Universidade de Lisboa
Faculdade de Ciências
Departamento de Biologia Animal

Environmental drivers on the life cycle strategies and distribution of cephalopods in the Portuguese coast

Ana Cristina Andrade Moreno Marques

Doutoramento em Biologia
Especialidade de Biologia Marinha e Aquacultura
2012

Universidade de Lisboa
Faculdade de Ciências
Departamento de Biologia Animal

Environmental drivers on the life cycle strategies and distribution of cephalopods in the Portuguese coast

Ana Cristina Andrade Moreno Marques

Tese orientada por
Professor Auxiliar com Agregação Henrique Nogueira Cabral
Doutor António Miguel Piecho de Almeida Santos

Doutoramento em Biologia
Especialidade de Biologia Marinha e Aquacultura
2012

Doctoral dissertation in Biology
(specialization in Marine Biology and Aquaculture)
presented to the University of Lisboa

Dissertação apresentada à Universidade de Lisboa
para obtenção do grau de doutor
(especialidade Biologia Marinha e Aquacultura)

Ana Moreno

2012

Contents

Abstract

The common squid *Loligo vulgaris* has a long spawning season within the upwelling system off NW Portugal. The meroplanktonic paralarvae develop under very different environmental conditions, which are the key to define the recruitment success of the new cohorts. Among these environmental variables, temperature is determinant in modulating the population dynamics and distribution. The common squid is studied as a model to understand the influence of environmental variables, on the life history strategies of cephalopods based on data from wild populations. The age-at-maturity, size-at-maturity, growth rates and growth curves, paralarvae growth and planktonic stage duration are analysed through countings and measurements of daily increments in statoliths of two groups of squid, the cold and the warm cohorts, hatched under distinct environmental conditions, namely the temperature during the first 3 months of life. These seasonal strategies are discussed in relation to global warming scenarios. The identification of essential habitats for each marine species has recently been recognized as an important component of the fisheries management. In particular, the distribution and abundance of planktonic paralarvae of several cephalopod species is analysed in relation to oceanographic mesoscale features, including currents, thermal fronts and coastal upwelling along-shore and cross-shelf transport, prevailing in the western Iberia or the Gulf of Cadiz systems. Additionally, the seasonal nursery grounds of the common octopus along the Portuguese coast are identified using georeferenced fishery independent data, to analyse the relationships between juvenile abundance and several environmental variables, as indicators of possible juvenile essential habitats, and suggest areas to be protected in future marine management options.

Key words: Cephalopods, squid growth, planktonic paralarvae, nursery grounds, environmental effects, upwelling

Resumo

A lula *Loligo vulgaris* apresenta um período de desova alargado no sistema de afloramento da costa ocidental portuguesa, pelo que as suas paralarvas meroplanctónicas se desenvolvem sob condições ambientais diversas que são a chave para a definição do sucesso do recrutamento das novas coortes. Dentro destas variáveis ambientais, a temperatura revela-se como determinante na modelação da dinâmica populacional e da distribuição. A lula vulgar é usada como modelo para a compreensão da influência de variáveis ambientais nas estratégias do ciclo de vida dos cefalópodes com base em dados das populações naturais. A idade e tamanho de maturação, taxas e modelos de crescimento, e duração da fase planctónica são analisados com base em contagens e medições de incrementos diários nos estatólitos de dois grupos de lulas (coorte fria e coorte quente) eclodidas sob diferentes condições ambientais, nomeadamente a temperatura durante os primeiros três meses de vida. As estratégias de crescimento destas coortes são discutidas no contexto de cenários de aquecimento global. A importância da identificação dos habitats essenciais das espécies marinhas tem sido reconhecida recentemente como uma componente importante da gestão das pescas. Em particular, é analisada a distribuição e abundância das paralarvas planctónicas de várias espécies de cefalópodes relativamente às principais características dos sistemas de oceanográficos da Ibéria ocidental e do golfo de Cádis, incluindo correntes, frentes térmicas e transporte relacionado com o afloramento costeiro. Por outro lado, são identificadas as áreas de viveiro do polvo *Octopus vulgaris* ao longo da costa portuguesa através de dados georreferenciados independentes da pesca e analisadas as relações entre variáveis ambientais e a abundância de juvenis, como indicadoras de habitats essenciais dos juvenis desta espécie e de áreas sensíveis a serem protegidas no âmbito de estratégias futuras de gestão marinha.

Palavras chave: Cefalópodes, crescimento, paralarvas planctónicas, viveiros, factores ambientais, afloramento costeiro

Resumo alargado

Loligo vulgaris Lamarck, 1798 é a espécie comercial de lula mais importante nas águas portuguesas, apresentando nesta região uma estrutura populacional complexa, fortemente influenciada pela desova ao longo de todo o ano (Moreno *et al.*, 2002) que é induzida pelas características ambientais do sistema de afloramento da costa noroeste portuguesa. Como resultado do extenso período de desova desta espécie e da marcada sazonalidade ambiental da costa portuguesa, as várias coortes sazonais sofrem diferentes influências ambientais durante o seu ciclo de vida, que afectam distintamente o crescimento e a maturação. Assim, esta espécie revelou-se um bom modelo para estudar a influência de factores ambientais na dinâmica populacional e nas estratégias de ciclo de vida dos cefalópodes. Este estudo foi realizado com base em dados das populações naturais, nomeadamente idade de maturação, comprimento de maturação, crescimento e duração da fase planctónica. Estes parâmetros foram analisados com base em contagens e medições de incrementos diários nos estatólitos, com o objectivo de compreender a sua relação com os factores ambientais.

Esta tese encontra-se organizada em 8 capítulos. O capítulo 1 corresponde à introdução à biologia e exploração dos cefalópodes e à importância do seu estudo. No capítulo 2 descrevem-se as principais características geomorfológicas, climatológicas e oceanográficas da área de estudo.

No capítulo 3 é analisada a influência do género e da época de eclosão na idade-de maturação, comprimento-de-maturação e investimento reprodutor de *L. vulgaris*. A estimativa das ogivas de maturação indica que os machos amadurecem um mês mais cedo (aos 277 dias) do que as fêmeas (aos 298 dias). As fêmeas amadurecem com um $CDM_{50\%}$ (comprimento dorsal do manto) de 17,6 cm, enquanto os machos amadurecem com um tamanho menor. Estes contudo evidenciam um maior grau de complexidade no tamanho de maturação, apresentando duas modas no comprimento de maturação. Em ambos os sexos, a maturação é mais dependente do tamanho do que da idade. O efeito da época da eclosão na idade de maturação, no comprimento de maturação e no investimento reprodutor foi analisado comparando dois grupos de lulas nascidos sob condições ambientais distintas, nomeadamente a temperatura que influenciou os primeiros 3 meses de vida: a coorte fria (CC), com eclosão entre Dezembro e Março e a coorte quente (WC), com eclosão entre Maio e Setembro. São observadas diferenças significativas entre as coortes relativamente à idade-de-maturação, ao comprimento-de-maturação e ao investimento reprodutor, indicadoras dos efeitos ambientais na maturação sexual. As lulas da coorte fria amadurecem cerca de 2 meses mais tarde do que as lulas da coorte quente, apresentando um comprimento significativamente maior. O investimento reprodutor medido pelo índice gonado-somático (GSI) é maior nas lulas da coorte quente. A maior parte da variabilidade na idade e no comprimento de maturação das fêmeas da população é devida a diferenças entre coortes. No entanto, o mesmo não se verificou nos machos, cujas estratégias reprodutivas são de origem essencialmente endógena.

No capítulo 4 são examinadas as influências do género e da época de eclosão no padrão (modelo) de crescimento de *L. vulgaris*. O crescimento dos machos e das fêmeas ajusta-se a modelos distintos, atingindo os machos comprimentos à idade superiores aos das fêmeas. Os machos apresentam taxas de crescimento crescentes, independentemente da época de eclosão, embora o comprimento à idade seja maior nas lulas eclodidas durante a estação quente. As fêmeas podem apresentar crescimento assintótico ou não, dependendo das condições ambientais a que estão expostas durante o seu ciclo de vida. As taxas de crescimento após a eclosão, verificaram-se ser menores nas fêmeas eclodidas durante a estação fria do que nas fêmeas eclodidas na estação quente. No entanto, as condições mais favoráveis de alimentação e temperatura que se verificam durante os meses seguintes de Primavera e de Verão, contribuem não só para aumentar as taxas de crescimento mas também para atrasar a maturação sexual. O maior comprimento à idade das lulas eclodidas na estação quente, observado em ambos os géneros, indica que a temperatura próxima da eclosão tem um impacto significativo no tamanho dos juvenis e sub-adultos. Contudo, este estudo também revelou que as condições ambientais têm influência significativa nas taxas de crescimento e na definição da forma do crescimento durante todo o ciclo de vida desta espécie.

O capítulo 5 aborda os efeitos de variáveis ambientais no crescimento durante o desenvolvimento embrionário (RH) e a fase pós-eclosão (R_{90d}), assim como a duração da fase planctónica, com base em medições dos incrementos diários em estatólitos de juvenis e adultos. A temperatura e salinidade foram usadas como indicadores da variabilidade ambiental e os seus efeitos em RH e R_{90d} explorados com modelos aditivos generalizados (GAM). A variabilidade no comprimento do estatólito na altura da eclosão foi fracamente explicada pelas variáveis no modelo, sugerindo que os locais de desova de Inverno e de Verão poderão ser localizados em áreas com temperatura e salinidade semelhantes. Por outro lado, os efeitos da temperatura de superfície e de fundo foram significativos no crescimento das paralarvas. Incrementos mais espessos são depositados nos estatólitos das lulas que vivem sob temperaturas mais elevadas, resultando em estatólitos maiores aos 90 dias nas lulas eclodidas no Verão. A análise do padrão de deposição dos incrementos no estatólito, realizada através do método de regressão linear "piecewise" (PLR), revelou uma alteração ontogénica na espessura dos incrementos, permitindo a estimativa da idade de transição da fase de paralarva para juvenil. A fase planctónica dura cerca de 60 dias a temperaturas superiores a 15ºC, estendendo-se até aproximadamente 90 dias quando as temperaturas são inferiores a 15ºC. A estratégia de crescimento adoptada sob condições quentes favorece potencialmente a sua própria sobrevivência, reduzindo a duração da fase planctónica vulnerável.

A importância da identificação dos habitats essenciais das espécies marinhas tem sido reconhecida na última década como uma componente importante da gestão das pescas. Dada a sensibilidade notória dos cefalópodes face a alterações no ambiente marinho, e as características oceanográficas da costa portuguesa (com regimes distintos na costa ocidental e na costa sul e padrões de circulação sazonal inversos), é fundamental determinar o efeito relativo dos processos oceanográficos no comportamento das espécies, em particular na dispersão e abundância das fases planctónicas e juvenis que vão condicionar a variabilidade no recrutamento e a abundância dos 'stocks'.

O capítulo 6 centra-se na análise da distribuição sazonal e espacial das paralarvas de cefalópodes na costa portuguesa. Os efeitos de variáveis temporais e ambientais na abundância das paralarvas da lula *Loligo vulgaris*, do polvo *Octopus vulgaris*, e das paralarvas da família Sepiolidae e Ommastrephidae são analisados recorrendo a modelos lineares generalizados (GLM). Os seus padrões de distribuição são discutidos relativamente a características oceânicas de meso-escala, incluindo correntes, frentes térmicas e transporte de "Ekman", típicos do sistema de afloramento ibérico ocidental. As paralarvas das espécies neríticas ocorrem durante um período do ano consideravelmente alargado, com dois ou três picos de abundância no sistema de afloramento mais produtivo da costa ocidental, o que contrasta com o que ocorre na costa sul que se localiza no sistema do Golfo de Cádis. Observa-se que a temperatura e o afloramento costeiro são as variáveis mais importantes na determinação da distribuição e sazonalidade destas paralarvas. A influência do ambiente físico é particularmente pronunciada nas paralarvas de *O. vulgaris*, que apresentam padrões distintos consoante a oceanografia dos sistemas ibérico ocidental e do Golfo de Cádis. As paralarvas das espécies oceânicas, as quais em muitos casos têm o seu limite de distribuição a norte na costa portuguesa, foram encontradas na parte sul da área amostrada. A distribuição destas espécies indica que as condições ambientais prevalecentes no sistema oceanográfico do Golfo de Cádis, especialmente frentes térmicas em conjunto com a própria temperatura, actuam como barreiras à dispersão geográfica, contribuindo para a existência de uma área com elevada biodiversidade de cefalópodes na zona sul da costa portuguesa.

O capítulo 7 refere-se à distribuição dos juvenis do polvo *O. vulgaris* e define as principais áreas de concentração de juvenis ("nursery grounds"), pré-recrutas à pesca comercial, ao longo da plataforma continental, com base na análise de dados de cruzeiros de investigação realizados no Outono e no Inverno. Os juvenis desta espécie distribuem-se ao longo de toda a costa portuguesa agregados em várias áreas de viveiro (ou de alimentação) localizadas a 80 m de profundidades média (possivelmente mais costeiras), e aproximadamente a 10-15 km da linha de costa, em zonas adjacentes a grandes rios e sistemas lagunares. A influência do tipo de sedimento, salinidade e pluviosidade na abundância dos juvenis é significativa e independente de efeitos sazonais. A abundância é consideravelmente maior na região sul, associada a águas de temperatura e salinidade mais elevada. O habitat dos juvenis é caracterizado por substrato de grão de maiores dimensões como o cascalho e a areia cascalhenta e também substrato lodoso com rocha, proporcionando abrigos de fuga à predação. A distribuição e abundância dos juvenis apresentam acentuada sazonalidade induzida pelas diferentes condições ambientais da costa ocidental e da costa sul, sendo a abundância maior no Inverno no sul e no Outono na costa noroeste. A área adjacente à Ria de Aveiro é provavelmente a principal área de viveiro do polvo na costa noroeste. Na região sudoeste são identificadas áreas de viveiro apenas durante o Inverno. A área de viveiro localizada a oeste da Ria Formosa parece ser a área de viveiro de *O. vulgaris* mais importante de toda a costa portuguesa, suportada pela elevada abundância de juvenis e pela persistência da sua localização ao longo da série analisada, consistentes tanto no Outono como no Inverno. Esta área é alvo de intensa pesca, quer artesanal com armadilhas, quer por arrasto de fundo e a sua importância deverá ser tida em consideração no âmbito de estratégias futuras de gestão marinha.

Finalmente, no capítulo 8 são delineadas as principais conclusões deste estudo e as perspectivas futuras de investigação, na sequência desta tese.

List of Papers

This thesis comprises the papers listed below, each corresponding to a Chapter, from 3 to 6. The author of this thesis is the first author in all papers and was responsible for the conception and design of the work, sample collection and processing, laboratory analytical procedures, data analyses and manuscript writing of all the papers. Remaining authors collaborated in some or several of these procedures.

Chapter 3. A. Moreno, J. Pereira, M. Cunha (2005) Environmental influences on age and size at maturity of *Loligo vulgaris*. Aquatic Living Resources, 18, 377-384.

Chapter 4. A. Moreno, M. Azevedo, J. Pereira, G.J. Pierce (2007) Growth strategies in the squid *Loligo vulgaris* from Portuguese waters. Marine Biology Research, 3, 49-59.

Chapter 5. A. Moreno, G.J. Pierce, M. Azevedo, J. Pereira, A.M.P. Santos (2012) The effect of temperature on growth of early life stages of the common squid *Loligo vulgaris*. Journal of the Marine Biological Association of the United Kingdom (in press).

Chapter 6. A. Moreno, A. Dos Santos, U. Piatkowski, A.M.P. Santos, H. Cabral (2009) Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia. Journal of Plankton Research, 31, 73-91.

Chapter 1

General Introduction

1. General Introduction

Cephalopods are among the most attractive of all invertebrates living in the sea, having received the attention of humans since at least the sixteen century BCE (Nixon and Young, 2003). They are apparently able to exploit most of marine habitats, but no cephalopod lives in fresh water.

Cephalopods can be considered subdominant predators that tend to increase in biomass when other species, particularly their predators and competitors for food, become depleted (Caddy and Rodhouse, 1998). World catches have increased steadily in the last 30 years, from about 1 million tons in 1970 to more than 4 million tons in 2008, alongside with a finfish generalized decrease (FAO, 2010).

Cephalopods occupy a middle position in the marine foodwebs (Clarke, 1996; Hunsicker *et al*., 2010). They are voracious, active predators that feed upon crustaceans, fishes, other cephalopods and, in the case of *Octopus vulgaris* Cuvier, 1797, on bivalved molluscs. On the other hand, they are components of the diet of many predators, including whales, dolphins, seals, seabirds, sharks, and other fish (Clarke, 1980; Guerra *et al*., 1993; Cherel and Klages, 1998; Santos *et al*., 2001). Their food conversion is highly efficient, especially in octopuses, where up to 50% of the food eaten can be converted into body mass. More active cephalopods like squids, however, need several times the amount of food required by octopuses and can eat from 3 to 15% of their body weight each day (Jereb *et al*., 2005). They are often considered 'ecosystem accelerators' to explain their main role in the oceanic system. Their high feeding rates and high turnover ratios mean that small increases in cephalopod standing stock result in a large increase in production, with consequent major effects on their predators or prey (Caddy and Rodhouse, 1998).

The reproductive systems are highly complex structures with ducts, glands, and storage organs. Fertilized eggs are embedded in one or more layers of protective coatings produced by the oviducal and nidamental glands and generally are laid as egg masses (Figure 1). Egg masses are benthic in Loliginidae and Octopodidae and pelagic in Ommastrephidae. Development of embryos is direct, without true metamorphic stages and hatchlings undergo gradual changes in proportions during development. To differentiate from the term ‘larva’, the term ‘paralarva’ was first introduced by Young and Harman (1988) for the early stages of cephalopods between hatchling and subadult, which differ morphologically and/or ecologically from the older stages.

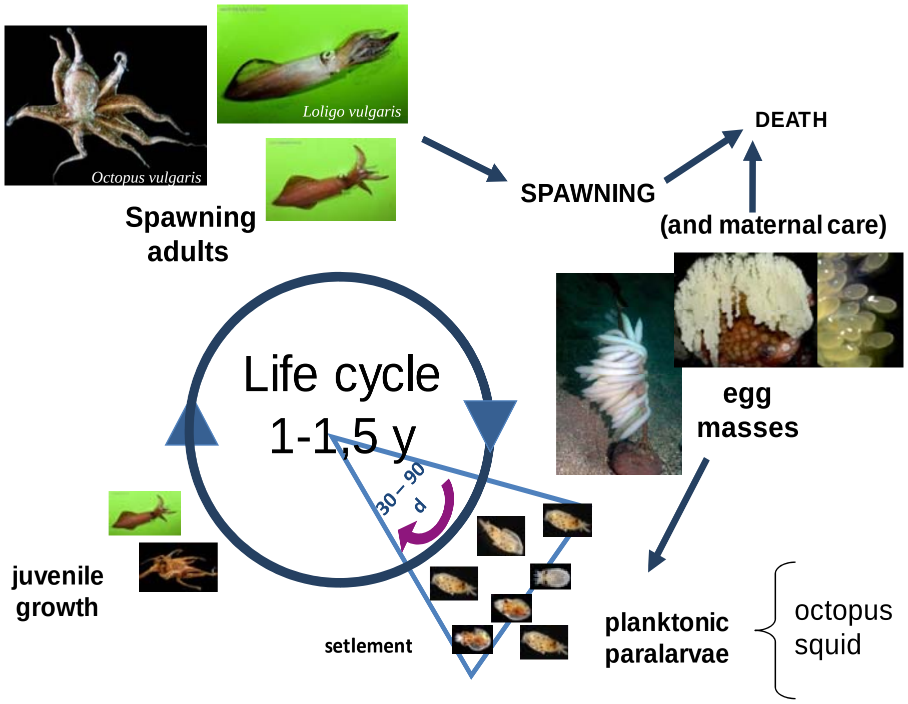

Figure 1 - Simplification of the *Octopus vulgaris* and *Loligo vulgaris* life cycles.

Cephalopods show great resilience to environmental change by having short lives, high turnover of generations, asynchronous growth and maturation, and extended spawning (Boyle and Boletzky, 1996). This gives the populations the potential to exploit available food resources and overcome short adverse environmental episodes more efficiently (Katsanevakis and Verriopoulos, 2006a). It is documented that populations of shelf species fluctuate enormously. They may decreasing sharply from one year to the next, but yet survive and flourish in later years (Clarke, 2007). The potential biomass of any cephalopod population is difficult to estimate because it is totally dependent on the success of recruitment, conditions for growth and exposure to predation in that year, as there is no overlap between successive generations (Boyle and Boletzky, 1996). Therefore, despite their great resilience to environment, cephalopod populations are also extremely vulnerable to large adverse fluctuations of biological and physical variables, in particular during the paralarva phase. To minimize this vulnerability and provide some temporal stability (O'Dor, 1998), many cephalopod populations are composed at any given time, of several groups of similar age, cohorts or microcohorts. This population dynamics results from the interaction of the temperature and the productivity seasonal cycles with the hatching season and subsequent animal growth (Grist and des Clers, 1999).

In European waters cephalopods have always been considered to be a minor fisheries resource, although undoubtedly they are of local economic importance, especially in southern Europe artisanal fisheries (Shaw, 1994). Cephalopods are important fishery resources in the UK, France, Spain, Portugal, Italy, and Greece in terms of quantities landed and in the trade market (Pierce *et al*., 2010). In Portugal, the economic importance of cephalopods is relatively high and their exploitation continues to increase along with the general decrease of the finfish catches (Figure 2a). Over the years, their landings and economic value increased gradually in importance in relation to those of other marine catches, indicating a greater dependence of fisheries economy on cephalopod landings (Figure 2b).

