

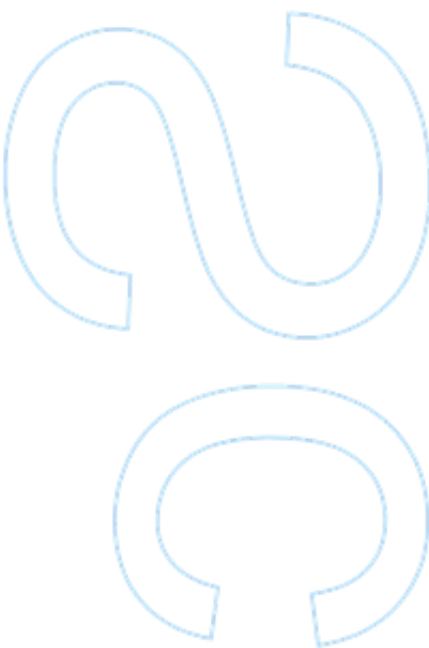
Interannual variability in early life history of *Pleuronectes platessa* (L.) using otolith microstructure

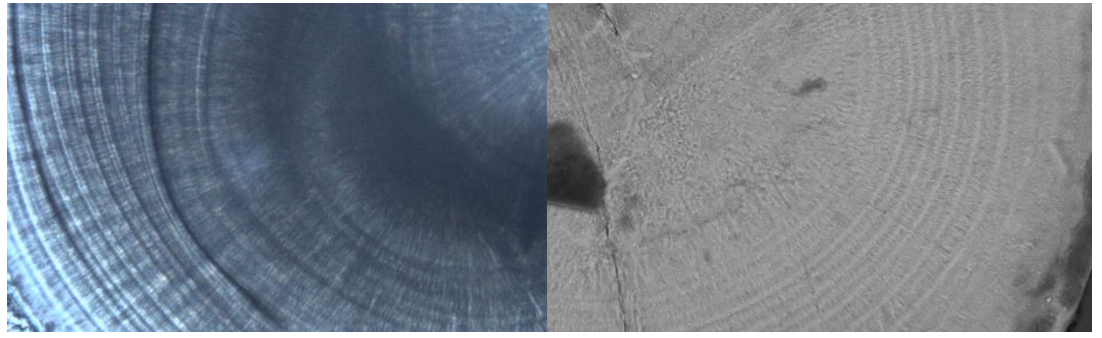
Joana Filipa Moreira de Sousa

Dissertação de Mestrado apresentada à
Faculdade de Ciências da Universidade do Porto no Mestrado em
Ecologia, Ambiente e Território
2015

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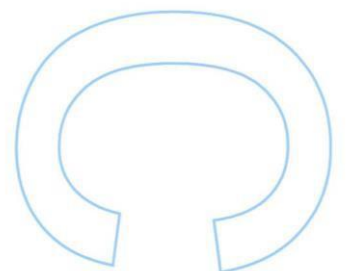
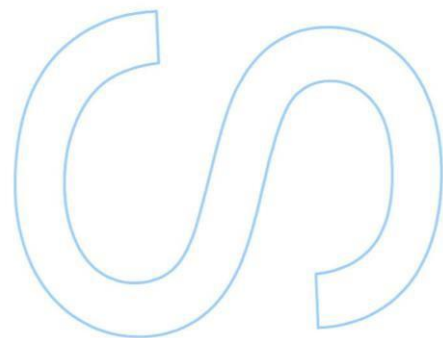
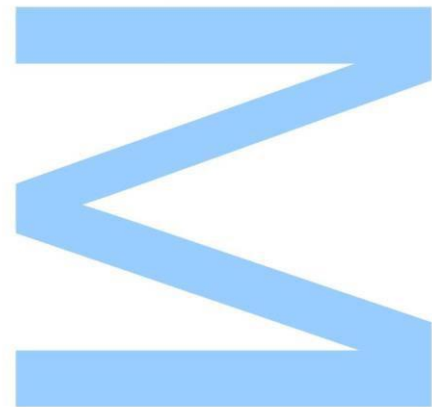
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Mestrado em Ecologia, Ambiente e Território
Departamento de Biologia da FCUP
2015

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Co-Orientador
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Cover Image: two microphotographs of a sagittae otolith from *Pleuronectes platessa*: left – optical microscope; right – scanning electron microscope

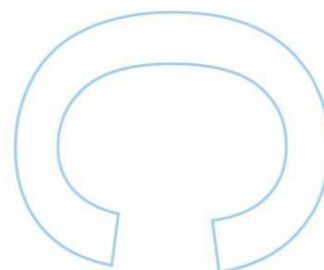
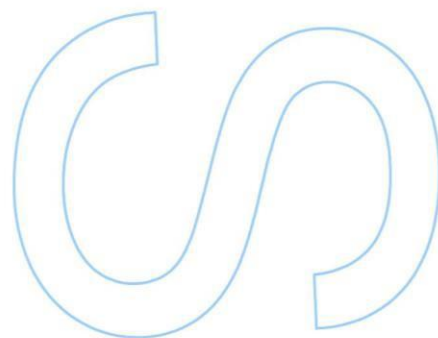
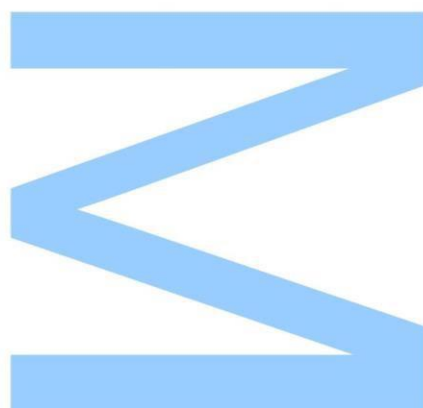




Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ____ / ____ / ____



Acknowledgments

First of all, I would like to thank to my parents to the education they gave me. Thanks to them I have learned to follow my dreams, even if that meant that I had to go against them. I learned to be self-independent and that without hard work we can't accomplish anything. It is, also, thanks to them that I was able to finish my degree and, now, my masters without depending on them. So, mom, dad and stepmom, thank you.

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Last but not least, I have to thank to all my friends that have helped me throughout this effort, and, especially to the ones that were present in the final stretch, where I did not had all the strengths to keep going with my thesis, because I had a lot going on in my life. Thank you guys, you were the best. And to the ones that are not present anymore, I also have to thank you too.

I just want to finish with a citation that means a lot to me and, probably, to everyone that had already felt that way, at least once in their life.

"It doesn't take a lot strength to hold on. I takes a lot of strength to let go."

J. C. Watts

Scientific Output

Throughout the development of this masters thesis, some of the results have been presented in several scientific meetings, like:

- Sousa, Joana, Freitas, Vânia, Santos, Paulo (2015). Determination of interannual variability in early life history of *Pleuronectes platessa* (L.) using otolith microstructure. *Front. Mar. Sci.* doi: 10.3389/conf.FMARS.2015.03.00132

- Sousa, Joana, Freitas, Vânia, Santos, Paulo (2015). Determination of interannual variability in early life history of *Pleuronectes platessa* (L.) using otolith microstructure. European Congress of Ichthyology, Porto, Portugal.

- Sousa, Joana, Freitas, Vânia, Santos, Paulo (2015). Interannual variation in early life history of *Pleuronectes platessa* (L.) in the northern distribution limit. 4th Iberian Congress of Ecology, Coimbra, Portugal.

- Sousa, Joana, Freitas, Vânia, Santos, Paulo (2015). Interannual variability in early life history of *Pleuronectes platessa* (L.) in a cold-water nursery ground. IJUP'15, Porto, Portugal.

Resumo

A solha, *Pleuronectes platessa* L. (1758), é uma das espécies de peixes chatos mais importantes comercialmente na Europa. Tal como outras espécies, esta já sofreu de sobre-exploração; contudo, atualmente a sua população encontra-se estável. Tem uma distribuição desde o Mediterrâneo até ao Mar Branco, sendo abundante no Mar do Norte. Apesar de haverem muitos estudos acerca dos estados iniciais do ciclo de vida para espécie, informações no limite norte da sua distribuição são escassas. Através da análise da microestrutura de otólitos é possível compreender melhor os estados de vida iniciais em juvenis de solha, nomeadamente obter estimativas sobre a data de nascimento, a data de assentamento, a duração da fase larvar, a duração da metamorfose ou até a taxa de crescimento de um dado indivíduo. O objetivo deste trabalho foi, então, estimar a idade total, a duração de cada uma das fases do ciclo de vida, o período de nascimento, bem como o de assentamento, relacionar o nascimento e o assentamento com a temperatura e ainda estimar a taxa de crescimento global, de indivíduos de *Pleuronectes platessa* amostrados em Valosen (Bodø, Noruega), durante os anos de 2005 e 2006, analisar a variação interanual para este local e comparar com outros locais mais a sul da distribuição da solha. Através de microscopia ótica foram analisados 30 otólitos de cada ano, pertencentes a indivíduos com tamanhos entre 14 e 72 mm. As idades para 2005 estimaram-se entre 41 e 99 dias e 50 e 129 dias para 2006. As datas de nascimento em 2005 decorreram entre abril e maio, enquanto em 2006 decorreram entre março e junho. Nos dois anos a duração da fase larvar foi semelhante. O assentamento ocorreu em junho, durante 2005, e estendeu-se de maio a julho em 2006. Relativamente à taxa de crescimento, ocorreram diferenças significativas entre anos, tendo os indivíduos capturados em 2006 uma taxa média de crescimento superior à de 2005. O facto de, em 2006, os picos de nascimento e assentamento terem ocorrido com aproximadamente um mês de diferença relativamente a 2005, pode estar relacionado com a diferença nas temperaturas da água do mar, pois esta foi, em média, superior à de 2005. A temperatura pode também ser uma possível explicação para a taxa de crescimento superior verificada em 2006. Também foi possível verificar através da comparação com dados de outros autores que existe uma tendência latitudinal em vários aspetos do ciclo de vida nesta espécie, uma vez que tanto o nascimento como o assentamento ocorreram mais cedo no ano em latitudes mais baixas, onde a temperatura da água do mar é superior, do que em latitudes mais altas, onde a temperatura da água do mar é mais baixa. No entanto, a

duração da fase larvar foi semelhante a outros locais contrariando a tendência positiva para um aumento da fase larvar com a latitude.

