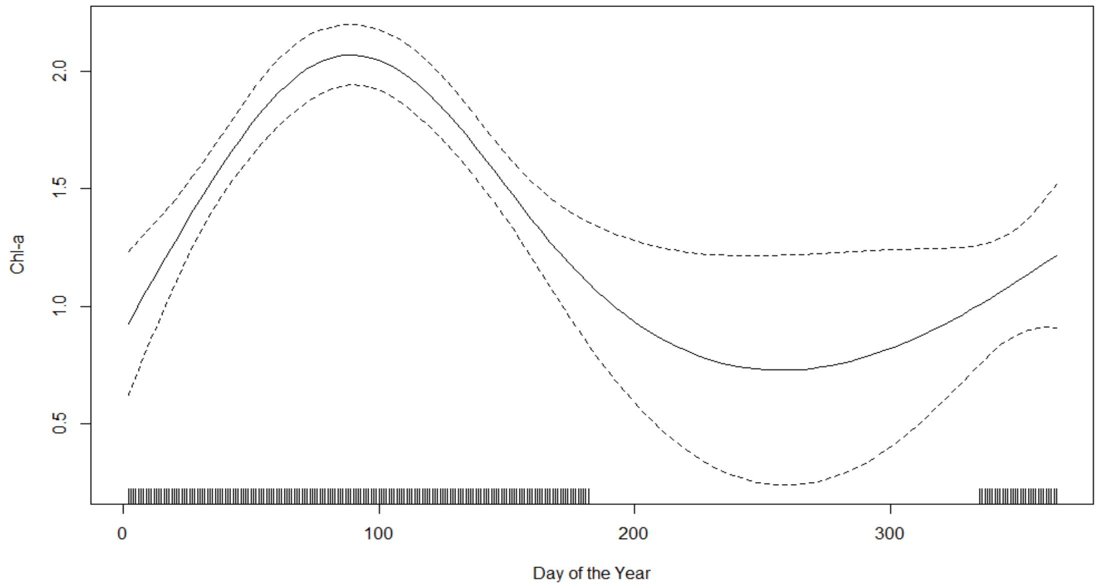


Figure 12 - Result of the Generalized Additive Models (GAM), showing the partial effect of Day of the Year on the Concentration of Chl-*a*. Dotted lines indicate 95% confidence intervals, and tick marks along the X-axis below each curve represent effect values where observations occurred.

The upwelling index is represented in Figure 13A from September 2013 to June 2014. We could analyse that from September 2013 until the next spring, in May 2014, downwelling events were preponderant, although with some upwelling peaks (in November 2013, and January 2014). From May 2014 onwards, upwelling events turns more prevalent, matching upwelling seasonality in these latitudes (upwelling predominant winds from spring to early-autumn, and downwelling predominant winds during the rest of the year).

The higher temperatures and low Chl-*a* concentrations registered in Berlengas Archipelago with respect to the rest of the sampling locations, might be indicating the presence of an upwelling front between the Island and mainland. In order to identify the existence of that front and its relationship with upwelling, we have analysed the index *DifTemp* (i.e. the difference of temperature between Berlengas and each different locations in mainland) (Figure 13B), and its relation to the upwelling index and its persistence (UI averaged 5 and 10 days).

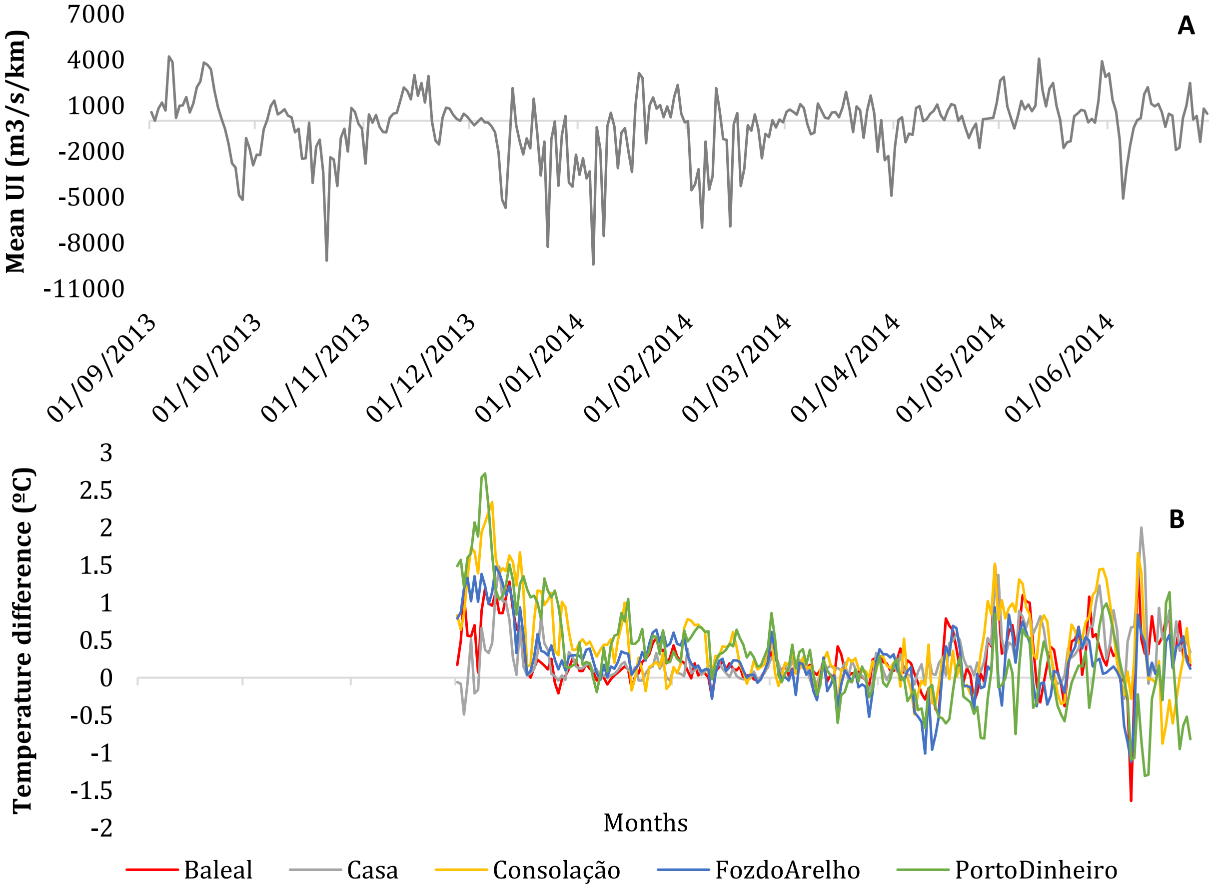


Figure 13 - At the top (A) is represented the upwelling index (m^3/s/km) from September 2013 to June 2014, while in the bottom (B) is the temperature difference (temperature of Berlengas minus the temperature of each mainland location - ºC), along the sampling months.

A GAM (Table 4, Table 5, and Figure 14) was performed in order to analyse that interaction between the upwelling index (mean of the previous 5 and 10 days) and the temperature difference in each mainland location in relation to Berlengas. Regarding to location, the baseline applied to perform the model was Baleal. This model could explain 17.7% and 20.5% of the variability observed (for the 5 and 10 days averaged UI respectively). UI10d and Location are the combination that explains better the variability observed in *DifTemp*, but using both analyses we can compare the differences between more prevalent up-down events and those which are less prevalent.

We could observe significant interactions between upwelling and the different locations ($p<0.01$, in both cases with the mean UI of the previous 5 and 10 days; Tables 4 and 5). For the places located to North of Cape Carvoeiro, Foz do Arelho and Baleal, we observed a linear relation between the difference of temperature and UI when UI > 0 (Figure 14A, B). The higher the upwelling index, the higher the difference in sea water temperature between Berlengas and those locations, both using the averaged upwelling index for 5 days or 10 days, which means that in Berlenga's locations the temperature becomes higher in relation to Foz do Arelho and Baleal. That might indicate that even during persistent upwelling events (10 days) the upwelling front cannot reach the Island, and the water remains warmer in the Island than in the coastline. During downwelling events (i.e. negative values of UI), in Baleal there is almost no variation in temperature difference, although in Foz do Arelho the D*ifTemp* increases to positive values once again (Figure 14A, B).

At Casa, located in the Cape Carvoeiro, the temperature difference increases to positive values with the upwelling index until it reaches a plateau, when we use the previous 5 days UI average (Figure 14C). When the previous 10 days UI average is used, the relation become more linear (Figure 14C), indicating that more persistent upwelling or downwelling events have a stronger effect in temperature differences at this location. With downwelling events of 5 days the temperature difference tend to zero, becoming similar in Berlengas and Casa. But with events of 10 days persisting, the difference of temperatures becomes linearly negative, indicating colder waters in the Island than in mainland.

In the locations south of the Cape, Consolação and Porto Dinheiro, also can be observed an increase in temperature differences during upwelling events until those differences reach a plateau (Figure 14D, E). When we look to more persistent upwelling events (mean previous 10 days UI), differences in temperature between the Island and southern locations tend to disappear when the upwelling index is high (Figure 14D, E). This might indicate some kind of recirculation of upwelled water south of the cape, which might reach the Island simultaneously to mainland only when upwelling index is strong and persistent. Nevertheless, when looking at 10 day averaged UI, Porto-Dinheiro also showed a particular behaviour. When intermittent upwelling is prevalent (mean UI 10d around 0) differences in temperature tend to be negative, with colder water closer to the island in relation to Porto Dinheiro (Figure 14E), this might indicate that the upwelled water under relaxation events, can reach the island.

Table 4 - Result of the Generalized Additive Models (GAM), showing the partial effect of Day of the Year on Upwelling Index (mean of 5 previous days - $m^3\ s^{-1}\ km^{-1}$) in the temperature difference at each mainland location. Dotted lines indicate 95% confidence intervals, and tick marks along the X-axis below each curve represent effect values where observations occurred.

Parametric coefficients	**Estimate**	**Std. Error**	**t**	**Pr(>|t|)**
Intercept (Baleal)	0.209	0.027	7.690	3.66×10^{-14} ***
Casa	0.053	0.039	1.374	0.169
Consolação	0.140	0.039	3.630	2.98×10^{-4} ***
Foz do Arelho	-0.066	0.039	-1.720	0.086
Porto Dinheiro	-0.063	0.039	-1.644	0.100

Approximate significance of smooth terms	**edf**	**F**	**p-value**
s(mean5d):Baleal	1.968	5.219	3.27×10^{-3} **
s(mean5d):Casa	3.037	3.915	4.55×10^{-3} **
s(mean5d):Consolação	5.217	8.415	3.26×10^{-9} ***
s(mean5d):FozdoArelho	2.981	5.294	5.2×10^{-4} ***
s(mean5d):PortoDinheiro	5.036	7.831	2.27×10^{-8} ***

Table 5 - Result of the Generalized Additive Models (GAM), showing the partial effect of Day of the Year on Upwelling Index (mean of 10 previous days - m^3 s^{-1} km^{-1}) in the temperature difference at each mainland location. Dotted lines indicate 95% confidence intervals, and tick marks along the X-axis below each curve represent effect values where observations occurred.