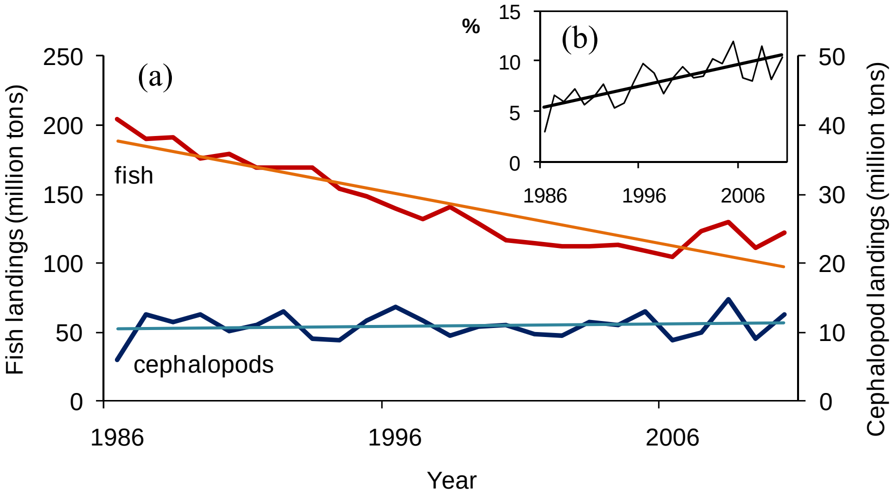

Figure 2 - Finfish and cephalopod landings from Portuguese continental waters (a) and percentage of cephalopod landings among marine fishery resources landings (b), between 1986 and 2010 (source: DGPA).

The main commercial cephalopod in Portugal is by far *Octopus vulgaris*. Octopus represents ~80% of the total cephalopod landings from Portuguese continental waters. It is also the most valuable species among all marine catches (17.4%) and the fourth more important species landed in weigh (7.5%) (DGPA, 2011). Along with cuttlefish, octopus landings have increased over the years (Figure 3). However, this was not the case of squids. Similarly to finfish, squid landings decreased severely during the last two decades.

A stock collapse of any of these resources will have significant economic and ecological impacts, in particular in some local communities very dependent on the small scale artisanal fishery. Consequently, there is a strong need to improve our understanding of the population dynamics in order to sustainably manage their fisheries.

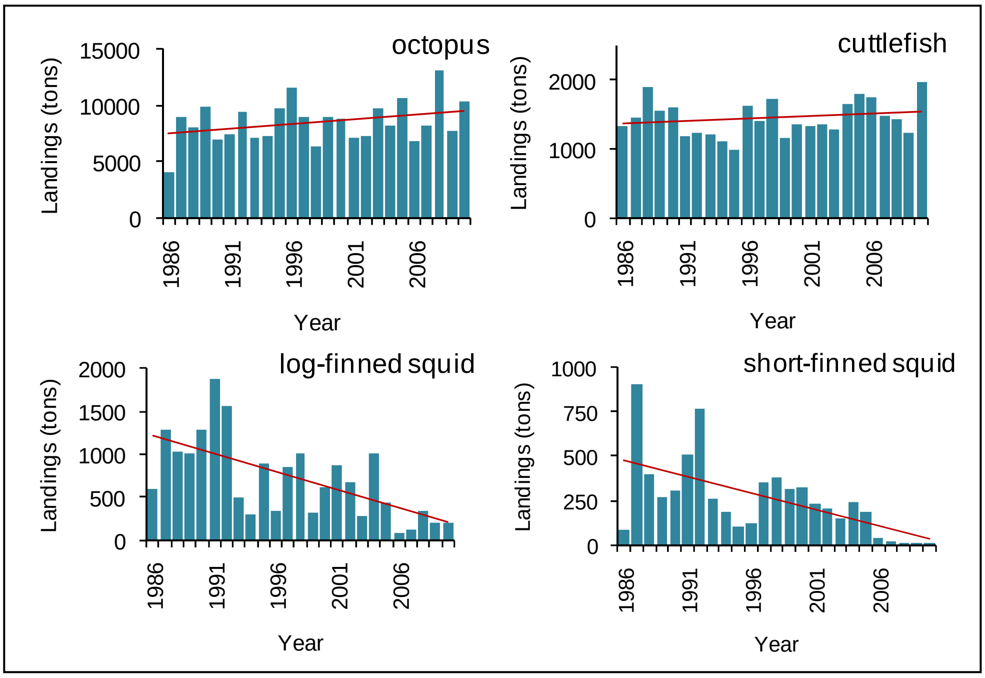

Figure 3 - Annual landings from the Portuguese continental waters and trends for the period 1986 to 2010 for the 4 cephalopod commercial groups of species (source: DGPA).

Cephalopod populations have several characteristics which make their exploitation management very different from the traditional approaches that manage the fisheries of "long-lived" finfish. The most relevant are that most commercial cephalopods live up to 1 year and all reproduce only once at the end of their short lives. They also exhibit exponential growth in their early life stages followed by rapid growth as juveniles and young adults (Forsythe, 1993).

Stock-recruitment relationships for these species are poorly defined and therefore relatively uninformative (Agnew *et al*., 2002; Pierce *et al*., 2010). Recruitment dynamics and abundance are highly variable and dependent on the paralarvae mortality rates during the early stages, which are in turn strongly influenced by environmental factors. During the last decade, special attention has been given to the role of several environmental variables, notably temperature, as drivers of distribution, recruitment and population dynamics and life cycle strategies of several cephalopod species, as well as for the identification of their essential habitats. The understanding of these complex relationships is still in a very incipient stage, but it is considered to be vital for the sustainably manage of any cephalopod fishery.

Aims

The studies in this thesis aim to contribute to the actual knowledge of the environmental effects on the cephalopod population dynamics, by accessing the environmental effects on the life cycle strategies and the early life stages distribution and abundance of some cephalopods species in the Portuguese coast, considering that this knowledge is an important step towards the sustainable management of their fisheries.

The main objectives are therefore:

1) To identify environmental variables which are relevant for the cephalopod population dynamics and distribution, namely to study,

2) The effect of temperature at hatching on the age and size at maturity of *L. vulgaris*;

3) The environmental effects on the growth rates and growth curves of *L. vulgaris*;

4) The temperature and salinity effects on growth during the embryonic and post-hatching phase of *L. vulgaris*, as well as on the duration of the planktonic phase;

5) The influence of the regional oceanography on the distribution of planktonic paralarvae

of neritic and oceanic cephalopods;

6) The nursery areas of *O. vulgaris* and the environmental characterization of the juvenile essential habitats

Thesis outline

This thesis is organized in 8 chapters. The chapter 1 comprises a general introduction focused on the biological characteristics of cephalopods and their exploitation on the Portuguese coast and presents also the aims and the structure of the thesis. The chapter 2 gives a characterization of the geomorphologic and environmental characteristics of the study area considered relevant for the following studies. The chapters 3 to 7 include five scientific papers, four of them published or in press in peer reviewed international journals and one yet to be submitted, each presenting an introduction to the specific study, the material and methods, the results and a discussion. The chapters 3, 4 and 5 analyze the effect of environmental variables on the life cycle strategies of the squid *L. vulgaris*, namely the age and size at maturity, growth rates, growth curves, growth during the embryonic phase and the duration of the planktonic phase. The chapters 6 and 7 analyze the effects of temporal and physical variables on the distribution of cephalopod paralarvae and on the *O. vulgaris* juveniles off the Portuguese coast, and the distribution patterns are discussed in relation to the regional oceanography of the western Iberia upwelling system and Gulf of Cadiz system. The chapter 8 includes the concluding remarks of the thesis and the perspectives for future research.

Chapter 2

Geomorphologic and environmental characteristics of the study area

2. Geomorphologic and environmental characteristics of the study area

2.1. Regional geomorphology and climate

The Portuguese continental shelf has an area of 28,000 km2. It can be divided into three regions, NW, SW and South, which have substantial differences in terms of geomorphology, water flow from the adjacent land and general climate (Dias, 1987). These differences affect marine species distribution, their density, and seasonality.

The NW region (between ~42º N and the Nazaré canyon ~39º N) is characterized by an extended continental shelf; the shelf break is located at ca. 50 km from the coastline and at ca. 160 m depth (Figure 4). The relief is smooth but incised by 3 submarine canyons. Fine and very fine sand are the dominant bottom sediments on the inner-shelf, gravely sand and gravel dominates most of the middle-shelf (Dias and Nittrouer, 1984) (Figure 5). A well-defined muddy area (Douro Mud Patch) is located northwest of the River Douro mouth (Jouanneau *et al*., 2002). Towards the south the outer-shelf is sandy. Rocky outcrops extend along the outer-shelf and on the northern part of the inner shelf region, making the seabed rough and irregular (Vanney and Mougenot, 1981). The Nazaré canyon, dividing at ca. 500 m from the coastline, marks a physical boundary to the north-south displacement of benthic species (e.g. Diekmann *et al*. 2005). The NW region has high rainfall levels (1000 mm/year) and strong river-runoff (Figure 6). Several of the main rivers flow into this region (65%) and the major river estuaries discharge very close to each other (~20 km). Therefore, the coastal waters tend to have relatively low salinities and are quite turbid. When these rivers flood during winter, the coastal waters are turned brown by the large amounts of suspended sediments being discharged in their plumes (Oliveira *et al*., 2002).

The SW region (between ~39º N and Cape S. Vicente ~37º N) is characterized by several capes and a relatively narrow shelf (< 25 km, until 180 m) (Figure 4). The outer shelf and slope is incised by 3 submarine canyons (Cascais, Lisboa and Setúbal). Sand is the dominant component of the shelf sediments (Figure 5), but large mud deposits are located in the middle shelf, linked to the supplies of the Tagus and Sado estuaries (Dias, 1987).

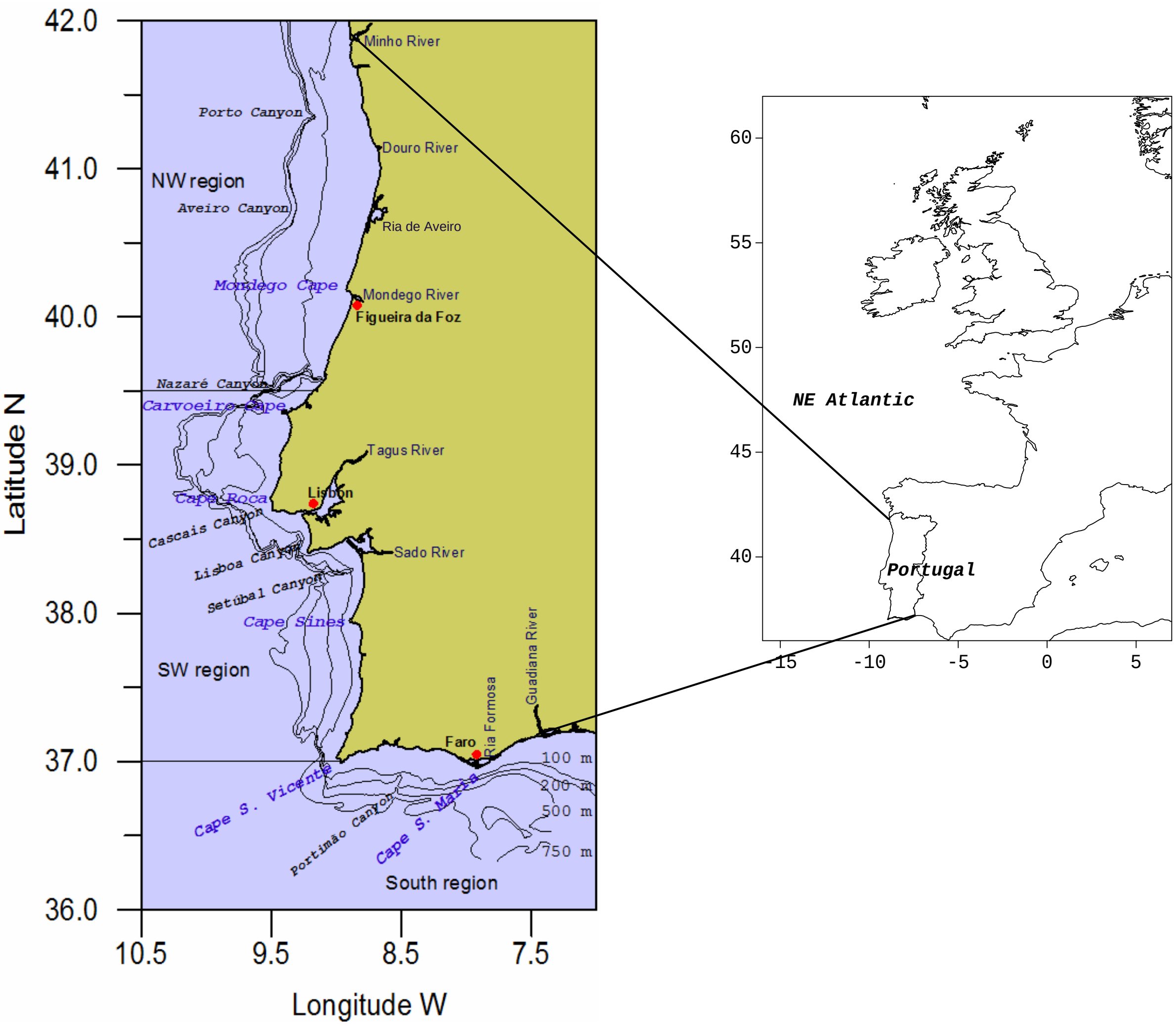

Figure 4 - General bathymetry and location of the main rivers, capes and submarine canyons.

Gravely sand deposits occur on the inner and middle shelf associated to several rocky outcrops, in particular south of Cape Sines. Fine sand also occurs on the inner shelf, where there are high energy littoral currents. On the outer shelf, near the shelf-break south of the Setúbal canyon, there are large bodies of sand and gravel (Jouanneau *et al.*, 1998). As in the other regions the rainfall is higher from October to January. Monthly rainfall is lower than up north and the summer is very dry (Figure 6a). The northern part of the SW region is affected by the flow of two important rivers (Tagus and Sado), but the drainage of fresh water south of Cape Sines is meaningless (Figure 6b).

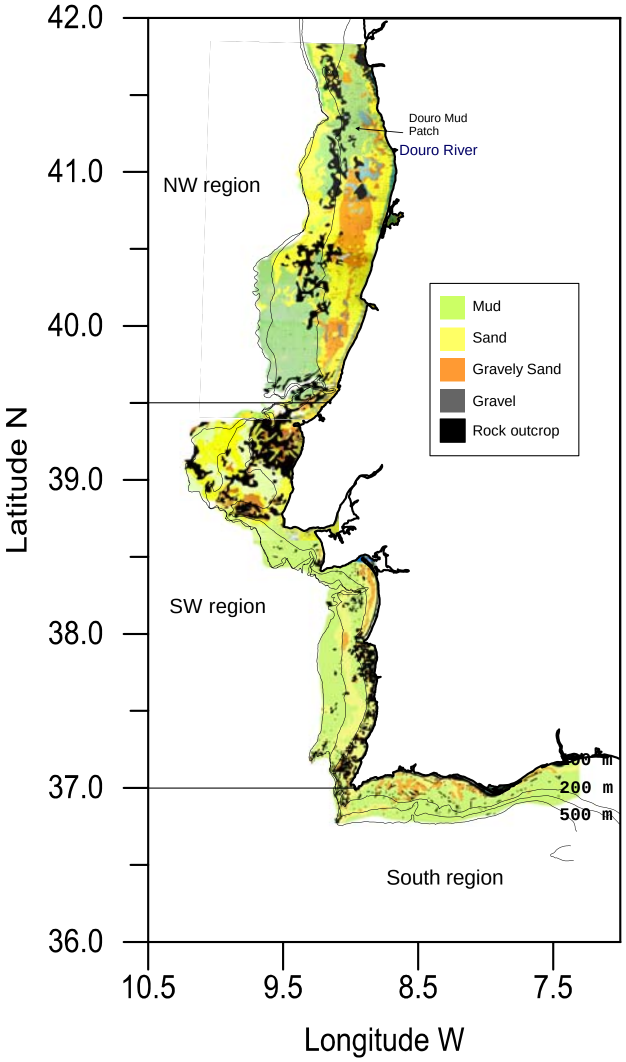

Figure 5 - Bottom sediments of the Portuguese continental shelf and slop down to 500 m deep (source: Cartas de Pesca do Instituto Hidrográfico).

In the NW and SW regions the general climate is temperate, with rainy autumns and dry and mild summers. These western regions have a high-energy shelf environment exposed to NW swells from the North Atlantic, due to the general N-S orientation of the coastline. The swells in winter have mean wave heights of about 3-4 m in the NW and 2 m in the SW (Costa, 1994/3/4). This exposure to marine processes is the main reason why the western shelf has coarser-grained sediments than the southern shelf.

The South region is located between Cape S. Vicente on the west and the Guadiana River estuary on the east ~ 7.4 ºW (Figure 4). The continental shelf is narrow, between 8 and 28 km, until 150 m of depth (Vanney and Mougenot, 1981). The slope is incised by 4 submarine canyons: S. Vicente, Lagos, Portimão and Faro. The prominent Cape S. Maria divides the South region in two sectors of different topography and oceanographic dynamics (García-Lafuente and Ruiz, 2007). The eastern sector is dominated by the Ria Formosa Barrier Islands System enclosing a lagoonal body along ca. 55 km.

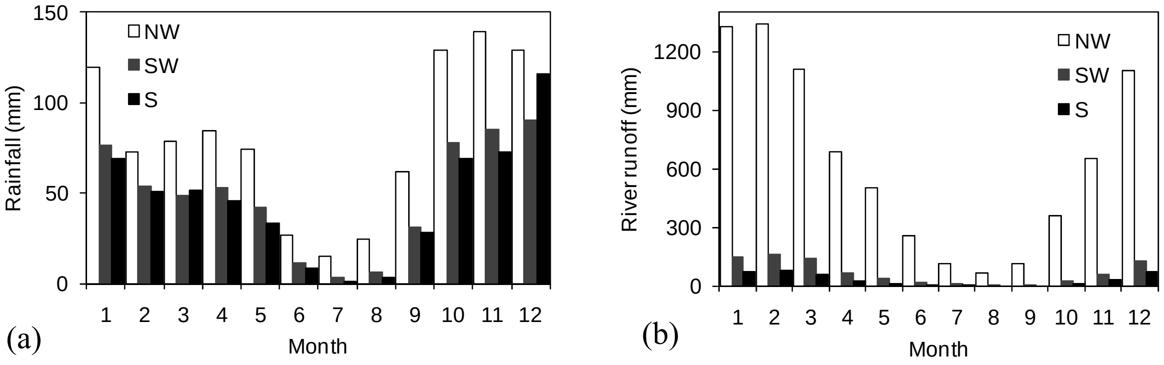

Figure 6 - Monthly mean rainfall (a) and mean river runoff (b) in the northwest (NW), southwest (SW) and south (S) regions (source: SNIRH, Sistema Nacional de Informação de Recursos Hídricos).

The inner-shelf on the South region consists mostly of sand with varying amounts of biogenic gravel, but with ~10% of terrigeneous components. The middle-shelf is covered with mud and muddy sand (mostly derived from the Guadiana River in the southeastern sector), with important patches of gravely sand located on the southwestern sector (Figure 5). Mud dominates the outer-shelf and slope sediments, although some sand and gravel patches may be found. Close to Cape S. Vicente, rock outcrops are abundant (Dias *et al.*, 1980). The South region has a low-energy regime, with swells predominantly from the SW, S and SE and average wave heights of 1 m (Costa, 1994/3/4). The climate is temperate with dry and warm summers, similar to a Mediterranean climate. The rainfall is generally higher in December (Figure 6a) and the river runoff in the following February (Figure 6b).

2.2. Large scale and regional oceanography

Both the NW and the SW regions are located in the western Iberia region, the northernmost part of the Canary Current upwelling system, one of the four major eastern boundary current systems of the world that stretches from Cape Finisterra (NW Spain; ~43º N) to the south of Cape Vert (Senegal; ~10º N), which is characterized by optimum conditions for phytoplankton blooms and consequent zooplankton richness (Aristegui *et al.*, 2006; Valdés *et al.*, 2007). The werstern Iberia is characterized off-shore by slow broad equatorward gyre re-circulation, a meridional alignment of coastlines and a predominant equatorward wind during summer. These winds force an offshore Ekman transport in the upper layer and the consequent decline of the sea level towards the coast. As a result an equatorward jet is formed under northerlies, transporting cold and nutrient rich upwelled water over the shelf (Relvas *et al.*, 2007), increasing the primary production and the ecosystem productivity (Figure 7). Downwelling occurs when poleward winds induce net onshore surface Ekman transport, resulting in surface coastal convergence and, to compensate, deeper waters flow offshore.

The Portugal Current, that roughly marks the northern extent of the Canary Current, is the main feature of the large scale circulation of the western Iberia. This is a broad equatorward slow current that extends from about 10° W to about 24° W longitude (e.g., Pérez *et al.*, 2001; Martins *et al.*, 2002) and connects the North Atlantic Current (Dietrich *et al.*, 1980) or at least the inter-gyre (subarctic and subtropical) transition zone (Krauss, 1986) with the Azores Current. The eastern branch of the Azores current (ACb), transports eastwards warm and salty sub-tropical water into the Gulf of Cadiz (Fernandez and Pingree, 1996; Martins *et al.*, 2002). There are some evidences that this current could recirculate northward in the vicinity of Cape S. Vicente and form a south branch of the Iberian Poleward Current - IPC (Peliz *et al.*, 2005).