Abstract

Plaice, *Pleuronectes platessa* L. (1758), is one of the most important commercially exploited flatfish species in Europe. Like other species, it has suffered from over-exploitation; however, currently its population is stable. It has a distribution range from the Mediterranean until White Sea, and is particularly abundant in the North Sea. Although there are many studies about the early life stages of this species, information on the northern limit of its distribution are scarce. Through the analysis of otolith microstructure it is possible to gather information about the early life stages in juvenile plaice, namely the hatching date, the date of settlement, the duration of larval stage, the duration of metamorphosis or the growth rate of a given individual. The objective of this work was then to estimate the total age, duration of each phase of the life cycle, the timing of hatching, as well as the settlement timing, relate hatching and settlement with water temperature and also estimate the global growth rate of individuals from *Pleuronectes platessa* sampled in Valosen (Bodø, Norway) during the years 2005 and 2006, analyse the interannual variability in this location and compare the results with places further south of the distribution of plaice. By optical microscopy 30 otoliths of each year were analysed, belonging to individuals with sizes between 14 and 72 mm. The ages for 2005 were estimated between 41 and 99 days and 50 and 129 days for 2006. The timing of hatching in 2005 took place between April and May, while in 2006 took place between March and June. In both years the duration of larval stage were similar. The settlement took place in June, in 2005, and was extended from May to July in 2006. Regarding to growth, significant differences between years were found, with individuals caught in 2006 having a higher growth rate than the ones caught in 2005. The fact that, in 2006, hatching and settlement peaks had occurred approximately one month apart from 2005, can be related to the difference in sea water temperatures, since this was, on average, higher in 2006. Temperature may also be a possible explanation for the higher growth rate observed in 2006. It is also possible to say that, when comparing with data from other authors, a latitudinal trend was observed for this species, in several features of the life cycle, since hatching and settlement occurs first in the year at lower latitudes, where the sea water temperature is higher than at higher latitudes, where the sea water temperature is lower. However, the duration of the larval stage was similar to other locations, opposing the positive trend for an increase of the larval duration with latitude.

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List of Abbreviations

PLD – Pelagic Larval Duration

AP – Accessory primordia

B - Metamorphic stage

C – Benthic stage

SGR – Somatic Growth Rate

OL – Otolith Length

TL – Total Length of the fish

SST – Sea Surface Temperatures

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1. General Introduction

Temperate estuarine and shallow coastal systems are key habitats in the context of life cycle closure in marine populations (*sensu* Sinclair 1988). These provide important nursery grounds for many marine fish species of the continental shelf and, therefore, processes affecting these areas, either linked to human activities or to natural causes, can influence the recruitment. This will contribute to the continuous decrease of adult populations (Ciotti et al. 2014). These nursery areas, used by many marine species, like flatfishes, on estuarine and shallow coastal habitats (Selleslagh and Amara 2012; Martinho et al. 2013) are very important for early life stages growth and survival, because of abundant food resources, protection from predators and high water temperature (Freitas et al. 2010; Freitas et al. 2012; Selleslagh and Amara 2012). In these environments, both habitat quantity and quality are very important and can vary substantially. Habitat quantity refers to the amount of suitable habitat available, while habitat quality includes abiotic - temperature, sediment type, salinity and oxygen - and biotic – availability of food, density of newly settled fish- conditions on the nurseries, (Freitas et al. 2010; Selleslagh and Amara 2012). Individuals that have faster growth or are larger, are more suited to avoid predators and are more efficient at feeding (Haynes et al. 2012). So, the higher the quality of these habitats, the more juvenile reaches adult populations, since nursery grounds provide a bigger contribution to the recruitment of adult population compared to other juvenile habitats (Geffen et al. 2011; Haynes et al. 2012). Recruitment is the number of fish which reach a fishable size or stage (Miller et al. 1991). Furthermore, there are annual variations on the number of individuals arriving on a nursery ground, as well as the settlement patterns or timing (Geffen et al. 2011). The European plaice *Pleuronectes platessa* (L.) has long been used as a model for studying the processes occurring in the nursery ground and understanding the functional role of these areas in population renewal (Ciotti et al., 2014).

The arrival of plaice larvae to the nursery areas (normally in relatively shallow waters) (Geffen et al. 2011) depends on oceanographic connectivity with the spawning grounds (Fox et al. 2014), often occurring in pulses, with some interannual variability in the time of arrival (Geffen et al. 2011; Selleslagh and Amara 2012). Regarding to habitat quantity, larger habitats seems to provide higher numbers of juvenile plaice to the adult population (Van Der Veer et al. 2000). Some aspects of habitat quality are also very important, because the structure and the exposure of the habitat influences larval supply

and level of predation after settlement, thus affecting juvenile plaice densities, and sediment type is important because of prey availability (Haynes et al. 2012).

Plaice mortality on nursery grounds is usually density-dependent. Predation appears to be the main cause of mortality of juvenile plaice on nursery grounds (Nash and Geffen 2012). Growth rate in this species vary among regions (Ciotti et al. 2014), and this is also related to mortality: there is an inverse relationship between the size of an individual and its risks of mortality (Sogard 1997). Also, colder winters tend to produce large year classes and elevated mortalities, which leads to low growth rates. However, low growth rates are also a direct result of colder water temperatures (Nash and Geffen 2012). Warmer temperatures provides a reduction on the duration of the larval stage, but can also boost the number of predators during the early stages of the development, contributing for the reduction of the year class strength (Hovenkamp and Witte 1991; Fox et al. 2007). Over a large spatial scale, such as along plaice distributional range, variations in abiotic and biotic factors can also occur causing heterogeneity on growth and mortality patterns and, consequently, on recruitment. Latitude, for instance, influences temperature, seasonality and insolation, which are correlated and interact among them. This, and in particular, temperature, will affect the life cycle of fishes, because it helps setting the fundamental rates of metabolism, energy uptake, storage and use, and also, has impact on spawning, growth and reproduction migrations (Martinho et al. 2013).

Most studies on plaice recruitment have been done in temperate areas of the north-east Atlantic (Haynes et al. 2012), and studies on the north distribution limits of this species are still scarce. Although this species prefer shallow soft bottom areas as nursery grounds, bays and sandy beaches located in the inner parts of Norwegian fjords are also used by juvenile plaice (Freitas et al. 2010). Freitas et al. (2010) studied settlement and growth of juvenile plaice in a cold water nursery in Norway and found some evidence for interannual differences. However, in the previous study, settlement and growth patterns were based on shifts in mean length of the population over time and hence, these estimates can be biased by size-selective processes that affect the size structure of the population like size-selective predation and migration (both immigration and emigration). An alternative is the use of otolith microstructure which, besides providing growth estimates, can also inform on the timing of early life history events.

In this study the work by Freitas et al. (2010) was extended, using otolith microstructure as a tool to examine hatching, settlement timing and growth rates in juvenile plaice in a cold water nursery. Using the two year data available, the interannual

variability was analysed in early life history events. The specific goals of this work were to:

- determine the age of the analysed individuals in days, as well the age at the end of the different stages (larval, metamorphosis and settlement);
- estimate the hatching and settlement dates;
- analyse the relationship between hatching and settlement timing to water temperature;
- estimate the somatic growth rate (mm/day);
- compare the results of both years;
- compare the results to other nursery grounds further south.

1.1 Species Description

Plaice is a boreal flatfish species from the Pleuronectidae family (IUCN 2014). It is very important commercially in Europe and its distribution ranges from western Mediterranean along European continental shelf waters until the White Sea, being especially abundant in the North Sea (Hoarau et al. 2002; Comerford et al. 2013, FAO 2015). It has a compressed, oval body and like other flatfishes, both eyes are on the right side of the body. Adults and juvenile colour is from brownish to greyish, with large, rounded red or orange spots dispersed over the body (Figure 1).



Figure 1 – Example of an adult plaice (MarLin 2015)

The pigmentation on plaice larvae is different from plaice juveniles which seems to be protection measure from the different predators that prey upon these two different life stages (Christensen and Korsgaard 1999).

These fishes are found in waters with less than 1 m of depth to about 100 m, with a temperature range of 2 to 15°C, preferring temperatures around 6°C to reproduce. Adults normally reach 50 cm and weight 1.1 kg (IUCN, 2014; FAO 2014). Males can live up to 25 years, reaching maturation at 2-3 years, while females can live up to 30 years reaching maturation at 4-5 years. Also, like other pleuronectids, at higher latitudes, growth and maturation are slower, so the individuals get older and larger than at lower latitudes (Albert et al. 1998).

Plaice normally feed on molluscs, sandeels, some crustaceans and also a wide variety of benthic invertebrates, such as polychaetes (Rijnsdorp and Vingerhoed 2001; IUCN 2015). In respect to plaice predators, the most important ones are invertebrates, such as brown shrimp *Crangon crangon* and crabs *Carcinus maenas*, that predate young plaice, and some fish species, such as weever fish. As individuals get older, there is an increase in the avian predation. In addition, cannibalism can also occur (Nash and Geffen 2000; Nash and Geffen 2012).

Plaice are gonochoristic and oviparous, with high fertility, with up to 600 000 externally fertilized, pelagic eggs being expelled during the spawning season (Hoarau et al. 2002; Augley 2007; Bremm 2011). Spawning generally occurs between December and May, on the continental shelf and the pelagic eggs hatch after 1 or 2 weeks (Hoarau et al. 2002; Haynes et al. 2012; Comerford et al. 2013). The duration of egg and larval stage are temperature dependent, as high water temperature decreases the development time (Martinho et al. 2013). Eggs and larvae are pelagic, so they drift with current, during approximately 3 to 4 months, before reaching shallow waters (Hoarau et al. 2004). Once they reach shallow waters, larvae experience metamorphosis and settle at a size between 10 and 15 mm, on nursery grounds, remaining there for several years, and progressively moving offshore as they grow (Figure 2) (Veer and Witte 1999; Haynes et al. 2012; Comerford et al. 2013; IUCN 2015).

