



UNIVERSIDADE D
COIMBRA

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INTERANNUAL VARIABILITY IN EARLY LIFE
HISTORY AND ESTUARINE COLONIZATION OF
EUROPEAN SEABASS *DICENTRARCHUS LABRAX*

Dissertação no âmbito do Mestrado em Ecologia, orientada pelo Doutor
Filipe Miguel Duarte Martinho e pelo Professor Doutor Miguel Ângelo do
Carmo Pardal e apresentada Departamento de Ciências da Vida da
Universidade de Coimbra.

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Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia
da Universidade de Coimbra

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Resumo

Entender como é que o peixe marinho é afetado por variáveis ambientais e oceanográficas durante as fases iniciais do ciclo de vida é fulcral dada a sua importância nas dinâmicas e conectividade da população. O objetivo deste trabalho foi determinar influência destes processos na variabilidade interanual nas datas de eclosão e padrões de crescimento do robalo, *Dicentrarchus labrax*, ao longo de um período de sete anos, desde 2011 a 2017. De forma a atingir este objetivo, foi utilizada a microestrutura de otólitos para determinar as datas de eclosão do robalo e para desenvolver cronologias de crescimento. Na maioria dos anos, o período de eclosão ocorreu entre fevereiro a abril, com duas exceções: no ano 2012 a eclosão começou no início de janeiro e em 2016 ocorreu um período de eclosão excepcionalmente longo. Através da utilização de Modelos aditivos Gerais (GAMs) verificou-se que a temperatura da água do mar e o índice da Oscilação do Atlântico Norte (NAOi) foram os principais fatores responsáveis pela variabilidade observada nas datas de eclosão do robalo, enquanto que a Clorofila-a teve impacto reduzido. A relação entre a temperatura da água do mar e as datas de eclosão foi positiva, enquanto que a relação entre o NAOi e as datas de eclosão do robalo foi negativa. O desenvolvimento de cronologias de crescimento permitiu identificar os principais efeitos intrínsecos e extrínsecos responsáveis pelo crescimento de juvenis. Estas cronologias foram desenvolvidas através de modelos lineares generalizados mistos (GLMM) e incluíram como co-variáveis a temperatura da água do mar e o NAOi. Em 2011 e entre 2013 a 2017, foram observados padrões de crescimento semelhantes, que começaram com crescimento acima da média durante um curto período, seguidos de uma queda acentuada até ao ponto de menor crescimento e depois, à medida que a colonização estuarina progrediu, o crescimento aumentou de forma estável até atingir o ponto máximo em torno do fim de junho e início de julho. Em 2012, o crescimento nos primeiros dias esteve abaixo da média, possivelmente devido à eclosão antecipada, em condições desfavoráveis. Uma vez que a temperatura da água do mar e o NAO são os principais fatores responsáveis pela variabilidade no recrutamento e crescimento do robalo, o seu ciclo de vida pode vir a sofrer alterações significativas devido ao aquecimento dos oceanos e ao aumento de instabilidade climática.

Palavras chave: microestrutura de otólitos, datas de eclosão, cronologias de crescimento, análise a longo prazo, alterações climáticas, modelação com efeitos mistos, robalo.

Abstract

Understanding how marine fish early-life history is affected by environmental and oceanographic factors is critical given their importance to population dynamics and connectivity. This work aimed at determining the influence of these processes on the interannual variability in hatch dates and growth patterns of European seabass *Dicentrarchus labrax*, over a seven-year period, from 2011 to 2017. To accomplish this, otolith microstructure analysis was used to determine seabass hatch dates and to develop growth chronologies. In most years, the hatching period occurred from February to April, with two exceptions: In the year 2012 hatching started in early-January and in 2016 an exceptionally long hatching period occurred. Through the use of Generalized Additive Models (GAM), it was observed that sea surface temperature and the North Atlantic Oscillation index (NAOi) were the main drivers behind the inter-annual variability in seabass hatch dates, while Chlorophyll-a's impact was reduced. Sea surface temperature had a positive relation with hatch dates whereas NAOi's was negative. Development of growth chronologies allowed to assess the effects of intrinsic and extrinsic factors on juvenile growth. Said chronologies were developed using Generalized linear mixed models GLMM and included sea surface temperature and NAOi as covariates in the models. In 2011 and from 2013 to 2017, similar growth patterns were observed, starting with above average growth rates, for a short period, followed by a sharp decrease until the lowest growth point, and then as estuarine colonization progressed, growth steadily increased until peaking near June's end to early July. In 2012 initial growth was below average, possibly due to an earlier hatching under unfavourable mid-winter conditions. Since seawater temperature and the NAO were the main drivers for seabass recruitment and growth, its life cycle may suffer important changes due to oceans warming and an increasingly unstable climate.

Keywords: otolith microstructure, hatch dates, growth chronologies, long-term data, climate change, mixed effects modelling, seabass.

1 - Introduction

1.1- Connectivity between coastal areas and estuaries and recruitment in marine fish

Many marine fish have complex life histories, in which transitions between life stages often coincide with shifts in habitat use (Able, 2005). These life history characteristics are shared by several coastal species, in which spawning takes place offshore, after which newly-hatched larvae are transported into shallow coastal and estuarine areas, where they remain for varying amounts of time, prior to their migration back into coastal areas. In this sense, the exchange of individuals amongst geographically separated units, i.e. connectivity, is a key feature for the population dynamics of these species (Cowen, 2000; Gillanders et al., 2012). The rates at which the exchange of individuals occurs are very important, as they can affect the population in its resiliency to external pressures or ability to colonize different areas (Thorrold, 2001). Still, quantifying the scale and extent at which fish larvae are able to disperse, remains a challenge (Morat et al., 2014). Being able to move across different habitats is fundamental for many marine species, especially fish (Thorrold, 2001). In a given life cycle stage, environmental conditions some fish species require are variable (Able, 2005), and, as a consequence, many of these perform migrations, searching for conditions best suiting development. As such, connectivity plays a key role in the recruitment process for fish.

Recruitment consists of “the number of individuals that reach a particular age to join a specific part of the population” (Rijnsdorp et al., 1995), and currently its variability is one of the most important issues in fisheries ecology (Rijnsdorp et al., 2009). Recruitment is influenced by both density-independent and density-dependent factors (Miller et al., 1991; Rijnsdorp et al., 1995; Cabral et al., 2007). In the ocean, fish survival, growth and recruitment is mostly influenced by density-independent factors (Rijnsdorp et al., 1995; Bento et al., 2016), such as sea surface temperature (SST) (van der Veer et al., 2000; Martinho et al., 2009), the North Atlantic Oscillation (NAO) (Martinho et al., 2009), upwelling and Eckman transport (Santos et al., 2004), offshore advection (Santos et al., 2004), and the extension of river plumes into coastal areas (Tanner et al., 2017).

The North Atlantic Oscillation index (NAOi) has been recognized as one of the major drivers for long-term ecological measures, including fish stocks, ocean productivity and growth of marine organisms (Attrill and Power, 2002). A large-scale climate system affecting Europe and North America, the NAOi is determined from the difference in sea-level pressure between Reykjavik (Iceland) and the Azores (Portugal) (Hurrell, 1995). The NAOi has a high influence on climate both at large and local scales (Lindegren et al., 2010; Bento et al., 2016): At large scales, the NAOi has a more indirect effect, being responsible for climate patterns (Hurrell, 1995). In southern Europe, years where NAOi is positive are generally milder and drier (Hurrell, 1995; Rodwell et al., 1999), while negative NAOi years are characterized by harsher conditions,

including heavy rain and storms. For instance, in the Baltic and North Sea, (Lindegren et al., 2010) reported that a transition of NAOi from a positive phase to a negative one in 1987-1989 caused a dramatic shift in the ecosystem regarding salinity, oxygen and temperature, which affected the cod stock.

The metabolic activity in most living organisms is heavily influenced by temperature. In marine organisms this no exception and thus, changes in ocean temperature will have influence over certain marine species. Water temperature is a factor able to affect fish reproduction (Politikos et al., 2015), spawning (Beraud et al., 2018; Vaz et al., 2019) and egg's survival (Cabral et al., 2001; Vinagre et al., 2009a). *Dicentrarchus labrax* is an example where SST has been linked to reproduction and to an optimal temperature towards egg-laying, in the North Sea (Beraud et al., 2018). When deviating from this threshold, fewer eggs are released (Beraud et al., 2018). As a consequence of SST having such an impact on fish since the earliest life-stages, a form of cascade effect may occur throughout fish life cycle.

Food availability is also known to be responsible for variations in growth and survival of early life stages in marine fish (Le Pape and Bonhommeau, 2015). Indeed, several papers have related species abundance/survival to primary production (the base of the food chain), which can be determined using Chlorophyll-a (CHLa) as a proxy (Martino et al., 2019). Indeed, Cushing (1975), pointed out that larvae survival can be determined through a synchronization (or lack of) between hatching and favourable environmental conditions, which is known as the match-mismatch hypothesis. According to this hypothesis, a good match means that reproduction will occur at a time where larval hatching will coincide with a food abundance period, and thus increasing larvae survival odds.

Because larvae have little to no swimming capabilities, they are susceptible to the effects of oceanographic dynamics, which can include upwelling and Ekman transport. These two events are the result of water displacement due to winds blowing on the ocean surface. Upwelling consists on the rise of colder water to replace that which moved away, while Ekman transport consists of a water sinking in the ocean in a spiralling effect. To resist these effects, larvae are able to perform vertical migration (Leis, 2006; Tanner et al., 2017; Beraud et al., 2018), which allows them to remain near shore. Comerford et al., 2013 and Fox et al., 2006 suggested that larval vertical migration is a very important behaviour to increase chances of settling in suitable locations. Still, when Ekman transport and upwelling are too intense, they may retain or cause larvae advection (Santos et al., 2004), thus hampering migration towards estuaries and hindering recruitment (Santos et al., 2007).