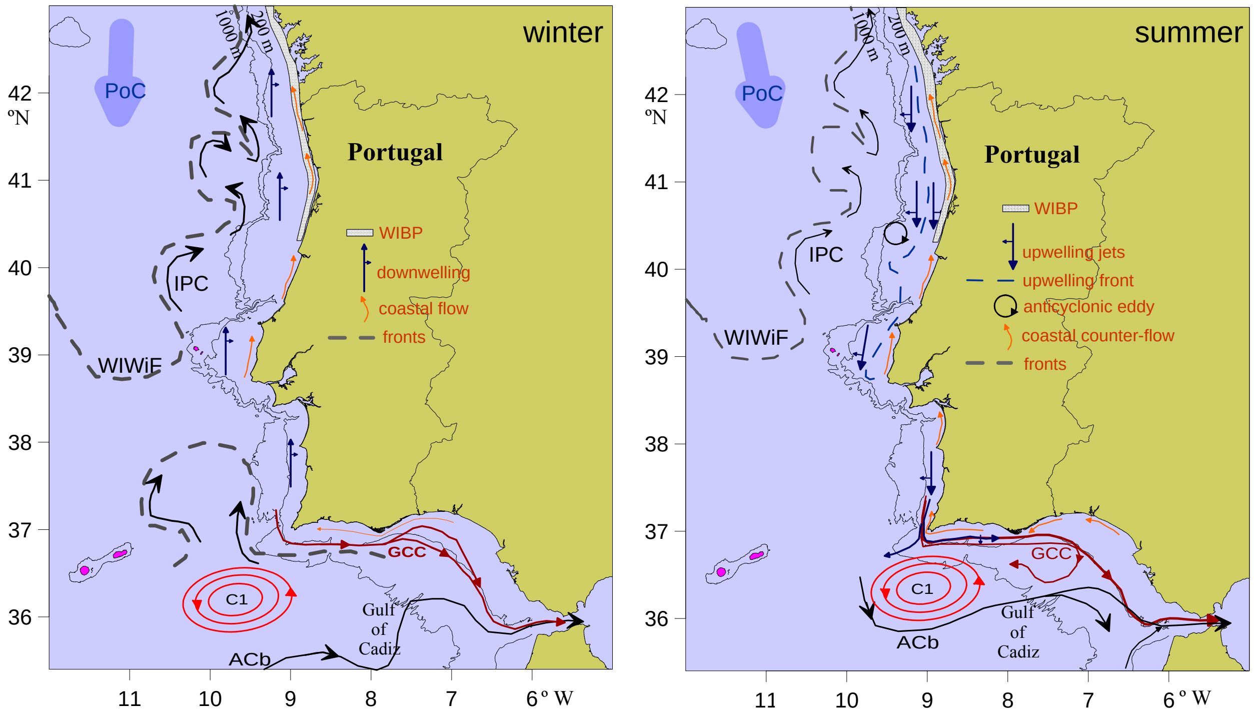

Figure 7 - Sketch of the surface circulation and oceanographic features in the western Iberia and Gulf of Cadiz in winter (and autumn) and in summer (and spring), based on Peliz *et al.* (2002, 2005, 2007a), García-Lafuente *et al.* (2006) and Relvas *et al.* (2007). PoC = Portugal Current, IPC = Iberian Poleward Current, WIBP = Western Iberia Buoyant Plume, WIWiF = Western Iberia Winter Front, ACb = Azores Current eastern branch, C1 = Cyclonic cell, GCC = Gulf of Cadiz Slope Current.

The IPC is a persistent feature of the winter circulation off the western Iberia (Wooster *et al.*, 1976; Frouin *et al.*, 1990; Haynes and Barton, 1990). Although still a subject of debate, there is some evidences that during the rest of the year the IPC is still present but weaker and flowing more offshore (e.g., Peliz *et al.*, 2002, 2005). The IPC is a warm and salty poleward current driven primarily by the interaction of a meridional density gradient with a meridionally oriented slope and shelf. This poleward flow, typical of eastern boundary upwelling systems, is important in forming a convergence zone over the shelf-break, which is of significant importance for the retention of nutrients and plankton over the shelf (Álvarez-Salgado *et al.*, 2003; Santos *et al.*, 2004; Ribeiro *et al.*, 2005). The generation of the IPC is closely related with the location of the Western Iberia Winter Front (WIWiF), which is a recurrent thermal front located at about 39 - 40º N, separating colder northern waters from southern warmer ones (e.g. Peliz *et al*., 2005).

The NW and SW regions are also characterized by a marked seasonality, largely dominated by medium size oceanographic structures (mesoscale activity), such as jets, meanders, ubiquitous eddies, upwelling filaments and countercurrents, obscuring the large scale climatological patterns (Relvas *et al*., 2007). Upwelling and downwelling patterns are very important on the cross-shelf circulation, which redistributes heat, salt and nutrients. These flows act also as a source of cross-shelf and along-shore dispersion of the plankton, depending on location and/or vertical migration in the water column (Marta-Almeida *et al*., 2006; dos Santos *et al*., 2007; Peliz *et al*., 2007b). The main upwelling season occurs during spring and summer, between April and September, with maximum offshore Ekman transport between June and August (Figure 8).

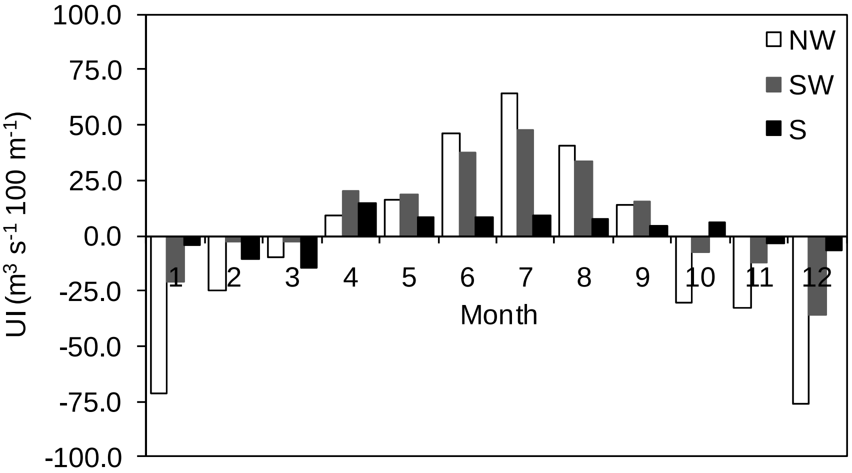

Figure 8 - Seasonality and intensity of the upwelling on the northwest (NW), southwest (SW) and south (S) Portuguese shelf (mean for the period 1986-2004, source: NOAA-NMFS-Pacific Fisheries Environmental Laboratory, http://www.pfel.noaa.gov).

Despite the surface westward transport, an upwelling front may form, associate to the high speed current flowing equatorward over the continental shelf (upwelling jet), acting as a barrier to offshore dispersal away from the continental shelf (Bakun, 1996). On the wider northwest shelf, a double frontal system is recurrent. The main upwelling front is meridionally oriented along the mid-shelf in summer, slightly off the 100 m isobaths (Peliz *et al*. 2002). The cold equatorward current of upwelled water is limited off-shore by the main slope poleward warm flow and at the inner-shelf side by the poleward warm flow, forming a secondary upwelling front inshore.

In autumn and winter the prevailing direction of the winds is poleward (south-southwesterlies) leading to the predominance of general coastal convergence conditions. Under downwelling-favourable winds, passive particles at the surface tend to move inshore until they enter the inner-shelf region, beyond the downwelling front, where they may be advected polewards by river plumes associated with maximum rainfalls and the buoyant plume off western Iberia (WIBP), which is present throughout the year (Peliz *et al*., 2002). On the contrary, deeper particles tend to move offshore towards the open ocean, where they may be retained by the shelf-break front or advected polewards by the IPC beyond the front (Peliz *et al*., 2005). Winter upwelling may also be important in some years (Vitorino *et al*. 2002), in association with positive values of the North Atlantic Oscillation (NAO) index (Borges *et al*., 2003), inducing important interannual biological variation in some species (Santos *et al*., 2004).

The South region is located in the northern Gulf of Cadiz, between the North Atlantic and the Mediterranean Sea. It is limited at the west by Cape S. Vicente, where the shoreline changes orientation from north to east at an almost right angle, and at the east by the mouth of the river Guadiana. Since equatorward winds are dominant during a substantial part of the year, this change in the orientation of the coastline has important implications for the coastal oceanography, mainly in relation to coastal upwelling. The Gulf of Cadiz separates the “western Iberian upwelling” from the “African upwelling” systems, between which continuity of flow is thought to be absent (Barton 1998). The slope current system above the Mediterranean water outflow of this area is mainly anticyclonic (García *et al*., 2002, Sánchez and Relvas, 2003) and affected by the eastern branch of the Azores Current and by the Mediterranean inflow-outflow coupling, resulting in a persistent equatorward current along the upper slope between Cape S. Vincent and the Strait of Gibraltar, the Gulf of Cadiz Slope Current (GCC) (Peliz *et al*., 2009). A cyclonic recirculation cell is centered offshore in the mid-latitude northeast Atlantic off the Gulf of Cadiz, at approximately 36°N and 10°W and extending to 15°W westwards (Lamas *et al*., 2010).

The east-west orientation of the southern Portuguese coast does not favour upwelling under northerly winds. Here, local upwelling events under westerly winds tend to be weak, intermittent and less frequent (Relvas and Barton, 2002) (Figura 8) and the circulation is mainly wind forced and influenced by the local orography. The prominent Cape St^{a} Maria divides the continental shelf in two shelves of different shape that hold different oceanographic processes. The wider southeastern shelf (~50 km) is more productive due to important inputs from the Guadiana River and tidally-driven processes, which are independent of wind and represent a continuous source of nutrients (García-Lafuente and Ruiz, 2007). The narrower southwestern shelf (~15 km) cut by the steep Portimão submarine canyon is more oligotrophic. However, the northern Gulf of Cadiz is partially influenced by the equatorward-jet of nutrient rich cold waters upwelled further north in the western coast, which extends around Cape S. Vicente (Relvas and Barton, 2002; 2005) flowing eastward with the Gulf of Cadiz slope Current (GCC) along the southern Portuguese coast (Peliz *et al.*, 2007a; Peliz *et al.*, 2009). Inshore of the upwelling jet, a counter current of warmer and salty water flow poleward from the South region to the SW region (Sánchez and Relvas, 2003; Relvas and Barton, 2005). Additionally, a current flowing westwards is recurrent on the eastern inner-shelf of the northern Gulf of Cadiz. Right after upwelling relaxation and under easterlies it flows beyond Cape St^{a} Maria invading the western shelf providing transport of biological material from the east to the west and a biological connection of the entire southern Portuguese shelf (García-Lafuente *et al.*, 2006, Teles-Machado *et al.*, 2007). In this case, the eastward extension of cooler water beyond Cape S. Vicente is less pronounced, and warmer water occupies a larger extension of the south Portuguese shelf. The conditions on the northern Gulf of Cadiz during winter are different from summer. Cold and low saline water (from Guadiana and Guadalquivir rivers runoff) flows westwards over the shelf. On the shelf break, the GCC flows to the east towards the Mediterranean Sea (Peliz *et al.*, 2004; Sánchez *et al.*, 2006).

Ocean temperature is one of the most important environmental variables driving the sea-life dynamics, because it is a vital physical variable with a very direct relationship with the organism's metabolism. The ocean temperature at surface (SST) generally follows the seasonal pattern related with the atmospheric temperature. However, SST in a given geographic location is also influenced by the water masses circulation (main currents, upwelling and river runoff currents, etc.), which may input warmer or colder water to that location, independently of the local atmospheric temperature. Therefore, over the Portuguese continental shelf the seasonal SST follows distinct patterns at the NW and South regions, not only related with their different latitudes (mean SST higher at the south), but also related to their distinct oceanographic regimes. At the South region temperature is ~2 ºC higher (SSTyr = 18.0 ºC; SBTyr = 15.4 ºC) than at the NW region (SSTyr = 16.1 ºC, SBTyr = 13.8 ºC). In general, higher SST occurs between June and October and the lowest SST in February (Figure 9). On the NW region a decrease in SST after the maximum in June is notorious, following the upwelling of cold waters to the shelf. SST rises again in September with the decrease in the upwelling intensity (Figure 8).

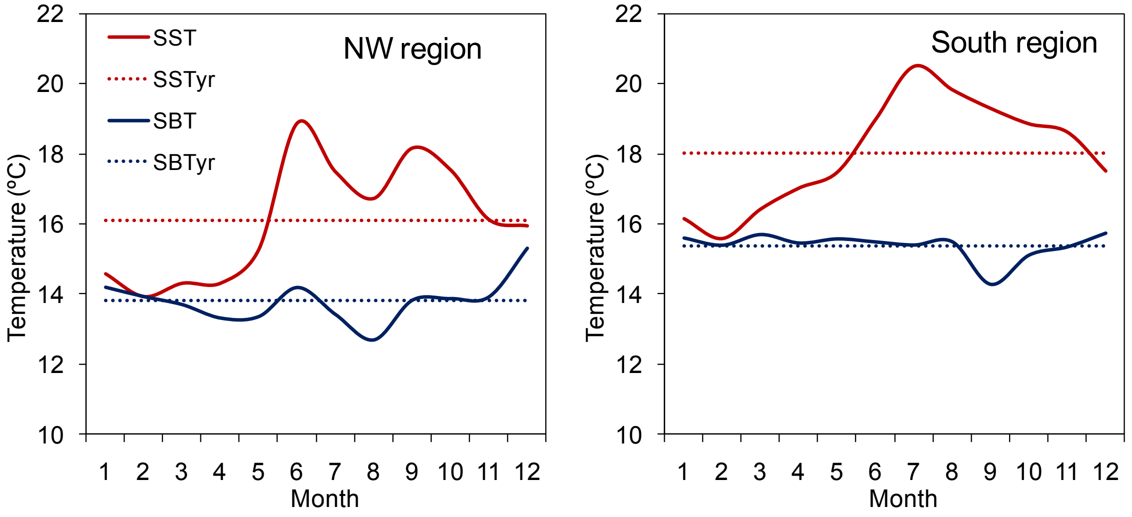

Figure 9 - Mean monthly values of sea temperature, at surface (SST) and near de bottom (SBT) at the NW and South continental shelves. Dotted lines represent the annual mean (SSTyr and SBTyr) for each variable. (Source: SST and SBT from CTD casts between 1986 and 2008 extracted from IPIMAR, NODC and ICES databases).

On the South region mean monthly SST follows primarily the seasonal atmospheric temperature pattern with maximum value in July. Temperature decreases below the surface and down to the bottom, but not in a linear way. The temperature close to the ocean floor (SBT) depends on bottom depth and water column mixing. Its seasonal pattern is less variable than that of SST. Mean SBT over the shelf is higher in autumn and lower in summer at both NW and South regions. During winter (from December until

March), the water column is well mixed and SST and SBT are of similar magnitude.

Oceanic salinity may range between 34 and 37 and its variability is mainly related with the precipitation-evaporation dynamics. Salinity at the coastal areas is more variable and may decrease considerable in the vicinity of estuaries. The surface salinity (SSS) seasonal pattern in coastal waters is very influence by both rainfall and river runoff levels, and ocean SSS may also decrease considerably when fresh water plumes flow offshore (Peliz *et al*., 2004). In general, salinity increases from the NW to the South coast, related to the higher rainfall, river runoff and lower atmospheric temperature (less evaporation) of the NW region.

Chapter 3

Environmental influences on age and size at maturity of *Loligo vulgaris*

A. Moreno[1], J. Pereira[1], M. Cunha[1]

1 IPIMAR, Avenida de Brasília, 1449-006 Lisboa, Portugal

Aquatic Living Resources (2005) 18, 377–384

3. Environmental influences on age and size at maturity of *Loligo vulgaris*

3.1. Introduction

Loligo vulgaris Lamarck, 1798 is the most abundant and commercially important loliginid squid in Portuguese waters. It is a short lived species, males attaining larger sizes and females undergoing “partial ovulation”, i.e. there are oocytes at various stages of development in the ovaries and egg-laying occurs in separate batches during the spawning period (Melo and Sauer, 1999). Energy for reproduction is probably obtained directly from ingested food rather than stored reserves. In many cephalopod species, this was hypothesized because the composition of energy storage organs is not significantly changed with maturity (e.g. Ho *et al*., 2004).

In north-west Portuguese waters *L. vulgaris* presents a complex population structure strongly influenced by year round spawning (Moreno *et al*., 1994; Moreno *et al*., 2002). The extended spawning season and the marked environmental seasonality in this geographic area mean that squid hatched in distinct seasons will be exposed to different influences during their life cycle, which will affect growth and maturation rates in different ways. It was already observed that *L. vulgaris* living under different water temperature regimes display high variability in reproductive and growth parameters (Moreno *et al*., 2002).

Growth estimates in *L. vulgaris* have revealed high variability in size at a given age, and significant differences between growth rates of seasonal groups (Arkhipkin, 1995; Bettencourt *et al*., 1996; Raya *et al*., 1999; Rocha and Guerra, 1999). If there is a specific minimal size before maturation could take place, regardless of age, as observed in *Lolliguncula brevis* by Jackson *et al*. (1997), differences in age-at-maturity between seasonal groups, due to different growth rates, are also to be expected. On the other hand, it has been shown experimentally for animals in captivity that temperature, photoperiod, and food availability directly influence maturation rates (Mangold, 1987). Therefore, distinct environmental conditions are likely to result in differences between seasonal groups.

This study aims to describe age- and size-at-maturity in *L. vulgaris* from north-west Portuguese waters and to analyse these parameters, as well as the reproductive investment, in two groups of squid hatched in distinct periods of the year. Squid were grouped based on the average temperature experienced during the first three months after hatching, the cold cohort (CC) and the warm cohort (WC), given the assumption that temperature at an early life stage has a major impact on growth rates and adult size (Forsythe, 2004). Besides temperature, each of those cohorts was obviously subjected to other distinct environmental conditions considered relevant to maturation processes, such as varying photoperiod and food supply. The differences in age- and size-at-maturity and reproductive investment between cohorts and the environmental influences on sexual maturation are discussed.

3.2. Material and Methods

3.2.1. Biological sampling

Specimens of *L. vulgaris* landed in ports on the North-West Portuguese coast, between January 1993 and December 1994 were collected monthly from the commercial fishery. Additional samples were collected from survey cruises and from the commercial fishery between February 1995 and March 2000. All samples were kept frozen for up to one month and thawed overnight before examination in the laboratory. Specimens were sexed and measures were obtained of the dorsal mantle length (ML), body weight (BW), the combined testis and Needham complex weight (TNC) in males, and the combined ovary and oviducal complex weight (OOC) and combined nidamental and accessory nidamental glands weight (OOCN), in females. A maturity stage was assigned on a scale ranging from 1 to 5 (Boyle and Ngoile, 1993).

3.2.2. Age readings

Statoliths were extracted and a transverse section of each obtained by grinding on both sides for age readings. In each statolith the total number of increments was counted from the nucleus to the tip of the rostrum. Readings were made using an image analysis system linked to a light microscope. The total number of increments was assumed to represent the age of the animal in days, based on the assumption that increment deposition in the statoliths has a daily rhythm, which was previously validated for paralarvae of *L. vulgaris* (Villanueva, 2000), juveniles and adults of the sub-species *L. vulgaris reynaudii* (Lipinski *et al*., 1998) and adults of other loliginid species (e.g. *Sepioteuthis lessoniana*, Jackson *et al*., 1993; *Lolliguncula brevis*, Jackson *et al*., 1997). Statoliths were discarded if less than 80% of the reading trajectory was readable. Two or more independent readings were performed in 10% of the statoliths. The coefficient of variation (CV=sd/mean.100) between independent readings indicated a high precision in ageing (mean CV=3.2%). No relationship was observed between mean age and precision in ageing (linear regression, r2=0.002, n=50). A total of 436 specimens were successfully aged (194 females, 23 unknown sex, 219 males).

3.2.3. Data analysis

Maturity ogives were estimated by fitting the logistic model $Y=1/(1+\exp(-a-bX))$, to the proportion of mature squid (Y) by age or size class (X). Parameters **a** and **b** were calculated by non-linear estimation. The age and size at which 50% of females or males were mature, respectively $t_{50\%}$ and $ML_{50\%}$, were derived as -a/b. The effect of sex and hatching cohort on age- and size-at-maturity was tested by comparing, between data-sets, the best fitted values of the parameters **a** and **b** of the maturity ogives using an F-test.

Hatching dates were back-calculated as the capture date minus the age in days. The temperature to which each specimen was exposed was determined as the average of the sea surface temperature (SST) during the first three months of life (i.e. the month of hatching plus the two following months). Hatching cohorts based on temperature were defined as follows: cold cohort CC (n=124; 28.4% of total sample) comprising squid that experienced average SST below 15 ºC during the first 3 months of their life (i.e. hatching in December to March, depending on annual variation in SST); warm cohort WC (n=153; 35.1% of total sample) comprising squid that experienced average SST above 17 ºC during the first 3 months of life (i.e. hatchings in May to September, depending on annual variation in SST). Average SST was computed from weekly data for North-West Portuguese waters (38.5º to 41.5º N and 8.5º to 9.5º W) extracted from the “IGOSS nmc” database (Reynolds and Smith, 1994).

The relationships between the weight of reproductive organs (OOC and TNC) and ML or age were assessed by Spearman Rank Order Correlations. Mean OOC and TNC by age group (t) were estimated for each cohort and maturation rates (mr) calculated as $mr=OOC_2-OOC_1/t_2-t_1$ or $mr=TNC_2-TNC_1/t_2-t_1$. Gonadosomatic indices (GSI) were computed for each male and female at maturity stages 2 to 5, as GSI=OOC/(BW-OOC)*100 or GSI=TNC/(BW-TNC)*100.

The effect of hatching cohort on maturation was analyzed by comparing the mean GSI, OOC, OOCN and TNC using ANCOVA, with ML as the covariate, since reproductive organ weight was highly dependent on the size of specimens. These analyses were performed only on mature specimens (stages 4 and 5), to minimize the effect of differences in the proportion of maturing vs. mature squid between cohorts.

3.3. Results

3.3.1. Age and size at maturity

Sex could be identified macroscopically in squid from 4 months old (121 days). The beginning of gonad maturation (maturity stage 2) was observed from 5 months in males and 6 months in females (Figure 10). Minimum age observed at maturity in males was 5 months. In less than one month from the beginning of the maturation process, specimens of either sex can be fully mature. Spawning took place at a mean age of 10 months (298 days) and males reached maturity at a mean age of 9 months (277 days). Variability in the maturity cycle was high in both sexes, with standard deviations of 1-1.5 months in squid in maturity stages 1 to 4 and up to 1 month in squid in maturity stage 5. About 92% of females and 86% of males were mature before the age of 1 year.