The timing between spawning and hatching and its coincidence with biotic and abiotic factors influence the likelihood of plaice reaching the nursery areas and the conditions in which each individual reaches these areas (Comerford et al. 2013). In plaice, metamorphosis and settlement occurs simultaneously at a size of about 13 mm (Raedemaeker 2012). The morphological and physiological changes are associated to metamorphosis, while the behavioural and ecological modifications associated to the transition from the pelagic stage to the benthic period are associated to settlement (Nash and Geffen 2012). When settlement occurs, juvenile plaice starts to experience a demersal or benthic existence instead of the pelagic phase that larvae experienced (Geffen et al. 2011; Comerford et al. 2013). Once juvenile plaice reach sexual maturation (about 2-5 years) they undergo spawning migrations offshore, in order to join the reproductive population (Raedemaeker 2012).

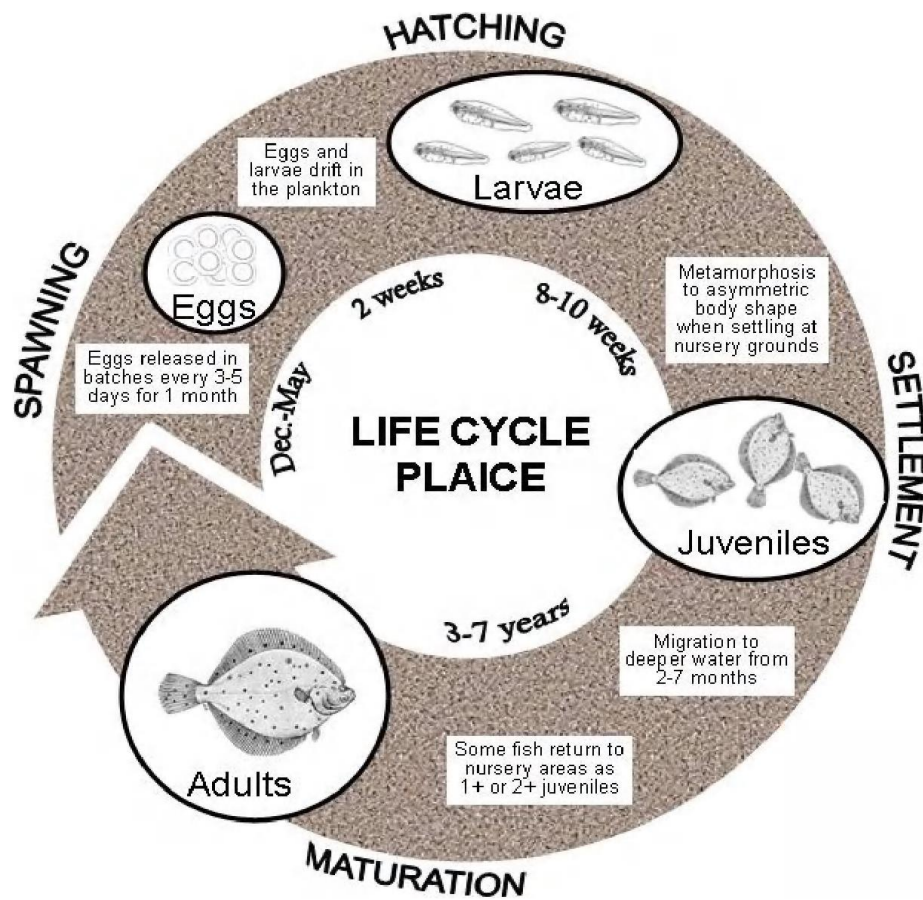


Figure 2 - Life cycle of *P. platessa* (from, Raedemaeker 2012)

Plaice is the most important flatfish commercially exploited in Europe. Currently, there are 6 stocks (fish stocks can be defined as random group of fishes, with members having similar life history that are large enough to reproduce) of plaice according to ICES Stock Assessment Database (2015) (Begg and Waldman 1999).

Countries with largest catches in 2013 were the Netherlands and Denmark (FAO, 2015). Plaice is fished along with other flatfishes, like sole, which may affect juvenile plaice that could get caught due to small mesh size or are discarded due to a minimum landing size regulations. Its overexploitation led to a reduction on the catches since 1990. Recently, the catches started to increase again and seems that the stocks are recovering (figure 3) (van Keeken et al. 2007; van Walraven et al. 2010; FAO 2015).

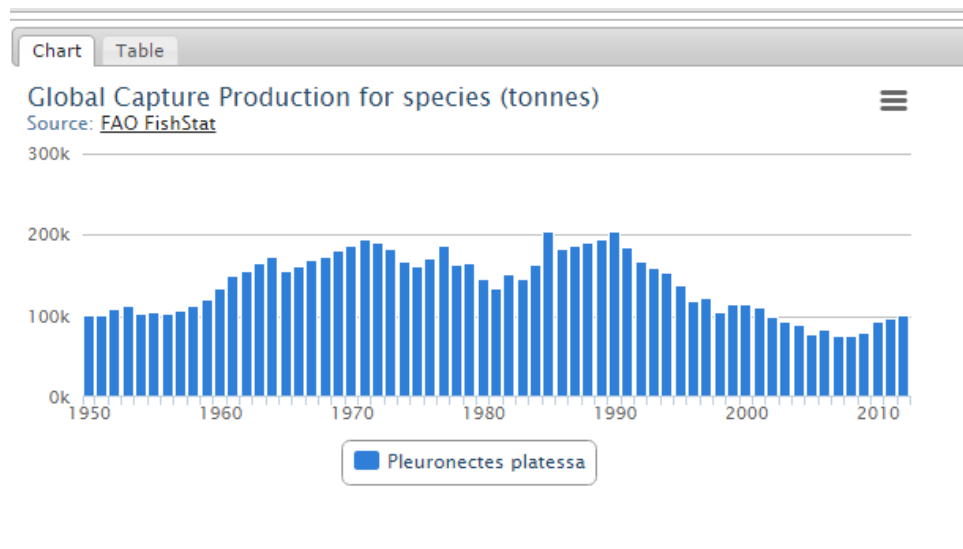


Figure 3 - *Pleuronectes platessa* landings from 1950 until 2013 (from FAO, 2014)

This raised questions about the number of stocks as well as on the overall genetic health of plaice (Hoarau et al. 2002). Studies about plaice population genetic structure have shown high genetic homogeneity among continental shelf populations from Norway to southern Britain, with samples from Iceland and Faroe being the exception, with significant population differentiation (Hoarau et al. 2002). This is in agreement with the fact that strong differentiation among plaice occurs between shelf and off-shore populations, but weak differentiation also within continental shelf samples (Hoarau et al. 2004). More recent studies also supported this, since Was et al. (2010) found genetic homogeneity among samples collected from Irish coasts and from Baltic Sea, but also from the majority of North Sea samples. According to the IUCN Red List to Threatened Species, *P. platessa* is considered as “Least Concern”.

Regardless, it is important to have a well-defined management of plaice stocks, in order to contribute to the population stability, even though the species may sometimes occurs throughout many marine protected areas. Anthropogenic activities on nursery areas may have a negative impact on this species, since plaice needs optimal conditions to survive, with plenty of space and without the negative consequences of human activities (like water pollution). With an increase of degradation on coastal areas, it is important to identify and protect high quality nursery areas, in order to not to affect negatively the recruitment of adult plaice population (Haynes et al. 2012). Plus, pollution may also be associated to lethal malformations and can too lead to mortality in the larval phase. Plaice nowadays is not only used for commercial fisheries, but also for sport fishing (Nash and Geffen 2012; IUCN 2015). Another factor that threatens this species is

climate changes. Due to the rising of sea water temperature (in part because of human activities), the abundance and geographic distribution of species are continuously changing. Each stage of fish life cycles could be affected in different ways by climate changes. This could imply changes in fish populations driven by a physiological response to changes in environmental conditions, such as temperature, behavioural responses, populations dynamics or ecosystem level changes in productivity and/or trophic interactions (Rijnsdorp 2009). In plaice, temperature may change egg mortality and can also affect metabolic costs and/or predation pressure (Van Der Veer et al. 1998; van Hal et al. 2010).

1.2. Understanding early life history using otoliths

The study of the dynamics of fish nursery grounds requires understanding the early life history including the larval and early juvenile stages. In this respect, otolith analyses have made significant contributions to this field of study.

Otolith microstructure is then a reliable tool in daily ageing estimation for plaice, in days.

The microstructure of plaice otoliths is a widely used tool to analyse growth conditions of this species, as well the age estimation of analysed individuals. This is important, because it allows to gather enough information to understand its population dynamics and to help manage its stocks.

Otoliths are biomineralized calcium carbonate bodies (figure 4), found in the internal ear of teleost fishes, important for hearing, equilibrium and orientation that are largely used to estimate fish ages, growth and behaviour (Lecomte-finiger 1999; Hüßy 2008; Fablet et al. 2011; Comerford et al. 2013). During initial phases of ontogenesis, they are the first calcified structures to be formed (Lecomte-finiger 1999). Dense rings and growth increments are the result of the accretionary process involved in otolith formation (Morales-Nin 2000; Fablet et al. 2009).

There are three pairs of otoliths found in fishes (sagittae, asterisci and lapilli), each of them differing in location, size, shape and microstructure. In most growth studies, usually sagittae are used (Jones, 1992; Secor et al, 1992).

Otolith microstructure is a powerful tool to estimate individual age, growth rate, hatch date, length of larval phase, post-larval ages and settlement date in larvae and juveniles of fishes, by counting and measuring the daily increments, since otoliths are the most

reliable structures providing information about daily events and annular events throughout life (Allen et al, 2008; Bustos et al. 2015; Jones, 1992).