Parametric coefficients	**Estimate**	**Std. Error**	**t**	**Pr(>|t|)**
Intercept (Baleal)	0.209	0.027	7.795	1.73×10^{-14} ***
Casa	0.056	0.038	1.473	0.141
Consolação	0.130	0.038	3.436	6.16×10^{-4} ***
Foz do Arelho	-0.071	0.038	-1.885	0.059
Porto Dinheiro	-0.089	0.038	-2.368	0.018 *

Approximate significance of smooth terms	**edf**	**F**	**p-value**
s(mean10d):Baleal	1.373	1.653	2.2×10^{-3} **
s(mean10d):Casa	1.000	1.000	8.41×10^{-3} **
s(mean10d):Consolação	5.477	6.626	6.18×10^{-10} ***
s(mean10d):FozdoArelho	3.453	4.300	2.29×10^{-3} **
s(mean10d):PortoDinheiro	6.849	7.952	4.51×10^{-14} ***

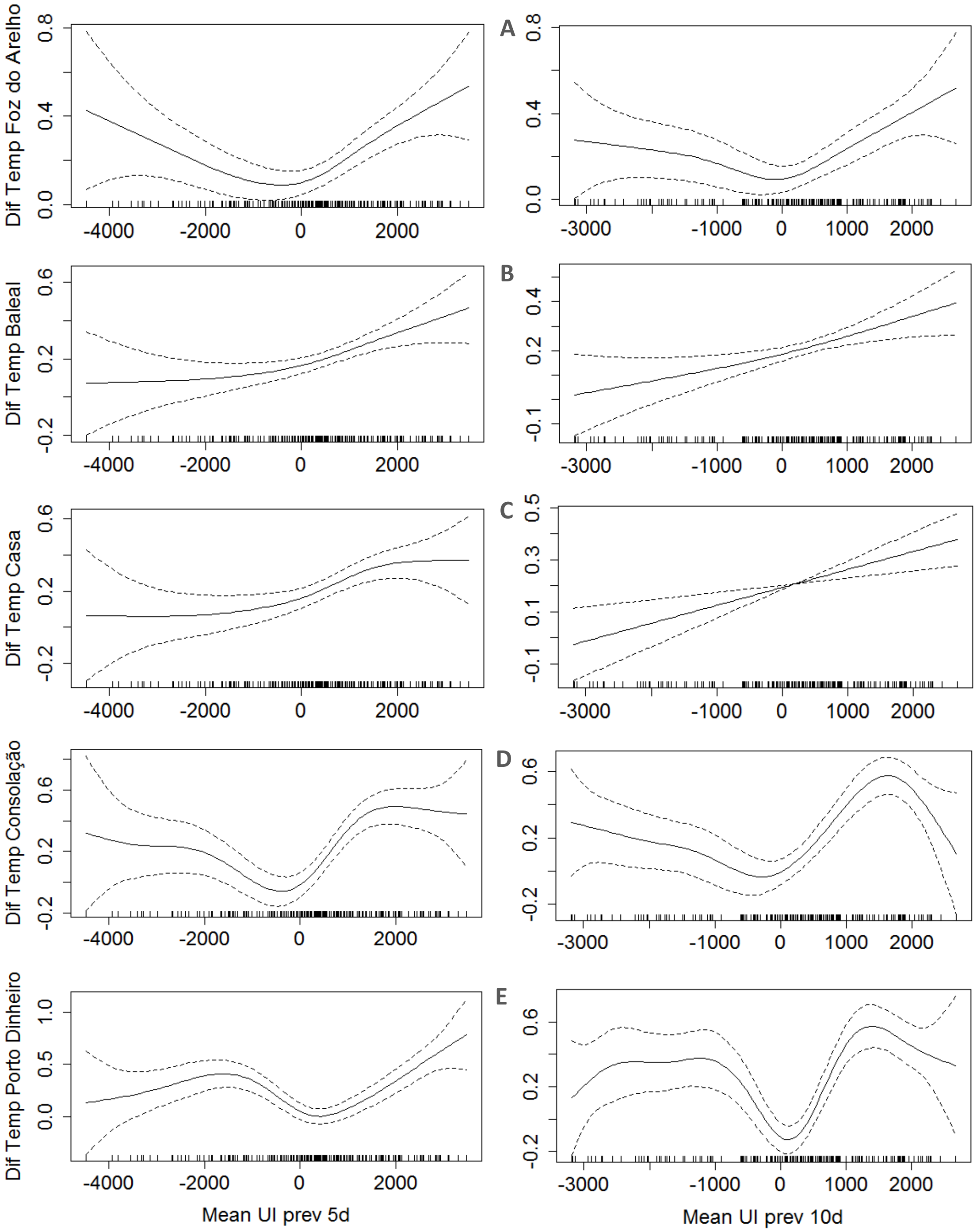


Figure 14 - Result of the Generalized Additive Models (GAM), showing the partial effect of Upwelling Index averaged during the last 5 and 10 days (m^3 s^{-1} km^{-1}) in the temperature difference (ºC) at each mainland location (from top to bottom, in the same way as from North to South: A) Foz do Arelho, B) Baleal, C) Casa, D) Consolação, E) Porto-Dinheiro). Dotted lines indicate 95% confidence intervals, and tick marks along the X-axis below each curve represent effect values where observations occurred.

Settlement time series (monthly sampling)

Different temporal and spatial patterns of *Mytilus galloprovincialis* densities per sampled area were observed according to the range of sizes considered.

Recently arrived individuals, with less than 15 days and with mean size less than 0.6 mm (Figure 15), show a common and intense peak in the beginning of May, except for the locations in the reserve island (Forte and Buzinas), which had the highest density in the end of winter (February and March) with 18.67±22.85 and 26.0±43.31 individuals/square, respectively. These two locations also had a second and smallest peak in the beginning of May with 9.0±7.94 e 9.67 ±8.62 indiv/square, in Forte and Buzinas respectively.

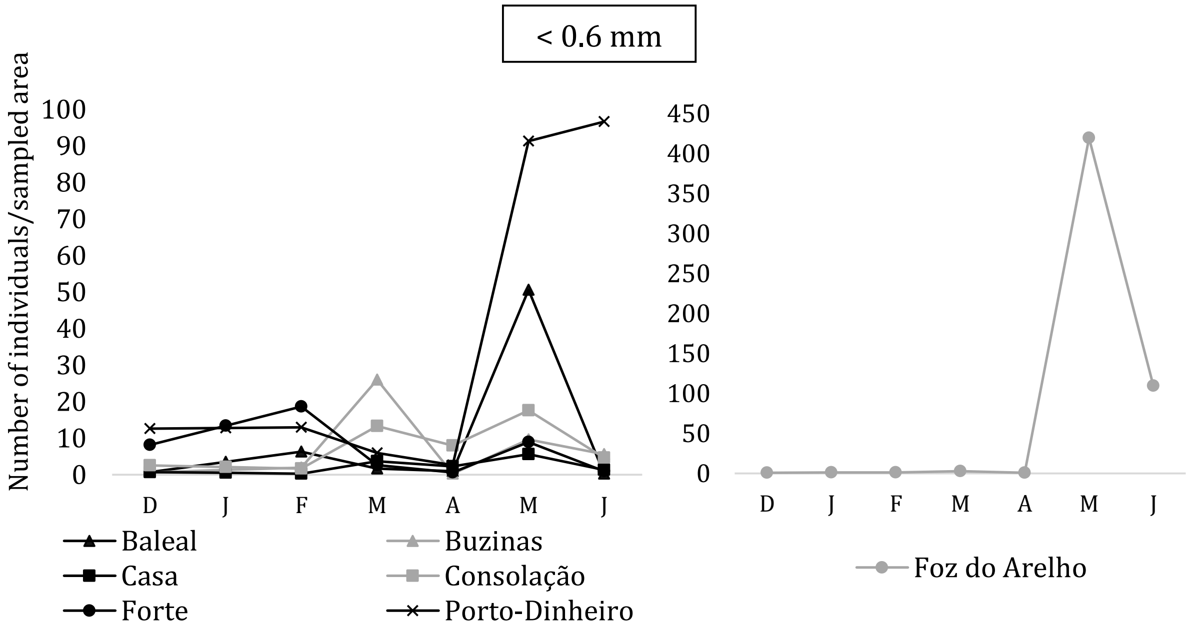


Figure 15 - Settlement time series, with the number of individuals with less than 0.600 mm per sampled area (36cm^2), between December 2013 and June 2014, in every sampling location. On the left are represented Buzinas, Forte, Baleal, Casa, Consolação and Porto-Dinheiro. On the right is only Foz do Arelho, with a totally different scale.

The values of mussel settlement were considerably higher in Foz do Arelho with 419±92.72 indiv/square in May and 109.67±96.67 indiv/square in June, while in winter and early spring settlement was almost inexistent. In Porto-Dinheiro early settlers had a maximum of density in June with 96.67±73.53 indiv/square and before in May with 91.33±71.06 indiv/square, and during the winter some settlement was registered with 12.60±11.55 indiv/square in December and 13±5.57

indiv/square in February. In Baleal the maximum of density was observed in May with 50.67±9.61 indiv/square, and with almost no record of early settlers in the rest of the sampling months, except in February with 6.33±6.11 indiv/square. Consolação had a maximum mean of settlement in May with 17.67±7.37 indiv/square, but also have shown similar values in March with 13.33±7.57 indiv/square. At the site located on the edge of the Peniche's peninsula, Casa, was recorded a maximum density of early settlers in May, although very reduced, with only 5.67±6.03 indiv/square.

The maximum correlations between settlement time series were found between Consolação and Casa, at lag0 (r=0.99). Another locations also presented maximum correlations at lag0, as Baleal and Foz do Arelho (r=0.95), Baleal and Casa (r=0.77), Casa and Foz do Arelho (0.77), and Foz do Arelho and Porto-Dinheiro (r=0.88). Buzinas, located in the Berlengas Island, have shown maximum correlations at lag+2 with Baleal and Foz do Arelho (r=0.94 and r=0.88, respectively). There weren't found any significant correlations between the rest of the locations.