Maturity ogives were estimated for each sex by age group (Figure 11a) and size class (Figure 11b). Maturity ogives by age group provided a good fit to the observed data, explaining 94.1% and 93.7% of the variance in maturity of females and males, respectively. Males matured ca. 1 month earlier than females and at a smaller size. Maturity ogives were found to be significantly different between sexes in relation to age group ($F_{2,18}$=4.69, p<0.01) and size class ($F_{2,34}$=7.32, p<0.01). The $t_{50\%}$ was estimated to be 7.6 months for males and 8.7 months for females. The smallest mature female measured 15cm (8 months) and the smallest mature male measured 8cm (5 months). The $ML_{50\%}$ of females was estimated to be 17.6cm. Size-at-maturity in males showed a high degree of complexity, with high proportions of small mature males and low proportions of mature males in some of the larger size classes. The $ML_{50\%}$ of males was estimated to be 15.3cm, but the adjusted maturity ogive by size class explained only 82.7% of the variance in maturity and estimated excessively high proportions of mature squid for the smaller size classes.

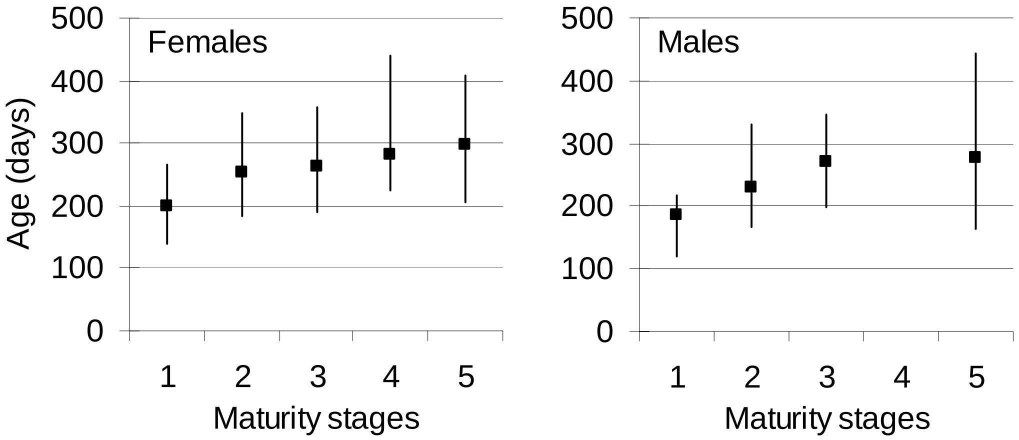

Figure 10 - Minimum and maximum age (lines) and mean age (dots) by maturity stage in male and female. Maturity stage 4 males were disregarded (n=3).

3.3.2. Maturity cycle by cohort

The effect of hatching season on age- and size-at-maturity was analysed by comparing two groups of squid hatched in distinct periods of the year, the cold cohort (CC) and the warm cohort (WC). Minimum age-at-maturity in the CC (8.5 months in females and 6.8 months in males) was higher than the average value for the whole population. The spawning season in this cohort was between September and January (mainly in autumn), with a peak in December (Figure 12). Males reach maturity between July and February, the majority in October and November.

Minimum age-at-maturity in the WC (7.3 months in females and 5.5 months in males) was lower than the average and near the minimum of the whole population. The spawning season in this cohort was between January and July (mainly in spring), with a peak in April (Figure 12). Males reach maturity throughout the year, the majority between December and April.

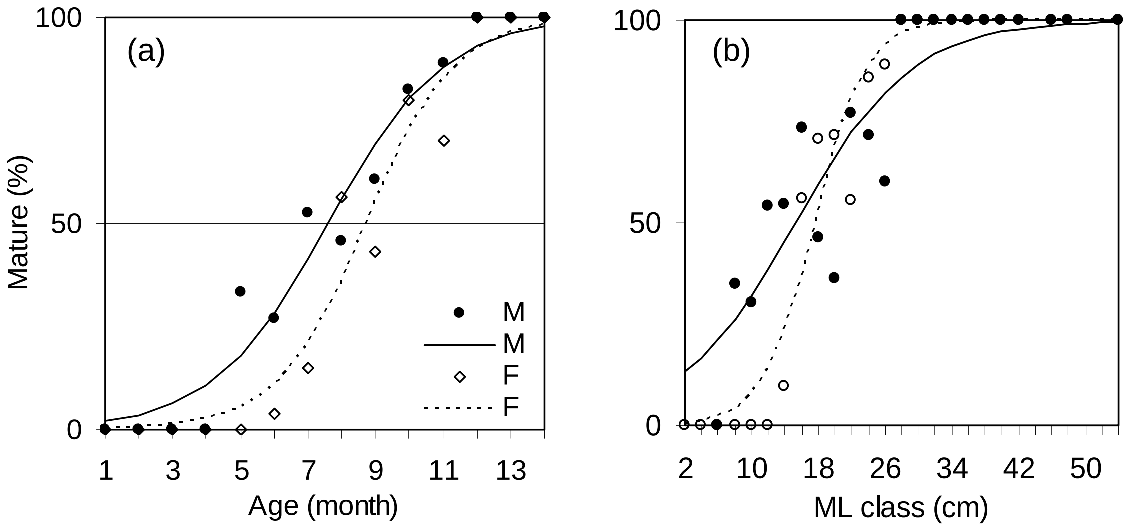

Figure 11 - Percentage mature by age group (a) or ML class (b) and estimated maturity ogives for females and males. $t_{50\%}$ or $ML_{50\%}$ are the estimated age or ML at which 50% of females (F) or males (M) are mature.

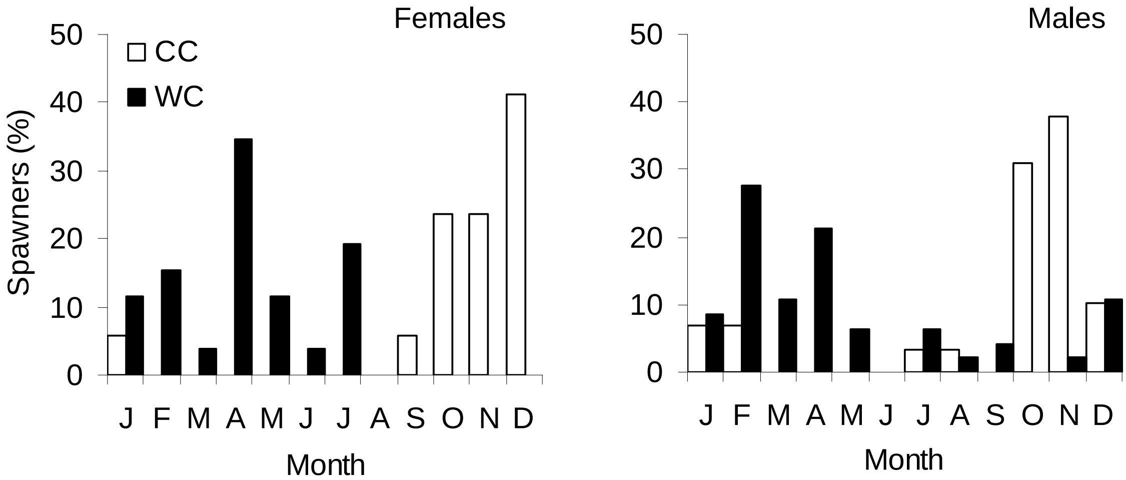

Figure 12 - Percentage of female or male spawners by month from the total spawners in the cold cohort (CC) and in the warm cohort (WC).

A significant influence of hatching cohort on age-at-maturity, as assessed by comparing the cohort's maturity ogives, was found in females ($F_{2,10}$=153.7, p<0.001) and males (($F_{2,14}$=8.88, p<0.01), indicating that CC squid mature later in life than WC squid. Females of the CC mature at $t_{50\%}$=9.5 months and females of the WC mature at $t_{50\%}$=7.3 months (Figure 13). Males of the CC mature at $t_{50\%}$=8.4 months and males of the WC mature at $t_{50\%}$=6.2 months. As for females, minimum age-at-maturity was greater in CC males (6.8 months) than in WC males (5.5 months). The variability in age-at-maturity was lower within cohorts than for the whole population in females but not in males.

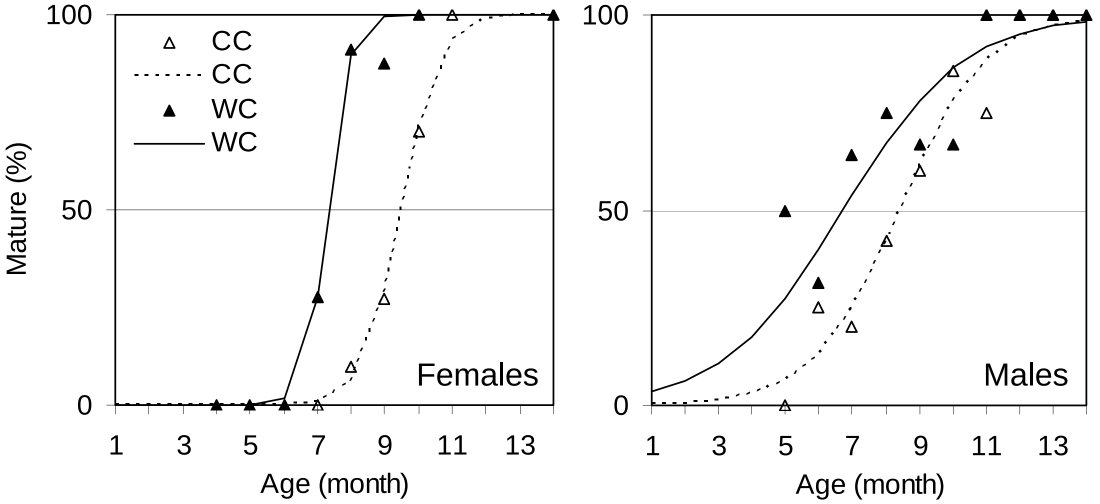

Figure 13 - Percentage of mature females and males by age group and estimated maturity ogives for the cold cohort (CC) and the warm cohort (WC).

Differences in size-at-maturity were also observed between cohorts. $ML_{50\%}$ was 19.1cm and 15.6cm in females of the CC and WC, respectively (Figure 14). Size-at-maturity in males was lower than in females, $ML_{50\%}$=14.1cm in the CC and $ML_{50\%}$=12.0cm in the WC. The variation in size-at-maturity of males was especially high and the fit of a maturity ogive to data on maturity by size class, for males of each cohort, was considered poor (r^2=0.70-0.79). In both cohorts high proportions of small mature males were observed as well as low proportions of mature males in some of the larger size classes. The high variability for the whole population and the co-existence of groups of small and big mature animals was not explained by differences between cohorts

($F_{2,27}=0.483$, $p>0.05$), two sizes-at-maturity still being present in each cohort. Fitting a single maturity ogive to the size-at-maturity data for both male cohorts combined gave a more satisfactory fit.

In maturity ogives by size class a better fitting of was observed when splitting females by cohorts, indicating the underlying significant differences in the size-at-maturity between cohorts. In fact, the comparison between maturity ogives fitted to each cohort indicated that WC females mature at significantly smaller sizes than those of CC ($F_{2,19}=40.51$, $p<0.001$).

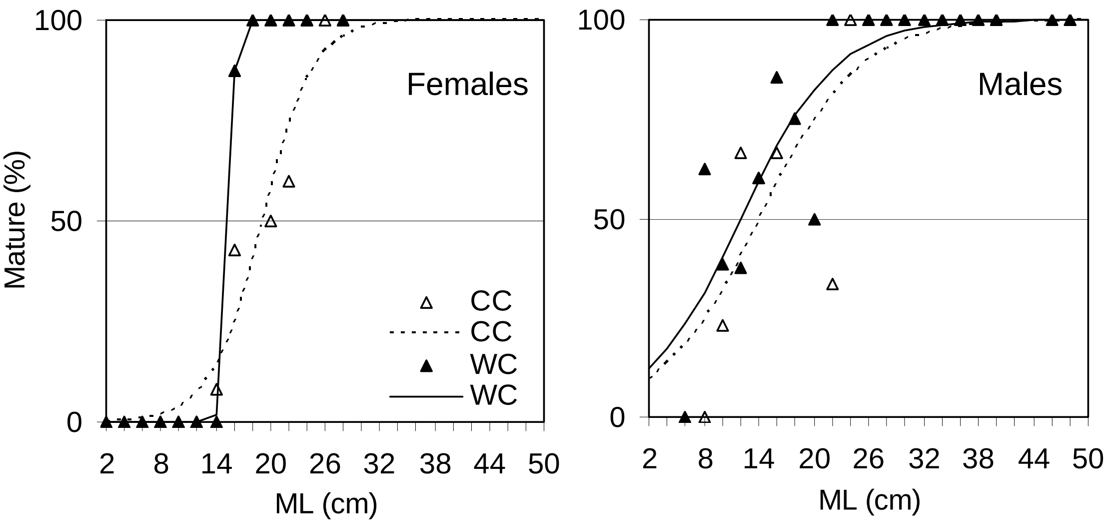

Figure 14 - Percentage of mature females and males by size class and estimated maturity ogives for the cold cohort (CC) and the warm cohort (WC).

3.3.3. Reproductive investment with age

As maturation started earlier in WC females, differences in mean gonad weight (OOC) and GSI with age in relation to CC females were apparent from 7 months old (Figure 15a,b). Within WC females the highest increase in OOC, i.e. the maturation rate (*mr*), occurred earlier (6 to 8 months) than within CC females (9 to 11 months). In both cohorts the highest *mr* were observed close to the respective $t_{50\%}$ and, by this age, the *mr* was much higher in the CC than in the WC (Figure 15a, Table 1), i.e. close to the main spawning time of each cohort the increase in gonad weight was faster in the CC.

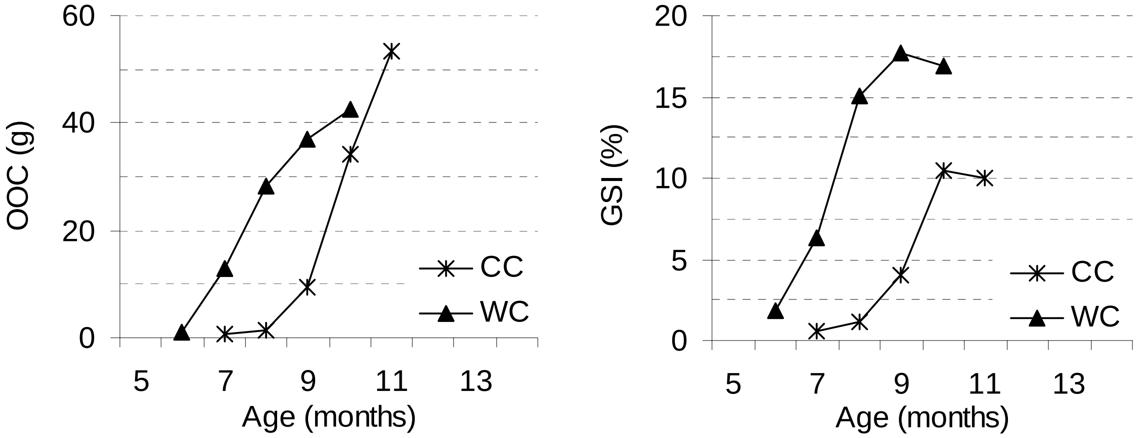

Figure 15 - Mean combined ovary and oviducal complex weight (OOC) by age group (a) and mean gonadosomatic index (GSI) by age group (b) in females of the cold cohort (CC) and the warm cohort (WC).

Table 1 - Maturation rates (g month $^{-1}$) of different age groups in females and males of the cold cohort (CC) and the warm cohort (WC).

Age group (month)	Females		Males	
	CC	WC	CC	WC
6-7	-	11.9	0.0	3.7
7-8	0.8	15.3	0.8	0.0
8-9	11.3	8.7	1.4	1.7
9-10	33.2	5.9	2.5	2.2
10-11	27.5	-	7.7	3.9
11-12	-	-	-	1.5

The reproductive investment in the WC females, revealed by GSI, increased with age and was highest at 9 months old (Figure 15b). In the CC females, GSI also increased with age and was highest at 10 months old. However, GSI was much higher at every age in WC females than in CC females. The variation of OOCN with age (Figure 16) showed similar trends and differences between cohorts as that of the OOC.

In males of both cohorts, the monthly increase in TNC (*mr*) was highest between 10 and 11 months. In the WC a high *mr* also occurred between 6 and 7 months, which resulted in mean TNC values between 7 and 10 months slightly larger than those in the CC males (Table 1, Figure 17a). GSI was greater in the WC males up to 8 months of age, and similar in both cohorts beyond that age (Figure 17b).

The combined ovary and oviducal complex weight (OOC) was better correlated with size (R=0.858) than age (R=0.493) of females. The increase in the combined testis and Needham complex weight (TNC) was also better correlated with increasing size (R=0.828) than age (R=0.700) of males.

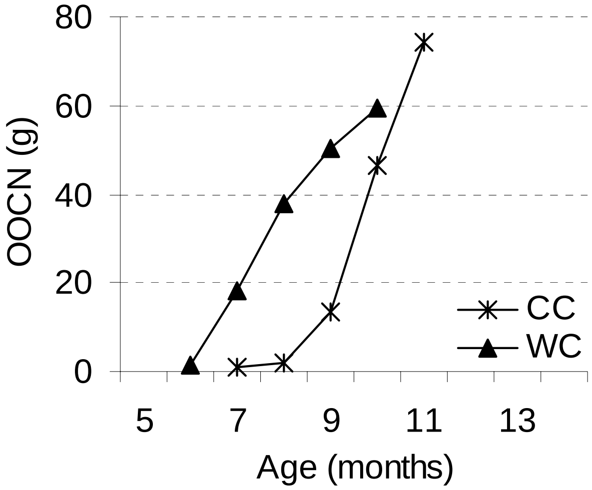

Figure 16 - Mean combined ovary and oviducal complex weight (OOC) plus combined nidamental and accessory glands weight by age group in females of the cold cohort (CC) and the warm cohort (WC).

The results of the ANCOVA tests indicated a significant influence of hatching cohort on the reproductive investment in each sex, as the mean GSI of mature squid of the WC was significantly greater than that of the CC (Table 2). The mean TNC in the WC mature males was also significantly higher than that of the CC. However, no significant differences were found between cohorts in mean OOC and OOCN of mature females.

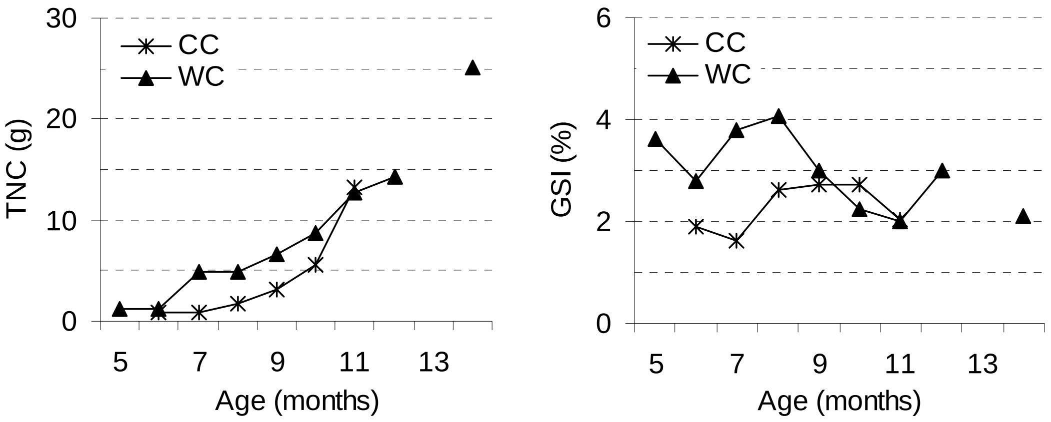

Figure 17 - Mean combined testis and Needham complex weight (TNC) by age group (a) and mean gonadosomatic index (GSI) by age group (b) in males of the cold cohort (CC) and the warm cohort (WC).

Table 2 - Influence of hatching cohort; cold cohort (CC), warm cohort (WC) on gonadosomatic indices (GSI, %), mean combined ovary and oviducal complex weight (OOC, g), mean combined OOC weight plus accessory and nidamental glands weight (OOCN, g) and mean combined testis and Needham complex weight (TNC, g). ANCOVA, mean ML (cm), ML as the covariate. Significant differences between cohorts marked in bold.

Sex	**Biological variables**	**CC**	**WC**	**Mean square effect**	**df error**	**Mean square error**	**F**	**p**
Females	GSI	12.2	17.0	211	40	48	4.4	**0.042**
	OOC	40.1	37.3	459	40	120	3.8	0.058
	OOCN	55.3	51.0	830	40	205	4.0	0.051
	ML	23.0	20.6					
Males	GSI	3.4	3.9	10	70	1	13.0	**0.001**
	TNC	5.5	8.1	35	70	6	6.1	**0.016**
	ML	19.3	21.7					

3.4. Discussion

3.4.1. Age and size at maturity

In analyzing age and size-at-maturity data it appears that a complex combination of factors may influence the exact shape of growth and maturation curves. Significant differences were observed in maturity ogives between sexes, highlighting the important differences in the maturation process that exist between males and females. In males, reproductive investment is lower and maturity is reached earlier and at a smaller size, while in females reproductive investment is higher and maturity is reached at older ages and bigger sizes. The fact that males mature at a smaller size than females has been observed across the geographic distribution of this species (Baddyr, 1991; Coelho *et al*., 1994; Guerra and Rocha, 1994; Moreno *et al*., 1994). The earlier maturation of males in Portuguese waters explains their seasonal maturity peak, which is around one month before that of females, as observed in several *L. vulgaris* and *L. forbesi* populations of the NE Atlantic (Collins *et al*., 1997; Moreno *et al*., 2002).