When near nursery areas, larvae are able to sense cues, such as small-scale variations in salinity, water temperature, and turbidity (Le Pape et al., 2003; Dixson et al., 2011; Tanner et al., 2017). Indeed, brackish and slightly warmer waters than those of the ocean can act as indicators of proximity for estuarine nursery areas (Martinho et al., 2009). In rainier years, higher river

runoff will form larger river plumes that allow attracting more larvae, which tends to result in a better recruitment, and thus in a higher abundance of 0-group fish in estuaries, as reported by several authors (Le Pape et al., 2003; Vinagre et al., 2009a; Martinho et al., 2009). In several estuaries in the Portuguese coast, a positive relationship has been determined between the abundance of juveniles of several marine species such as *Dicentrarchus labrax*, *Solea solea* and *Platichthys flesus* and river flow (Martinho et al 2009; Vinagre et al., 2009a). Similar results have been observed elsewhere: for instance, the abundance of 0-group sole in the Vilaine bay, France also increased with the size of river plume (Le Pape et al., 2003)s, thus highlighting their importance for the completion of the life cycle of marine fishes with juvenile migrations.

At local scales, individuals are affected mostly by density-dependent factors such as food supply, inter and intra competition and predation (Martinho et al., 2009). According to (Le Pape and Bonhommeau, 2015), juveniles tend to gather in locations with high productivity and food abundance, as these conditions are likely to boost their fitness through better feeding. However, this often leads to competition between individuals. In order to avoid this, and according to the principle of competitive exclusion (Britton, 1989), juveniles from different fish species, with similar diets, make use of spatial and temporal variations when feeding. By doing this, juveniles avoid competition, promoting their own growth (Laffaille et al., 2000). Being the interface between river and sea, estuaries are transitional areas, where the mixture between the saltwater and freshwater results in a very dynamic natural environment (Dolbeth et al., 2010). These environments provide excellent nursery services (Beck et al., 2001), due to the combination of three main factors: i) a high habitat diversity, derived from both salinity and temperature gradients, as well as the existence of both mudflats and channel zones (McLusky and Elliott, 2004); ii) high inherent productivity, which provides a continuous food supply (McLusky and Elliott, 2004; Martinho et al., 2007; Baptista et al., 2015) and iii) refuge from predators (Able, 2005; Dolbeth et al., 2010), which despite not being a limiting factor, can increase larvae survivability (Miller et al., 1991). As such, the use of estuaries by juvenile fish greatly improve their chance of survival, while also granting the necessary conditions for rapid growth (Cabral et al., 2007; Bento et al., 2016). Nursery services play a key role in

1.2- Otoliths as time keepers

Otoliths are calcium carbonate structures formed in the beginning of the ontogenic process (Stevenson and Campana, 1992), and are located on the equivalent to the inner ear in fish. They are crucial for detecting movement, maintaining balance and ultimately for orientation (Comerford et al., 2013; Morrongiello and Thresher, 2015). Otoliths possess two special properties, which are the base to many current applications in marine ecological studies. Firstly, otolith growth is non-skeletal and continuous throughout life, even during periods of sub-nutrition and when somatic growth is non-existent (Campana and Thorrold, 2001). This opens up the

possibility of analysing growth in a continuous way, daily or seasonally, throughout an individual's life (Campana and Thorrold, 2001). Secondly, and unlike scales or bones, otoliths are never subject to resorption even in periods of starvation, which allows them to retain all the information regarding past growth and environment (Campana and Thorrold, 2001).

Otoliths grow through biomineralization and accretion (Campana and Thorrold, 2001; Elsdon et al., 2008; Nanami, 2017) and in this process, growth rings are formed. Larvae and juvenile stages present daily growth rings, deposited with constant frequency, while in adult fish only annual rings can be seen (Stevenson and Campana, 1992). After hatching, the otolith is composed by a nucleus, derived from the fusion of primordia structures, themselves precursors to otoliths (Stevenson and Campana, 1992). The nucleus has a circular shape and as the otolith grows, growth rings will form around the nucleus. Otolith growth is proportional and dependent of somatic growth - and of any disturbances to the last, occurring on the day of deposition (or on the previous one) (Gutiérrez and Morales-Nin, 1986; Stevenson and Campana, 1992). In teleost fish, we can find three pairs of otoliths: sagittae, lapilli and asterisci (Stevenson and Campana, 1992). Sagittae otoliths are larger, easier to extract and have wider increments, providing a better resolution for their microstructural analysis (Campana and Thorrold, 2001).

In fisheries ecology, otolith microstructural analysis is a powerful tool to unveil many species' early life history traits, such as hatching day, mouth opening and first feeding, settlement, metamorphosis, age and growth patterns, and even migration (Rodríguez-Valentino et al., 2015; Nanami, 2017). Several studies used otolith microstructural analysis to gain insight on several species life-history. Some examples (on economically relevant species) are: *Pleuronectes platessa* (Fox et al., 2007), *Sardina pilchardus* (Santos et al., 2007), *Lateolabrax japonicus* (Islam et al., 2009), *Platichthys flesus* (Martinho et al., 2013; Smoliński and Mirny 2017), *Esox lucius* (Fey et al., 2018) and *Solea solea* (Vaz et al., 2019). Hatch days are determined by summing the number of total increments to allow the estimation of individual age, and are used to determine individual growth rates; Determining when metamorphosis occurs is especially important in flatfish species, since estuarine colonization usually occurs after it (Amara et al., 2000; Primo et al., 2013). Thus, determining the timing and duration of key events is very important towards understanding a given fish's life cycle and is useful information to have when considering fisheries management.

The above-mentioned life-history traits are recorded in bipartite structures on a microstructural level, daily growth bands or growth rings, which form from diel variations in calcium and protein deposition (Rodríguez-Valentino et al., 2015). Daily growth increments are the basis for age and growth backcalculation, which are considered to be among the most powerful applications of the otolith microstructure examination (Stevenson and Campana, 1992). Growth backcalculation allows the determination of both the size and growth rate of an individual fish for each day of its life. This is done by using the measured widths of a daily increment time

series, together with the fish length - otolith length relationship (Francis, 1990; Stevenson and Campana, 1992; Campana and Thorrold, 2001). When backcalculating growth, there are two assumptions that need be considered: firstly, the frequency of formation of the daily increments is constant, and secondly, the distance between consecutive features (increments) is proportional to fish growth.

Fish growth can be further explored by pairing it with environmental data, creating growth chronologies or biochronologies (Morrongiello and Thresher, 2015; Smoliński and Mirny, 2017; Martino et al., 2019). These chronologies do not directly reflect somatic growth changes over time, but can be used as proxy to determine it (Ashworth et al., 2016). Because fish growth is directly affected by temperature and the ramifications of temperature changes on the ecosystem (Rountrey et al., 2014), this technique can be used to reconstruct past climate conditions and even predict how future climatic changes may affect marine populations (Smoliński and Mirny, 2017). With this in mind, biochronologies can be very important tools in fisheries management, especially considering the rising ocean temperatures (Domingues et al., 2008; Cheung et al., 2013).

Connectivity studies are severely hampered by the intrinsic difficulty and dependency on mark and recapture. This is especially true on fish species where small pelagic offspring suffers from very high mortality rates in early life stages (Thorrold, 2001). A way to overcome this lack of knowledge comes in the form of natural tags from fish otoliths, as the elements otoliths incorporate to grow mainly derive from the water where an individual has been (Thorrold, 2001; Elsdon et al., 2008). Fish otoliths (earstones) have long been known as timekeepers, but interest in their use as a metabolically inert environmental recorder has accelerated in recent years (Campana, 1999). In part due to technological advances, applications such as stock identification, determination of migration pathways, reconstruction of temperature and salinity history, age validation, detection of anadromy, use as a natural tag and chemical mass marking have been developed, some of which are difficult or impossible to implement using alternative techniques (Campana, 1999; Secor and Rooker, 2005; Elsdon et al., 2008).

1.3- *Dicentrarchus labrax* - European seabass

The European seabass, *Dicentrarchus labrax*, is a demersal euryhaline and eurythermic species that can dwell in a variety of habitats such as coastal lagoons, estuaries as well as open sea and coastal waters (Cardoso et al., 2015; Bento et al., 2016), and has its geographical distribution ranging from the North Sea in Norway to the coast of Senegal, including the Mediterranean and Black seas (Pickett and Pawson, 1994; Cardoso et al., 2015). Over the course of its life cycle, individuals from this species perform ontogenic migrations (Pickett et al., 2004; Bento et al., 2016), moving from one habitat to the next as they mature, depending on which life

cycle stage the individual is in. The life cycle of *D. labrax* can be characterized as that of the typical marine fish with ontogenic migrations (Fig.1), with oceanic and estuarine (nursery) phases (Pickett et al., 2004) well delimited in time: adults spawn offshore and, as eggs begin to hatch into larvae, currents transport the offspring to coastal waters. By now larvae are developed enough to perform vertical migrations, which help them remain nearshore and counteract the upwelling effects. At this stage, they start entering nursery areas (often estuaries) and mature into juveniles. Juveniles remain inside the nursery areas until they attain sexual maturity, which happens 2-4 years after entering (Bento et al., 2016). Mature fish recruit to the stock, where they become fully developed adults (Jennings and Pawson, 1992). In the northern stock (composed by the North Sea, English Channel, Celtic Sea, and Irish Sea areas), seabass tend spawn eggs by winter's end to early spring, approximately between January and March, as the 9°C threshold seems to be the trigger for spawning (Beraud et al., 2018). However, regarding the southern seabass stock, how environmental conditions affect spawning remains largely unknown. This is relevant because as oceans slowly warm up (Domingues et al., 2008; Cheung et al., 2013), fish populations can be displaced towards higher latitudes. An example can be seen in the Wadden sea (the Netherlands), where over the last three decades there has been an increasing trend of seabass becoming more common (Cardoso et al., 2015). In the UK, the more frequent catch of *Mullus barbatus* - Red Mullet - is attributed to warming of the oceans (Cheung, et al., 2013), as this fish is typically found in warm waters.