In the scaled abundance of the individuals with less than 0.6 mm (Figure 16), similar results to the cross-correlations can be observed, although represented in an easier way. The peaks of abundance can be detected in different locations in winter and in spring, meaning that settlement is not synchronized among stations. During the winter the maximum abundances predominate in the southern locations and in the island (Consolação, Porto-Dinheiro, Buzinas and Forte). In the other hand, when spring begins can be observed a shift of the maximum abundances to the northern locations (Baleal, and mainly Foz do Arelho).

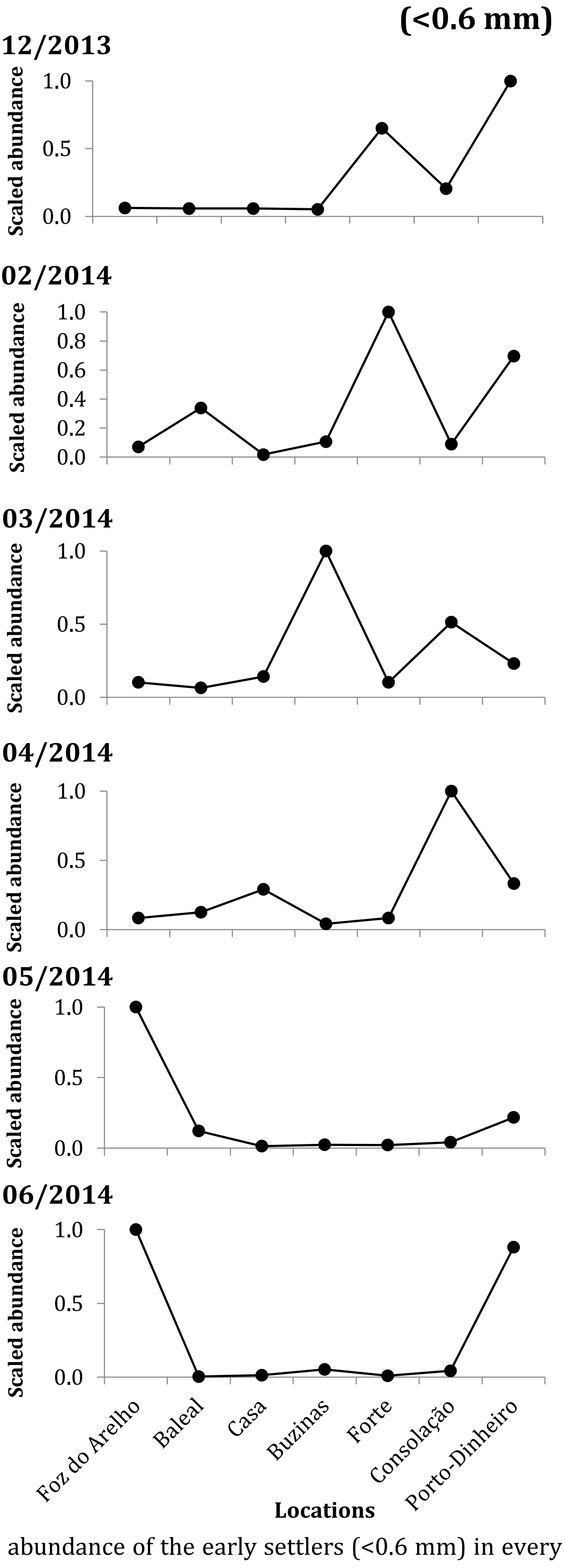


Figure 16 - Scaled abundance of the early settlers (<0.6 mm) in every locations, between December 2013 and June 2014. Locations: left to right, corresponds to North to South.

When analysing the time series of the second group mean density, a totally different pattern can be found (Figure 17). This group with the individuals comprehended between 0.6 mm and 2 mm, can be defined as plantigrades (Bayne 1976). Based on the growth rates calculated, these individuals are around 1-2 months old. Unlike early settlers, plantigrades have shown a clear high mean density in December, at least in 3 locations (Buzinas, Forte e Porto-Dinheiro), suggesting that in this 3 sites the settlement peak in Autumn is of great importance, while in the other locations (Foz do Arelho, Baleal, Casa and Consolação) it seems to have no relevance.

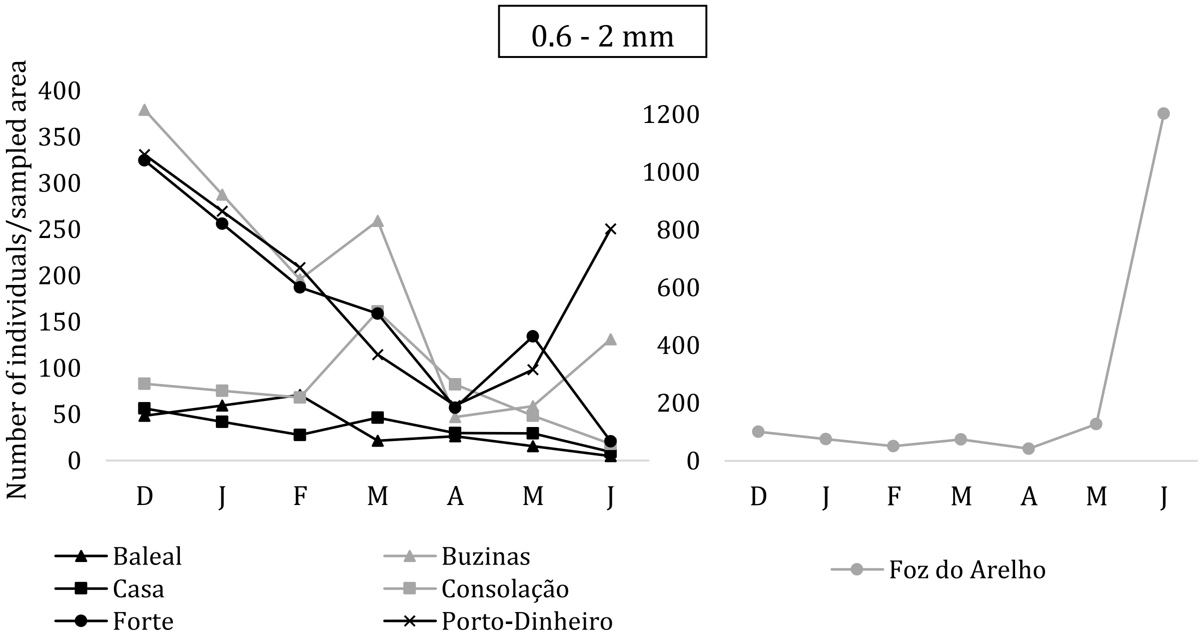


Figure 17 - Settlement time series, with the number of individuals between 0.600 mm and 2 mm, per sampled area (36cm^2), between December 2013 and June 2014, in every sampling location. On the left are represented Buzinas, Forte, Baleal, Casa, Consolação and Porto-Dinheiro. On the right is only Foz do Arelho, with a totally different scale.

In all the sampling sites was registered a higher mean density of individuals between 0.600 mm and 2 mm than of early settlers, in almost every months. Once this range of sizes comprehend individuals between 1 and 2 months old, each monthly samplings integrates individuals that arrived in the last 2 months, while the group of early settlers (<0.6 mm) arrived no more than 15 days ago.

In Baleal, the maximum density appears earlier now in February with 71±74.22 indiv/square. In Casa, the maximum appears in December with 56.25±32.42 indiv/square, although the mean density in the rest of the months doesn’t show much variation. In Consolação, appears a clear peak of mean density in March, with 161.33±15.63 indiv/square. Foz do Arelho has a larger peak with 1202.33±918.82 indiv/square, in June, one month later than the peak of early settlers. Porto-Dinheiro have shown a maximum density of plantigrades in December 330.80±167.91 indiv/square, decreasing until April, increasing again until the next peak in June with 250.67±81.88 indiv/square. In the Berlengas Natural Reserve, the peak in December is also evident, with 379.33±155.09 in Buzinas and 324.60±168.70 in Forte. In Buzinas a second peak happens in March (259.33±144.97 indiv/square), and in Forte after decreasing until April, a second smaller peak appears with 134.33±97.30 indiv/square in May.

Analysing the cross-correlations between the settlement time series of plantigrades in the Berlengas Natural Reserve and neighbour areas, a maximum correlation at lag0 can be found between the Island locations Forte and Buzinas (r=0.85). Maximum and significant correlations at lag0 were also found between Casa and Forte (r=0.85), Casa and Buzinas (r=0.75), and also between Porto-Dinheiro and Buzinas (r=0.75). Moreover, at lag+1 a significant correlation between Baleal and Porto-Dinheiro was registered (r=0.81).

In Figure 18, the scaled abundance of the individuals with length between 0.6 and 2 mm presents a similar pattern to the early settler’s. With maximum abundances in the southern locations and in the island during winter, and during spring the maximums are presented in the northern locations.

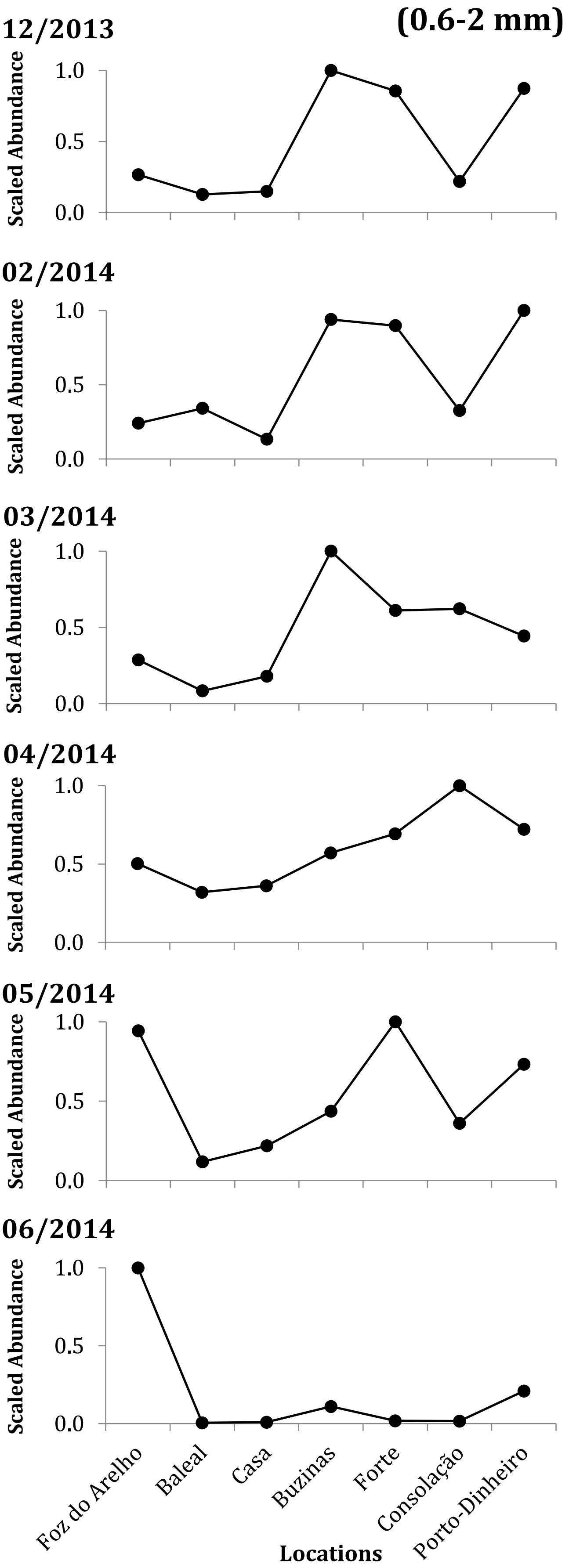


Figure 18 - Scaled abundance of the plantigrades (0.6 to 2 mm) in every locations, between December 2013 and June 2014. Locations: left to right, corresponds to North to South.