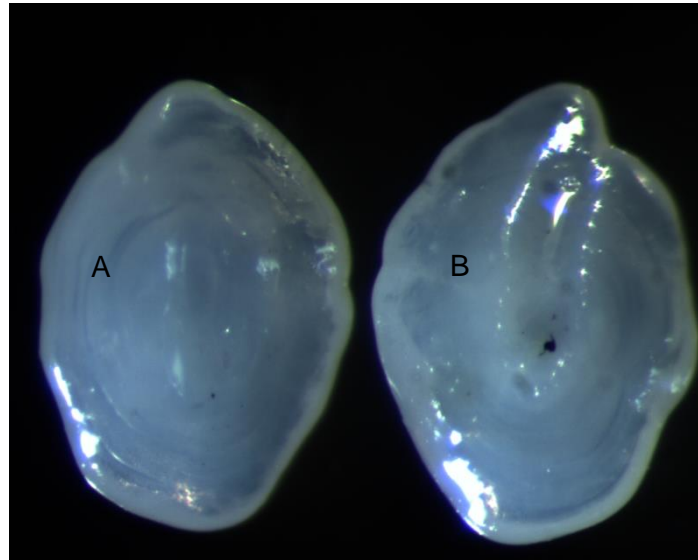


Figure 4 - Left and right *sagitta* otolith of an individual of *P. platessa* (reflected light on dark background), caught in 2006, in Valosen, Norway. Length of the individual: 31 mm. Length of the otoliths: A - 0.798 mm; B - 0.817 mm. Proximal surface.

In older fishes, age (in years) can be estimated by using, most frequently, scales and otoliths. In otoliths, age in years is determined by the counting of *annuli* – annular ring formations (Jones 1992; Campana 2001). In young fishes, who doesn't have their first *annulus* formed, otolith microstructure is used to estimate daily ageing (Campana and Neilson 1985; Campana 2001). In 1971, Panella was the first to describe daily incremental marks, in red hake, that could be used to estimate the age of fishes, in days.

Daily increments are ring-like structures (figure 5) that are dependent of a photoperiod induced by an endogenous circadian rhythm (Campana 1992; Lecomte-finiger 1999). Subdaily increments can also be formed, due to external factors, such as temperature fluctuations and feeding periodicity, being morphologically similar to daily increments, which makes difficult to distinguish between these two structures. While daily increments are formed at a continuous rate, subdaily increments may form at any time of the day (Campana and Neilson 1985; Campana 1992; Morales-Nin 2000). This can lead to a misidentification of age and somatic growth rate (Neilson 1992).

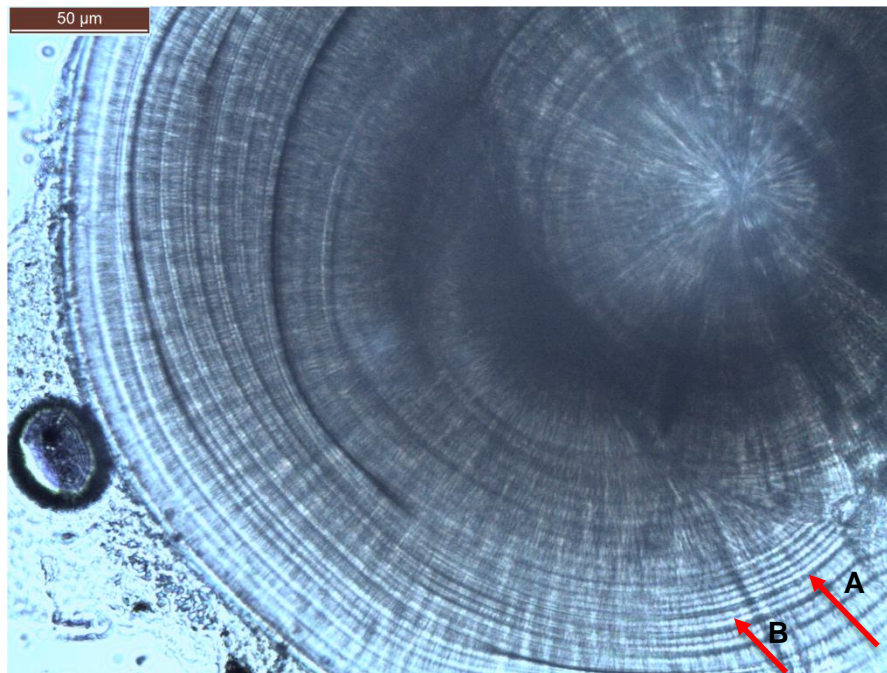


Figure 5 – Daily (A) and sub-daily (B) increments of a polished *sagitta* otolith of an individual of *P. platessa*, caught in Valosen, Norway, in 2006.

The time of the deposition of the first increment can vary from species to species. Normally it is distinguished by a distinctive feature, like a prominent check. In some species the first ring may correspond to the hatching day, and so, be called hatch check, being distinct from the other rings; in others species, the first increment may be formed in association with a specific developmental stage, like the first feeding check, in fishes with vitelline reserves (Wright et al 2002; Geffen et al. 2011)

With otolith microstructure, it is possible not only to estimate the age of the individuals, but also to identify distinct events of a fish life cycle, like developmental ones (hatching or metamorphosis) or behavioural (like settlement) (Geffen et al. 2011). During metamorphosis, distinct and wide rings start appearing and accessory growth centres begin being formed (Geffen et al. 2011). These are extra planes of growth, from which a new sequence of increments appear to emanate (Morales-Nin 2000). Accessory growth centres can also be called *accessory primordia* and their formation can be related to a transition in physiology, habitat and behaviour, which is a characteristic of species that experience a marked transition in habitat between the larval and the juvenile stage, like flatfishes (Morales-Nin 2000; Wright et al. 2002).

It is also known that as the fish grows, otoliths also grow, which shows a strong correlation between the fish size and the otolith size. However, in some rare cases, this correlation cannot be found, due to several factors such as starvation, low growth rate,

ontogenic development and supra-optimal temperatures (Campana and Jones 1992; Hüseyin 2008). Also, in order to make a proper estimation of the age of the fish, it is crucial to know the exact age of the deposition of the first increment and the accuracy of increments counts (Jones 1992).

The width of each growth band on otoliths is used to estimate the daily otolith growth, and this can be used to infer daily somatic growth (Jones, 1992). With that, there are a series of mathematical equations to have more precise and complete information about the growth of the individuals (Campana and Jones 1992).

Another important aspect of the analysis of otolith microstructure is the growth back calculation. Gathering information like the measured widths of a daily increment time series along with fish length / otolith length it is possible to infer the size and the growth rate of an individual fish for each day of its life. However this requires having a correct validation of the frequency of increment formation in order to have accurate growth back calculations (Campana and Jones 1992).

In *P. platessa*, validation of the daily increment deposition was done by Alhossaini and Pitcher (1988) which demonstrated that these structures can be used on the determination of plaice age, growth rates and mortality studies. In this species, growth increments are formed daily, with a distinct first increment, and it is known that the number of increments is age-dependent, while the increment width is growth-dependent (Karakiri et al. 1989; Geffen et al. 2011). Karakiri and Westernhagen (1989) showed that the first increment is deposited four to six days before hatching and environmental changes don't affect the process of deposition of daily increments. Moreover, besides ageing, otolith microstructure can also provide an indicator of environmental changes on the growth rate of young plaice (Karakiri et al. 1989). Somatic growth and otolith growth are correlated, and the average width of the increments of the otoliths can be used as an indicator of somatic growth (Hovenkamp 1990).

For plaice, otoliths increments are laid down daily during the larval stage and when the transition to demersal stage happens, four to six accessory primordia could appear (Allen et al. 2008), corresponding to metamorphosis. This kind of pattern is also known in sole (*Solea solea*), winter flounder (*Pseudopleuronectes americanus*) or flounder (*Platichthys flesus*). It is the presence of these accessory primordia that will allow the back calculation of settlement dates and the determination of the duration of the larval stage. The increments deposited during the larval stage are narrower than the ones deposited after this phase (Allen et al. 2008; Geffen et al. 2011).

Some researchers, as Karakiri et al. (1991), used otolith microstructure analysis to investigate variations in settlement and growth of 0-group plaice in the Dutch Wadden Sea. From various sampling sites, there were differences in the length of the larval stage and in growth rates among regions. They also observed the presence of subdaily increments due to tidal effects.

Allen et al. (2008), also used otolith microstructure to determine hatching times, larval duration, settlement and larval growth of plaice in Galway Bay. In this area, these authors found a tidal effect on hatching and settlement.

2. Methodology

2.1. Study area and sampling

Otoliths used in this study were previously collected during a sampling programme in Valosen, a small estuary in the Saltfjord, northern Norway (67°17'N, 14°37'E) (figure 6), carried out in 2005 and 2006 (for details see Freitas et al. 2010). Post-metamorphosed plaice were collected fortnightly from April onwards to determine the beginning and end of the estuarine colonization period. Fish were collected with a 1 m beam trawl (5 mm stretched mesh in the cod end, one tickler chain) operated from a rubber dinghy with an outboard motor during daytime. Fish samples were sorted, counted, measured to the nearest mm total length (TL) and weighed. Sagittae otoliths were removed from each fish, cleaned and stored in identified petri dishes with a drop of nail polish.

Water temperature in the study area was measured continuously (every 15 min) with temperature loggers (StowAway® TidbiT™) positioned in the bottom. Only the temperature data around high-water was considered. In addition, average monthly sea-surface temperatures from the adjacent coast were gathered from the International Comprehensive Ocean-atmosphere Data Set (ICOADS) online database (<http://dss.ucar.edu/pub/coads>, dataset 540.1, Worley et al. 2005), including the period of winter/spring which could encompass plaice spawning period.