A higher complexity in size-at-maturity than in age-at-maturity was found, especially in males, suggesting that in the case of Portuguese squid there are groups of animals with distinct growth rates, and that there may be different cohorts that are subjected to different environmental conditions as the animals grow. Temperature appears to be a determining factor for somatic growth, at least in an early phase (Grist and des Clers, 1999; Forsythe, 2004). Therefore, cohorts of animals that hatched at different temperature regimes have different somatic growth rates, reaching different sizes prior to the onset of maturation. However, because there is always a coupling between different environmental factors, temperature does not necessarily explain all the variability in growth and consequently in age- and size-at-maturity.

Sexual maturation, like growth, may be promoted by exposure to relatively high average water temperature, a favourable feeding regime (Richard, 1966; Rodhouse and Hatfield, 1990), or combinations of these or of these and other factors. It has been shown that the maturation rate is very responsive to changes in temperature (Jackson *et al*., 1997) and that there is a decelerating effect of long days (Mangold, 1987), the two factors having an effect on the age of spawning. In our case the CC individuals, that hatched in colder water, were exposed to an increasing photoperiod, which was the highest (13 to 15 hours of light) when squid had achieved the minimum age of maturation (5-6 months), and increasing food availability (higher in summer). These environmental factors together would contribute to the delayed maturation observed in this cohort, as lower temperatures experienced early in life decrease growth rates and, consequently, minimum size for maturity is reached later in life. At this time, maturation will be further delayed in the majority of the cohort by a long photoperiod and high food supply. On the other hand, in the WC age-at-maturity was lower. The faster growth induced by higher temperatures enables this cohort to attain the minimum size for maturity earlier (ca. one month). This cohort then faces favourable environmental conditions and the majority of individuals quickly reach maturity. It has also been shown that, among a seasonal group of *Illex coindetii*, under lower temperature and longer photoperiod, maturation started at older ages (less 20-40 days) than in another group, under the opposite environmental regime (Arkhipkin *et al*., 2000). In *Loligo chinensis* and *Lolliguncula brevis* differences were also found in age-at-maturity between seasonal groups, and were thought to be related to differences in growth rates and the minimum size to reach maturity (Jackson, 1993; Jackson *et al*., 1997).

The differences in age-at-maturity between cohorts resulted in a relatively short period between the main spawning peaks of each (December and April). It has been said that a certain degree of life cycle synchronization is achieved by an environmental induction of maturation (Moreno *et al*., 2002), possibly following temperature and photoperiod cues. These spawning cues will induce a rapid maturation in a given period of the year, if the minimum size threshold has been reached. From the recognisable onset of maturation, the process takes place at a very rapid rate and within less than a month full maturation is reached. This may allow the animal to take immediate advantage of favourable environmental conditions, switching from somatic to reproductive growth within a short time. The lowest sizes-at-maturity were observed during the main spawning seasons, and a significant negative correlation was found between seasonal trends of proportion mature and size-at-maturity (Moreno *et al*., 2002). This seems to indicate that at favourable spawning times most animals that have reached the minimum size threshold will begin the maturation process (as in the WC). Otherwise, they will delay maturation and continue the somatic development until the next environmental spawning cue (as in the CC).

In males, variability in age- and size-at-maturity could not be accounted for by assigning them to a temperature cohort. Other authors have shown distinct sizes-at-maturity for *L.vulgaris* and *L. forbesi* (Guerra and Rocha, 1994; Moreno *et al*., 1994; Pierce *et al*., 1994a), probably as a result of a “guard-sneaker” breeding strategy, as observed in *L. vulgaris reynaudii* (Sauer *et al*., 1997) or *L. pealei* (Hanlon, 1998). Males seem to display complex breeding strategies, perhaps derived from endogenous stimuli. These breeding strategies may help to maintain the genetic homogeneity of the population by co-occurring and interbreeding with different female cohorts.

3.4.2. Reproductive investment

Some of the characteristics of the brood, such as the number and quality of the eggs and sperm cells, and its relation to the reproductive investment, may be determined by the interval of time between reaching the minimum size for maturity and experiencing the cues (environmental or otherwise) that determine adequate breeding conditions.

The late maturing CC females experience favourable environmental conditions, namely high food supply and temperature, before and during the main spawning period (summer and autumn, respectively), which enables enough energy storage to be used for fast maturation rates even during their spawning peak in December, when temperatures are lower. The WC females spawn mainly during or soon after the adverse winter months; thus a lower amount of energy will probably be available to allocate for gonad maturation, as revealed by their lower maturity rates. On the other hand, the reproductive investment as indicated by GSI was significantly higher in the WC than in the CC females. This fact highlights the differences in the relative reproductive investment and is more closely related with the differences in the age- and size-at-maturity between cohorts, since the absolute reproductive investment (i.e. the weight of the reproductive system) did not differ significantly between them. In contrast, in *Sepioteuthis lessoniana* (Jackson and Moltschaniwskyj, 2002) and *Idiosepius pygmaeus* (Jackson and Choat, 1992; Jackson, 1993) the absolute reproductive investment (i.e. total mass of the ovary) was greater in the late maturing females. In any case, the differences in GSI between cohorts show that, at all ages, the amount of energy allocated for gonad growth in relation to body mass was higher in the WC females. In *Sepioteuthis lessoniana* it was also observed that the “hot” strategy squid invested a greater proportion of their total mass in reproduction than the “cool” strategy squid (Jackson and Moltschaniwskyj, 2002).

In the CC females, the energy for reproduction was probably mainly derived from ingested food rather than body reserves. In this strategy, there is an increase of an individual's ability to capitalise on favourable environmental conditions by rapidly converting food to fuel sexual maturation and somatic growth (Ho *et al*., 2004), favouring large size mature animals and longer life spans. On the other hand, in the WC, part of the energy must be re-allocated for reproduction at some cost to somatic growth. A decline in relative mass of the mantle during reproductive maturation was seen in *Illex argentinus* (Hatfield *et al*., 1992) and *Photololigo* sp. (Moltschaniwskyj and Semmens, 2000), and a decline in growth rates was seen in *Photololigo* sp. (Moltschaniwskyj, 1995).

In males of the WC both relative reproductive investment (GSI) and absolute reproductive investment (i.e. the total mass of the reproductive system) were higher than in the CC, demonstrating that temperature, photoperiod and perhaps other environmental factors are not the only important factors in maturation.

3.4.3. Implications for management

Back-calculated dates of hatching suggest that hatching, and therefore spawning, occurs all year round in *L. vulgaris*, assuring exposure of squid to all seasonal extremes. Given that growth, maturation and potentially also life span, are dependent on environmental factors that have a great seasonal variability in Portuguese coastal waters, it is not unexpected that we find a highly variable and fluctuating population structure. This has important implications for population modelling and fisheries management (Dawe and Beck, 1997; Pecl, 2004). There being a recognized plasticity in both age and size-at-maturity, an influence of those in the characteristics of the next generation and a fishery-induced differential mortality-at-length and mortality-at-age could induce a strong selection for early age of maturation. This could in the medium to long-term affect the yield from the fishery and in the long-term the sustainable management of the stocks may be placed at great risk (Murphy and Rodhouse, 1999). A question that arises from this study is whether the different reproductive strategies, which result in small mature females and large mature females with gonads of similar size, have any implications in their fecundity. Directed fecundity studies are needed in order to investigate this aspect, since potential differences in fecundity between cohorts would have major implications for the options available to manage this important resource.

Chapter 4

Growth strategies in the squid *Loligo vulgaris* from Portuguese waters

A. Moreno[1], M. Azevedo[1], J. Pereira[1], G.J. Pierce[2]

1 IPIMAR, Av. Brasília, 1400-038 Lisbon, Portugal

2 School of Biological Sciences (Zoology), College of Life Sciences and Medicine, University of Aberdeen, Tillydrone Avenue, Aberdeen AB242TZ, UK

Marine Biology Research (2007) 3, 49–59

4. Growth strategies in the squid *Loligo vulgaris* from Portuguese waters

4.1. Introduction

Cephalopod growth is highly variable, similarly to other aspects of their biology. This variability is believed to be a combination of intrinsic variability and the influence of several environmental factors, of which temperature and food intake have major roles (reviewed in Forsythe and Van Heukelem, 1987; Jackson, 1994).

Several studies investigating the impact of temperature on variability in size-at-age were conducted in the last decade in wild populations (e.g. Jackson *et al*., 1997; Hatfield, 2000; Jackson and Moltschaniwskyj, 2002) to corroborate the Forsythe hypothesis (Forsythe, 1993) or Forsythe effect (Forsythe, 2004), which states that because hatching in many species occurs over a period of continually changing temperatures, each cohort of hatchlings encountering warmer temperatures will grow significantly faster than those that hatched only weeks previously. Other studies in wild squid populations revealed significant differences in size-at-age between geographic areas (Jackson and Moltschaniwskyj, 2002; Chen and Chiu, 2003) and between seasonal cohorts (Arkhipkin *et al*., 2000; Villegas, 2001; Pecl, 2004). These differences in growth were mainly attributed to the geographic or seasonal prevailing temperatures during the early life stages.

Loligo vulgaris is a near-shore species distributed along North Eastern Atlantic waters and the Mediterranean, where it is commercially exploited. It is widely distributed along the Portuguese continental shelf, displaying yearlong spawning and recruitment (Moreno *et al*., 2002). On the North-West Portuguese coast *L. vulgaris* occur mainly from the shore to the 100 m isobath. In contrast to most other loliginid populations, spawners and juveniles co-occur both in time and space and are available to fisheries all year round (Cunha *et al*., 1995; Moreno, 1998). Nevertheless, some horizontal short-range mixing of animals from different spawning grounds may occur, since there is no significant evidence of population sub-structuring (Pierce *et al*., 1994b; Anon, 2004).

Currently no formal assessment of this resource is undertaken. Some exercises with cohort analysis (Royer *et al*., 2002; Challier *et al*., 2006a) and depletion methods (Young *et al*., 2004) have been conducted for loliginid squids, but for the most part, conventional models used in assessment of finfish stocks are not applicable (Pierce and Guerra, 1994). Nevertheless, if stock size predictions are to partly rely on temperature data (Robin and Denis, 1999; Pierce and Boyle, 2003; Chen *et al*., 2006), the understanding of age structure, growth, and its variability under fluctuating environmental conditions is of fundamental relevance.

There is a general consensus among squid researchers (Jackson, 1994) that squid growth does not follow the generalised von Bertalanffy model (Bertalanffy, 1938) often adopted to describe fish growth. Cephalopod growth is generally described as continuous and non-asymptotic (Jackson and Moltschaniwskyj, 2002). Nonetheless, the growth of many ommastrephid squid species has been modelled using Gompertz or logistic models (*Todarodes sagitattus*, Arkipkin *et al*., 1999; *Todaropsis eblanae*, Arkhipkin and Laptikhovsky, 2000; *Illex coindetii*, Arkhipkin *et al*., 2000; *Illex illecebrosus*, Hendrickson, 2004; *Dosidicus gigas*, Markaida *et al*., 2004), which suggests an inflexion in growth at some point. It seems that the power model best fits most *Loligo* size-at-age data, (e.g. *L. vulgaris*, Arkhipkin, 1995; *L. pealeii*, Macy and Brodziak, 2001; *L. gahi*, Villegas, 2001; *L. opalescens*, Jackson and Domeier, 2003). Other models, however, best fitted age data of some of the above species when applied to populations of different geographic areas, including exponential models (*L. vulgaris*, Rocha and Guerra, 1999; *L. forbesi*, Challier *et al*., 2006b) or double exponential models (*L. vulgaris*, Natsukari and Komine, 1992).

Whether squid grow asymptotically or not is not an issue in this manuscript. Instead, it is our intent to analyse the variability in the type of growth model within the population, between sexes and seasonal cohorts, and in particular whether there is an inflexion in growth and why. Analysis of age and size at maturity in *L. vulgaris* from NW Portuguese waters has already revealed that variability is closely related to the environmental conditions experienced by individuals through their life cycle, namely those affecting maturation rates of females (Moreno *et al*., 2005). The aim of this study is to further the understanding of life cycle plasticity in *L. vulgaris*, by analysing their growth variability.

4.2. Material and Methods

4.2.1. Biological sampling

Samples were collected monthly, between January 1993 and December 1994, along the NW Portuguese coast (38º30' to 42º N). Every month samples were obtained from either the trawl or the seine fisheries both operating within the same geographical area. In order to increase the sample size of large squid and to include pre-recruits in the size-age range, additional samples from the jig fisheries and survey cruises carried out in the same area, between March 1993 and March 2000, were also used. Dorsal mantle length (ML, mm), sex, age, and maturity stage were determined in 435 individuals (23 unknown sex (U), 193 females (F) and 219 males (M)). Maturity stages 1, 2-3 and 4-5 were used to classify individuals as immature, maturing and mature, respectively, following the five-stage maturity scale described in Boyle and Ngoile (1993). The individuals of unknown sex were all small, immature animals with insufficient gonad development to allow sex to be determined.

4.2.2. Age readings

Age was determined by increment counting in the statoliths following the methodology detailed in Moreno (2002). Deposition of increments in the statoliths was assumed to be daily, as was validated for paralarvae of this species (Villanueva, 2000). Ageing precision was evaluated by independent readings (same reader, distinct reading date) in 50 statoliths (more details in Moreno *et al.*, 2005, chapter 3), showing high precision (CV=3.2%).

4.2.3. Data analysis

Hatching date was calculated as capture date minus age. For some of the analyses, ages were grouped into 30-day classes. To examine the effect of sex on growth, the length-at-age data of the unknown sex specimens were added to data for each sex to increase the length-age range, assuming no significant sex-related differences in smaller specimens.

Average SST in the first 3 months of the life of each specimen was computed.

To explore the effect of SST during early life on growth, while controlling for hatch month and year we fitted a Gaussian GAM to (sqrt transformed) length-at-age data. SST during the first three months of life and month were treated as continuous variables and year (1992, 1993, 1994, other) as a nominal variable. Degrees of freedom for smoothers were estimated using cross-validation. This preliminary analysis showed a significant effect of temperature on length-at-age ($p << 0.001$ for both sexes).

To maximize SST differences, two groups of squid were selected, named Cold Cohort (CC, average SST<15 ºC) and Warm Cohort (WC, average SST>16.5 ºC), regardless of hatching year or season. Weekly SST data for NW Portuguese waters (38.5 to 41.5º N and 8.5º to 9.5º W) was extracted from the integrated Global Ocean Services System – Meteorological Center "IGOSS nmc" database (http://iridl.ideo.columbia.edu/ SOURCES /.IGOSS/.nmc/Reyn_SmithOIv1/.monthly/.sst/, Reynolds and Smith 1994) on a 1º latitude x 1º longitude grid.

Analysis of covariance (ANCOVA) was used to test the effect of cohort on the size-age relationship of each sex, using age as the covariate. Due to unequal sample size by age class between cohorts, the average length-at-age of the CC and WC groups was compared by sex using a data set generated by random sub-sampling of an equal number of specimens by age class in each cohort.

Six models were fitted to the (individual) length-at-age data, namely linear, exponential, power, Gompertz, logistic, and von Bertalanffy. The parameters of the models were estimated by the least squares method (linear and intrinsic linear models) and the iterative simplex method (non-linear models). The best-fit model was determined by the highest goodness of fit, the examination of residuals for any systematic pattern, and the reliability of the parameter estimates. Growth model fitting and ANCOVA were performed with R using the stats package (R development core team, 2006). GAMs were fitted using the R-interface statistical package BRODGAR (Highland Statistics Ltd).

Daily growth rates (DGR), by sex and hatching cohort and instantaneous growth rates (G), for each sex were determined in 30 day time intervals, after Ricker (1979):

$$DGR=(ML_{i+1}-ML_i)/(t_{i+1}-t_i);$$

$$G=(\ln(ML_{i+1})-\ln(ML_i))/(t_{i+1}-t_i)*100$$

where ML_i and ML_{i+1} and t_i and t_{i+1} are the ML (mm) and age (days) at the beginning and the end of each age class t, respectively. For time intervals t>30 days, the arithmetic means of DGR and G were calculated (avgDGR or avgG).

4.3. Results

4.3.1. Age composition

Mantle length of aged individuals ranged from 21 to 546 mm (21 to 72 mm for unknown sex, 31 to 332 mm for females and 64 to 546 mm for males). The youngest squid was 87 days old. Sex could be determined in specimens from 120 days old. Age of CC squid ranged from 93 to 387 days (93 to 109 days for unknown sex, 181 to 357 days for females and 165 to 387 days for males) and age of WC squid ranged from 88 to 446 days (88 to 213 days for unknown sex, 140 to 439 days for females and 154 to 446 days for males). In general, the maximum age observed was 439 days in females and 446 days in males and thus maximum longevity was estimated to be ca. 15 months in both sexes, though the majority of specimens were less than 1 year old.

4.3.2. Preliminary GAM modelling

The GAMs fitted to length-at-age data demonstrated that SST in the first 3 months of life significantly affected growth in both males and females ($p<0.001$ in both cases). In both sexes, the shape of the smoother for the effect of temperature indicated that squid reached higher length-at-age when SST was higher, at least up to around 17 ºC (Figure 18).

There were also significant effects of hatch month in both sexes ($p<0.001$ in females, $p=0.001$ in males), with the lowest length-at-age (for a given temperature experienced in early life) achieved by squid hatching during June to September. [Since these months tend to be warm months, squid hatched in these months would still on average achieve larger size at age.] Females hatched in 1993 reach larger lengths-at-age than females hatched in 1992 ($p=0.002$) but there were no other significant year effects. For the subsequent analysis, hatch month and year effects on growth were ignored.

It should be noted that, as in most growth studies, we infer patterns of individual growth from single measurements of length-at-age in a large number of individuals. Obviously the more that individual growth trajectories vary the greater chance there is that the composite growth model is a poor representation of the growth in any one individual. This caveat should be borne in mind in any discussion of alternative growth models.

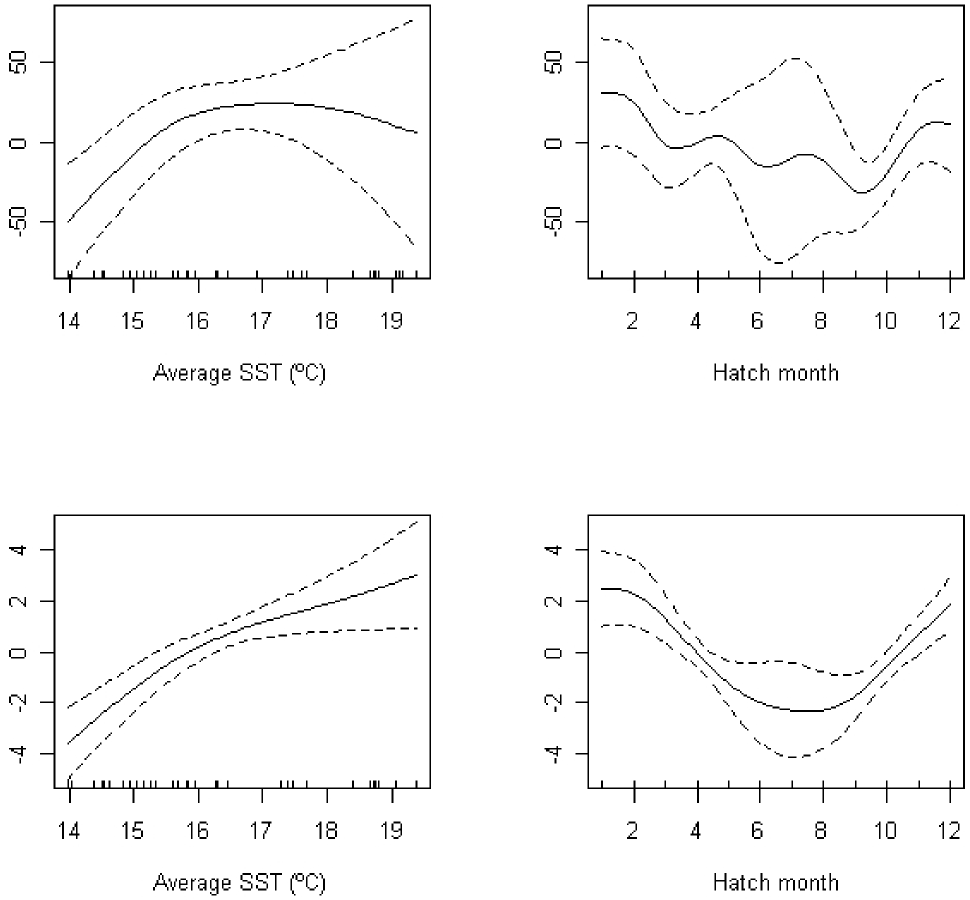

Figure 18 - Generalised additive model of length-at-age in *Loligo vulgaris*. Partial effects (solid line) and 95% confidence limits (broken lines) of smooth terms of average SST and hatch month for females (upper panel) and males (lower panel).

4.3.3. Gender differences in growth

Mantle length was significantly correlated with age ($p<<0.001$). This correlation increased when computed by sex showing that sex is a source of variability in length-at-age. The effect of sex on growth was significant (ANCOVA, $F_{1,409}=7.36$, $p=0.007$), showing that males grew faster than females. The models' goodness of fit was similar in females (0.75-0.82) and in males (0.74-0.78). The scatter-plot for females (Figure 19) shows an inflexion, suggesting that an asymptotic model could better describe growth (Table 3), and this is corroborated by the systematic patterns detected in the residuals of the non-asymptotic models, namely concerning smaller and/or larger animals.

Although the goodness of fit for both Gompertz and logistic models is of the same magnitude (0.82), the logistic model is adopted as better describing female growth, given that the estimated asymptotic ML is closer to the maximum observed ML (logistic: ML_∞ = 335 mm, S.E. = 23.3 mm; Gompertz: ML_∞ = 415 mm, S.E. = 55.9 mm) and the S.E. of the estimated parameters is lower. The ML-at-age data for males do not indicate any growth inflexion, thus asymptotic models present non-reliable parameter estimates. The power model is adopted (Figure 20, Table 3) as the best fitting growth model, with higher goodness of fit (0.78) and no systematic pattern in the residuals, in relation to the linear and exponential models.