Settlement time series (spring sampling – every other day)

During May, the peak of spring season, the settlement in two locations (inside and outside the Berlengas Natural Reserve) was observed every two days. The settlement patterns between the two locations were similar, despite the different scales (Figure 19). The fact that the maximum length we found on this samples was 0.60 mm indicates that we were dealing with 'primary settlement' (Bayne 1964). Buzinas had a maximum of settlement in the 6th May, with 2.33±4.04 indiv/square, while the maximum in Baleal was of 29.67±5.77 indiv/square on 20th May. No significant correlation was found between these two locations at any lag.

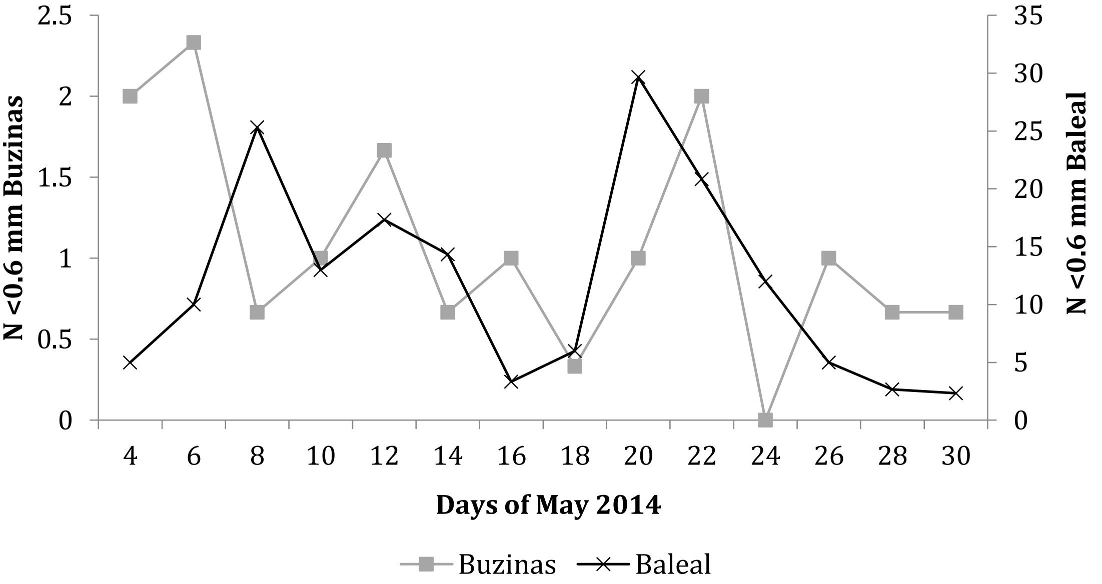


Figure 19 - Settlement time series during May (spring season) every two days in two locations, Buzinas (inside de reserve) and Baleal (outside the reserve).

The settlement time series of these two locations, Buzinas and Baleal, during the peak of spring season, were compared with the evolution of the environmental parameters during the same period (Figure 20). When analysing the upwelling index (UI) with the mean number of early settlers, it seems that there is a relation between the maximum of UI and the peak of early settlers a few days later, in both locations.

With regard to chlorophyll *a*, in Baleal this factor seems to have a clear influence on settlement, as the peaks of Chl-*a* match settlement peaks. In Buzinas this relations is not so evident, although the concentration of Chl-*a* in this location is lower than in Baleal. The temperature appears to have some influence in the mussel settlement as well, once the small increases and decreases of temperatures corresponds in a certain way to the increasing and decreasing of the early settler's number in both locations, although this factor is also related to the upwelling index. The small variations of salinity registered during the peak of spring season seems to have no effect in the number of mussel settlement per day, both in Buzinas and Baleal.

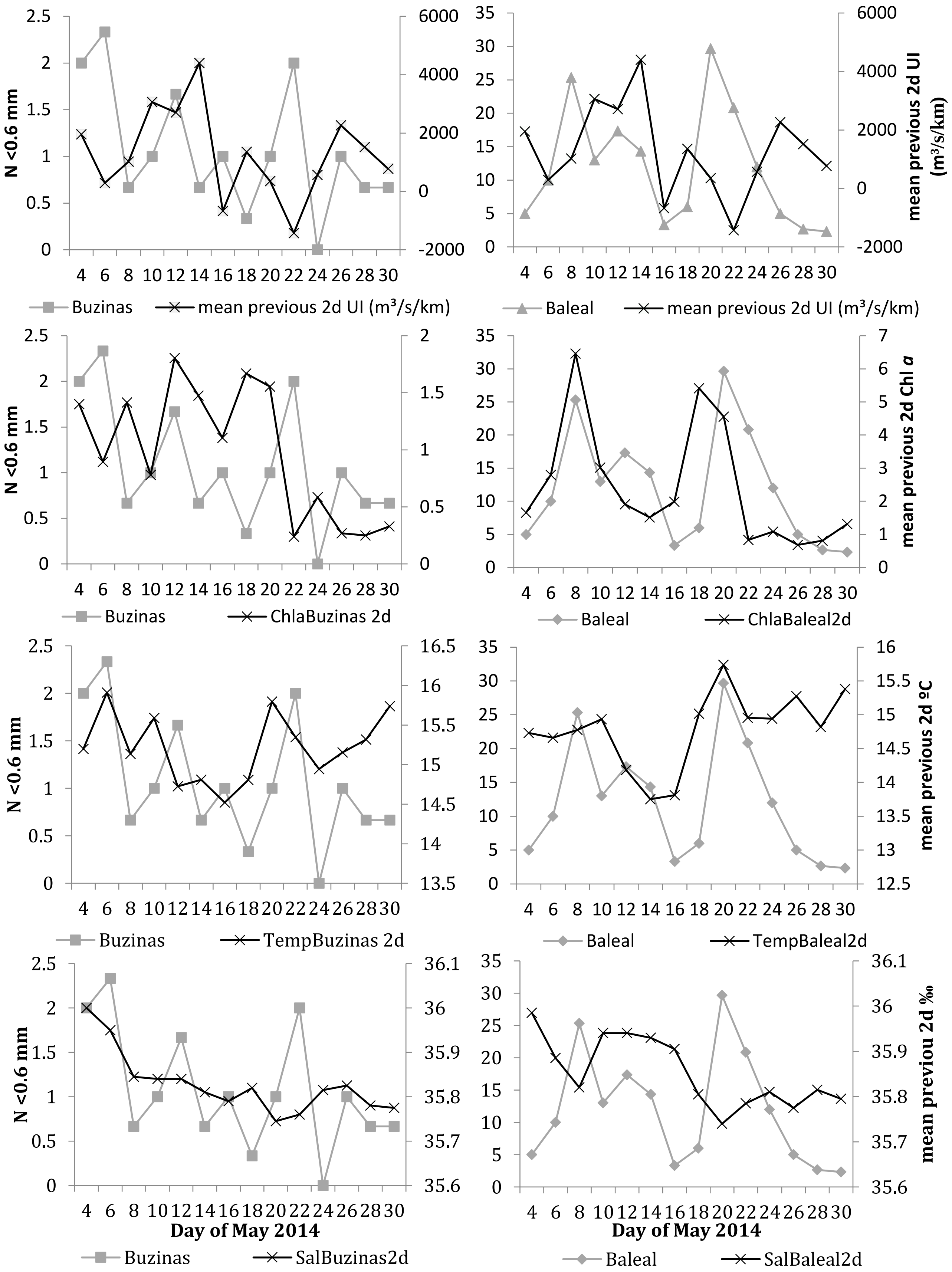


Figure 20 - Settlement time series during the spring season, every two days in May 2014, in Buzinas (left) and Baleal (right) and the evolution of the environmental parameters (UI, Chl-*a*, temperature and salinity) during the same period.

A Generalized Additive Model (GAMs) was built to test the significance of the relationships observed between the environmental variables and mussel settlement, According to the results, 64.8% of the settlement variability observed can be explained using Location as a factor and its interactions with the mean upwelling index during the previous 2 days and Chl-*a* concentration (Table 6, Figure 21A, B). The ANOVA showed a significant effect of Location ($p<0.001$), reflecting the significantly lower magnitude of settlement in Buzinas. UI and Chl-*a* concentration only showed a significant effect in Baleal (Table 6).

About the Chl-*a* concentration, there is a linear and positive relation in Baleal. The higher the concentration of Chl-*a*, the higher the *M. galloprovincialis* settlement in this location (Figure 21B). However, for UI the relation is positive and linear until reaching positive values of UI (upwelling situations). Once the upwelling index is positive, increasing the intensity will not have a higher effect on the settlement magnitude (Figure 21A).

Table 6 - Structure of the General Additive Model selected to describe the Upwelling Index (mean of 2 previous days) and Chl-*a* concentration in the mussel settlement each 2 days in May. S.E.: standard error; e.d.f.: estimated degrees of freedom.