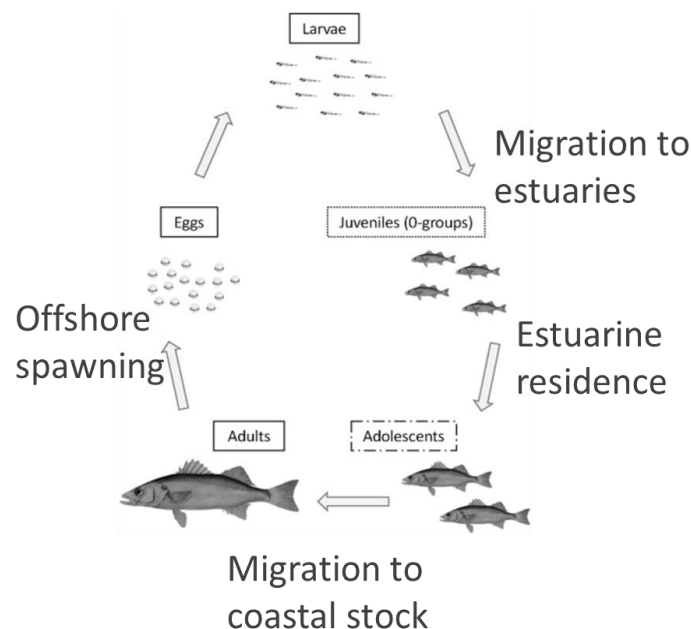


Figure 1- *Dicentrarchus labrax*'s life cycle representation - Adapted from (Bento et al., 2016).

In Portuguese estuaries, seabass populations are marked by seasonal abundance patterns (Martinho et al., 2007; Bento et al., 2016), related to the entrance of juveniles into the estuarine nursery habitats and the mature juveniles recruiting back to the stock. This is reinforced by

several studies which point to estuaries as important sources for adult seabass stock (Martinho et al., 2007; Baptista et al., 2010).

This species is very popular for recreational and professional fisheries, which grant it a high economic relevance. Despite its high economic relevance, few are the studies on its otolith microstructure (Gutiérrez and Morales-Nin, 1986; Planes et al., 1991; Regner and Dulcic, 1994), especially when considering that the previous studies are almost three decades old, and thus new insight in this area is required. In addition, and despite that previous studies have focused on the effects of water temperature and photoperiod of the spawning period of seabass (Vinagre et al., 2009b), and of the control of climate patterns on juvenile abundance in the Mondego estuary (Bento et al., 2016), there is clear lack of knowledge of the long-term effects of climate and oceanographic conditions on the hatch day distributions and growth in *Dicentrarchus labrax* on the northeast Atlantic.

1.4- Objectives

Unravelling species/climate interactions is key to the development of management and protection plans for fish and their fisheries, ensuring a sustainable harvest of marine resources. In this sense, this work focused on understanding the effects of climatic and oceanographic variability on the early life history of *Dicentrarchus labrax* in the Portuguese coast, over the period between 2011 and 2017. More specifically, the present work aimed at determining the interannual variability in sea bass hatch dates over a 7-year period, as well as the influence of environmental and oceanographic factors on hatch date distributions. Growth chronologies were also developed to assess the long-term influence of both intrinsic and extrinsic factors on juvenile growth during estuarine colonization.

2- Materials & Methods

2.1- Study area

Located in the west coast of continental Portugal, the Mondego estuary (Fig.2) is small and has an area around 8.6km², characterized by a continental temperate climate (Pardal et al., 2002). The estuary is composed by two arms - north and south, formed when the river splits in half, about 7km from the sea (Pardal et al., 2002). Near the river mouth, both arms are re-joined.

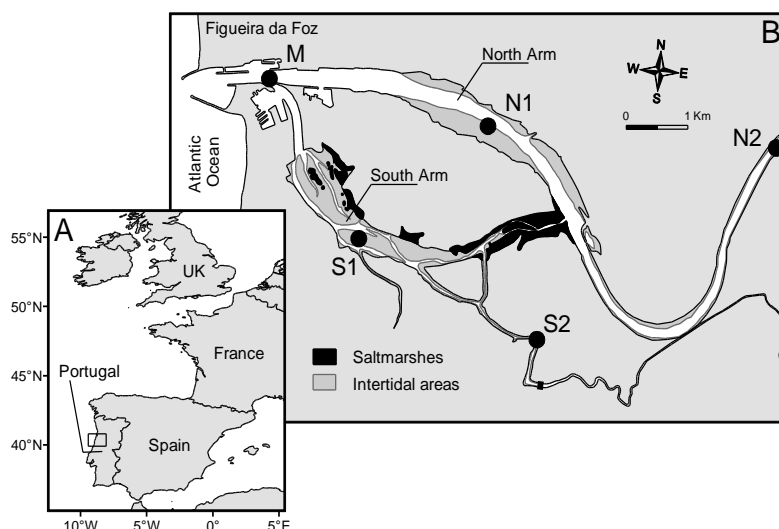


Figure 2- Geographical location of the Mondego estuary (A) and five sampling stations representative of the whole estuary (B). M corresponds to the station close to the river mouth. S1 is representative of Mondego's south arm, while S2 is representative of the Pranto river which itself holds influence over the south arm. N1 represents the north arm, while N2 represents the upper reaches of Mondego estuary.

The north arm is the deepest, with a 5-10m depth during the high tide. In the north arm, frequent dredging maintains depth, which allows shipping activity, making the north arm the main navigational channel (Pardal et al., 2002). The south arm is shallower, with a 2-4m depth during the high tide, and intertidal mudflats constitute about 75% of its area (Pardal et al., 2002). Freshwater flow occurs mostly in the north arm, as the location where the river splits in two arms is heavily silted, blocking most water from entering the south arm. As a consequence, south arm water circulation is mostly due to the tidal influx of seawater, despite receiving minor freshwater quantities from the tributary Pranto river (Pardal et al., 2002). In the year of 2006, the connection between the two arms was enlarged, which resulted in the south arm receiving more freshwater, increasing its hydrodynamics (Nyitrai et al., 2013).

2.2- Sample acquisition & processing

Seabass samples were obtained from a monthly monitoring programme that took place from January 2011 to December 2017, based on five different sampling stations to guarantee the representation of the whole estuarine system. For each fish campaign, three hauls were performed

per station with a 2-meter-wide beam trawl and at the speed of two knots, for an average duration of three minutes, covering at least 500m². Hauls were performed at night, so fish would not be able to see the net and evade it.

In the laboratory, all seabass specimens were measured (total length in cm; TL) and weighted (wet weight in g; WW). From the obtained samples, 0-group seabass with TL under 9cm were selected (n=215) to ensure that they were born in each respective year, and that a representative sample of the existing size range was obtained. From the selected fish, sagittae otoliths were extracted, cleaned and stored dry in eppendorfs until further analysis.

2.3- Otolith microstructure analysis

Sagittae otoliths were mounted in microscope slides with Crystalbond 509 resin and then polished in the sagittal plane using P4000 Buehler (5 µm) grinding paper until clear visibility of the daily rings was obtained from the core to the edge. Then, digital images of each otolith were obtained with a light microscope Leica DM2000 LED attached to a Leica ICC50 W camera connected to an external computer, using Leica LAS V4.8 software. At least two photos per otolith were taken: one for the otolith core with a magnification of 400x, and another for the outer otolith area, with a magnification of 100x (Fig.3). In larger otoliths, additional photos were required to capture the whole microstructure. Daily ring counts and increment measures were performed using the ObjectJ plugin for the ImageJ software (Schneider et al., 2012). A total of 28319 growth increment measures were obtained.

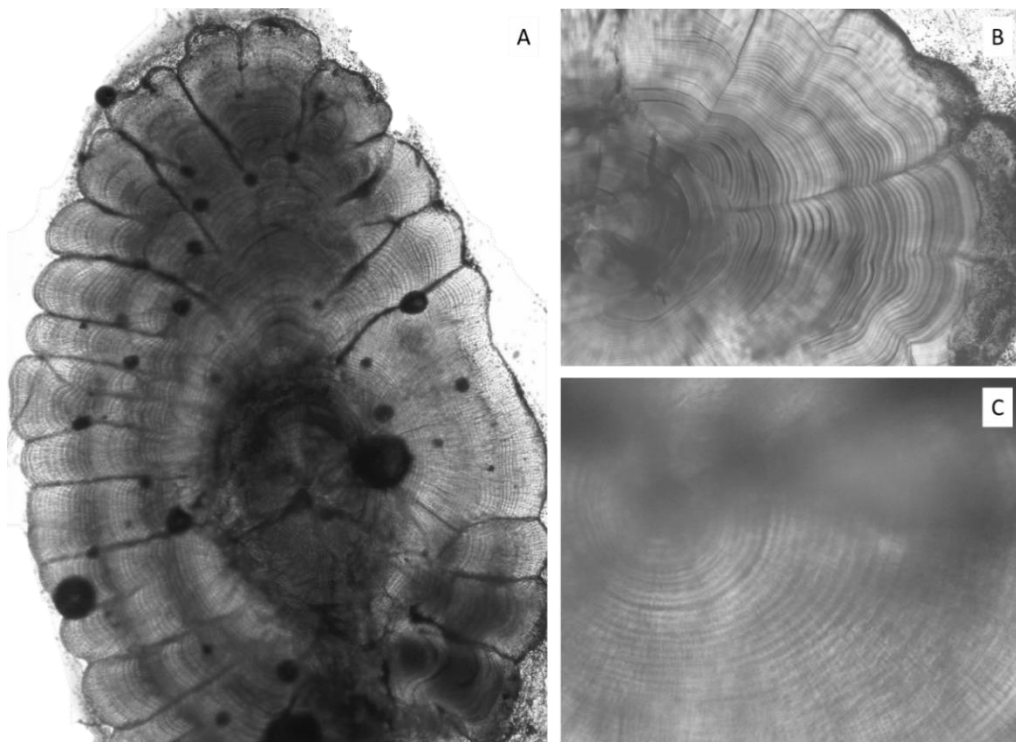


Figure 3- Photograph of a seabass otolith, highlighting its microstructure in terms of daily growth rings. Observation of an entire otolith microstructure on a 50x magnification (A); otolith edge growth rings, at a 100x magnification, with the core visible at the centre left (B); otolith core and the first daily growth rings, at a magnification of 400x (C). 18

2.4 - Environmental data acquisition

Several environmental variables, with different time resolutions were obtained from various online internet sources. This had the purpose of evaluating their effect on fish life-cycle in different temporal resolutions, thus attempting to increase prediction accuracy.