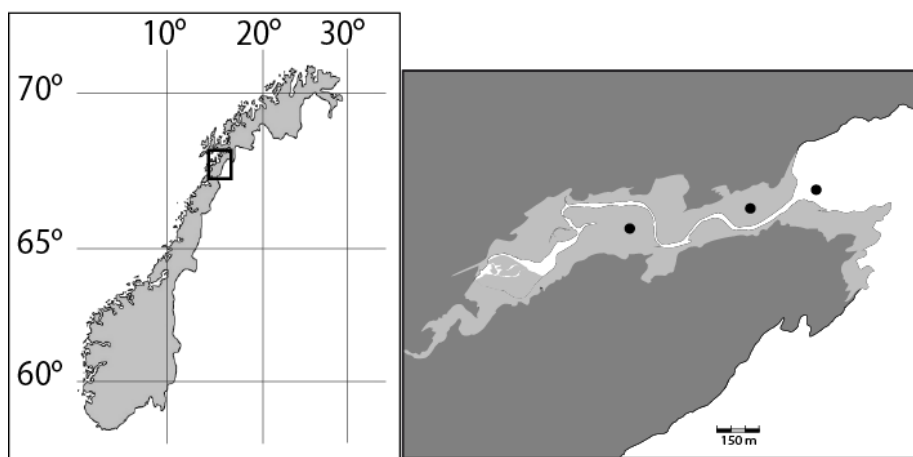


Figure 6 - Map of the Norwegian coast with a square indicating the study site (Valosen, NW Norway). On the right, dots indicate the three sampling stations on the study area.

2.2. Otolith preparation and examination

For each year, 30 otolith pairs from a subsample of juvenile plaice were chosen randomly from the range of sizes available. For the first part, and whenever the pair was available, each otolith was cleaned with acetone and mounted separately in microscope slides, with the concave side down, using thermoplastic resin (Crystalbond™) (figure 7). After that, lengths and widths of each otolith were measured, with an image analysis software (LEICA Application Suite (LAS), version 3.7.0 [Build: 681]), connected to a stereomicroscope.



Figure 7- Sagitta otolith of *P. platessa* being mounted on a microscope slide

To recognize daily growth increments, the otoliths required polishing (Allen et al, 2008), so after the biometric measurements, only one otolith from the pair was selected to be polished, since both otoliths are identical in terms of microstructure (Karakiri et al, 1989). In this work, whenever possible, left *sagitta* otolith was chosen to maintain greater consistency.

Smaller otoliths (otolith length inferior < 1 mm) were hand-polished using fine-grit (3, 1 μm - 3M lapping film, 3micrometer), while bigger otoliths (otolith length larger than 1 mm) were polished using silica carbide paper (1200, 4000 - Struers Waterprof Silicon Carbide Paper) and on both sides. Otoliths were polished in the sagittal plane to the central core and this process was made carefully, with the help of a microscope, in order to obtain a clear continuous image of daily growth increments since the nucleus until the outer edge of the otolith (Karakiri and von Westernhagen 1988).

In order to examine the otolith microstructure, otoliths were examined through a Leica light microscope, connected to a camera, with x 10, x 40 and x 100 (oil) magnification. Subsequent analysis were carried out with the help of LEICA Application Suite (LAS), version 3.7.0 [Build: 681]) image analysis software.

Otolith daily growth increment were counted at least twice by the same reader and the age of the fish was regarded as the average of the two counts (Allen et al. 2008; Martinho et al. 2013). Three different life stages were distinguished: pelagic larval duration (PLD), the metamorphic stage (B) and the benthic stage (C). Pelagic larval duration (or larval stage) corresponded to the inner area from the hatch check (which is approximately at 10 μm from the centre of the otolith (Allen et al, 2008)), until the occurrence of the first accessory growth centre (also known as accessory primordium, AP). Metamorphic stage corresponded to the daily growth increments among the innermost and the outermost accessory primordia. Benthic stage corresponded to the first daily growth increment after the outermost AP until the edge of the otolith (figure 8) (Martinho et al. 2013). To sum up:

- Larval Stage (days): PLD = from hatch check until the appearance of first AP
- Metamorphic stage (days): B = innermost ring of the first AP until the outermost ring of last AP
- Benthic stage (days): C = outermost of last AP until the edge of the otolith
- Total age of the fish (days) = PLD + B + C

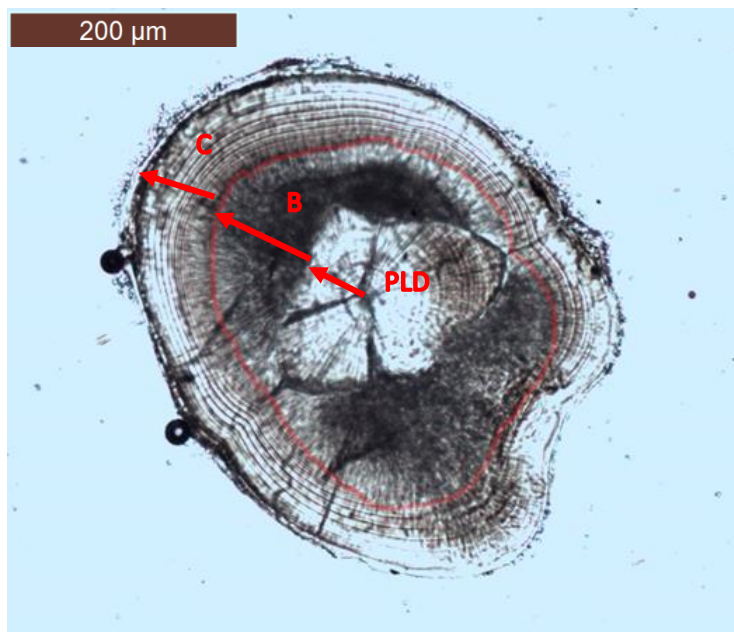


Figure 8 – Microphotograph of a *sagittae* otolith of *P. platessa* showing the three different life stages (PLD: larval stage; B: metamorphic stage; C: benthic stage; Red contour indicates the end of stage B and the beginning of stage C)

A photographic record of all of the 3 stages was made and widths, lengths and otoliths radius were measured.

The average somatic growth rate G (mm d^{-1}) was determined for each individual fish by dividing the total length (TL, mm) by the total age (in days).

The hatch date of individual juvenile plaice was obtained by subtracting the total age in days, to the date of capture. For plaice, it has already been used by some authors, such as Al-Hossaini et al. (1989), and validated by others (see Modin et al. 1996), the metamorphosis to estimate the beginning of settlement, so the date of settlement was obtained by adding the days of larval stage to the date of birth.

- Hatching date: Sampling date - Total age of the fish
- Settlement date: Hatching date + A

2.3. Data Analysis

Linear regression analyses were performed between otolith length against body length and body length against fish age (in days). These linear models were compared among years (2005 and 2006) with analysis of covariance (ANCOVA). Differences in growth rates, timing of hatching, settlement and timing and duration of the pelagic and metamorphic stages were evaluated by means of ANOVA. All statistical tests were done in R software (R Development Core Team, 2012) using a significance level of 0.05.

3. Results

The time of 0-group plaice peak density and the length frequency distribution in each sampling date (see annex 1) were used to determine the end of the estuarine colonization period. Peak densities were observed in early July and at this time, length distributions were approximately normal suggesting that main colonization had ceased. Therefore, for otolith microstructure analyses, selected otoliths in 2005 were from 5th of July (n = 13) and 18th of July (n = 17), while for 2006, all animals selected were from the sampling date of August 7th. The total length of fish ranged from 14 to 52 mm in 2005 and 19 to 72 mm in 2006. The *sagittae* otoliths polished varied in lengths between 0.384 mm and 1.595 mm and only in 12 of them wasn't possible to analyse the left *sagitta*, and, in those cases, right *sagitta* was analysed.

As expected, the length of the otolith (OL) showed a significant linear relationship with the fish total length as can be seen in figure 9a ($R^2_{2005} = 0,980$ $R^2_{2006} = 0.956$). These relationships also did not vary between years (ANCOVA: $p = 0.625$).

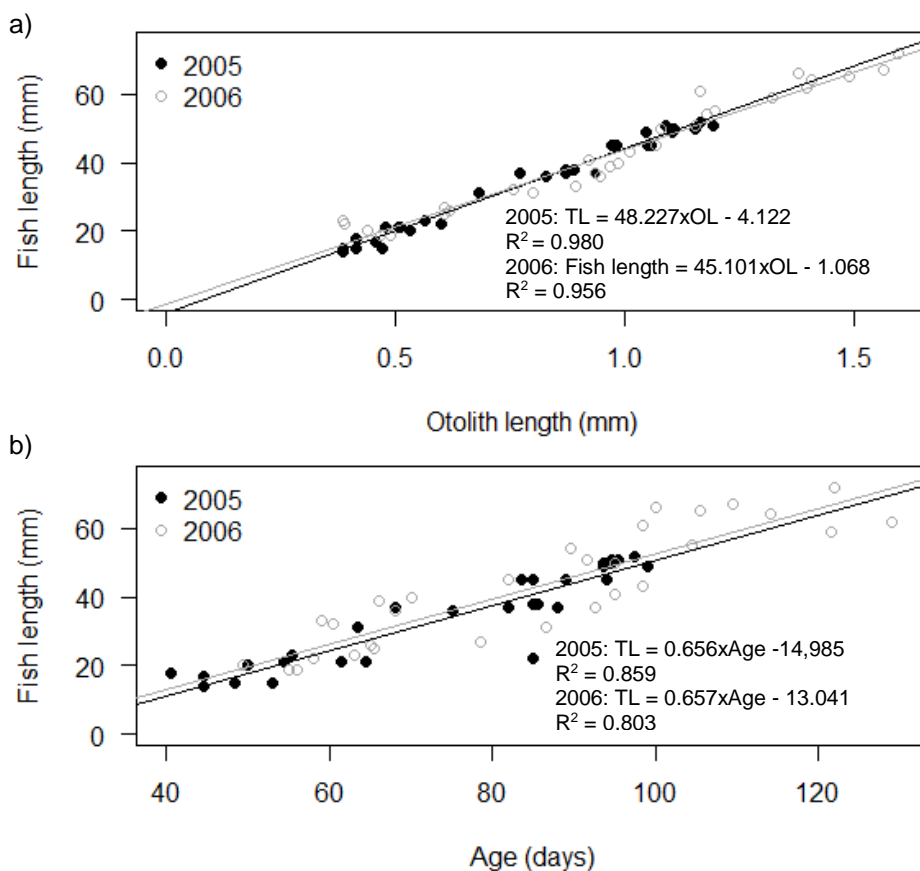


Figure 9 – Linear relations. A) Linear relation between the length of the fishes (mm) and their *sagittae* otolith (mm). B) Linear relation between the fish length (TL, mm) collected for this study and their age (in days).