Parametric coefficients	**Estimate**	**Std. Error**	**t**	**Pr(>|t|)**
Intercept (Baleal)	0.757	0.062	12.282	$<2x10^{-16}$ ***
Buzinas	-0.515	0.102	-5.056	$3.22x10^{-6}$ ***

Approximate significance of smooth terms	**edf**	**F**	**p-value**
s(mean2d):Baleal	5.111	3.197	$7.9x10^{-3}$ **
s(mean2d):Buzinas	1.000	0.000	0.990
s(Chl-a):Baleal	1.000	13.255	$5.03x10^{-4}$ ***
s(Chl-a):Buzinas	1.000	0.016	0.898

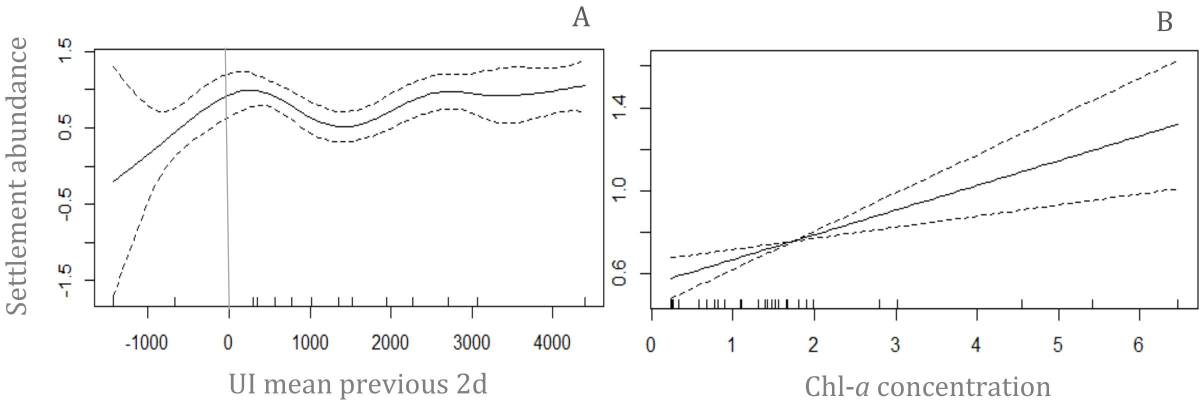


Figure 21 - Result of the Generalized Additive Models (GAM), showing the partial effect of the variables UI mean of the previous 2 days (A), Chl-*a* concentration(B) on settlement abundance in Baleal. Dotted lines indicate 95% confidence intervals, and tick marks along the X-axis below each curve represent effect values where observations occurred.

Histograms, cohort analysis and growth rate

In this chapter we analyse cohort progression and growth rates of recently settled *Mytilus galloprovincialis* based on the monthly algal turfs scrapped at each location. For this purpose, we started by making histograms for every sampling months in each sampling site (Figures 22-25), after what we have made cohorts analysis with the software FISAT II (Figures 26-29), and with the means of this identified cohorts, the growth rate for each location was calculated (Tables 7-8).

The size frequency distribution along the sampling period are presented by "regions". First the northern locations, Foz do Arelho and Baleal (Figure 22), followed by the Berlengas island locations, Buzinas and Forte (Figure 23), the central location, Casa in the Cape Carvoeiro (Figure 24) and ultimately Consolação and Porto-Dinheiro (Figure 25) south of the Cape. Based on these size frequency distribution, we observed that most of the individuals are below 1.5 mm with a few exceptions. Those are Foz do Arelho in December, when the mode is in the size class [2.50; 2.75], Casa in December and February presented a mode in classes [2.00; 2.25] and [1.50; 1.75] respectively. The mode at Buzinas in March is in class [1.50; 1.75], and at last, Forte in June shows the mode in [1.75; 2.00].

We can also observe through the histograms the spread of the data in the range between 0 and 20 mm, and all the locations have, although not always in every month, individuals that cover a large range of values. Only in Casa's samples, we can see that the collected individuals do not cover a wide range of classes, which can be indicating a higher post-settlement mortality or migration (also named as secondary settlement) in this location.

Based on FISAT II routine, a series of cohorts were identified for each sampling month and location. The progression of the modal classes considered for each cohort is plotted in Figures 26-29. Eleven juveniles cohorts of *M. galloprovincialis* were identified in Foz do Arelho and nine in Baleal (Figure 26). For Buzinas and Forte, eleven and six cohorts were identified respectively (Figure 27). Eight cohorts were recognized both in Casa and Consolação (Figure 28), and six cohorts in Porto-Dinheiro (Figure 29). The analysis in FISAT II indicated that the settlement of *M. galloprovincialis* occurred every month from December/2013 to June/2014 in every locations.

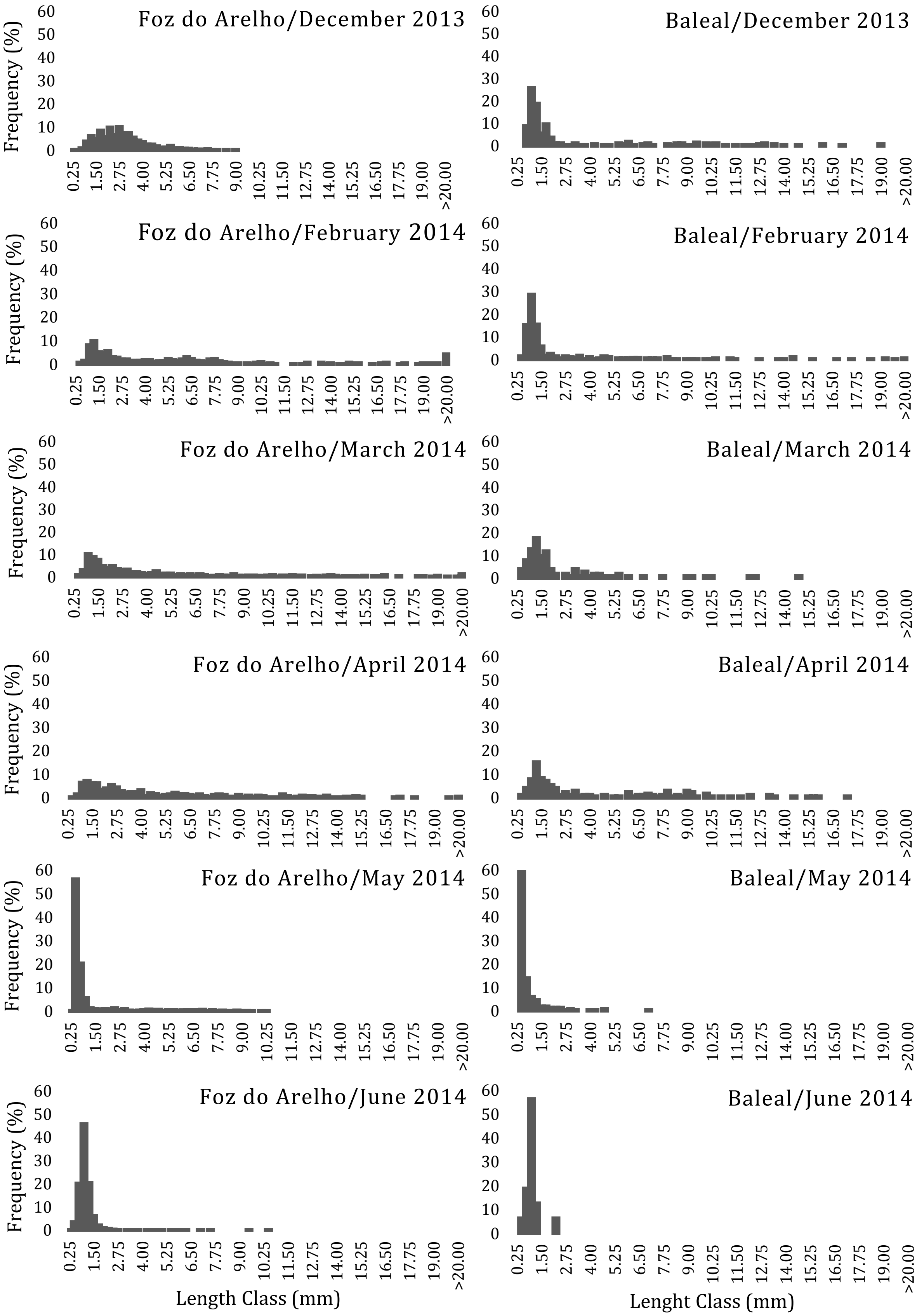


Figure 22 - Size frequency distribution of *Mytilus galloprovincialis* collected in Foz do Arelho (left) and in Baleal (right) through the same period. In the x-axis is the shell length classes and y-axis is the mean frequency of mussels per sampled area.