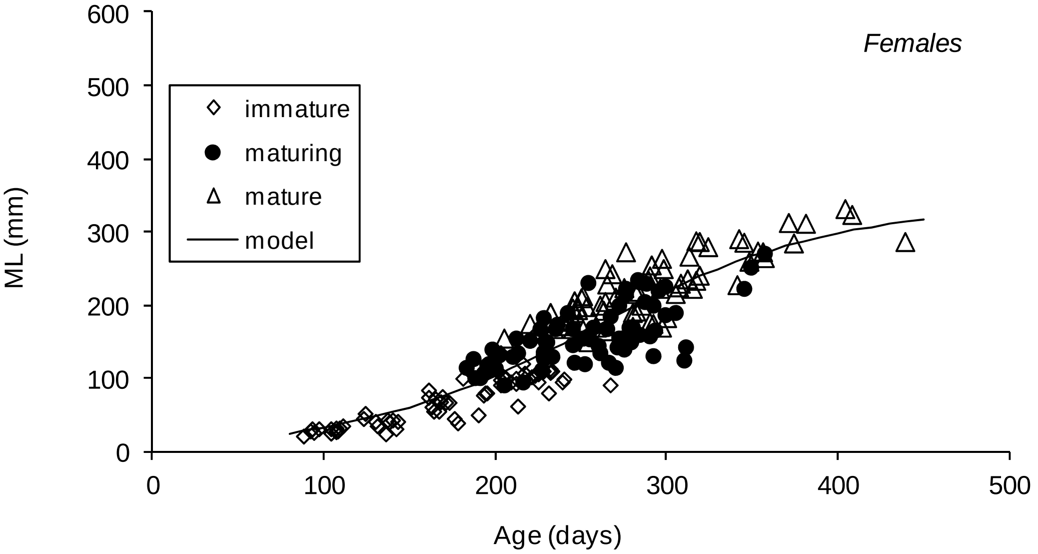

Figure 19 - The relationship between age and mantle length for *Loligo vulgaris* females. The fitted curve is the logistic model.

Female DGR increased from 0.42 mm/day in the 4th month of life (90-120 days) to a maximum of 1.16 mm/day in the 9th month (240-270 days), around the growth inflexion point (257 days), and subsequently decreasing (Figure 21). On the other hand, males showed a steady increase in DGR until death. Daily growth rates were similar between sexes until squid were 240 days old ($AvgDGR_{90\text{-}240F}$ = 0.77 mm/day and $AvgDGR_{90\text{-}240M}$ = 0.83 mm/day).

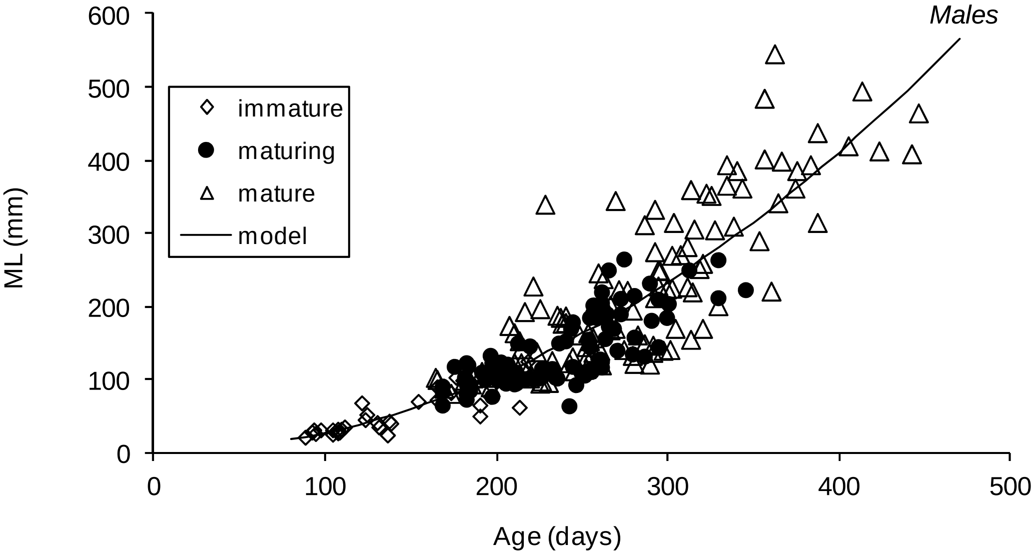

Figure 20 - The relationship between age and mantle length for *Loligo vulgaris* males. The fitted curve is the power model.

Sexual differences became more apparent when the squid were 270 days old (ca. 5 mm difference in ML), reaching a difference of ca. 65 mm in ML when they were about 1 year old and a difference of ca. 150 mm ML when squid reach 420 days old. In this period of life, the growth rate of males is much higher ($AvgDGR_{240\text{-}450M}$ = 1.70 mm/day) than that of females ($AvgDGR_{240\text{-}450F}$ = 0.77 mm/day). Between 90 and 450 days of age, females grew at an average rate (avgDGR) of 0.77 mm/day and males at an average rate of 1.34 mm/day. The instantaneous growth rate (G) decreased until death in both sexes. This growth rate was always higher in males ($AvgG_{90\text{-}450M}$ = 0.86 %/day) than in females ($AvgG_{90\text{-}450M}$ = 0.65 %/day), explaining the larger sizes reached by males.

Table 3 - Parameter estimation of the general growth model for females and males.

Females Growth Model		**ML∞**	**k**	**ti**
	Estimate	335.4	0.014	256.5
Logistic model	Standard error	21.7	0.001	10.2
ML=ML∞/(1+exp(-k*(t-ti)))	t-value (df=213)	15.5	11.8	25.0
r^2=0.82	p-level	0.00	0.00	0.00
	lower confidence limit	292.6	0.012	236.3
	upper confidence limit	378.1	0.017	276.7
Males Growth Model		**a**	**b**	
	Estimate	0.003	1.98	
Power model	Standard error	0.001	0.07	
$ML=a*t^b$	t-value (df=240)	2.4	27.3	
r^2=0.78	p-level	0.02	0.00	
	lower confidence limit	0.001	1.84	
	upper confidence limit	0.005	2.12	

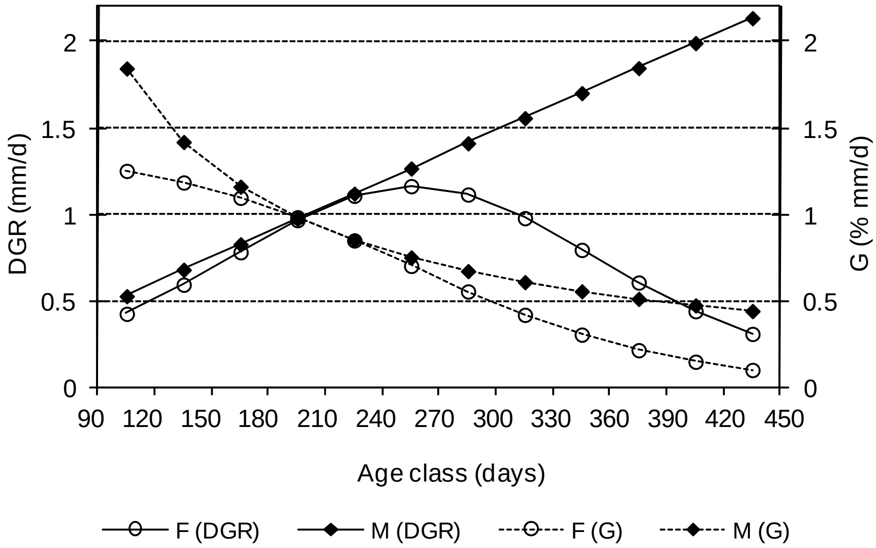

Figure 21 - Variation of Daily Growth Rate (DGR) and Instantaneous Growth Rate (G) through the life cycle for *Loligo vulgaris* females (F) and males (M). Growth rates calculated in 30-day time intervals.

4.3.4. Cohort differences in growth

Growth variability in the CC was higher than in the WC for both females and males. ANCOVA tests confirmed that the effect of hatching season on growth was significant for both sexes (Females, $F_{1,83}$=17.1, p<<0.001; Males, $F_{1,111}$=8.1, p<0.01), showing that mean length-at-age, in the period between 180 days of age and the end of the life cycle, was higher in the WC (175 mm for females, n=43 and 224 mm for males, n=59) than in the CC (145 mm for females, n=43 and 174 mm for males, n=55). In squid younger than 180 days, no significant differences in mean mantle length were found between cohorts (WC: 56 mm, n=30 and CC: 42 mm, n=11) (ANCOVA $F_{1,38}$=2.5, p>0.05).

The best fitted growth models for each seasonal female cohort showed differences between cohorts (Table 4). The growth of the WC females was asymptotic, following a logistic model with an inflexion point at 237 days and asymptotic ML at 303 mm, whereas the growth of the CC females followed a power model (Figure 22a,b). The growth models indicate that after 350 days, CC females are larger. However, all observed CC females were less than 1 year old. The fit of asymptotic models to the CC females proved inadequate, yielding very high ML_∞ values and growth inflexions close to the maximum age.

On the other hand, in WC females growth was clearly asymptotic (even if the three oldest specimens are discarded) and the non-asymptotic models showed clear systematic patterns of the residuals and lower goodness of fit (Figure 23a,b). The logistic model was adopted because it showed higher r^2 and a slightly better fit in the lower ages than the Gompertz model.

In the case of males there was no evidence of asymptotic growth in either cohort and non-asymptotic models were adopted to describe growth of both seasonal cohorts (Table 4). The growth of the CC males (Figure 24a) is better fitted by an exponential model (acceptable residual pattern and higher goodness of fit), whereas the growth of WC males (Figure 24b) is better fitted by a power model (all other models present systematic patterns in residuals and a lower goodness of fit). The CC males had lower ML-at-age than the WC males until 360 days old, and higher ML-at-age above this age.

Monthly variation of growth rates was such that the DGR of the WC females increased sharply until a maximum was reached during the 8th month (corresponding to the growth inflexion point) and decreasing thereafter (Figure 25a). On the other hand, the DGR of the CC females increased steadily until the end of the life cycle. The WC growth rate was higher until squid were 270 days old ($AvgDGR_{90:270WC}$ = 0.96 mm/d, $AvgDGR_{90:270CC}$ = 0.74 mm/d) and the opposite was true after that age ($AvgDGR_{270:360WC}$ = 0.84 mm/d, $AvgDGR_{270:360CC}$ = 1.13 mm/d). As in females, the DGR of the WC males (Figure 25b) was higher until they reached 270 days old ($AvgDGR_{90:270WC}$ = 0.91 mm/day, $AvgDGR_{90:270CC}$ = 0.66 mm/day) and the opposite was verified after that age($AvgDGR_{270:360WC}$ = 1.47 mm/day, $AvgDGR_{270:360CC}$ = 1.88 mm/day).

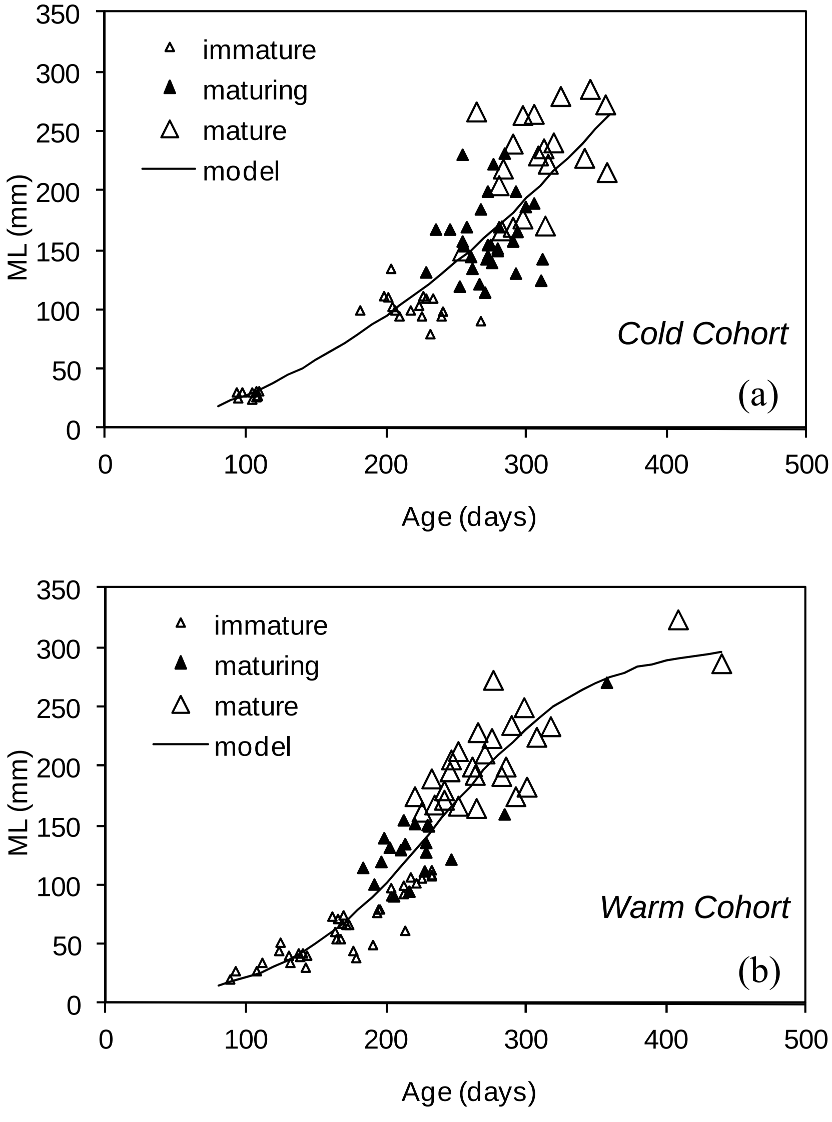

Figure 22 - The relationship between age and mantle length for *Loligo vulgaris* females of (a) the Cold Cohort (CC) and (b) the Warm Cohort (WC). The fitted curves are the power model for the CC and the logistic model for the WC.

Table 4 - Parameter estimation of the adopted growth models for the females of the Cold Cohort (FCC), females of the Warm Cohort (FWC), males of the cold Cohort (MCC) and males of the Warm cohort (MWC). * standard error of of ln(a), parameter estimation with ln transformed data.

FCC Growth Model		**a**	**b**	
	Estimate	0.009	1.75	
Power model	Standard error	0.37*	0.07	
$ML=a*t^b$	t-value (df=76)	-12.6	25.7	
$r^2=0.90$	p-level	0.00	0.00	
	lower confidence limit	0.004	1.61	
	upper confidence limit	0.019	1.88	

FWC Growth Model		**ML∞**	**k**	**t_i**
	Estimate	302.6	0.019	236.8
Logistic model	Standard error	18.7	0.002	8.1
$ML=MLoo/(1+exp(-k*(t-t_i)))$	t-value (df=82)	16.2	10.8	29.4
$r^2=0.87$	p-level	0.00	0.00	0.00
	lower confidence limit	265.3	0.015	220.8
	upper confidence limit	339.9	0.022	252.9

MCC Growth Model		**a**	**b**	
	Estimate	17.5	0.008	
Exponential model	Standard error	3.1	0.001	
$ML=a*exp(b*t)$	t-value (df=78)	5.7	14.3	
$r^2=0.75$	p-level	0.00	0.00	
	lower confidence limit	11.4	0.007	
	upper confidence limit	23.7	0.009	

MWC Growth Model		**a**	**b**	
	Estimate	0.007	1.84	
Power model	Standard error	0.003	0.09	
$ML=a*t^b$	t-value (df=97)	2.0	21.5	
$r^2=0.85$	p-level	0.045	0	
	lower confidence limit	0.000	1.67	
	upper confidence limit	0.013	2.00	

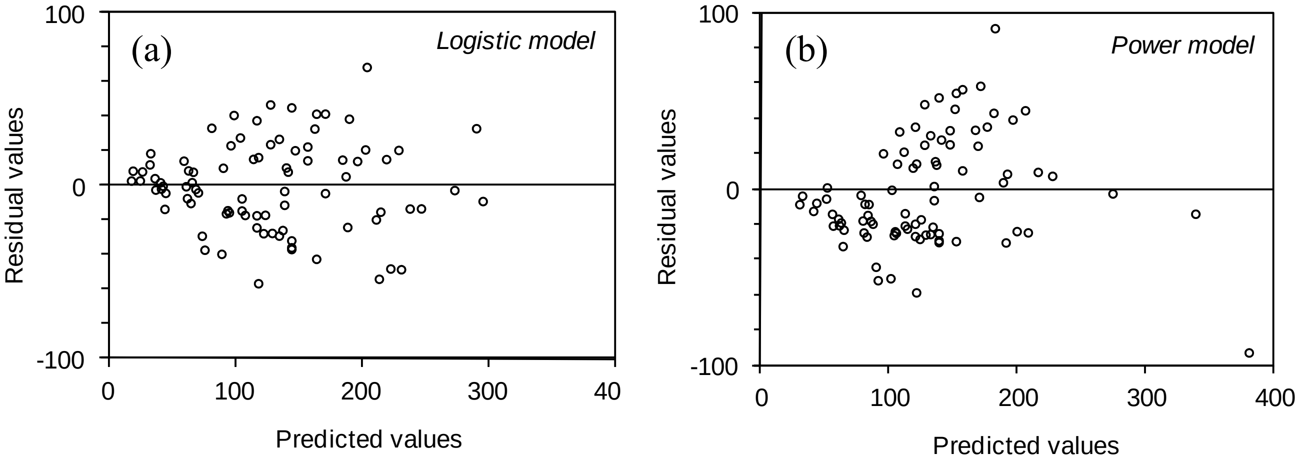

Figure 23 - Example of residual versus predicted values from the fitting of (a) an asymptotic growth model or (b) a non-asymptotic growth model to data for the *Loligo vulgaris* Warm Cohort females.

4.4. Discussion

The preliminary GAM modelling indicated that, for squid, which experienced SST up to around 16.5 ºC during the first three months of their life, the effect of SST on length-at-age was positive. Above this temperature there was no further consistent increase in length-at-age related to increased SST. The analysis also revealed significant effects of hatching month and year, even after the effect of SST was taken into account. For the purpose of fitting parametric growth models we have ignored these latter effects and defined two "cohorts" representing "cold" and "warm" portions of the SST range.

In spite of the similar sizes reached in both cohorts, a longer lifespan was detected in the WC squid, which spend their adult life in colder waters than those occupied by the CC. This leads to the hypothesis of intraspecific geographical variation in life span, with a tendency for lower longevity in the populations living in warmer waters. This is corroborated by the higher maximum age of *L. vulgaris* in the NW Portuguese waters (ca. 15 months) than that observed in the NW African coast (10-13 months, Raya *et al.*, 1999, Arkhipkin, 1995), where the average annual SST is ca. 4 ºC higher (Moreno *et al*., 2002).

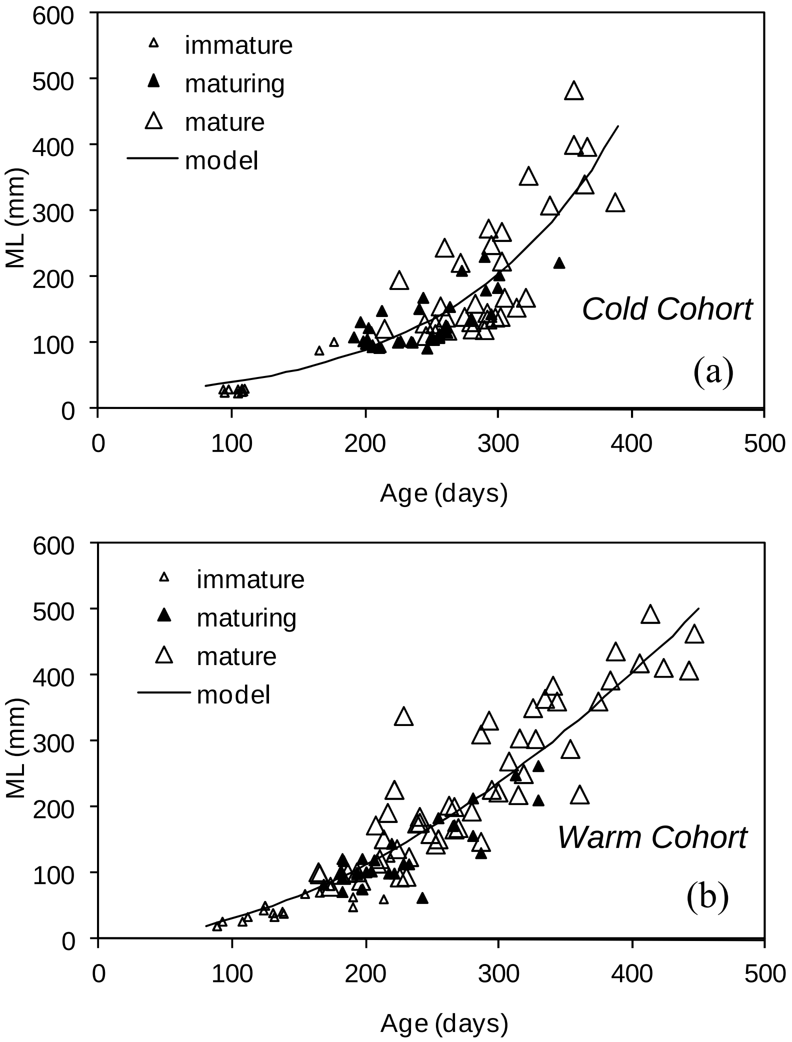

Figure 24 - The relationship between age and mantle length for *Loligo vulgaris* males of (a) the Cold Cohort (CC) and (b) the Warm Cohort (WC). The fitted curves are the exponential model for the CC and the power model for the WC.