The NAOi (calculated from the difference in atmospheric pressure between Azores, Portugal, and Reykjavik, Iceland), was chosen as a predictor for hatching and fish growth due to being a large-scale environmental predictor influencing western Europe and North Atlantic. NAOi data was composed by three different temporal resolutions: 30-day (30d) and 8-day (8d) means were used for the purpose of explaining hatch date variability, while daily NAOi data was used for the growth predicting models. All data concerning NAOi were acquired from the National Weather Service, Climate Prediction Centre (<https://www.cpc.ncep.noaa.gov/products/precip/CWlink/pna/nao.shtml>; accessed on the 29th of April of 2019).

Sea surface temperature (SST) (°C) data was chosen as a predictor for both hatch dates and fish growth. This choice derives from the extensive research data pointing to SST as an influencer in *Dicentrarchus labrax* life cycle (Jennings and Pawson 1992; Henderson 2002; Vinagre et al., 2012; Cardoso et al., 2015; Beraud et al., 2018). SST data, in a radius of 20km off the coast of Mondego estuary, were also obtained in three temporal resolutions: 30d means and 8d means were used to explain hatching in seabass, while daily data was used as a one of the growth predictors. 30d and 8d means data were obtained from NASA Oceancolor Web; (<https://oceancolor.gsfc.nasa.gov/13/>); accessed on the 12th of May of 2019. Daily SST data was generated using Copernicus Marine Environment Monitoring Service (CMEMS; <http://marine.copernicus.eu/>; accessed on the 16th of May of 2019).

Chlorophyll a (CHLa) can be used as a proxy for primary productivity, as it is considered the base for the marine food web. Because of this, CHLa was used to explain hatching in seabass. CHLa data was not used in the growth models as daily data was unavailable at the time. CHLa (mg m⁻³) data, concerning a zone off the coast of Mondego estuary in a 20km radius, were obtained from NASA Oceancolor Web (<https://oceancolor.gsfc.nasa.gov/13/>; accessed on the 12th of May of 2019).

Upwelling is known to affect larval transport (Santos et al., 2004; Santos et al., 2007), thus it may influence seabass to reproduce at a time where hatched larvae are less likely to run into strong upwelling events. Upwelling (m³ s⁻¹ km⁻¹) data were obtained from the Spanish Oceanographic Institute (IEO) (http://www.indicedeafloramiento.ieo.es/index_UI_es.html; accessed on the 11th of May of 2019), corresponding only to 30d values.

2.5 - Data analysis

2.5.1- Biological data

For all individuals (n=215), fish age was determined by summing the total increment number of daily increments. Linear regressions were fitted to the relationship between total length (cm) and fish age (days). Differences between the slopes for each year were analysed with a linear model and an estimated marginal means (EMMs) for specified factors was computed using the lsmeans (Lenth, 2016) package in R software (R Development Core Team, 2018). Differences between years existed whenever the interaction between Age and Year was significant. Model validation was performed through visual inspection of residuals. Differences in hatch day distributions between years were assessed by Kruskal-Wallis ANOVA, after which a pairwise Wilcoxon test for multiple comparisons (with Bonferroni correction), to relate hatch days.

2.5.2- Effect of environmental data on hatch dates distributions

For each fish, hatch dates were calculated from otolith microstructure analysis. This was done by summing the number of increments counted and then subtracting that number to the capture date. To assess the relation between biological and environmental variables several GAMs were developed. However, before GAM development itself, any collinearity between environmental predictors was evaluated with Pearson correlation coefficient; from this analysis, Upwelling data were excluded due to a high positive correlation coefficient with the NAOi. During the indicial model development process, CHLa was log-transformed to reduce skewness. When assessing the influence of environmental parameters (NAO, SST and CHLa) on hatch date distributions per year, GAM development was performed in a way that allowed testing of all possible variable combinations. This resulted in the development of several GAMs for both 30d and 8d means. Model selection was done by choosing the model with lower AIC. All analyses were performed using the mgcv (Wood, 2011) package in R software, with a level of significance of 5%.

2.5.3- Growth analysis

A series of increasingly complex mixed-effects models (Generalized Linear Mixed Effects; GLMM) was used to explore growth variation in juvenile seabass, based on the methodology of (Morrongiello and Thresher, 2015). In this work, the original methodology was adapted from an annual to a daily analysis and instead of comparing growth among different areas, growth was compared between years. These models allow partition between intrinsic (biological data) and extrinsic factors (environmental data) influencing growth over time. This approach also has the advantage of considering within-fish and within-year correlations and variability, through addition of random slopes and/or intercepts, often overlooked by other

sclerochronological analysis (Martino et al., 2019). All analyses were performed using the lme4 (Bates et al., 2015), AICcmodavg (Mazerolle, 2019) and Effects (Fox et al., 2018) packages in the statistical program R, version 3.5.1 (R Development Core Team 2018). To satisfy model assumptions, all data (except year of capture; YOC) was log transformed and all predictor variables were mean-centred to facilitate model convergence and interpretation (Morrongiello and Thresher, 2015). Then, random intercepts were added to every unique fish identifier (FishID), and the interaction between YOC and AgeJD (fish age at each Julian day), allowing for correlation between growth increments within individual fish, and all increments formed at a given day for each year (AgeJD and YOC interaction). The random age-slope and fishID intercept allows for different growth patterns in each individual fish, and a random intercept for each Julian day induces a correlation between all increments formed in the same day.

For each year, optimal models were selected and then the best intrinsic models were further developed by analysing all intrinsic fixed effects (Age; Age at capture - AAC) with several random effects (combinations of random intercepts from FishID, AgeJD, YOC and/or slopes). After this process, model selection was based on Akaike's information criterion corrected for small sample size (Burnham and Anderson, 2002; AICc). AICc values were rescaled as the difference between each model and the model with the lowest AICc (Delta-AICc; DAICc). The best models were reanalysed using REML (restricted maximum likelihood), to produce unbiased parameter estimates (Zuur et al., 2009). Chronologies were then built by extracting Julian Day (Age JD) effect for each year to obtain otolith standardized growth per day, over the year.

To understand how environmental variables influenced fish growth, extrinsic factors were added to the best intrinsic models. The best models, including both intrinsic and extrinsic data, were ranked by AICc to determine the model that best explained fish growth over time. GLMM variance was assessed through the calculation of two R^2 metrics: Marginal and Conditional. Marginal R^2 describes the variation of the fixed effects while Conditional R^2 explains the model variation in both fixed and random effects.

3- Results

3.1- Environmental conditions

During the analysed period (2011-2017), the NAOi was mostly positive (Fig.4A): 2015 was the most positive year (mean 0.43), while 2012 had a negative mean value (-0.46). In 2016 mean NAOi was close to 0 (-0.04).

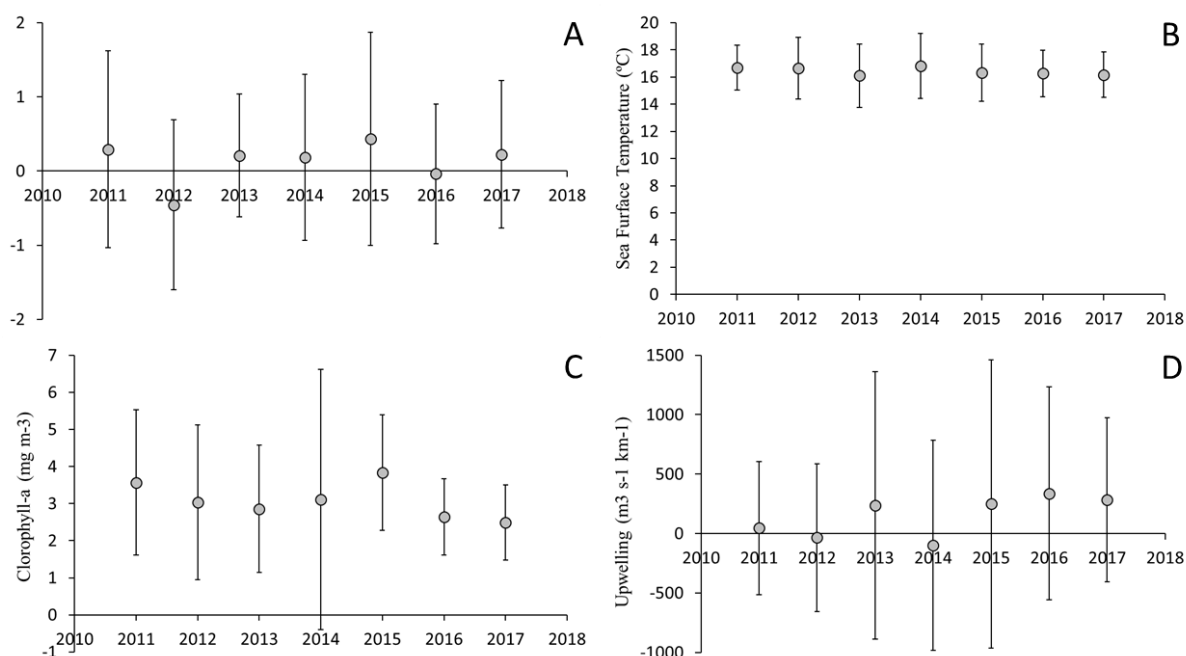


Figure 4- Representation of the yearly average for the four environmental variables used - North Atlantic Oscillation index (A), Sea Surface Temperature (B), Chlorophyll a (C) and Upwelling index (D) - from 2011 to 2017. The values for B, C and D were measured in an area of 20km off the coast of Figueira da Foz.

Mean SST (Fig.4B) was higher in 2014 (16.82°C) and colder in 2013 (16.11°C). Chlorophyll-a concentration (mg m⁻³) (Fig.4C), had its peak yearly average in 2015 (3.84 mg m⁻³) and the lowest yearly average in 2017 (2.49 mg m⁻³). In 2014, variability in Chlorophyll-a was much higher than in any other year. Upwelling index (Fig.4D) ranged from -98.63 m³ s⁻¹ km⁻¹ in 2014 to 338.12 m³ s⁻¹ km⁻¹ in 2016.

3.2- Otolith microstructure analysis

In Fig.5 we can see the results for the linear regression for the years of 2011 to 2017. In the obtained model, both Age and Year of capture were significant ($p = 2.2e-16$ and $p = 8.095e-10$, respectively), as well as their interaction ($p = 8.660e-05$), meaning that the slope was different between years, as follows: 2012 was different from 2013 ($p = 0.0002$), 2012 was different from 2017 ($p = 0.0318$) and 2013 was different from 2016 ($p = 0.0044$).