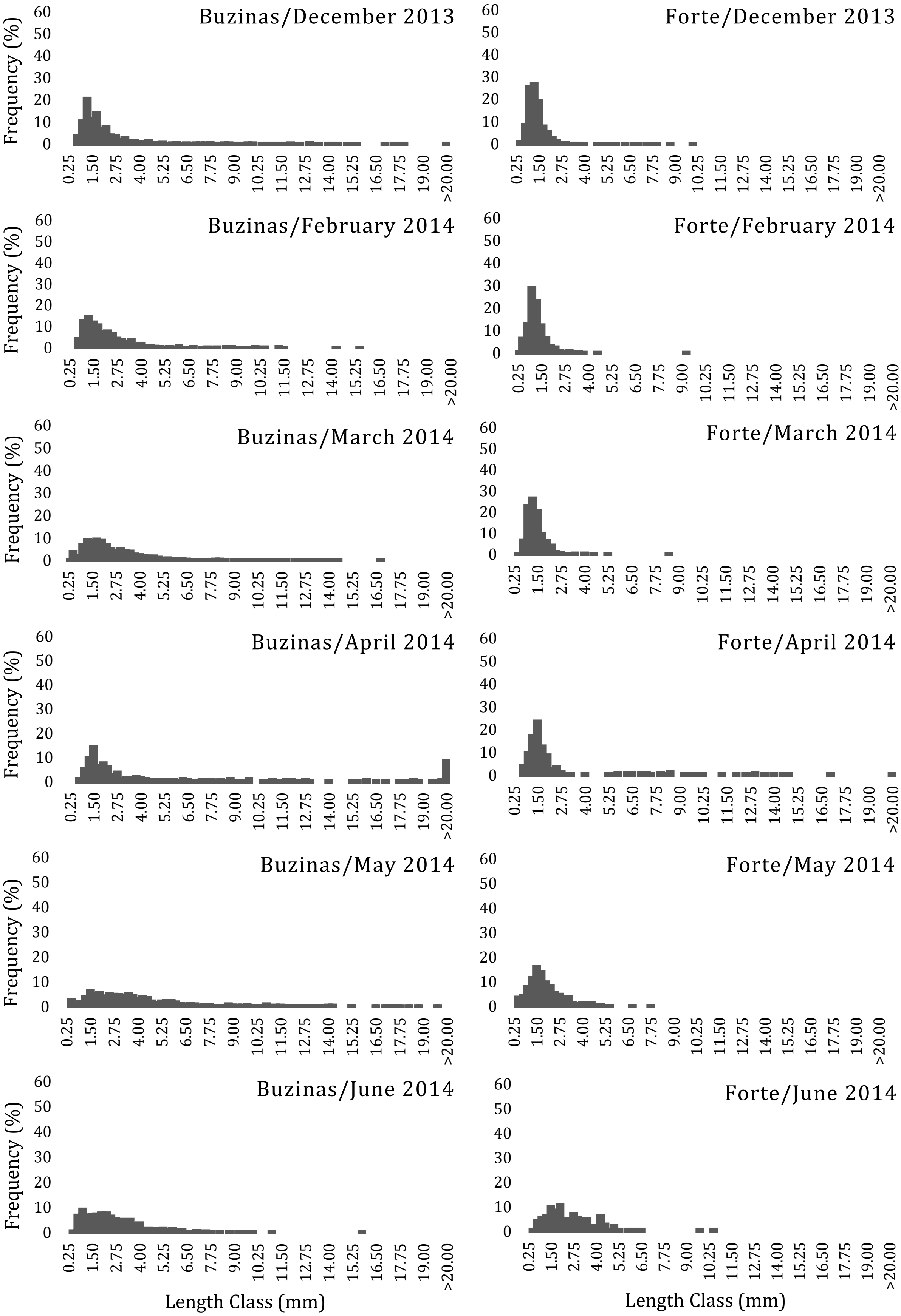


Figure 23 - Size frequency distribution of *Mytilus galloprovincialis* collected in Berlengas - Buzinas (left) and Forte (right) through the same period. In the x-axis is the shell length classes and y-axis is the mean frequency of mussels per sampled area.

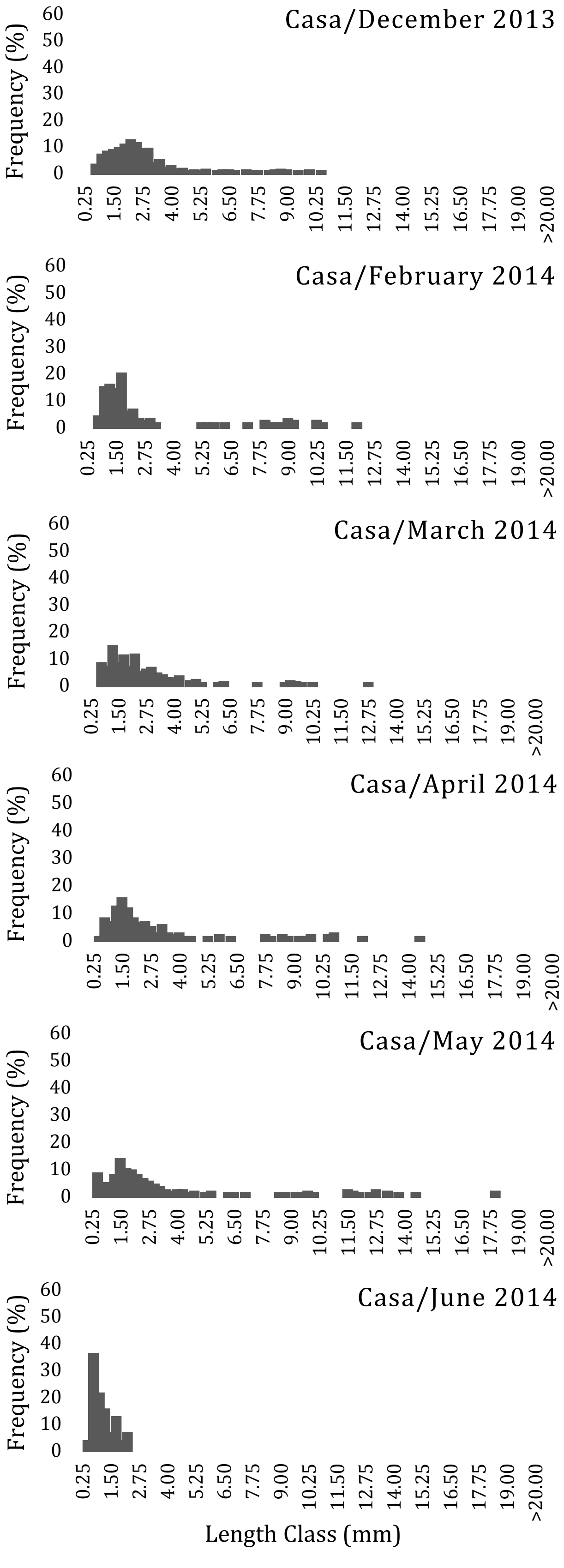


Figure 24 - Size frequency distribution of *Mytilus galloprovincialis* collected in Casa, Cape Carvoeiro. In the x-axis is the shell length classes and y-axis is the mean frequency of mussels per sampled area.

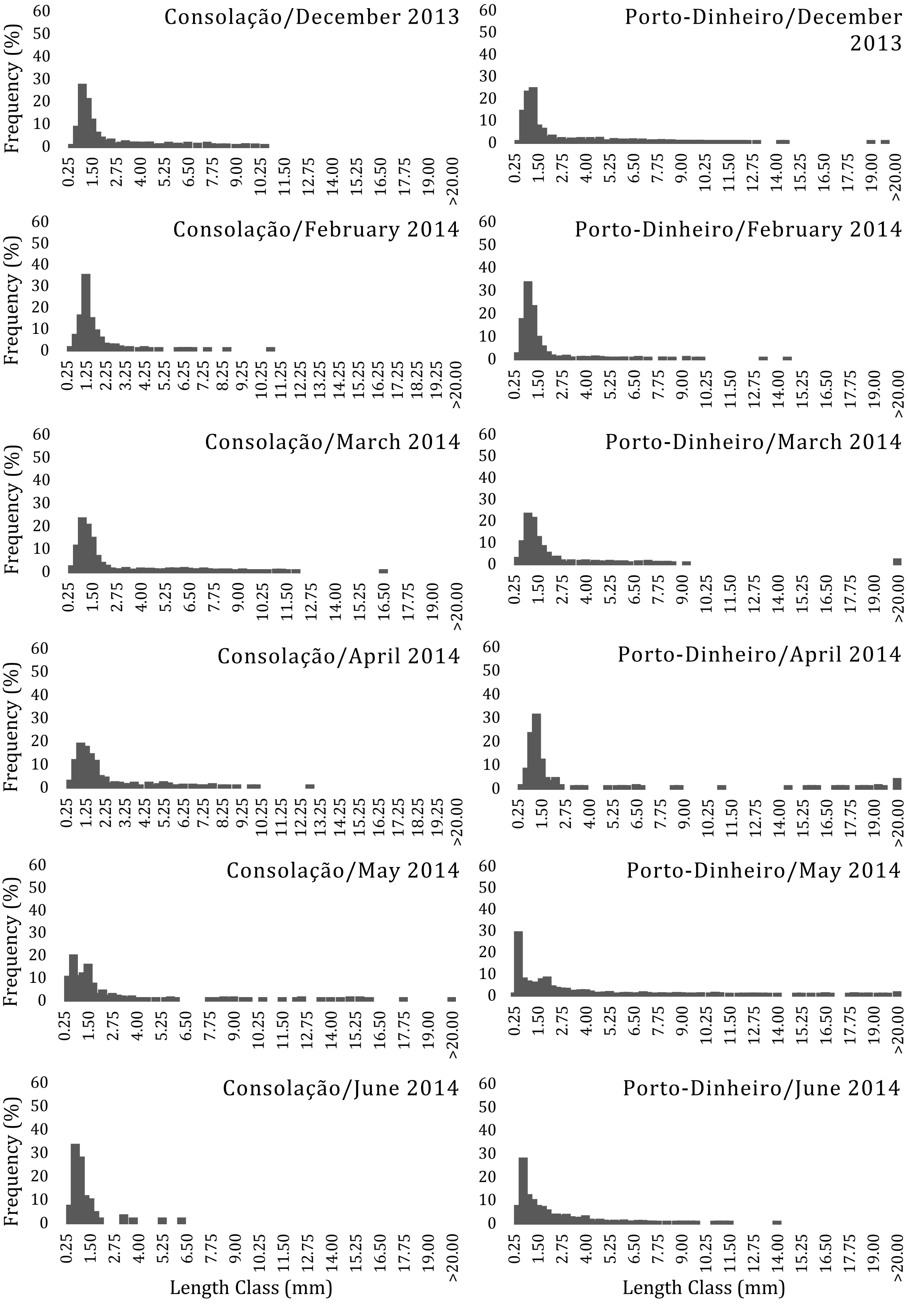
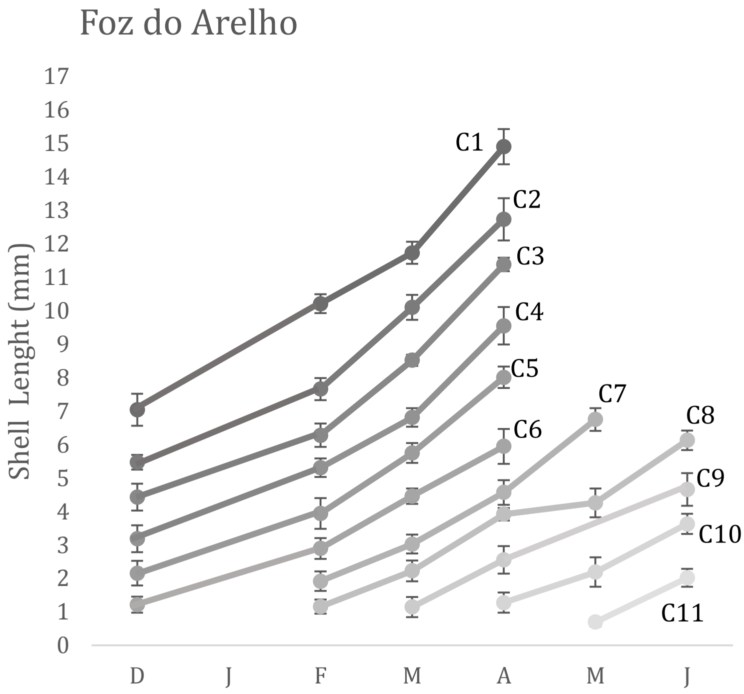


Figure 25 - Size frequency distribution of *Mytilus galloprovincialis* collected in Consolação (left) and in Porto-Dinheiro (right) through the same period. In the x-axis is the shell length classes and y-axis is the mean frequency of mussels per sampled area.