4.4.1. Early growth

The analysis of growth rates (DGR) showed that juvenile squid grow to 75 mm ML in 170 days. This growth rate is slower than that observed for the same species by Turk *et al.* (1986) in captivity (75 mm in 112-140 days), but similar to what Arkhipkin (1995) observed in nature on the NW African coast (70-80 mm in 150-160 days). Higher growth rates up to 270 days of age were detected in squid hatched under warm temperatures, reflecting the influence of environmental factors. Water temperature itself may be the most important factor because of accelerated metabolism, but other factors such as food availability, may be limiting, while others, such as the photoperiod may act as cues to trigger internal responses. Zooplankton abundance on the NW Portuguese coast is, in general, higher between May and October and lower between December and February (Cunha, 1993), thus providing more favourable feeding conditions for the early stages of the WC than for those of the CC. In culture experiments, Villanueva (2000) observed a higher ML (double after 50 days) in *L. vulgaris* hatched and maintained under high temperatures (19.5 ºC) compared with those hatched and maintained (also for 50 days) under low temperatures (12.2 ºC). In our study, in spite of the differences in growth rates between cohorts, the observed differences in the size-at-age of young squid were not statistically significant.

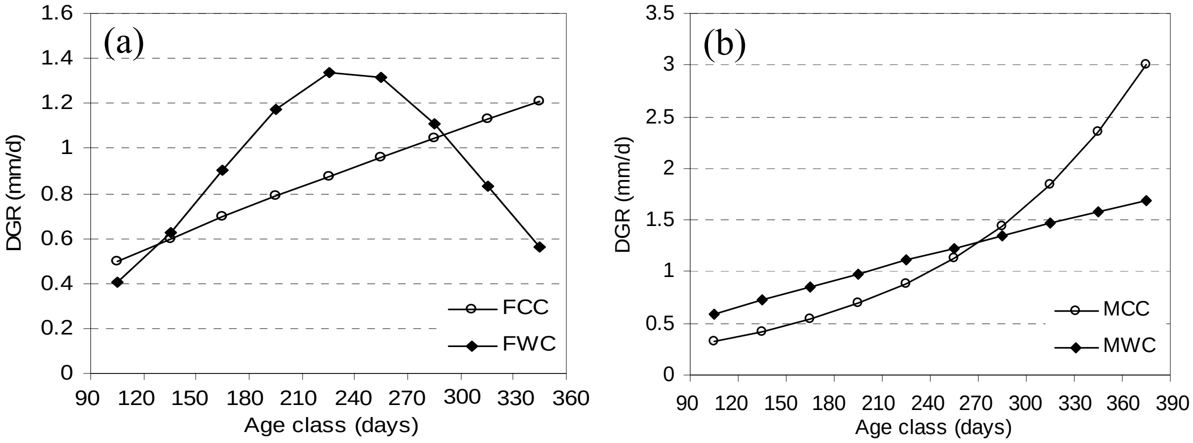

Figure 25 - Variation of Daily Growth Rate (DGR) through the life cycle for *Loligo vulgaris* (a) females and (b) males of the Cold Cohort (FCC and MCC) and the Warm Cohort (FWC and MWC). Growth rates calculated in 30-day time intervals.

One explanation for this is that WC squid are smaller in size-at-hatching, masking the result of a higher early growth rate at higher temperatures (Pecl *et al*., 2004). After some time, larger hatchlings growing slower may reach similar sizes to those reached by smaller hatchlings growing faster. Moreover, in a study based on wild caught specimens, individual variability may also mask the differences between cohorts, particularly when the absolute difference in growth is necessarily of a small magnitude due to the smaller size of the specimens. Nevertheless, it should be noted that our ability to detect significant differences might have been compromised by the low sample size of the young squid from the CC; thus further research may be required to support the above hypotheses.

4.4.2. Gender differentiation

The short life span of squid and their ability to generate new muscle fibres throughout their lives (Moltschaniwskyj, 1994) have been cited as the main features supporting continuous and non-asymptotic growth. In fact the majority of growth models used so far to describe the growth of *Loligo* species were linear or near linear, hence, with no inflexion point or asymptotic length. Based on a wide range of individual sizes and ages from our study, *L. vulgaris* growth along the NW Portuguese coast differs between the sexes. Female growth clearly showed an inflexion point followed by a decrease in the growth rate, best described by a logistic model, while males exhibited growth according to a power model. Although females grow asymptotically (even if they only approach the asymptotic ML by the end of lifespan), both sexes demonstrate continuous growth. Raya *et al*. (1999) also observed a decrease in the growth rate of *L. vulgaris* females in the NW African coast beyond a certain age and, because sampling data included few old females, the hypothesis of asymptotic growth in females was not excluded. More importantly however, our study revealed that apart from sexual differences in size-at-age, mainly in the second half of the life cycle, the growth strategy of *L. vulgaris* is also sexually differentiated. Depending upon the environmental conditions to which they are exposed through their life cycle, females may exhibit asymptotic growth or not. On the other hand, male growth rates always show an increasing trend and their reaction to environmental conditions, is revealed mainly by the magnitude of that increase. A description of these strategies is presented below and the reason why they differ is discussed in light of endogenous and exogenous factors.

4.4.3. Growth strategies in males v. females

Differences in the ML-at-age between sexes at a certain age were previously reported for *L. vulgaris* (e.g. Arkhipkin, 1995; Rocha and Guerra, 1999). In our results, sexual differences in growth were detected mainly after the age of 8 months, when males continue to increase growth rates, whereas some of the females experience a growth inflexion. The inflexion point of female growth (8.6 months) coincides with their t50% (8.7 months, Moreno *et al*., 2005), indicating that maturation is closely related to the decrease in the somatic growth rate. This is similar to the results obtained by Smith *et al.* (2005) for *Loligo forbesi* and agrees with the sexual difference in squid reproductive investment that can be seen from the very high differences between the gonadosomatic indices of males and females (Stearns, 1992).

In wild populations, faster growth of summer hatchers and slower growth of winter hatchers was reported by Raya *et al*. (1999) in *L. vulgaris* from the Saharan Bank. Hatfield (2000) and Natsukari *et al*. (1988) also observed a significantly higher length-at-age in squid hatched in the warmer season in relation to those hatched in the cold season, in wild populations of *Loligo gahi* in Patagonia and *Photololigo edulis* in Japan, respectively.

When analysing the growth of each cohort, we observed that environmental conditions throughout the life cycle may also strongly influence the growth strategy of either sex, which fits distinct growth models. The growth inflexion of the general female population was not detected in the females of the CC, in which growth after 90 days follows a power model. As a result of the low early growth rates, the minimum size at maturity (15 cm, Moreno *et al*., 2005) is attained later in life in the CC, when these squid are approximately 8 months old, during the summer months. The summer environmental conditions of higher temperature, food availability and day-length favour the delay of maturation and high growth rates. Because significant reproductive investment is only made later in life, after 10 months of age (Moreno *et al*., 2005), and the transfer of metabolic resources from somatic to reproductive growth can be compensated by the favourable autumn conditions when many prey items become more abundant (IPIMAR unpublished data, Pierce *et al*., 1994c), there is no significant change in growth pattern. The females of the WC cohort on the contrary, grow initially under favourable spring and summer environmental conditions, maturing younger, as soon as they reach the minimum size threshold, which explains the lack of overlap between maturing and mature individuals shown in Figure 22b. However, as this minimum size at maturity happens mainly during winter and early spring under more adverse environmental conditions, they then experience a shortage of resources when they must spend more energy in maturation.

The association of both exogenous and endogenous factors will thus contribute to the observed decrease in their growth rates beyond 7 months of age, i.e. from the age at maturity when reproductive investment increases significantly ($t_{50\%\ WC}$ = 7.3 months, Moreno *et al.*, 2005). Ultimately the exogenous factors appear to be the most important factors affecting growth of juvenile (immature) females. The growth of sub-adult and adult (maturing and mature) female is more dependent on a coupling between exogenous and endogenous factors, which regulate the maturation process. Male growth rates in both seasonal cohorts show an increasing trend. Male somatic growth is little affected by the maturation process and, because of this; males are able to adjust resource allocation to suit their ambient environment more closely than do females (Steer and Jackson, 2004). Thus the magnitude of their growth rate is more directly related to the influence of environmental conditions.

The “second phase of growth” of cephalopods has been frequently considered as a temperature-independent phase (Grist and des Clers, 1999). However from our results, it seems quite evident that environmental conditions, although not as dramatically as in the paralarval phase, play a significant role in the rate and shape of growth of squid through the life cycle. The existence of differences in growth rates, rates of maturation and longevity, among others, mediated by environmental factors, emphasizes the need to take environmental data into consideration in fisheries models, particularly in times of acknowledged environmental global change.

Chapter 5

The effect of temperature on growth of early life stages of the common squid *Loligo vulgaris*

A. Moreno[1], G.J. Pierce[2], M. Azevedo[1], J. Pereira[1], A.M.P. Santos[1]

1 IPIMAR, Av. Brasília, 1400-038 Lisboa, Portugal

2 University of Aberdeen, Oceanlab, Main Street, Newburgh, Aberdeenshire, AB41 6AA, UK

Journal of the Marine Biological Association of the United Kingdom (2012) in press

5. The effect of temperature on growth of early life stages of the common squid *Loligo vulgaris*

5.1. Introduction

The common squid *Loligo vulgaris* Lamarck, 1798 is a commercially important species in the ICES area, and is particularly valuable in the Portuguese market where it attains the highest first sale value of all finfish and mollusc species (Pilar-Fonseca *et al*., 2009). This long-finned squid is a fast-growing shallow water species found in the Mediterranean Sea and in the Atlantic Ocean from the North Sea (55º N) to the African coast (20º S). There is considerable geographical variability in life history traits across its distribution range. The physiology of squid enables them to rapidly change their growth rates in response to environmental changes (O'Dor and Webber, 1986; Jackson and Domeier, 2003). This plasticity in growth results in squid changing their life-history features in accord with seasonal changes in their environment (e.g. *L. gahi*, Hatfield, 2000; *L. pealei*, Brodziak and Macy, 1996; *Sepioteuthis lessoniana*, Jackson and Moltschaniwskyj, 2002; *L. forbesi* and *L. vulgaris*, Rocha and Guerra, 1999).

In waters of the northwest Portuguese shelf, located within the Canary Current Upwelling System, *L. vulgaris* has two main spawning seasons, one in late autumn/winter and the other in late spring (Moreno *et al*., 2002), which results in two main hatching seasons (or cohorts) (Moreno *et al*., 2009). Consequently, the early life stages of the two cohorts develop under very different environmental conditions. Previous studies have shown important differences between the so-called "cold cohort" and "warm cohort", namely in terms of age at maturity (Moreno *et al*., 2005), growth trajectories (Moreno *et al*., 2007), fecundity and egg size (Boavida-Portugal *et al*., 2010). The results of these studies, which analyse the effects of environmental conditions on the juvenile and adult phases, raised questions regarding the impact of such environmental factors (notably temperature) on growth during the early life stages of *L. vulgaris* in Portuguese waters.

Experiments in captivity have shown that the duration of the embryonic development of *L. vulgaris* is negatively correlated with temperature (Villanueva, 2000a; Sen, 2005a). At the same time, the weight, mantle and statolith length of newly hatched paralarvae are positively correlated with the duration of the embryonic development (Villanueva, 2000a,b). Salinity is another important environmental variable that may impose physiological limits on the embryonic development of cephalopods (Cinti *et al.*, 2004). Within the tolerance limits, lower salinity values have been reported to maximize the hatching success of *L. vulgaris* eggs and to produce larger hatchlings in captivity (Sen, 2005b). However, embryo survival appears to be drastically reduced at salinities below 32 or above 38 and any large fluctuations in salinity may also be lethal for the eggs (Sen, 2005b). Steer *et al.* (2003) demonstrated that larger hatchlings of *Sepioteuthis australis* within a given cohort have better survivorship than smaller hatchlings.

Post-hatching growth in length is usually described as exponential and is strongly correlated with temperature, with the initial growth rate ranging between 0.05 mm.d^{-1} at 11 ºC and 0.17 mm.d^{-1} at 19.2 ºC (Villanueva, 2000b). As hypothesized by Forsythe (1993), small changes in temperature during the early growth phase (~90 days) of the life of a squid can result in marked differences in size-at-age.

L. vulgaris hatchlings range in size from 2 to 3 mm mantle length (ML) and are planktonic for at least 3 months (González *et al.*, 2010). Squid swim actively by jet propulsion as soon as they hatch, and fin beating plays only a minor role in the general locomotion (Boletzky, 2003). In loliginids, the transition from the paralarval to the juvenile phase is not characterized by an abrupt morphological change (as in ommastrephids) or a marked habitat change (as in octopodids), yet involves important anatomic and physiological changes. There is an ontogenetic shift in the squid locomotory system, from the dominance of mitochondria-rich and fatigue-resistant muscle fibres, which power the jetting movements of paralarvae, to the use of mitochondria-poor muscle fibres for escape- and attack-jetting movements in the nektobenthic juveniles and adults, a shift which delivers the increased acceleration power required by larger individuals (Preuss, 1997) The arrangement and volume of intramuscular collagen fibres of the outer tunic also changes during ontogeny, affecting the kinematics of mantle movement and elastic energy storage during jet locomotion (Thompson and Kier, 2001). In *Loligo opalescens* the relative proportion of the different types of muscle fibres typical of adults is achieved by 8 weeks post-hatching (Preuss, 1997) and in *Sepioteuthis lessoniana* the organization and volume fraction of collagenous connective tissues of adult is achieved at 15 mm ML (Thompson and Kier, 2001).

Although squid paralarval growth and survival depend mainly on temperature and food availability in culture conditions (Vidal *et al*., 2002), in the wild, the faster the hatchlings grow the greater their chances of avoiding predation. As the duration of planktonic (jetting) paralarval phase (PLD, by analogy to fish) is apparently to some extent size-dependent, it will be indirectly dependent on water temperature. Therefore, the PLD of each *L. vulgaris* cohort is expected to be different, with implications for their survival.

Environmental changes and life cycle transitions affect individual metabolism and/or growth, and are thus recorded chronologically in the microstructure of calcified structures, such as the squid statoliths. The lack of resorption or alteration of the material deposited within the statoliths, allows features of their microstructure to be used as natural tags, as has been the case for fish otoliths (Campana and Neilson, 1985). Individual history can be analysed either visually (e.g. checks, increment width and contrast) or chemically (e.g. minor elements, trace elements and stable isotopes) (Arkhipkin, 2005; Semmens *et al*., 2007). Villanueva (2000a,b) examined the statolith microstructure of *L. vulgaris* embryos and age-validated paralarvae (up to 60 days old), demonstrating the usefulness of these structures to reconstruct events during the early part of the squid life cycle in wild populations. Villanueva *et al*. (2003) went on to examine the part of the statolith corresponding to the embryonic stage and to make inferences about the ambient temperatures experienced during embryonic development in wild *L. vulgaris* populations.

Based on their results on ages of *L. vulgaris* paralarvae obtained from the wild, González *et al*. (2010) suggested that squid age may be underestimated by as much as 3 months when counting the increments in statoliths of adult specimens. Implicit in their conclusion was the idea that some increments corresponding to early growth become obscured as the squid becomes older.

Ontogenetic shifts have been identified in the statolith growth pattern of some squid species, apparently related to the transition from the paralarval stage to the juvenile stage (Arkipkin, 2005). The age at which a change is seen in the width of daily increments (i.e. the growth inflection) in fish otoliths is widely used as an indicator of the duration of the planktonic larval stage (Sponaugle, 2010). By analogy, analysis of the statolith accretion pattern could represent a useful tool to determine PLD in *L. vulgaris*.

In the present study we aim to provide a better understanding of recruitment processes in *L. vulgaris* in northwest Portuguese shelf waters, using juvenile and adult statoliths as records of early-life events. We ask two main questionss: (1) what are the effects of temperature and salinity on embryonic and post-hatching growth in *L. vulgaris*? and; (2) is there evidence of a growth inflection in the statolith accretion pattern (i.e. the sequence of increment widths), which might represent the ontogenetic shift from planktonic to nektobentonic life and could thus represent a measure of the planktonic paralarval phase duration (PLD)? In addition, we examine evidence that statolith increments representing early growth become unreadable, resulting in underestimates of age, as proposed by González *et al*. (2010).

5.2. Material and Methods

5.2.1. Biological data

Juveniles and adults of *L. vulgaris* (ML ranging between 21 and 465 mm) were captured every month between January 1993 and March 2000 over the northwest Portuguese shelf, and their statoliths removed. To facilitate age readings, a transverse section of each statolith was obtained by grinding on both sides. Counts and measurements of increments were made on digitized mosaic images of statoliths, using the image analysis software TNPC 4.1 (Noesis). Prepared statoliths were screened to identify those in which the natal ring (the first prominent check, which is formed at hatching) was identifiable and the increments in the rostrum area were clearly visible. In addition we aimed to obtain adequate samples of squid living under distinct environmental conditions (>25 statoliths per hatching trimester, independently of hatching year). This led to a final selection of 161 statoliths. For each statolith 5 to 9 images, with slight changes of the focus plane, were used to build a mosaic, which guarantees that the final mosaic image allows correct interpretation of the statolith microstructure (avoiding two increments being interpreted as one or incorporation of sub-daily increments, etc.). The standardised procedures for age determination of squid using statoliths are detailed in Moreno (2002).

In each statolith (N=161) the total number of increments was counted from the natal ring to the tip of the rostrum. Age was determined as the total number of increments, assuming that statolith increment deposition has a daily rhythm, as previously found in *L. vulgaris* paralarvae (Villanueva, 2000b) and in cultured juveniles and adults of the subspecies *L. vulgaris reynaudii* (Durholtz *et al*., 2002). The hatching date of each individual was back-calculated as the capture date minus the age in days. The distance between the focus and the natal ring (Figure 26), i.e. the statolith radius at hatching measured along the rostrum (RH, mm), was used to estimate growth during the embryonic phase.

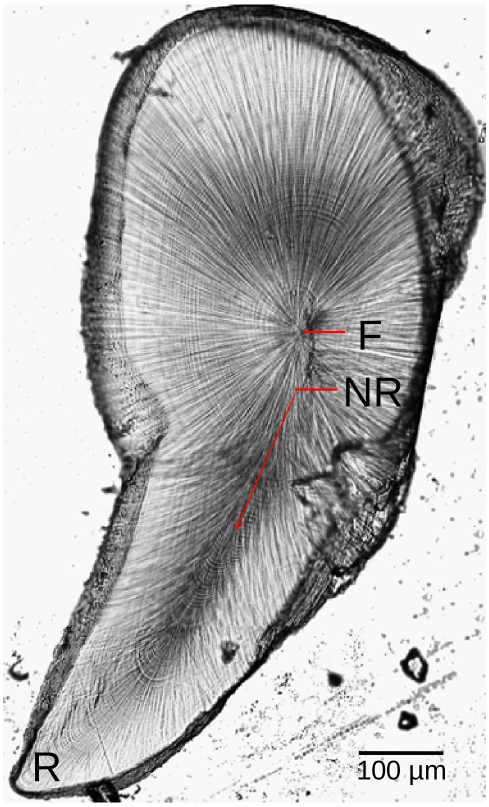

Figure 26 - Light micrograpth of a *Loligo vulgaris* statolith showing the focus (F), the natal ring (NR) and the growth axis where increments counting and measurements were made on the rostrum (R).

The statolith accretion pattern after hatching was analysed by measuring the maximum increment widths from the natal ring to the 126th increment (~18 weeks old) along the rostrum growth axis. At this stage the sample was reduced to 130 statoliths. Individuals younger than 126 days (incomplete record) and those in which the microstructure was not clear along the rostrum growth axis were not included.

The significant correlation between age, statolith size and dorsal mantle length in *L. vulgaris* suggests that somatic growth and statolith growth are related (Moreno *et al.*, 2007), as in other squid species (Hatfield, 2000). Therefore, measurements of the daily growth increments provide a measure of individual growth rates from hatching. The statolith growth in the first 3 months of life (R90d, mm) was estimated for each individual (N=130) as the sum of the first 90 post-hatching increment widths. The choice of this number of days was based on the modelling of temperature effects on early growth by Forsythe (2004) and on the maximum age of *L. vulgaris* paralarvae recorded in plankton samples by González *et al.* (2010).

5.2.2. Temperature and salinity data

Average sea bottom temperature (SBT_H) and bottom salinity (SBS_H) were computed for the month before hatching of each individual. This was used as a proxy of the conditions for the duration of the embryonic phase, to test for effects on RH. Average sea surface temperature (SST) and average sea bottom temperature (SBT) were also computed for the first 30, 60 and 90 days post-hatching (SST_{30d}, SST_{60d}, SST_{90d}, SBT_{30d}, SBT_{60d} and SBT_{90d}), to test for effects on statolith growth during early life (R_{90d}).

SBT and SBS data were obtained from CTD casts extracted from IPIMAR, NODC and ICES databases. Weekly (8 days) mean satellite-derived SST data were obtained from the AVHRR Pathfinder V.5 provided by NASA-JPL-Physical Oceanography Distributed Active Archive Center (PO.DAAC). These data were spatially averaged for a region over the Western Iberia continental shelf between 41.5º-38.5º N and between the 35 m and 125 m isobaths using NOAA PMEL Ferret software.

5.2.3. Data analysis

We tested the proposal by González *et al.* (2010) that early increments are "lost" due to their close proximity to each other rendering them unreadable by calculating the relationship between the number of increments counted in the first 0.36 mm post-hatching (NIR_1) and statolith size (hatching ring to the tip of rostrum, R), since we would expect a decrease in NIR_1 as a function of (increasing) R if there was a loss of readable rings.

To explore the effect of temperature and salinity on growth during embryonic development and early life, Generalized Additive Models (GAMs, Hastie and Tibshirani, 1990) were fitted to RH and R_{90d} data. Hatching year (Hyear) was included in models (as a nominal variable) to account for interannual differences unrelated to temperature or salinity. Histograms of the response variables were used as guidance to the appropriate family distribution and link function: a normal distribution seemed to be appropriate and an identity link function was therefore used. The assumptions of homogeneity of variance and normality (of the RH and R_{90d} series) were verified, and data were checked for influential observations (Zuur *et al.*, 2010). Bi-variate and multivariate collinearity between the explanatory variables were checked. Effects of explanatory variables were fitted as smoothers, using the thin plate regression spline method and limiting the number of "knots" (*k*) in the smoother by setting a maximum value of 5 (which limits degrees of freedom to 4 or less) to avoid fitting unrealistically complex relationship (i.e to avoid overfitting). Smoothing parameters were estimated using Generalized Cross Validation (GCV) (Gu and Wahba, 1991) subject to the above-mentioned constraint on *k*. To validate each model, residuals were checked for patterns and departures from normality.