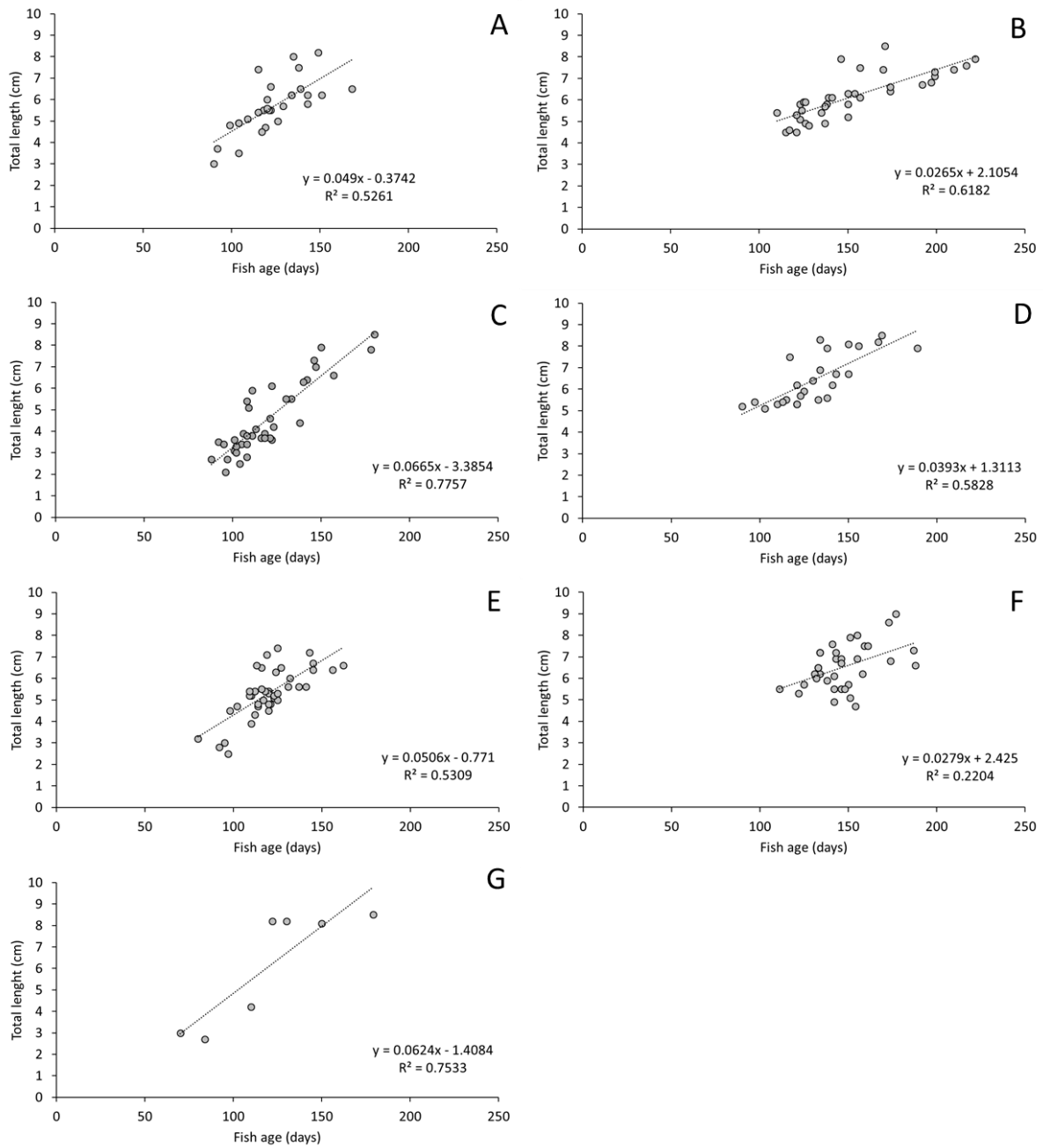


Figure 5- Relationship between total length (cm) and estimated age (days) for 0-group juvenile *Dicentrarchus labrax* from Mondego estuary for the years of 2011 to 2017, A to G respectively.

3.2.1- Hatch date distributions

Seabass hatch dates were significantly different between years ($H=65.128$; $p<0.001$) (Fig.6). The pairwise Wilcoxon test for multiple comparisons (with Bonferroni correction) indicated that hatching in the year 2011 was different from hatching in the year 2012 ($p=0.00076$) and similar to hatching in remaining years; in 2012 hatching was different from hatching in every other year ($p=6.4e-08$ [2013], $p=1.4e-07$ [2014], $p=9.1e-06$ [2015], $p=2.5e-06$ [2016], $p=0.00447$ [2017]); hatch dates in 2014 were different from hatch dates in 2015 ($p=0.01175$) and hatching in 2015 was different from hatching in 2016 ($p=0.02933$). In Julian days, the shortest hatching period, was observed in 2015, from day 44 to day 85, with a total duration of 41 days. In contrast, the widest hatching period was recorded in 2016, from day 14 to day 148, with a total duration of 141 days. In 2012 the earliest median hatch day was recorded, while in 2016, the latest median hatch day occurred.

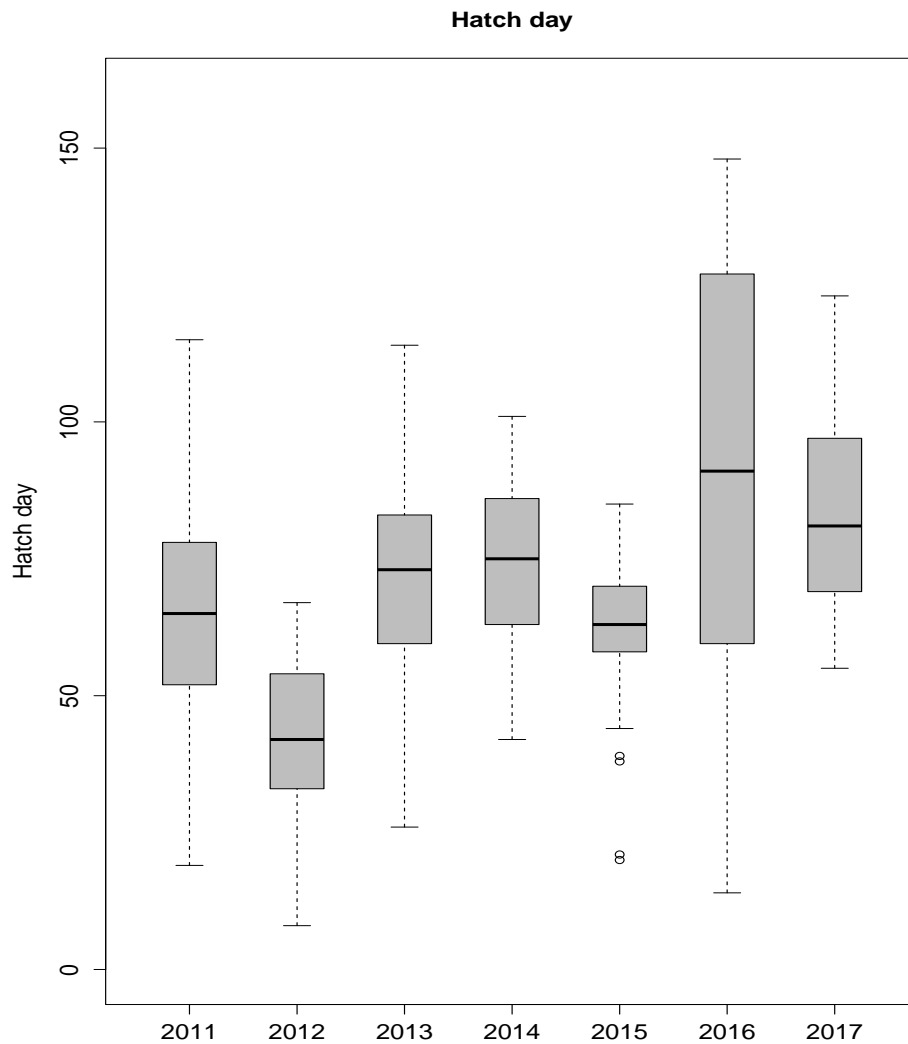


Figure 6- Box plot representing hatch days distribution for the years of 2011 to 2017. Hatch days are represented in Julian days.

The influence of environmental factors on hatch dates was evaluated with GAMs (Fig.7). Overall the generalized additive models presented a good predicting capacity. The 30d (30d) GAM (Fig.7 B, D & F) explained 71% of the deviance, while the 8d GAM (Fig.7 A, C & E) explained 24.5% of the deviance. Overall, the NAO had a negative relationship with hatch dates while SST presented a positive relation with hatching in seabass. In both GAMs, hatch dates occurred sooner with lower SST and higher NAOi values, and were delayed by higher SST and lower NAOi values. In the 30d GAM, higher LogCHLa values slightly delayed hatching, while in the 8d GAM LogCHLa was not significant.