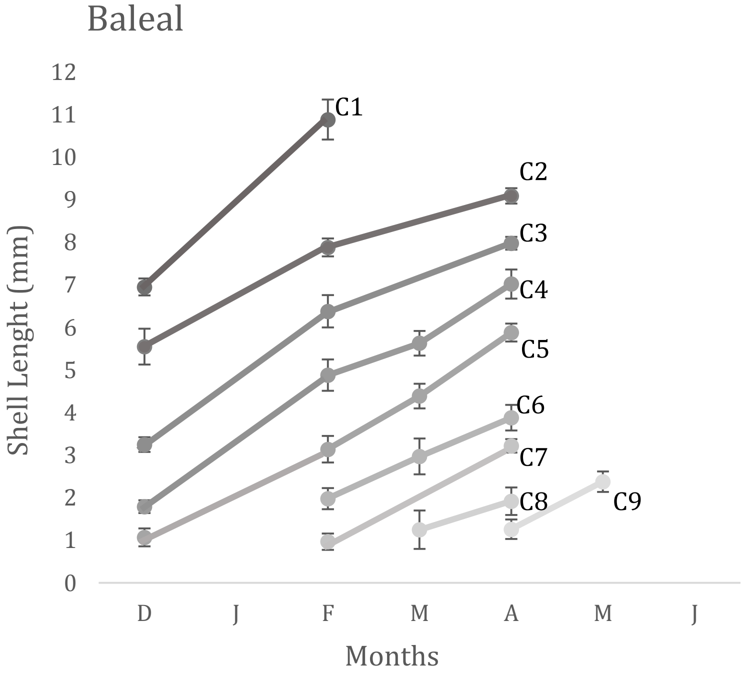
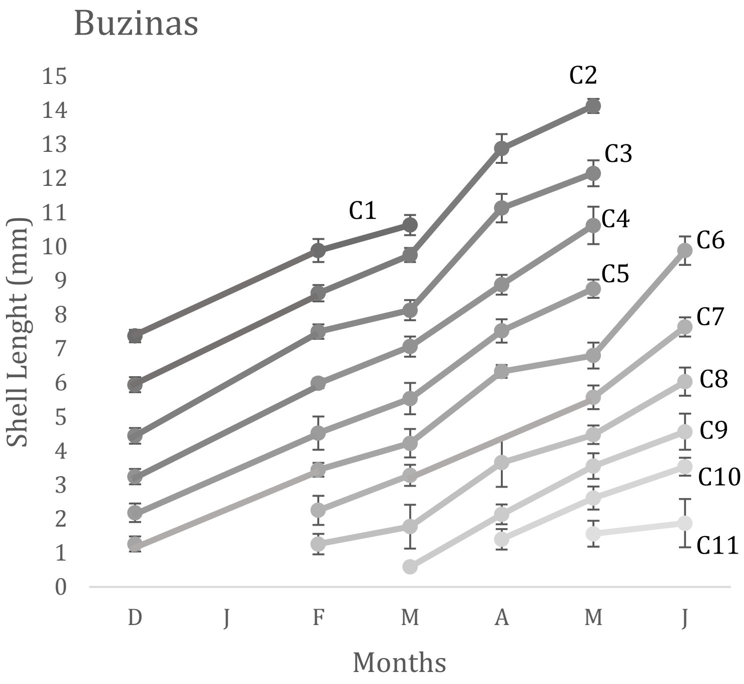


Figure 26 - Mean cohort length of *M. galloprovincialis* in Foz do Arelho and Baleal with indication of 11 and 9 cohorts respectively identified by FISAT II (C1-C11; C1-C9).



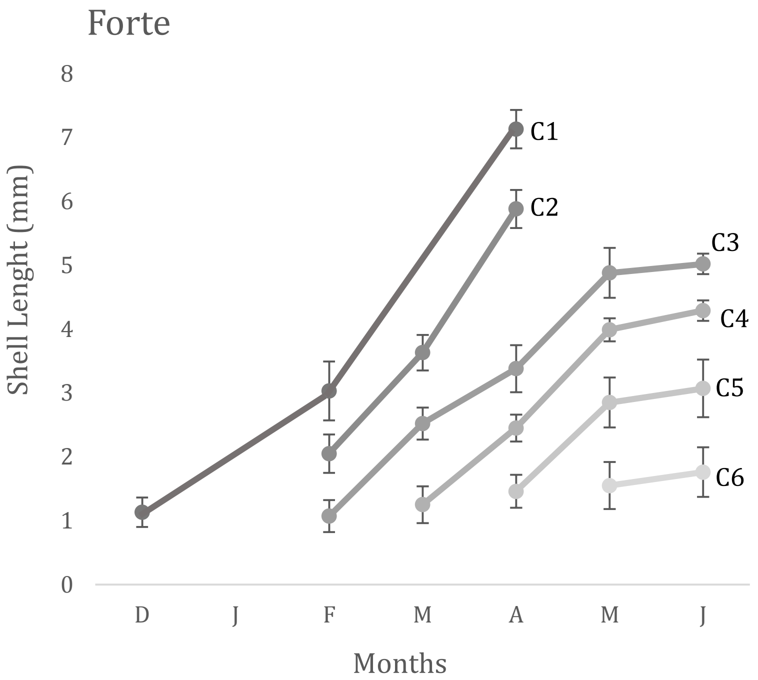
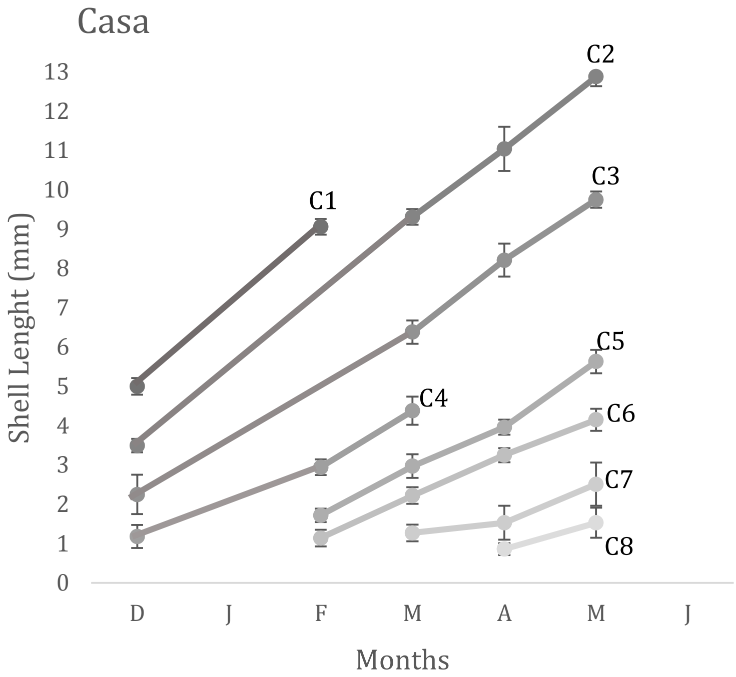


Figure 27 - Mean cohort length of *M. galloprovincialis* in Buzinas and Forte with indication of 11 and 6 cohorts respectively identified by FISAT II (C1-C11; C1-C6).



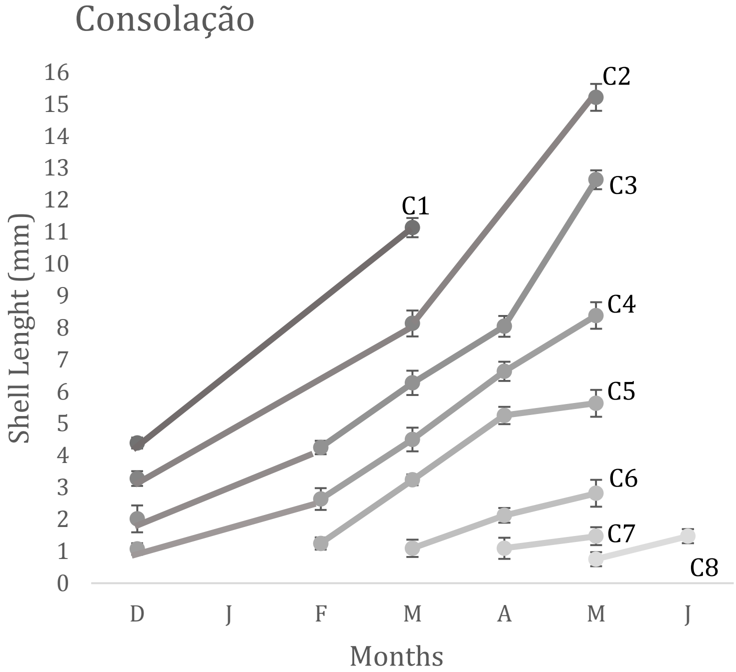


Figure 28 - Mean cohort length of *M. galloprovincialis* in Casa and Consolação with indication of 8 cohorts in both locations identified by FISAT II (C1-C8).

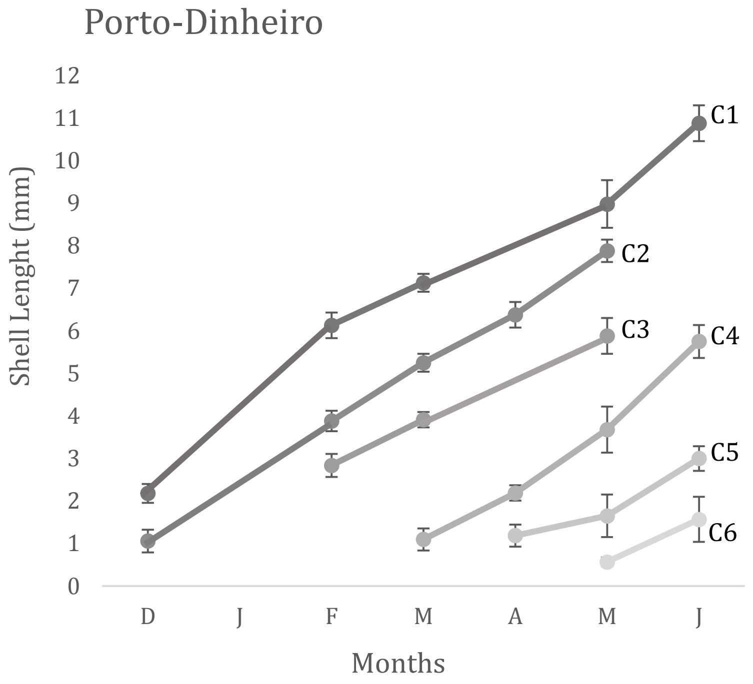


Figure 29 - Mean cohort length of *M. galloprovincialis* Porto-Dinheiro with indication of 6 cohorts identified by FISAT II (C1-C6).