Linear relationships were also obtained for fish total length (TL, mm) and age (days) (figure 9b) but no differences were observed between years (ANCOVA: $p = 0.241$).

The estimated ages for 2005 ranged between 41 and 99 days, while in 2006 the individuals presented ages between 50 and 129 days. In both years a few metamorphosing individuals were still found (0 rings in the benthic phase). This means that in the analyses it should have been chosen animals of posterior dates, but this could only be concluded after only after otoliths analyses have been conducted.

Birth dates in 2005 were between 10th of April and 25th of May and, in 2006, birth dates were between 31st of March and 18th of June (figure 10).

In 2005, on average, individuals hatched on 118th day of year (SD = 14), while in 2006 individuals hatched, in average, on 133rd day of the year (SD = 23) (figure 11a) and significant differences in time of hatching were found between years (F value = 9.852; $p < 0.05$).

Regarding to the duration of the larval stage, this lasted between 25 days and 65 days in 2005, with an average of 47 days (SD = 10) and between 27 days and 66 days in 2006, with an average of 46 days (SD = 10) (figure 11b). No significant differences between years (F value = 0.028; $p = 0.868$) were found.

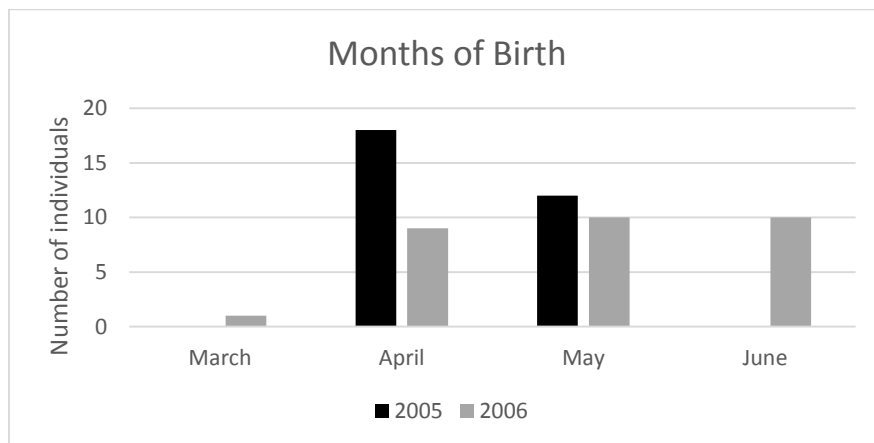


Figure 10 - Distribution of hatching dates of *P. platessa*, in 2005 and 2006, in Valosen (NW Norway)

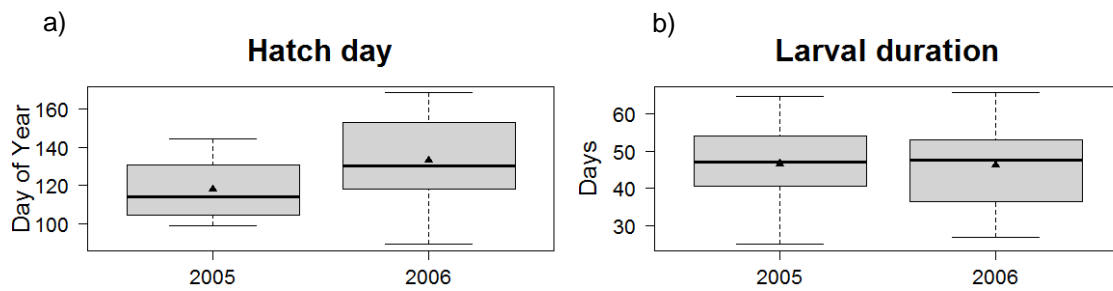


Figure 11 – Box Plot. a) Day of the year of hatching for *P. platessa* caught in Valosen (NW Norway), in 2005 and 2006; b) Estimated larval duration of *P. platessa* caught in Valosen (NW Norway), in 2005 and 2006. Thick horizontal bars represent medians, boxes indicate the upper and lower quartiles, whiskers represent the range, and triangles indicate mean values.

Settlement dates occurred between the 2nd of June and 27th of June, in 2005, and between 15th of May and 25th of July, in 2006 (figure 12). In 2005 individuals settled on average in the 164th day of the year (SD= 7), while individuals from 2006 settled in the 179th day of the year (SD= 16) (figure 13a). Significant differences were found between years ($F = 22.672$; $p < 0.05$) with settlement occurring later in the year in 2006 and over an extended time compared to 2005.

The duration of the metamorphic stage ranged between 8 and 20 days in 2005 and between 5 and 25 days in 2006 (figure 13b). On average, individuals from 2005 spent 12.7 days (SD = 3.2) in the metamorphic stage, while individuals of 2006 spent, on average, 16.1 days (SD = 5.0) in that stage with significant differences between years ($F = 10.356$; $p < 0.05$).

Regarding the benthic stage, this was smaller in 2005. During 2005, it ranged between 0 and 34 days, with an average of 15 days (SD = 10). During 2006, it ranged between 0 and 59 days, with an average of 23 days (SD = 14) (figure 13c). There were significant differences between years ($F = 6.0216$; $p < 0.05$). This means that most of the animals caught on 2006 were, probably, more developed than the ones from 2005. This is corroborated by the average post-larval age (number of days in metamorphic stage + number days in benthic stage) which was 28 days (SD = 11) in 2005, and 39 days (SD= 16) in 2006 (figure 13d) and with significant differences between years ($F = 10.371$; $p < 0.05$).

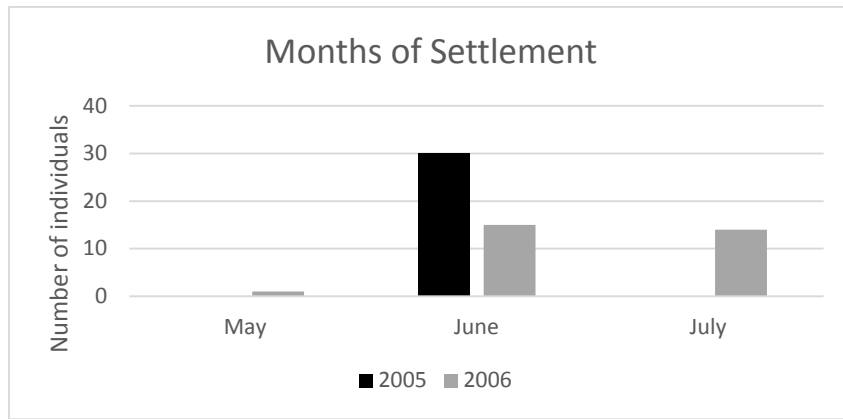


Figure 12 - Distribution of settlement dates, throughout the months of settlement, of *P. platessa*, in Valosen (NW Norway)

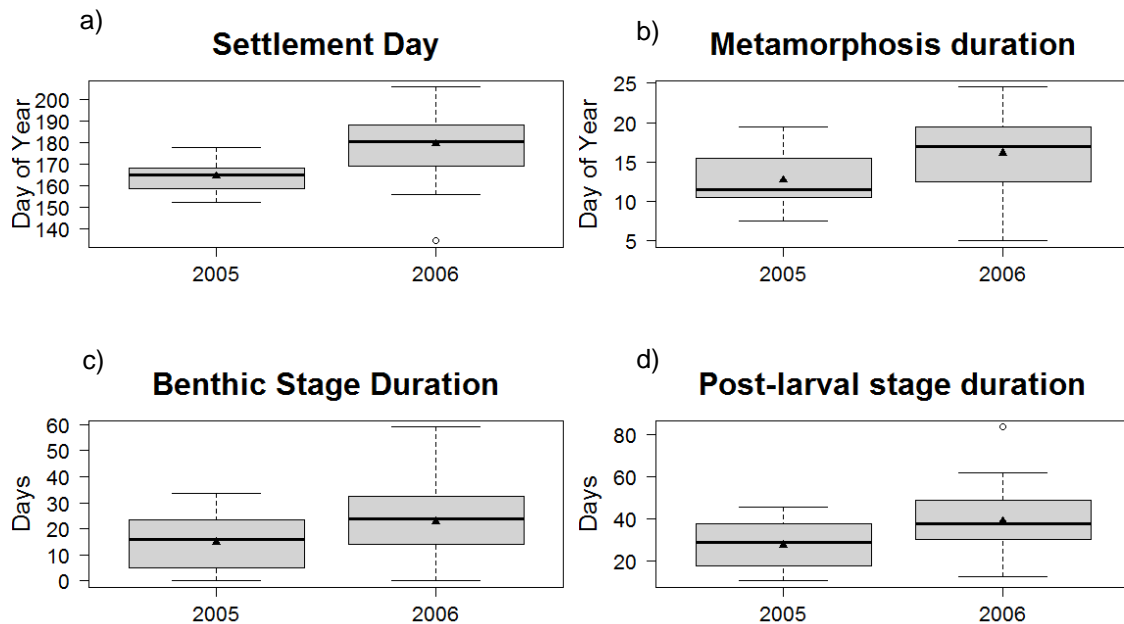


Figure 13 – Box plot. a) Day of the year of settlement for *P. platessa* caught in Valosen (NW Norway), in 2005 and 2006; b) Duration of metamorphic stage, in days, of *P. platessa*; c) Duration of benthic stage, in days, of *P. platessa*; d) Post-larval phase duration, in days, of *P. platessa*

Regarding to the overall individual growth rates, significant differences were found between years ($F = 4.622$; $p < 0.05$) with larger growth in 2006 than in 2005. On average, individuals in 2005 had a growth rate of 0.439 mm per day (SD = 0.091), while in 2006 this average was of 0.492 mm per day (SD = 0.100) (figure 14).