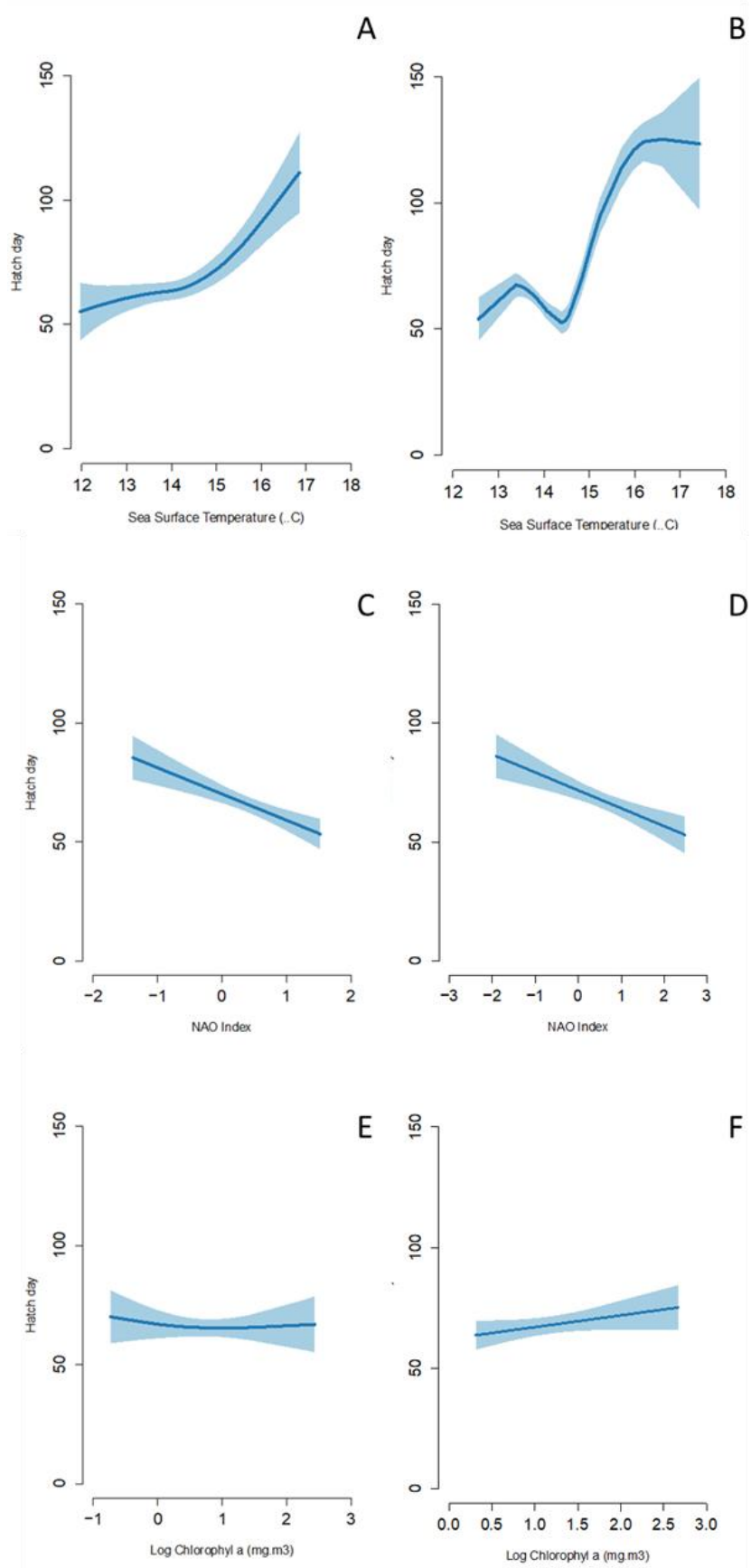


Figure 7- Effects of environmental processes on hatch date distributions, obtained by GAM. A, C & E represent the effect of 8d means for SST, NAOi and CHLa respectively, while B,D & F represent the effects of 30d means for these same variables.

3.2.2 – Growth analysis

Distinct growth patterns were observed in otolith growth when comparing the individual years of the time series. Still, otolith growth was similar among years for the first 50 days, after which sharply increased at different rates (Fig.8). In most years (2011, 2014, 2015, 2016 and 2017), growth increased steadily until around day 100, after which it declined (2015, 2016, 2017) or remained relatively stable (2011, 2014). In both 2012 and 2013, peak otolith growth was displaced until days 130-140, declining afterwards (Fig.8).

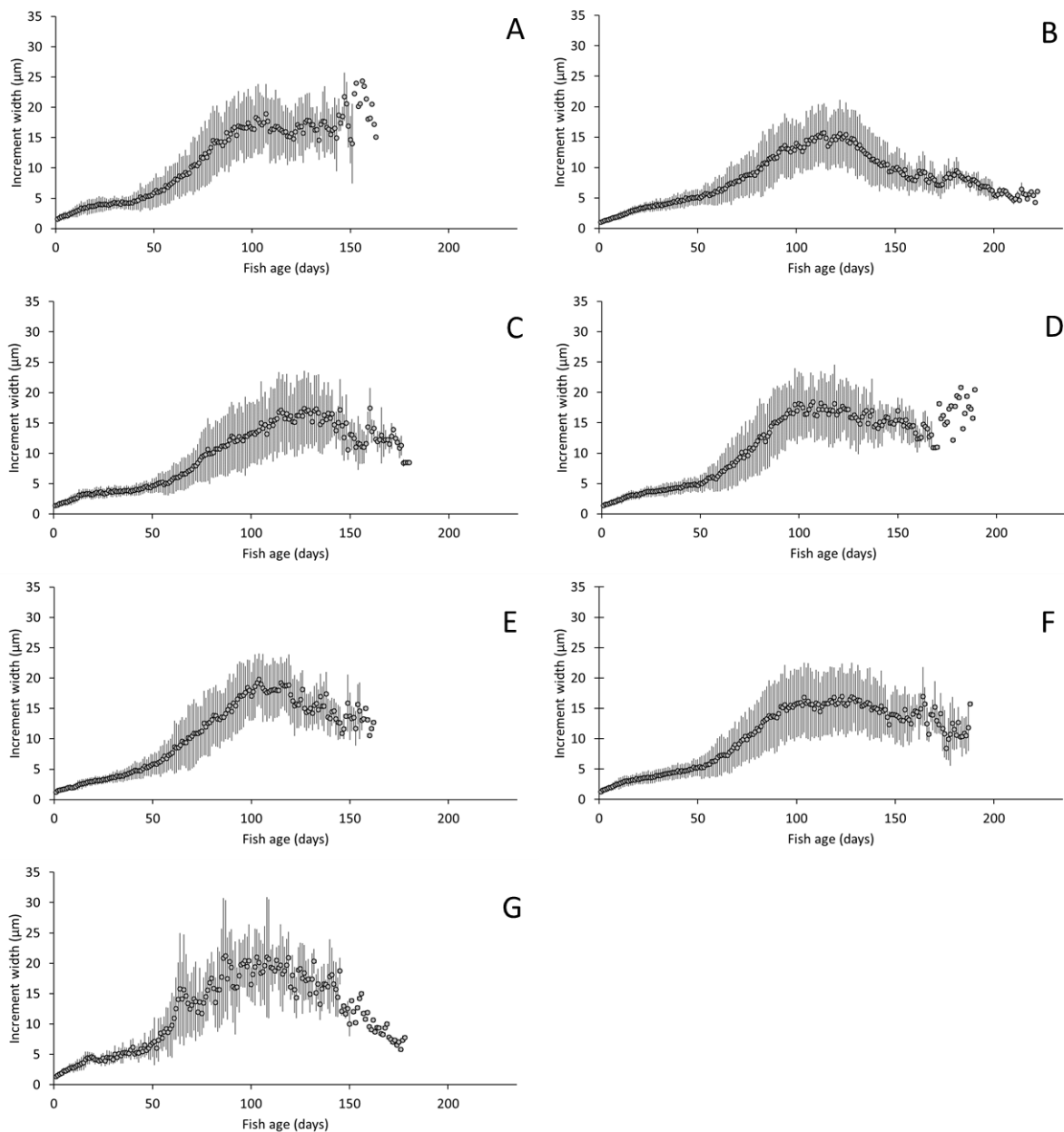


Figure 8- Seabass otolith daily growth increments (μm) 2011(A), 2012(B), 2013(C), 2014(D), 2015(E), 2016 (F) and 2017 (G). Circles represent the mean value for each day, and the respective standard deviation (vertical bars).

Similarly, the chronologies obtained by the extracting the Julian day effect of the selected model depicted differences in seabass otolith growth patterns between years. The final model used to obtain the chronologies was:

$$\log(\text{Increment}) \sim c.(\log(\text{Age})) * \text{fYOC} + c.(\log(\text{AAC})) + (c.(\log(\text{Age}))|\text{FishID}) + (1|\text{fYOC:fAgeJD})$$

A similar growth pattern occurred in all years except in 2012 and 2017 (Fig.9). When analysing growth in this type of chronologies, it should be noted that data is centred and standardized, meaning that the growth average is 0 and above average values mean above average growth while below average values mean below average growth. Concerning the growth pattern for the years of 2011, 2013, 2014, 2015 and 2016 (Fig.9), growth was well above average during the initial days, and then plummets until reaching its lowest point. This decrease in growth was at first very sharp but gradually slowed down over February and March, until reaching its lowest point, which generally happened between middle to end of March (~days 70 to ~90). From the lowest growth value onwards, a sharp and steady growth period took place. Growth increased until peaking around late June to midJuly (~days 180 to ~195), with growth in 2016 peaking near July's end (day ~210). For these years, after growth peaked it started to steadily decrease, although at different rates, with growth in the year 2014 being the only exception, where it remained near peak values despite being very variable.

Growth pattern in the years 2012 and 2017 (Fig.9) was different from the remaining years, especially before peaking. In the year 2012 a very high initial growth was not observed. Instead, growth starts off earlier than usual and very slowly, being the only year where starting growth was under average. Lowest growth in the year 2012 was registered near January 10th. From here growth sharply increased until around January 20th, and remained stable until around April's beginning (day ~95). From here, growth pattern in 2012 was similar to that of the remaining years. In the year 2017 initial growth was close to the average and started later than in any other year, around mid-February (day~50). From this point growth steadily declined until reaching its lowest point near April's end (day~115). After reaching the lowest point, growth pattern in the year 2017 is similar to those of the remaining years. GLMM variance was calculated through R² metrics had the following values: Marginal R² of 0.4589448 and Conditional R² of 0.8007028.