Based on the progression of the modal classes, the growth rate of *Mytilus galloprovincialis* was calculated for every locations (Table 7), ranging between 1.017 mm/month minimum in Forte and 1.211 mm/month maximum in Consolação, coinciding with the lowest concentrations of Chl-*a* in Berlengas, and the highest in Consolação. In accordance with ANCOVA results (Table 8), the locations with significant different growth rates between them were Forte and Consolação, Forte and Porto Dinheiro, Forte and Casa, Baleal and Consolação, and finally Consolação and Buzinas.

Table 7 - Estimated growth rate of the *M. galloprovincialis* populations at each sampling station, calculated based on the progression in time of the cohorts identified by FISAT II.

Local	**Log Growth Rate (mm/month)**	**Std. Error**	**R^2**
Forte	1.017	0.009	0.934
Baleal	1.077	0.022	0.974
Buzinas	1.101	0.019	0.970
Foz do Arelho	1.112	0.020	0.978
Casa	1.155	0.021	0.952
Porto-Dinheiro	1.186	0.021	0.957
Consolação	1.211	0.022	0.959

Table 8 - ANCOVA results testing the effect of time (log Time) and location (Loc) and its interaction (log Time x Loc) on size (log Size).

	log (size)				
Source of variation	**d.f.**	**SS**	**MS**	**F**	**p**
log (time)	1	27.856	27.856	6469.345	< 0.001
Loc	6	0.029	0.005	1.141	0.339
log (time) x Loc	6	0.057	0.010	2.214	< 0.05
Residuals	227	0.977	0.004		

Discussion and Conclusions

There is a high variability of processes that lead to larval settlement, since a large number of factors can influence at different scales, temporal and spatial, the amount of competent larvae present at one moment, in one place (Pineda 2000, Porri *et al.* 2006, Peteiro 2009).

The mussel settlement patterns observed with this study are suggesting the existence of different larval delivering mechanisms to North and South of Cape Carvoeiro, located in Peniche. The highest relative abundances of early settlers (<0.6 mm) were found in different locations during winter and spring (Figure 15), which lead us to assume that settlement is not occurring at the same time in all stations of the study area. During winter, the peaks of abundance were mainly found in the island and in the southern locations (Forte and Buzinas, Consolação and Porto-Dinheiro). While during spring, the maximum relative abundances were detected primarily in the northern locations (Baleal and Foz do Arelho). The plantigrades relative abundance (individuals with length between 0.6 and 2 mm) (Figure 17) presented a very similar pattern to the early settlers already described.

In our study area, located in the Western Iberia Upwelling Ecosystem, the autumn/winter season is usually related to downwelling events, when prevalent currents circulate from North to South, while during spring and summer are typically present the upwelling events, with flows from South to North (Fiúza 1983, Queiroga *et al.* 2007).

Larval delivery can be determined by the upwelling regime and by the presence of upwelling fronts, as we could see in several works as Morgan and Fisher (2010) and Pineda *et al.* (2007). It is known that upwelling systems are considered very dispersive, however there are mechanisms, behaviour mediated, which may help larvae to keep close to the coast (Morgan *et al.* 2009b, Morgan *et al.* 2009c, Shanks and Shearman 2009), and some of this mechanisms are related to the formation of fronts (Shanks *et al.* 2000, McCulloch and Shanks 2003). Since we could observe a lack of delivery to the island locations during the upwelling favourable season, this could be related to the formation of an upwelling front between the island and mainland which prevent larval delivery to the island. The upwelled water seems to interact with the Carvoeiro cape, limiting larval delivery south of the cape or to the Island during upwelling favourable events. Those locations are receiving larvae mostly during downwelling favourable season. This might indicate a switch on source-sink interactions coupled to the upwelling-downwelling regime. As we can see in Figure 13, the sampling in the beginning of December was preceded by negative values of the upwelling index (indicating a downwelling event, with lower temperatures near shore) the same way as positive values of the UI were registered before the May sampling (representative of an upwelling event), although not intense.

The warmer temperatures associated with the lower concentrations of chlorophyll-*a* in the Island (Tables 1 and 3) also seems to indicate the presence of this upwelling front in between the Archipelago and mainland. Oliveira *et al.* (2009) describe all the upwelling process for this region. The cycle of an upwelling event starts with the intensification of northerly winds that lead to SST decrease and an increase in chlorophyll-*a* concentration near the coast, with the currents circulating from North to South. If the event persists, the cold water filament becomes wider until a certain point when it can reach the island. A recirculation area is formed to South of the Archipelago and embrace Consolação and Porto-Dinheiro areas. The stronger the upwelling event, the larger is the recirculation area, reaching southern locations. The patterns of the chlorophyll-*a* concentration and sea surface temperature are always related to each other (Oliveira *et al.* 2009).

Warmer temperatures in the Island with regard to the mainland (*DifTemp*), and its relationship with *UI5d* and *UI10d* also agree with the hypothesis of an upwelling front between locations (Figure 14). The upwelling front might overpass the Island under intensive and persistent upwelling events, as has been described previously (Oliveira *et al.* 2009) but during our study period, from winter to early Spring, upwelling was not that intense and persistent. So that, delivery to the Island might also be related to relaxation or downwelling events.

Limitation of larval supply during upwelling events is also supported by the every other day settlement time series in May, where we could observe an order of magnitude higher settlement in mainland (location - Baleal) than in the Island (location - Buzinas) (Figure 19). In addition, with the Generalized Additive Model of settlement versus the upwelling index of the previous 2 days per location and the chlorophyll-*a* concentration per location (Table 6), we obtained significant effects of both environmental variables on settlement at Baleal but not at Buzinas. The linear relationship observed between the UI and settlement until UI reaches positive values indicate that settlement at Baleal is favoured by upwelling situations (Figure 21). Nevertheless, once UI reach positive values, the intensity of upwelling don't show an effect on settlement anymore (Figure 21). The relationship observed between density of early settlers in Baleal and UI might be related with a delivery of larvae from northern locations. Once upwelling is present, and a flow from North is stablished, the intensity is not that relevant (Figure 21). With regard to Chl-*a*, it might be a direct effect because of the effect of Chl-*a* on larval survival and development, or indirect because it is possible that the same factors controlling phytoplankton (Chl-*a*) accumulation might be controlling larvae accumulation as well.

One reason for these differences between mainland and the island might be that larvae are delivered from the North with the upwelled water, and only reach the island under intense upwelling episodes. In the study months there were no records of intense and extended periods of upwelling, so that the front might be always in between the island and mainland. This could affect the delivery of larvae to the island, once the colder waters didn't get there.

During a downwelling event, warmer waters come from the south, from the Sesimbra/Lisbon area where we know to be a strong source of *M. galloprovincialis* larvae (Ferreira *et al.* 2013). This waters reach the southern locations of our study area (Porto-Dinheiro and Consolação) as well as the Island locations (Forte and Buzinas), but hardly have an influence to northern locations, suggesting that this warmer water get displaced from the shore due to the cape topography influence. This explains the high density of plantigrades in Porto-Dinheiro, Buzinas and Forte, and not in northern locations during the winter. The GAM of the mean upwelling index of the previous 5 and 10 days in the difference of temperature, per location (Figure 14) also demonstrates that the warmer waters don't reach the northern locations (Foz do Arelho), once the temperature difference is always increasing positively (warmer waters in the island). Baleal and Casa seems to be in the range of these warm waters once the temperature difference in relation to the island is almost zero with downwelling events. The warmer current seems not to be near the shore in the beginning of the event, as we can see in the southern locations the temperature difference is increasing until it stabilizes, probably with the waters spreading.

Roughgarden *et al.* (1988) formulated the hypothesis that larvae should be transported offshore during upwelling by Ekman's transport, having a passive particles behaviour once their swimming speed was lower than cross-shelf currents, and carried onto the shore with downwelling events. Although, contrary to this predictions, there are reports of larval settlement during both upwelling and downwelling events, similarly to our results (Morgan *et al.* 2009a, Shanks and Shearman 2009). This diverse results weren't taking into account the larval behavioural component capable of determining whether the settlement occurs (Pernet *et al.* 2003). Even for being retained in the upwelling front, larvae need to develop certain behaviours. Or they are capable to keep their position in between the coast and the front, or they are being accumulated inside the front itself. Either way, larval behaviour is determinant for their final position (Franks 1992, Shanks and Shearman 2009).

Shanks and Brink (2005) observed that bivalve larvae was nearest the coast during an upwelling event, with larvae remaining below the thermocline. In the other hand, in Ma (2001)'s work, also with bivalves, the larval settlement occurred during downwelling events, and the larvae were found above the thermocline. The temperature below the thermocline in Shanks and Brink (2005) and above it in Ma (2001) was similar, which suggests a preference of the species for some temperatures, as demonstrated by Ruiz *et al.* (2008) for *Mytilus galloprovincialis*, and for other bivalves (Hidu and Haskin 1978, Mann and Wolf 1983). So, it has been concluded that the effect that upwelling and downwelling have on larval distribution cannot be predicted without knowing their vertical position in the water column (Shanks and Shearman 2009, Pfaff *et al.* 2011).

Even if upwelling is not playing a fundamental role in cross-shore transport, might be much more relevant in along-shore dispersal, as has been suggested previously (Wing *et al.* 1995). From an evolutionary point of view, it is also more important for larval and juvenile survival to maintain a position close to shore, rather than keep a position close to the parental habitat.

Differences in settlement abundance between locations are most likely linked to differences in larval delivery and source-sink relationships according to upwelling regime and predominant along-shore flow directions than to differences in habitat quality, once the growth rates are similar between all locations. Post-settlement mortality was not considered because of the enormous variability observed, which we consider to be related with secondary settlement behaviour, where plantigrades have a preferential habitat different than early settlers (Bayne 1964, Caceres-Martinez *et al.* 1994, Navarrete *et al.* 2015). Therefore, we did not found adult mussels in the algal turfs, however, the fact that growth rates are similar, indicates similar performance in every locations. Predation upon newly recruited individuals can also offer an explanation to the high variability in the distribution of mussel juveniles (Dobretsov and Miron 2001). Another factors strongly affecting mussel populations are disturbances by excessive sand and storms (Erlandsson *et al.* 2006, Zardi *et al.* 2008), both common occurrences in the study area over the past few years.

The mussel populations in the Berlengas archipelago may be limited by the larvae arrival occurring only during downwelling episodes, which are more frequent during the winter, when low temperatures, availability of food, storms, among others, may hinder survival. In this case, the reserve does not have a role as a "source" to other locations but as "sink". The fact that there is protection in the islands can facilitate the establishment of adult populations, since the potential for survival increases without disturbances. This can have a major importance not only for mussels' populations, but also for other species associated with them.

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