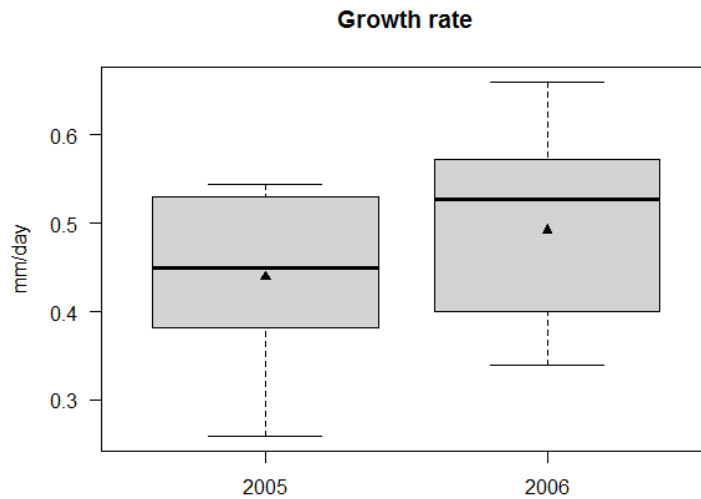


Figure 14 - Growth rate (mm day⁻¹) of larval stage of *P. platessa*, in 2005 and 2006, in Valosen (NW Norway).

When comparing hatching periods from both years to water temperature, on both years hatching started when average SST were around 6°C. This corresponded to April in 2005 and to March in 2006. However, in 2005 most of the individuals hatched in April, while in 2006 the peaks of hatching were in May and June, with temperatures between 8 and 9.5°C (figure 15). Estuarine temperatures (figure 16) also differed between years during the time of settlement (June and July), especially in June with higher values in 2006 than 2005. This temperature difference could explain the larger individual growth rates found in 2006.

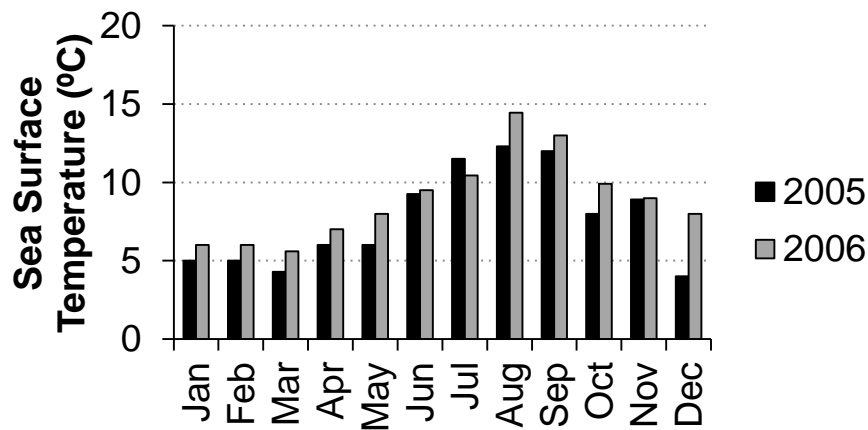


Figure 15 - Average sea surface temperature (SST) during 2005 and 2006, in Norway. (From Worley et al. 2005)

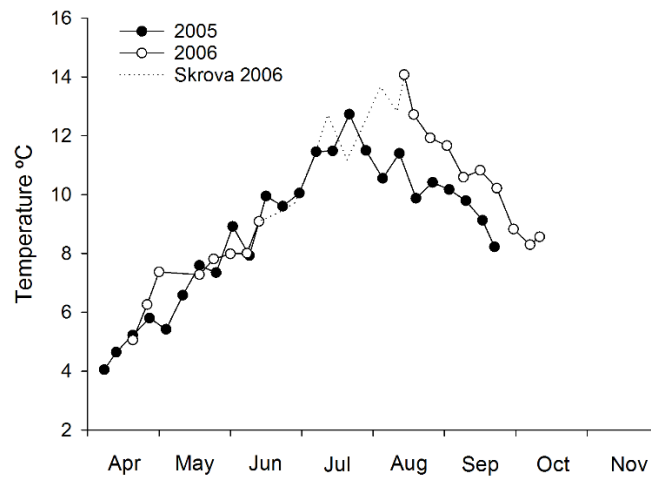


Figure 16 - Mean weekly seawater temperature (°C) observed in Valosen from April to October 2005 and 2006. Dashed line corresponds to temperature data derived from Skrova station in 2006 (from Freitas et al. 2010)

Data about hatching, settlement and growth rate of *Pleuronectes platessa* along the distribution range of the species, was collected from articles from various authors in order to detect any similarity with the other locations (table 1). The results obtained in this thesis are in agreement with the ones found by other authors.

Table 1 - Timing of hatching, settlement and growth rate of *Pleuronectes platessa*, in several nursery grounds along the species distribution range. Authors marked with * refers to otolith derived growth rate

Author	Location	Hatching Period	Settlement Period	Growth Rate
Present study*	Valosen, Norway	2005: April – May	2005: June – 2006: May	2005: 0,44 mm/day
		2006: March – June	July	2006: 0,49 mm/day
Al-Hossaini et al. 1989	Red Wharf Bay (North Wales)	1986: February – April	1986: Mid-April – late June	
		1987: Late January – Early April	1987: late March – Early June	
Allen et al. 2008	Galway Bay, Ireland	Late January – Late March	Early March – Late April	
Amara and Paul 2003*	French Coast		Mid-March – Early June	
Comerford et al. 2013	- Waden Sea	- January	- March	
	- Irish Sea	- March	- April	
	- West Ireland	- April	- May	
Freitas et al. 2010	Northern Norway			2005: 0.5 mm/day
				2006: 0.46 mm/day
Freitas et al. 2012	Gullmar Fjord, Sweden			1.3 mm/day
Fox et al. 2014	Scottish West Coast			From 0.38 to 0.50 mm/day
Geffen et al. 2011	Northern Irish Sea		Late April – Early July	From 0.3 to 0.8 mm/day
Gunnarsson et al. 2010	Iceland	Mid April – Mid June		
Karakiri et al. 1991	Dutch Waden Sea	February	April – May	
Van der Veer and Witte 1990	Southern Bight of North Sea		March – May	

4. Discussion

Most studies on fish recruitment focus on those areas where populations are under commercial exploitation, typically the centre of the species distributional range, leaving some gaps on marginal populations occurring at the extremes of species distribution. This study provides additional data from a northern cold-water nursery that allows analysing life history variation over a wider geographical range and complements information gathered previously for the same area (Freitas et al. 2010), in this case using otolith microstructure.

Otoliths showed the typical ring like structures that allowed to estimate the age of the fish and the typical patterns for flatfishes, with the transition from the pelagic stage to the benthic one being characterized by the formation of accessory primordia, as observed in other works (e.g. Al-Hossaini et al. 1989; Karakiri et al. 1991). Since the daily increments deposition has already been validated for larvae and juvenile plaice (Karakiri and von Westernhagen 1988), it was possible to use the counting of the increments to identify the three stages studied in this work (larval stage, metamorphic stage and benthic stage) and, therefore, the estimation of hatching and settlement timing, as well as the growth rate. Allen et al. (2008), also used in their work otolith microstructure of plaice to determine hatching times, larval duration, settlement and larval growth in Galway Bay (W Ireland).

Body length - otolith length relationships have been extensively used in fisheries research to calculate the growth of fish. However, the relationship between otolith length and fish length could be influenced by several factors, such as food availability, temperature or metabolic rates (Hovenkamp, 1990). Here a linear relationship between otolith length and fish length, without significant differences between years, was found. Hovenkamp and Witte (1991) found a relation of linearity between plaice larval size and otolith diameter, but with variability between years, with otoliths being larger relative to body size in 1987 and smallest in 1988.

Through the relation between the age of the fish (days) and the length of the fish, it was possible to obtain the growth equations for each year. Geffen et al. (2011) used this relation to estimate the growth rates. Comparing to him, the slope of the linear relation between age and total length of the fish was higher in Valosen, presuming that somatic growth rate will be higher comparing to his results. It was not found significant differences between years.

Comparing the hatching dates to other studies, it is possible to find some resemblances in places with similar latitudes to Valosen, and some differences when comparing to sites further south. For instance, in the Dutch Waden Sea hatching was on February (Karakiri et al. 1991), in the Red Warf Bay hatching started in February, in 1986, and in late January, in 1987 (Al-Hossaini et al. 1989). In other regions (Waden Sea and Galway Bay), hatching also started earlier in the year, in January (Allen et al. 2008; Comerford et al. 2013). However, in the Icelandic coast, Gunnarsson et al. (2010) report that hatching occurred from April until June. The results obtained by this author were consistent with the results obtained for Valosen, since the two sites have similar latitudes. Since temperature has a direct effect on the growth of fish larvae (Comerford et al. 2013), it is expected that hatching will start earlier at lower latitudes, where water temperatures are warmer. Duration of spawning period (estimated as the difference between hatch dates of the oldest and youngest individuals) in Valosen was however different between years with spawning lasting 1 ½ months in 2005 and 2 ½ months in 2006. Comparing both years regarding to the day of the year that each fish has hatched, 2006 was a lot more variable year. Warmer temperatures on this year could also be the reason for this variability. The reasons for such extended spawning period in 2006 are not clear but it might suggest that juvenile plaice examined in this study might have originated from different spawning grounds. A similar interannual variability has been observed in juvenile Baltic cod with differences between eastern and western stocks (Rehberg-haas et al. 2012). Unfortunately, it is presently unclear where the spawning locations of *P. platessa* along the Norwegian coast are to evaluate this hypothesis.