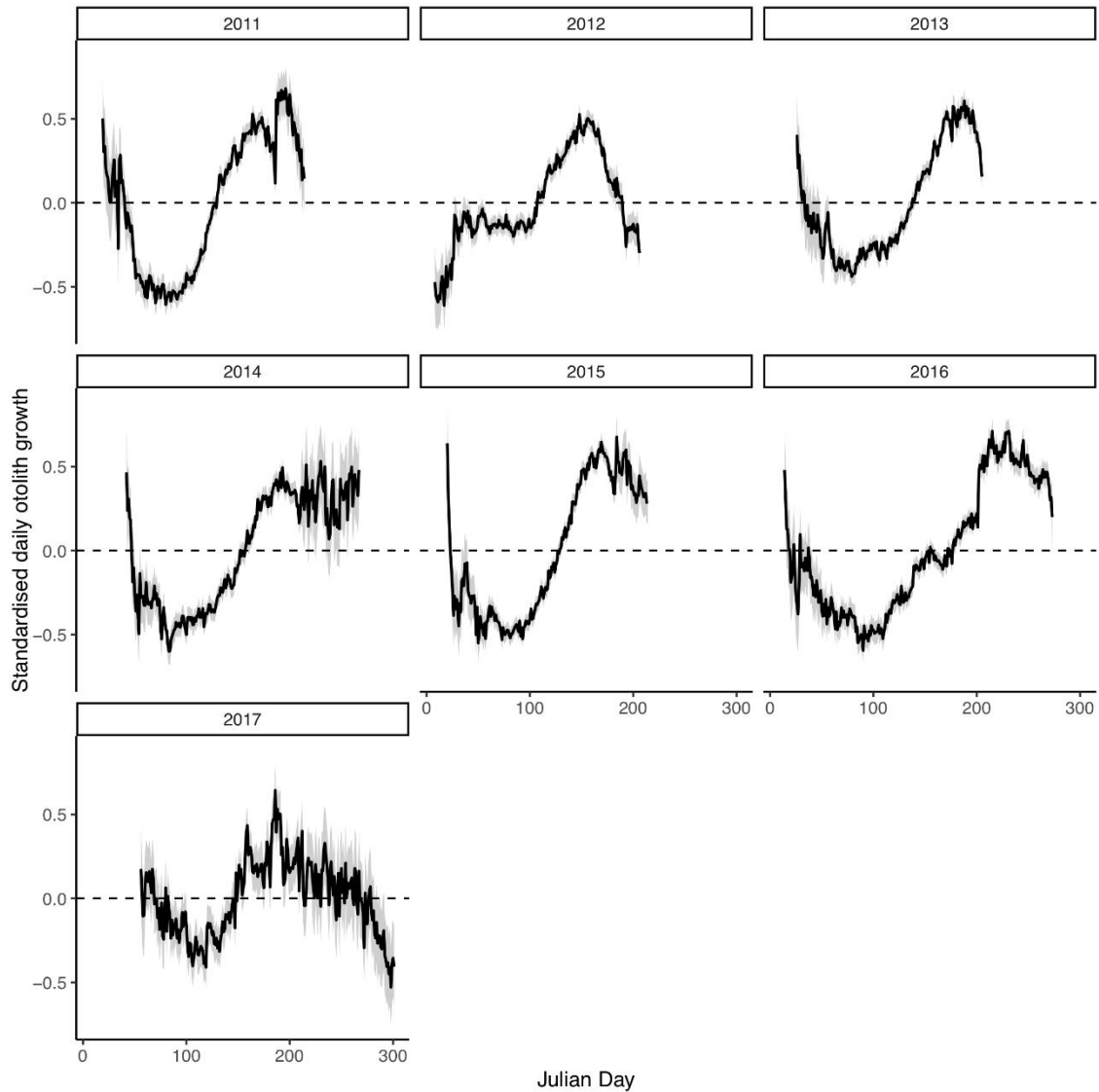


Figure 9- Growth chronologies for the years of 2011 to 2017. Chronologies were constructed by means of a Mixed Effects model, incorporating both intrinsic and extrinsic (environmental) factors to fish.

Both the SST and NAO were significant extrinsic predictors of juvenile seabass growth between 2011 and 2017 (Fig. 10). In more detail, SST showed a strong negative relationship with long-term daily growth patterns. In contrast, the NAO was positively related with seabass daily growth trends.

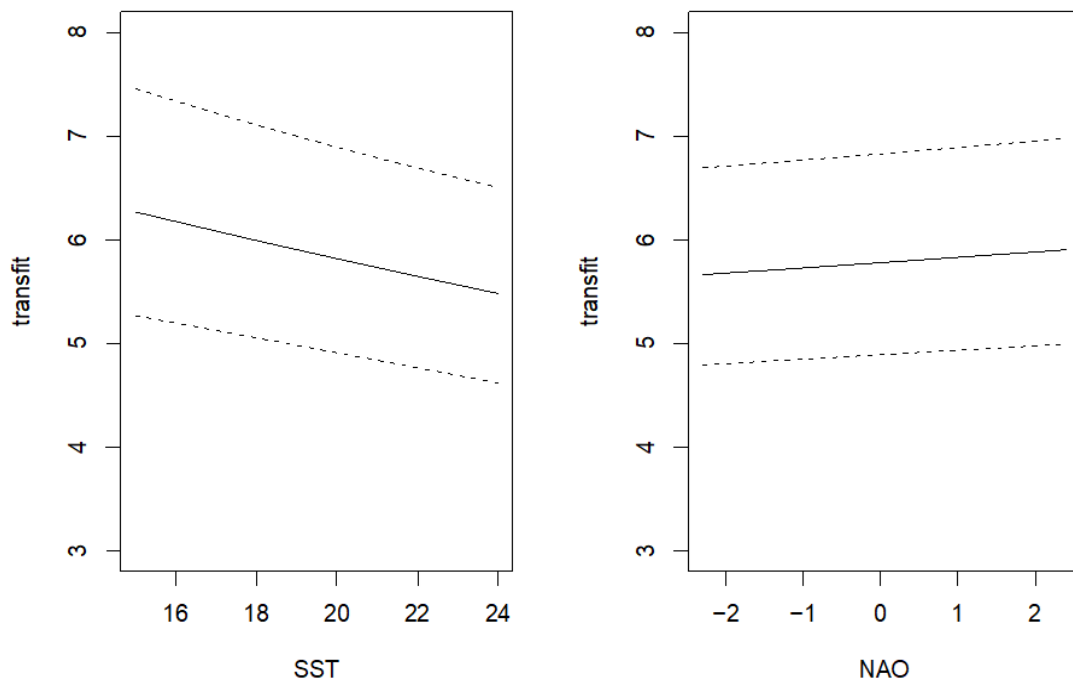


Figure 10- Extrinsic effects (SST; NAOi) on seabass juvenile growth.

4- Discussion

The present research focused on analysing European seabass *Dicentrarchus labrax*'s early-life-history over a 7-year period using otolith microstructure analysis. Studies on hatching and growth are essential towards understanding early-life-history of marine fishes, yet they remain relatively scarce, especially on seabass. This work is the first to use otolith growth chronologies based on daily otolith increments to delve on the mechanisms driving juvenile seabass growth on the Portuguese coast, where sea surface temperature and the NAO are presented as variables with a strong influence on both hatch dates and juvenile fish growth.

4.1 - Role of environmental variables on seabass hatching

In this study, seabass juveniles hatched mainly from late February to late March, between day 55 to 80, except in 2012 where hatching occurred mainly before day 50. In 2015 hatching period for seabass was shortest while in 2016 hatching period was longest. SST was the most important environmental variable driving the observed variability in hatch dates between 2011 and 2017. Results showed that at SST values near 13.5°C, hatching occurs after day 50 (mid-February), near winter's end and early spring. This agrees with previous data from the Portuguese coast, with seabass spawning occurring around winter's end and early spring, when SST is decreasing (Vinagre et al., 2009b). In the Portuguese coast, lowest SST values overlap with this period, linking seabass reproduction with a drop in water temperature near winter's end and early spring, before it starts rising again (Vinagre et al., 2009b). This decrease in SST can act as a trigger for seabass spawning, similarly to what has been documented for the North Sea populations, where seabass spawning was not only linked with a drop in water temperature, but also to increased egg production (Beraud et al., 2018).

The second explanatory variable that influenced the hatch date distributions was the NAO index. The NAO is as a major climactic system affecting Western Europe and North Atlantic (Hurrell, 1995; Trigo et al., 2002). In more northern latitudes, the NAO has been successfully correlated with several biological attributes in marine and estuarine fishes, such as community composition, growth and abundance (Attrill and Power, 2002). However, the influence of the NAO on fish in the Portuguese coast has been pointed as more indirect one (Martinho et al., 2009; Bento et al., 2016). This indirect influence may derive from NAO's ability to influence ocean temperature (Rodwell et al., 1999), as well as wind circulation patterns (Hurrell, 1995). In the present work, there was a negative relationship of the NAO with seabass hatching, meaning that a lower NAO (usually negative values) delays hatching, while the contrary is observed for positive NAO conditions. In this study, NAO may also have had influence in the extended hatching period observed in 2016. During years of high NAO, moisture transport occurs in a more southwest to northeast axis, reducing its content in southern Europe and increasing it in northern Europe (Hurrell, 1995). In southern Europe, stronger eastward flow of moisture causes drier conditions,

increasing evaporation, which results in a cooler SST (Rodwell et al., 1999). As in 2016 NAOi index was slightly negative, occurred less eastward flow, meaning that SST was not cooled. According to the GAM, higher SST and lower NAOi values delay hatching in seabass, thus in 2016, the low NAO likely is the cause of said extended hatching period. In Bento et al., 2016, a previous work with *Dicentrarchus labrax* in the Mondego estuary, NAOi with 1 year lag had a significant influence over variables as 0-group density, production and day of peak abundance. In a similar manner, using a lag period (one week/month) could provide complementary information towards understanding how seabass hatching is triggered in a given year.

Our results agree with Bento et al., 2016 in the way that seabass life-cycle is clearly climate-driven, as changes in SST and/or the NAO in a given year having a significant effect hatching of this species. Thus, variations in the timing of seabass hatching have the potential to hamper juvenile recruitment. A striking example can be found in the 2012 cohort, where hatching occurred earlier than expected, which led to much lower growth on the initial stage of the life-cycle when comparing with the remaining years. Changes in seabass hatching pattern (be it delay or anticipation) can result in a loss of synchronization between larvae, their prey and/or their optimal environmental conditions. Larvae survival in the ocean can be described according to the match-mismatch hypothesis (Cushing (1975), which states that if a mismatch occurs between larval prey and larvae hatching, larvae are not likely to find enough food to survive (Cushing 1975). In seabass, if hatching is linked to such an event, changes in its pattern might have dire consequences, and much of the offspring may probably die due to lack of food. For instance, early hatching may lead to a reduction in larval size that can ultimately hamper their ability to complete their migration into estuarine nurseries. After seabass larvae hatch, ocean currents have the role of dispersing larvae (Beraud et al., 2018), to nearshore locations where they are exposed to varying hydrographic conditions, such as upwelling events and coastal currents such as the Western Iberia Buoyant Plume (WIBP), before being able to enter nursery areas. These factors have shown particle retention capacity and are able to cause larvae advection (Santos et al., 2004). Fox et al., 2006 and Comerford et al., 2013 suggested that larvae vertical migration as a very important behaviour to increase chances of settling, thus these vertical migrations likely act as a tool used by larvae to move up and down in the water column, and counteract the influence of both upwelling events and WIBP northward currents. However, if larvae were to hatch later, as a response to warming of the sea, it is likely that they would also arrive later to coastal waters and, because of this late arrival, larvae may not have enough time enter nursery areas before upwelling intensifies in spring (Santos et al., 2007; Otero et al., 2008). If larvae are exposed to these intensified conditions, they are likely to be advected into offshore waters (Santos et al., 2004), which can compromise the juvenile recruitment. Hence, upwelling and coastal currents may act as a barrier between larvae and the estuarine mean, but this must be confirmed with more

directed studies towards the impact of hydrodynamics and coastal circulation on seabass larvae and juveniles.