The duration of the pelagic larval stage of plaice in Valosen was more variable compared to other studies, but within the results obtained by those authors. In the Dutch Waden Sea, Karakiri et al. (1991) found a larval duration range between 50 and 62 days, while in the Irish Sea, a duration between 31 to 51 days (Fox et al. 2007) and up to 59 days (Al-Hossaini (1989) has been reported. In Iceland, the larval phase ranged from 44 to 78 days depending on the region, with significantly shorter larval periods in warmer areas compared to northern, colder ones (Gunnarsson et al. 2010). Such variations could be explained by differences on prey availability and water temperature during the larval stage that could provide faster or slower growth for larvae (Comerford et al. 2013), and, therefore, a shorter or longer larval stage duration. Assuming a positive relationship between larval stage duration and latitude, it would be expected longer larval duration in this study site compared to other areas but this was not observed and, juvenile plaice in Valosen had an average larval phase shorter than the one reported for the southern

regions of the Icelandic coast, at similar latitudes. Also, in both years there were individuals with a larval duration inferior to 30 days. This pattern in pelagic stage duration was very consistent between years even though water temperatures influencing larval development might have been higher in 2006 compared to 2005. A better characterization of prevailing water temperature conditions in the coastal areas is, nevertheless, necessary to examine the influence of temperature on larval duration. Between 2005 and 2006 there were not found differences in the larval duration, for plaice in Valosen; larval durations were also approximately the same in the North Frisian Waden Sea in 1981 and 1982 (Karakiri et al. 1989).

Regarding to the metamorphic stage, Hovenkamp and Witte (1991) found that fast-growing larvae and slow-growing larvae were approximately of the same size at metamorphosis, but the ones with intermediate growth are larger at metamorphosis, which indicates that development is more related to temperature than to size, since the age at metamorphosis declined with temperature. The duration of the metamorphic stage is in agreement to the described for plaice, where the combination with the larval and the metamorphic stage lasts approximately 10 weeks (Christensen and Korsgaard 1999). In plaice, growth varies significantly throughout metamorphosis (Christensen and Korsgaard, 1999) and once individuals reach suitable size for metamorphosis, they could delay settlement for a few days if food conditions are not ideal; however they could only delay it for a few days, otherwise individuals will get lost from the population (Comerford et al. 2013). Settlement is a process that seems to be induced by optimal food conditions (Van Der Veer 1990) and, in plaice, it is considered to start at the same time of metamorphosis, just like in flounder *Platichthys flesus* (Martinho et al. 2013). All individuals settled in June, in 2005, but in 2006 settlement occurred mainly in June and July. This was expected as the spawning season was also extended in 2006. For other locations, settlement occurred between April and July in the Irish Sea (Geffen et al. 2011), for instance, and between early March to late April in Galway Bay, in Ireland (Allen et al. 2008). In places further south, settlement started earlier in the year, in March, in the French coast (Amara and Paul 2003). Differences between the day of the year that settlement occurred, would be expected, since settlement appears to vary from year to year and occurs in pulses (Hyder and Nash 1998). Also, tides may also have an effect on hatching and settlement, as Allen et al. (2008) had found, but here that hypothesis has not been tested. Regarding to the benthic stage, it is not possible to make a direct comparison to other authors, since there was not found any paper that has discriminated the 3 stages. It was found statistical differences between years regarding to benthic

stage, but that could be expected since 2006 had a bigger range of ages and a higher number of animals being older than the ones from 2005. Nevertheless, in both years were found animals with 0 days in the benthic stage (4 from 2005 and 2 from 2006). Post-larval stage (B+C), as expected, was also more variable in 2006. Al-Hossaini et al. (1989) for Red Warf Bay, found animals between 0 and 15 days for the post-larval stage, for the early samples. For the late samples, in September, they obtained a post-larval stage age between 147-161 days. Logically, the later the sample, the older the individuals will be.

Comparing both hatching and settlement dates to other geographical locations, there seems to be a latitudinal trend, since both hatching and settlement starts earlier at southern locations of the species range.

Regarding to growth rate, the values obtained were similar to the ones found by other authors. Fox et al. (2014), for the Scottish West Coast had values of growth between 0.38 and 0.50 mm/day, which is within the range of the growth rates of this study. In the Northern Irish Sea growth rates varied between 0.3 to 0.8 mm per day (Geffen et al. 2011). Freitas et al. (2010) also found similar growth rates for the same area, 0.50 mm/day in 2005 and 0.46 mm/day in 2006, although these were estimated from shifts in mean length of the entire population instead of otolith microstructure. The results obtained for growth rate are in agreement to the ones described by other authors. Yet, it would be expected that growth rate would vary among the distribution of the species, since higher temperatures are correlated with faster growth rates (Comerford et al. 2013) and populations from the north should have lower growth rates compared to the ones at southern locations. This could mean that plaice in the northern distribution ranges experiences a counter gradient growth compensation. Counter gradient growth compensation refers to adaptation to latitudinal gradients in the length of the growing season or in temperature. This means that individuals from higher latitudes may grow up faster than the ones from lower latitudes, compared to the same temperature (Conover and Present, 1990), having higher growth performance (Imsland et al. 2000). This was already observed in flounder *P. flesus* (Martinho et al. 2013), where individuals compensated the lower water temperatures by growing up much faster during favourable environmental conditions. Other authors, such as Imsland et al. (2000), had results that partly supported the counter gradient variation in growth, in turbot *Scophthalmus maximus*, where shorter growing seasons for populations at higher latitudes are compensated by higher growth performance. For artic charr, *Salvelinus alpinus*, results consistent with countergradient variation were also observed (Niloshini Sinnatamby et

al. 2015), with northern populations having higher growth rates and higher indices of metabolic rate than southern populations. The similarity in growth rate across different latitudes, could also be related with photoperiod, since in the north, during summer, there are greater number of hours of light per day. This may improve better visibility for plaice to feed and, therefore, led to higher ingestion rates with, consequently, higher growth rates. Nevertheless, there was variability in growth rates between years that could be explained by small variations in environmental factors at the same nursery for the two years, such as the observed in water temperature.

5. Conclusions

It was possible to estimate the age of all individuals and to distinguish the three life stages visible on otolith microstructure: larval (pelagic), metamorphic and benthic stage.

Even though hatching timing differed between years, this did not affect the duration of larval stage. Settlement timing was also different between years, and there seems to be a latitudinal trend for timing of life history events in this species, with hatching and settlement starting earlier from south to north.

Somatic growth rates were similar among the distribution of the species, which might indicate that plaice takes advantages of a smaller opportunity window in time, in its northern distribution range, to grow up. However small variations among the same nursery ground, like different food availability or presence or absence of predators, are sufficient to observe variability between years.

Temperature has a determinant role in the early life history of *Pleuronectes platessa* but, in order to characterize more accurately its early life history, more variables such as food availability, abundance of predators and environmental conditions should be gathered and increments widths should also be measured to determine growth in each stage and to connect that directly with environmental features, namely temperature.

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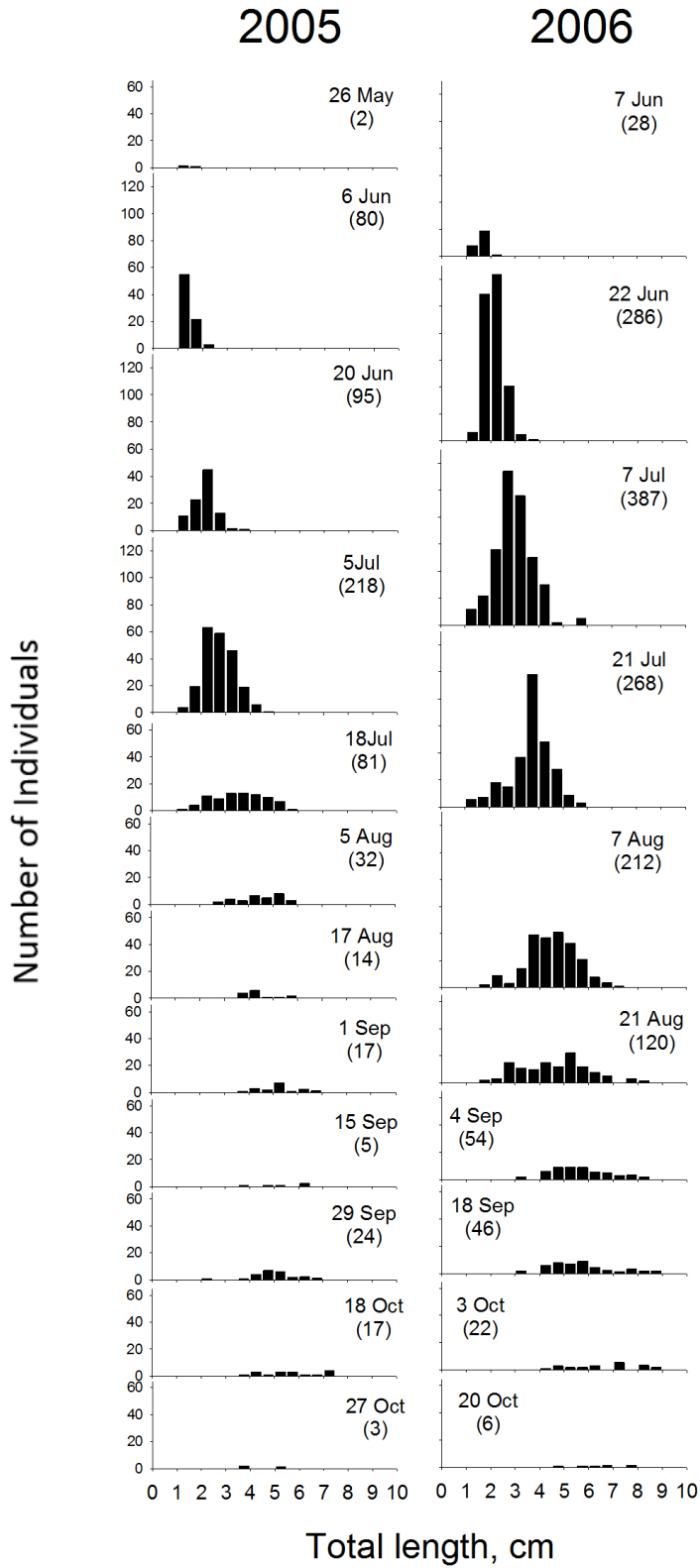
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Annex 1



Supplementar Figure 1 - Time of 0-group plaice *P. platessa* peak density and the length frequency distribution in each sampling date, in Valosen (NW Norway) in 2005 and 2006