Even if larval transport and migration into nursery zones were to remain unchanged by a shift in hatch dates, it is possible that this shift would cause seabass larvae to enter nursery areas later. The main purposes for fish like *Dicentrarchus labrax* to use nursery areas is to find shelter and a suitable place for rapid growth (Blaber and Blaber, 1980; Vasconcelos et al., 2010). Seabass have an optimal temperature from 18°C to 25°C (Almeida et al., 2015), at which growth is most favourable, and past this point, temperature increases induce thermal stress (Vinagre et al., 2012). This stress results in increased metabolic and mortality rates, while fish condition and growth rates decrease (Vinagre et al., 2012). From June's end onwards, in Mondego estuary, water temperatures often go over 25°C (personal observation), thus, likely to be the reason for juvenile seabass growth decrease after this period, as observed in the growth chronologies. This means that there is a limited time window to be exploited by seabass, where juveniles are able to grow fast, which will be essential in providing juveniles with over-wintering capabilities. Hence, late estuarine arrival may limit this time window where fast growth occurs, and in this scenario, fish may attain smaller body size heading into autumn/winter, which can undermine juvenile's abilities to survive until the next year.

According to our results, the main threat for juvenile recruitment in seabass seems to be related to late hatching due to the warming of the oceans (Domingues et al., 2008; Cheung et al., 2013). In a hypothetical future where the exception becomes the rule, and seabass hatching pattern changes dramatically by moving forward in time, odds of larvae facing harsher oceanographic conditions will increase, what may undermine the species survival in the Portuguese coast.

In this study, CHLa was selected as a proxy for primary productivity, in an attempt to relate it with seabass hatch dates yet, no such link was observed. However, this predictor has been related with several species abundance and growth patterns: for instance, primary productivity has been correlated to growth in snapper *Chrysophrys auratus*, (Martino et al., 2019), and Cushing (1975), proposed that fish larvae hatching is linked to primary productivity in a match-mismatch hypothesis.

4.2- Growth

The chronologies developed for juvenile seabass were able to reconstruct growth in seven consecutive cohorts (2011 to 2017). Growth followed a general pattern in most years, with the exception of 2012 and 2017. This pattern was characterized by a short and well-above average growth, which quickly diminished until reaching the lowest point, mostly within the first 50-70 days. From this point onwards, growth increased until peaking, and after peaking, high

interannual variability was observed. Most of the years followed this growth pattern, suggesting a life-stage specific growth patterns, as changes in the last seem to be similar between years.

Right after hatching, seabass larvae experienced a brief period of intense growth, which is likely connected to the consumption of energy reserves; once these are depleted, growth quickly diminishes. This is further supported by the inability of larvae to feed in the first few days, as their mouth has not yet opened (Islam et al., 2009). The purpose of a very high initial growth might be the increase of larvae's chances of survival through the migration towards nurseries, and improve their overall chances to recruit. As previously stated, in the year 2012, this first growth peak was not present. It is possible that the lack of this initial growth peak may cause a cascade effect through seabass life-cycle, affecting growth at later stages. Indeed, the 2012 cohort seems to have registered the lowest overall growth: lower otolith growth, smallest period where growth was above average, slightly lower growth peak, less days where growth was above average and perhaps most importantly, it was the only year where growth was under average around mid-July (day ~195).

Life history changes such as metamorphosis are recorded in otoliths (Stevenson and Campana, 1992). Although *Dicentrarchus labrax* does not undergo through a true metamorphosis such as in flatfishes (Geffen et al., 2007), the transition from larvae to a juvenile is likely to be recorded in otoliths in a comparable manner to that of flatfishes, with clear marking/changes in otolith microstructure. However, few studies that extensively analyse these markings in *Dicentrarchus labrax* otolith microstructure exist (see Gutiérrez and Morales-Nin, 1986; Regner and Dulcic, 1994). In this study, when analysing *Dicentrarchus labrax*'s otolith microstructure, transition marks were observed generally about 55 to 70 days from hatching in all years. This period of 55 to 70 days is likely to have corresponded to the duration of the pelagic larval stage, from hatching locations all the way to the Mondego estuary. Larval transport duration is also subject to change, reflecting better or worse transport conditions. In *Dicentrarchus labrax*, larval to juvenile transition and estuarine entry seem to have matched the time where growth started to increase again and otolith growth further increased. This is further supported by the findings of Regner and Dulcic, 1994, where the authors state that because no morphological criteria had been established regarding the transition from larvae to juvenile seabass, the best way to understand when this transition happens is to look for a change (increase) in growth. In this study, the growth increase was consistent with the markings in the otolith microstructure. In this manner, growth increase most probably corresponded to young juveniles having encountered favourable conditions inside nursery areas, which allowed increased growth until peaking near late June and July. *Dicentrarchus labrax* shares similar life-cycle characteristics with *Platichthys flesus* and *Solea solea* (Vinagre et al., 2009a). In the Mondego estuary *Platichthys flesus*, and *Solea solea*, larval to juvenile transition occurs generally around day 50 (see Martinho et al., 2013 for *P. flesus* and Vaz et al., 2019 for *S. solea*), which is close to the observed age for *Dicentrarchus labrax*

larval to juvenile transition. This means that for these species who have a similar life cycle, transition from larvae to juvenile happens around the same time age.

As previously discussed, after the beginning of summer, water temperatures in Mondego estuary can often surpass 25°C. After prolonged exposure to temperatures of 25°C, juvenile seabass swimming capabilities diminish (Almeida et al., 2015). Reduction in seabass's swimming may serious consequences, such as loss of schooling behaviour, pray capture and predator avoidance (Almeida et al., 2015). This decrease of swimming capabilities might be a consequence from saving energy for other important metabolic activities such as waste products recycling (Almeida et al., 2015). In addition, during heatwaves, estuarine water temperatures can reach up to 28°C (Vinagre et al., 2012), which can result in growth rates decreasing and increased mortality. Thus, in the Mondego estuary, high water temperature in summer months above 25°C are likely the cause for the growth reduction seen in this study from June's end / July onwards.

Growth patterns in 2012 and 2017 were different from those in the other years. Considering that seabass reproduction often coincided with a decrease in SST, before it started to rise again (Vinagre et al., 2009b), larval hatching was likely expected to coincide with rising ocean temperature conditions, which should favour growth. This condition was not verified in 2012, when spawning occurred earlier than expected. As a consequence, larvae also hatched earlier and into colder seawater that were still on the decreasing trend, which probably led to conditions that were likely unfavourable towards growth. This are the most probable series of events behind the very slow growth on the first few days of 2012, which were not seen in any other year.

In the year of 2017, growth in the first few days was very close to the average. This might be linked with the overall later hatching occurred in the year 2017, because as previously discussed, later hatching may force larvae to face harsher oceanographic conditions, especially stronger upwelling in spring (Otero et al., 2008). This is further supported by the very sharp growth increase seen once juveniles were inside the estuarine mean. As expected, after peaking, growth kept going on a decreasing trend as fish headed into autumn-winter.

Juvenile seabass growth was significantly influenced by SST and the NAO, and both environmental variables presented a good forecasting capacity towards fish growth: higher NAOi values stimulated growth, showcasing the positive relation between the NAO and fish growth, while SST was negatively related to fish growth, with high SST values hindering growth.

The main environmental variables had contrasting relations with hatch dates and growth. In this study, the NAO exhibited a negative relation with hatch dates and a positive relation with growth. However, in NAOi, a positive phase was, in both cases, more favourable: hatching happened earlier during high NAOi and growth was also higher during high NAOi periods.

The contrast between the effect of SST on hatching and growth is more complex. The first thing to consider is that larvae hatching is more momentary while growth is influenced for a

period of over 200 days with a transition between two different environments. SST values for hatching and growth also have different ranges. However, inside their respective ranges, both hatching and growth are negatively affected by high temperatures, as high SST values delay hatching and slow down growth, with the possible consequences of facing unsuitable oceanographic conditions and a later effect on energy metabolism within estuarine nurseries, as discussed previously.

Despite that the developed chronologies allowed for inferring interannual variations in juvenile seabass growth, more samples from 2017 could be added to improve this model, since the chronology for this year was built using data from only the seven individuals, resulting in higher variability and likely lower accuracy and precision in growth prediction, when compared to the remaining years. Another way to improve the model outputs would be by obtaining daily data for estuarine water temperature to be used as an extrinsic predictor for the period when seabass larvae entered the estuary. In this case, data concerning estuarine water temperature were only available monthly, which could not be used in the GLMM. To overcome this issue, daily SST was used as a proxy for the water temperature that seabass larvae and juveniles experience throughout their first year of life.

4.3 – Conclusions & Final considerations

Otolith microstructural analysis allowed for age and growth estimations with high temporal precision. This dataset covered a seven-year-period, and allowed developing early life growth chronologies, providing unprecedented insight on seabass growth by drawing information from both intrinsic and extrinsic variables. Overall, seabass early life cycle is climate-dependent, with the NAO and SST as the main drivers. With this in mind, potential climatic changes that alter the NAO and ocean temperature patterns may bring alterations into its life cycle, which in turn can jeopardize the species' recruitment success and survival. Considering its economic relevance, it is important to understand how seabass responds to a changing climate scenario, and thus providing useful information for fisheries management.

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