A backwards selection process was used for the model of RH, starting with a full model (i.e. including all candidate explanatory variables: Hyear, SST_H, SBT_H and SBS_H) and sequentially removing explanatory variables with non-significant partial effects. There were very few observations for years 1995, 1997 and 1998 (n=9 in total) and these were excluded to allow evaluation of interannual differences. For R_{90d}, due to the larger number of candidate explanatory variables and the high collinearity of the different temperature series, a stepwise forwards procedure was carried out, fitting models sequentially with one, two and three explanatory variables, choosing the best model at each step as the building block for the subsequent step. In this case, post-hatching sea surface temperature and sea bottom temperature were included in models at different time lags (30, 60 or 90d), to determine which time-lag had the strongest effect on R_{90d}. For both SST and SBT, only one of the three possible time-lags could be included in the model (due to high collinearity in SST at different time-lags, as for SBT). Explanatory variables with non-significant partial effects were dropped from models. The Akaike Information Criterion

(AIC) was used to choose the best fitting model (lowest AIC) for each response variable, subject to the other criteria mentioned previously.

The statolith accretion pattern in the first 126 days of life (~18 weeks) was analysed to determine if the increment widths changed abruptly at a given age, which could be related to the age of the transition from paralarvae to juvenile (A_{LJT}). To achieve this, the sample was split into 1 ºC SST_{90d} classes (i.e. classes based on the explanatory variable with strongest effect on R_{90d}), thus creating 5 classes (SST_{90d} =13, 14, 15, 16 and >17 ºC). For each of these groups, a piecewise (segmented) linear regression (PLR) was fitted to the mean statolith increment width (IW) by age (A) data, to test the hypothesis that the data can be represented by two linear segments with different slopes, connected at A_{LJT}:

$$IW=\beta_o+\beta_1 A \qquad \text{when } A \leq A_{LJT}$$

$$IW=\beta_o+\beta_1 A+\beta_2(A-A_{LJT}) \qquad \text{when } A>A_{LJT}$$

where β_o is the intercept, β_1 is the slope of the left linear segment, β_2 is the difference in slopes, and A_{LJT} is the breakpoint. The model parameters were estimated by means of simple iterative fitting of linear models as described in Muggeo (2003, 2008). Smoothed scatter plots of the *IW~A* relationships (for each SST_{90d} class) were used as a guide to setting appropriate starting values for A_{LJT}. Wald-based 95% confidence intervals (CI 95%) were calculated for each parameter.

Computations and analyses were performed using R (2008, http://www.R-project.org) and the packages mgcv (Wood, 2000) and segmented (Muggeo, 2008).

5.3. Results

5.3.1. Growth during the embryonic phase

The statolith radius at hatching (RH), measured along the rostrum, ranged between 0.052 and 0.118 mm, with slight monthly variation (Figure 27). No significant bi-variate or multi-variate collinearity was found between possible explanatory variables, and weak correlations were seen between each explanatory variable and the response variable

RH.

These relationships were explored further with GAMs, using a Gaussian distribution with identity link. A full GAM model showed no significant effects of temperature, either SST_H or SBT_H, on the statolith growth during the embryonic development (Table 5). The best-fit GAM explains about 16% of deviance and included effects of year and bottom salinity. It has the form: RH ~ 1 + factor(Hyear) + s(SBS_H).

A significantly larger statolith radius at hatching was seen in 1993 and 1994, compared to 1993. SBS_H has a negative linear (partial) effect on RH. The model indicates faster embryonic statolith growth at lower salinities, although the relationship is clearest (confidence limits are narrowest) in the 35.75 - 35.95 range, i.e. where we have most data.

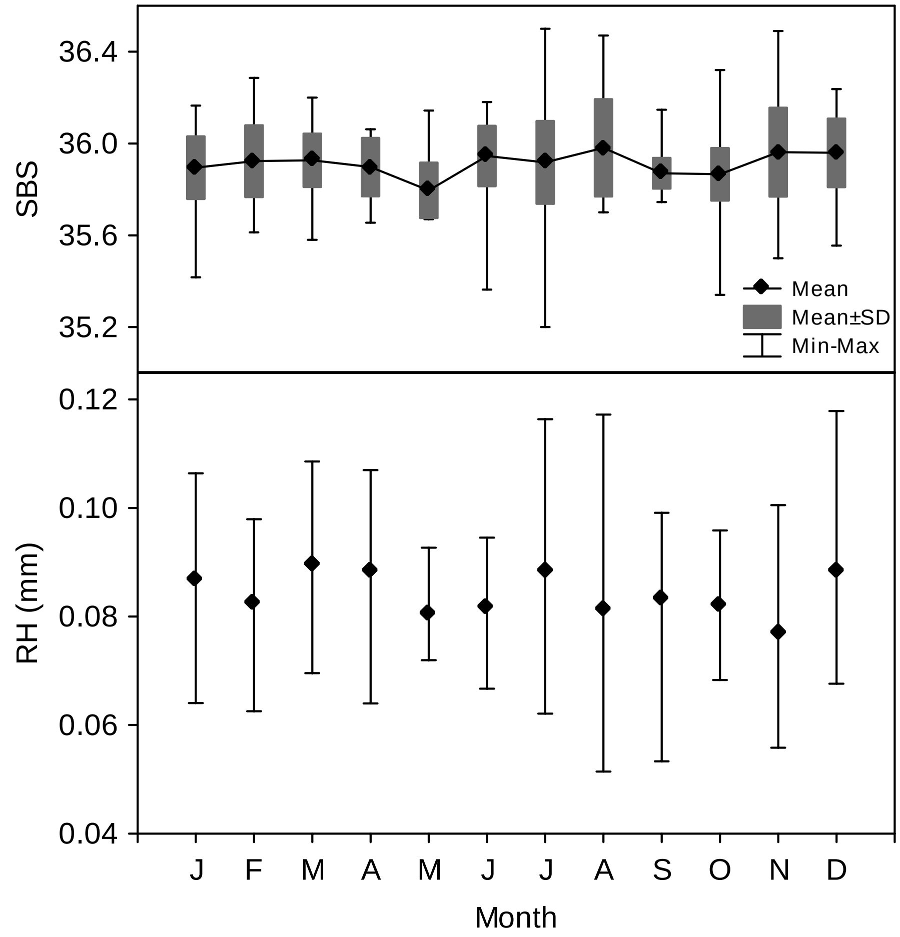

Figure 27 - Monthly variation of the statolith rostrum size at hatching (RH) and sea bottom salinity (SBS).

Table 5 - Results for the GAMs fit to RH. Estimates, SE, t-ratio and associated parameters are given for the nominal variable Hyear (hatching year); 1992 is the reference Hyear. Edf, F-statistic and associated probabilities are given for the smoother and for overall Hyear. SE, standard error; Edf, estimated degrees of freedom; AIC, Akaike Information Criterion.

Explanatory variables	**Estimate**	**SE**	**t-ratio**	**p-value**	**Edf**	**F-statistic**	**p-value**	**Deviance explained**	**AIC**
Hyear					4	4.910	0.0009***	16.2%	-869.01
1993	0.0143263	0.0043874	3265	0.00137**					
1994	0.0127897	0.0039794	3214	0.00162**					
1996	0.0002994	0.0056604	0.053	0.95788 *ns*					
1999	0.0086206	0.0048994	1760	0.08063 *ns*					
SBS_H					1	7.154	0.0083**		

5.3.2. Growth during the first 3 months

In relation to the hypothesis of González *et al.* (2010), we found no significant relationship between the number of increments counted in the first 0.36 mm post-hatching (NIR_1) and statolith size (hatching ring to the tip of rostrum, R). The slope parameter (b=7.813) not only was not-significant (N=126, r^2=0.023, p=0.0862), it was also not negative. We therefore assume that we did not underestimated the age of juvenile and adult specimens.

Seasonal variation can be observed in early statolith growth, with higher R_{90d} seen in squid hatched in summer months (Figure 28). The SST variables at different time-lags were highly correlated with each other and this was also true for SBT. Therefore, a series of models was fitted, each including one of the SST or SBT variables, enabling us to determine which SST and which SBT had the strongest effects on R_{90d}. Additionally, strong collinearity was verified between SST_{30d} and SBT_{90d} and between SST_{60d} and SBT_{90d}, and these pairs of variables were therefore not included in the same model.

The models implemented with one, two and three explanatory variables and the criteria for the selection of the optimal set of explanatory variables are listed in Table 6.

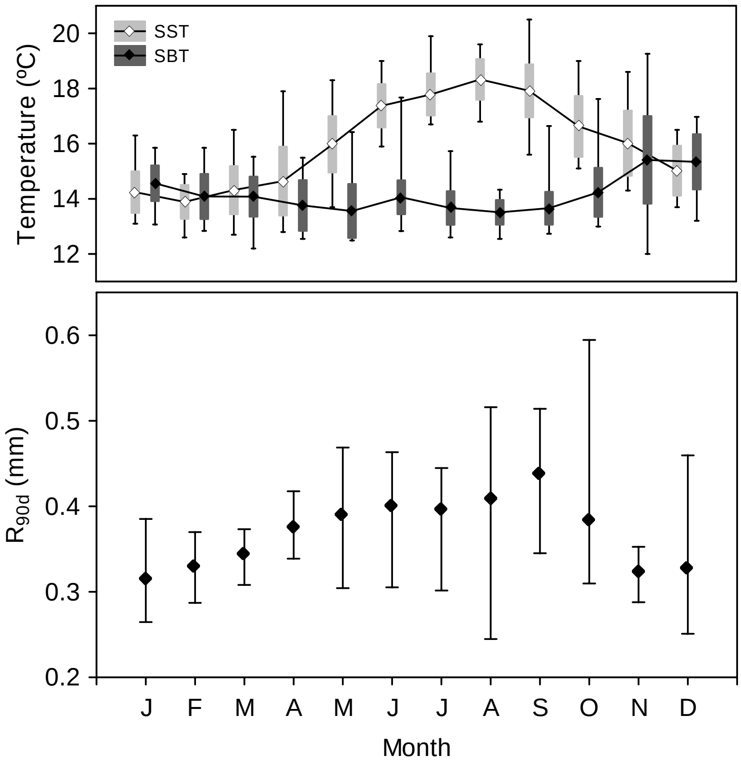

Figure 28 - Monthly variation of the statolith growth during the first 90 days post-hatching (R_{90d}), sea surface temperature (SST) and sea bottom temperature (SBT). Dots represent the mean, the whiskers the size range and the boxes the standard deviation.

The best-fit GAM for R_{90d} explains about 57% of deviance and has the form: R_{90d} ~ 1+ factor(Hyear) + s(SST_{90d}) + s(SBT_{90d}). Residuals were not exactly normally distributed although all other diagnostic plots indicated a good model fit. Transformation of R_{90d} (e.g. square root or log) did not improve their distribution and we therefore continued with untransformed data. Ideally interaction terms should be tested. Indeed, inclusion of an interaction between the effects of SST_{90d} and Hyear (by allowing separate smoothers for SST_{90d} in each Hyear) improved goodness of fit (F-test, $p<0.0001$). However there were insufficient data in some years to adequately describe the effect of temperature and we therefore present only the simpler model.

Table 6 - Results for the GAMs fit to R_{90d}. Estimates, SE, t-ratio and associated parameters are given for the nominal variable Hyear (hatching year); 1992 is the reference hatching year. Edf, F-statistic and associated probabilities are given for smoothers and for overall Hyear. SE, standard error; Edf, estimated degrees of freedom; AIC, Akaike Information Criterion.

Model variables	**Estimate**	**SE**	**t-ratio**	**p-value**	**Edf**	**F-statistic**	**p-value**	**Deviance explained**	**AIC**
Hyear					6	7.4	0.0001***		
1993	0.0203	0.0166	1.219	0.2254 *ns*					
1994	0.0441	0.0151	2.923	0.0042 **					
1995	0.0765	0.0318	2.403	0.0178 *				57.3%	-446.84
1996	0.0960	0.0189	5.067	<0.0001***					
1997	0.0251	0.0276	0.885	0.3779 ns					
1999	0.0564	0.0191	2.942	0.0039 **					
SST_{90d}					1.182	17.14	<0.0001***		
SBT_{90d}					3.134	5.79	0.0008***		

Inter-annual differences were detected and there was significantly higher statolith growth at 90 days post-hatching in squid hatched in 1994, 1995, 1996 and 1999 than in 1992 and 1993 (Table 6). The strongest effect of temperature on R_{90d} was observed when considering temperatures (both SST and SBT) measured 90 days post-hatching. The partial effect of SST_{90d} on R_{90d} is a positive and almost linear (df =1.2), indicating higher statolith growth during the early life under the highest sea surface temperatures (Figure 29a). Similarly, the partial effect of SBT_{90d} is also positive, between 13.3 and 14.4 ºC, but shows a peak around 14.5 ºC, i.e. statolith growth during the first 90 days of life is fastest when SBT 90 days after hatching is around 14.5 ºC. Outside this range of SBT_{90d} there were too few data to be confident about the form of the relationship, nevertheless the best fit for SBT is clearly nonlinear (Figure 29b). The partial effect of SST_{90d} on the post-hatching growth is stronger than that of SBT_{90d}, consistent with paralarvae being pelagic.

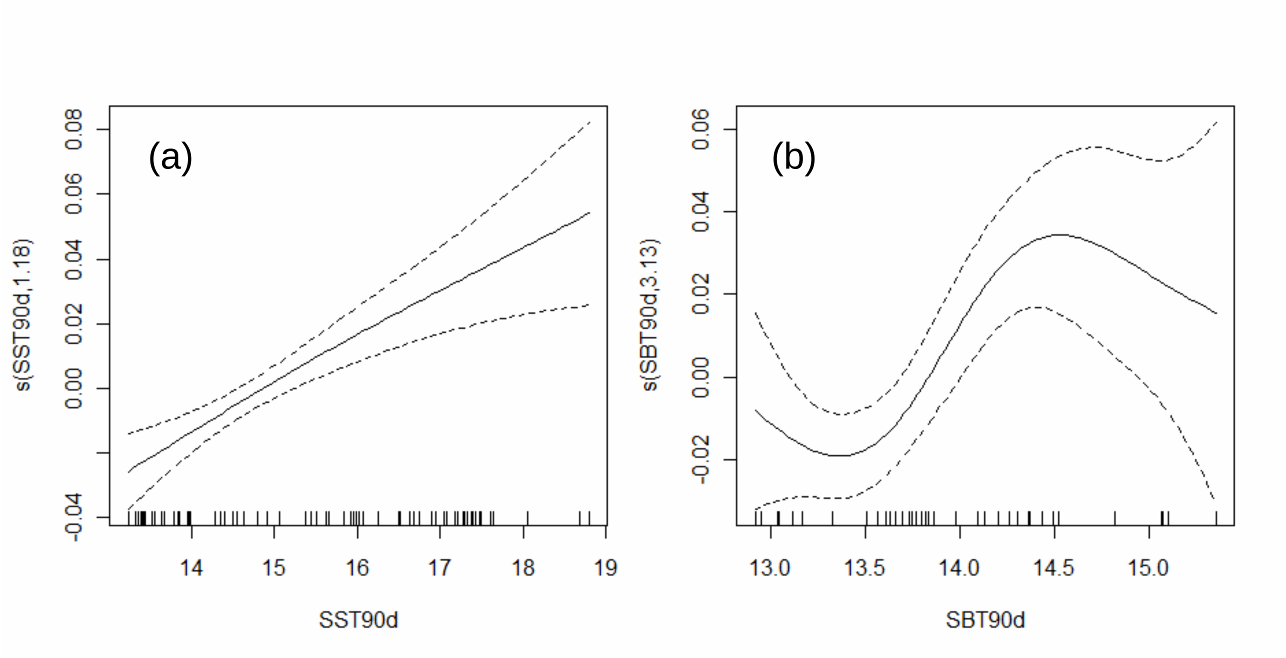

Figure 29 - GAM result: smoothing curve for partial effect of (a) sea surface temperature (SST_{90d}) and (b) of sea bottom temperature (SBT_{90d}) on the statolith growth during the first 90 days post-hatching (R_{90d}). Dashed lines indicate 95% confidence intervals around the main effects.

5.3.3. Duration of the paralarvae stage

Analysis of the statolith accretion pattern in the first 126 days of life (~18 weeks) showed a similar pattern in all the different SST_{90d} classes, with an increase in the increment width post-hatching to a maximum, followed by a decrease (Figure 30). Average statolith increment widths varied with temperature: increments are wider (faster growth) in squid living under higher temperatures ($SST_{90d} \geq 16$ ºC) and narrower (slower growth) in squid living under lower temperatures ($SST_{90d} \leq 14$ ºC).

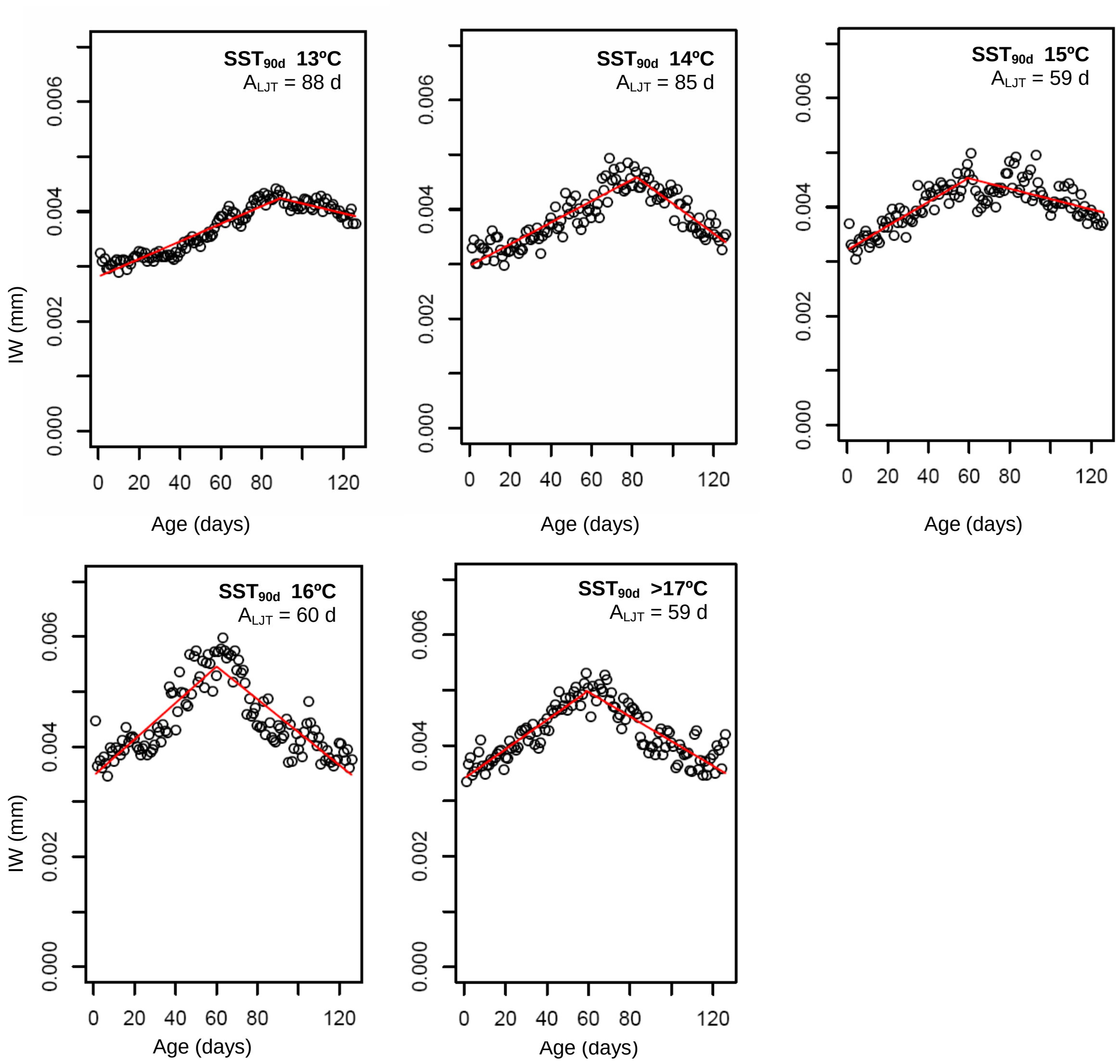

Figure 30 - Statolith growth pattern during the first 18 weeks post-hatching. Segmented regression models fitted to statolith increment width and squid age data for animals grouped by SST_{90d} 1 ºC classes. Breakpoints for each relationship (A_{LJT}) are highlighted.

These differences are clearly seen during the first 90 days of post-hatching growth: statolith growth is generally slow during the first month after hatching and reaches a maximum after two to three months, depending on temperature. The A_{LJT} values estimated from the break-point in the fitted segmented regressions were 85-88 days for squid which experienced SST_{90d} < 15 ºC and 59-60 days for squid which experienced $SST_{90d} \geq 15$ ºC (Table 7).

Table 7 - Summary of parameter estimates for the segmented (piecewise) regressions (PLR) fitted to the statolith increment width pattern for each SST_{90d} group. A_{LJT}, age of the transition from paralarvae to juvenile; CI (95%), Wald-based 95% confidence intervals; β_1, slope of the left linear segment.

SST_{90d} (ºC)	**A_{LJT} (days)**	**CI (95%)**	**β_1**	**CI (95%)**	**variance explained (%)**
13	88	84.32 - 92.27	$1.60e^{-5}$	$1.49e^{-5}$ - $1.71e^{-5}$	92
14	85	78.89 - 85.40	$1.97e^{-5}$	$1.78e^{-5}$ - $2.16e^{-5}$	82
15	59	53.52 - 63.61	$2.21e^{-5}$	$1.86e^{-5}$ - $2.56e^{-5}$	69
16	60	56.39 - 63.73	$3.31e^{-5}$	$2.82e^{-5}$ - $3.79e^{-5}$	75
17 - 18	59	55.42 - 61.98	$2.64e^{-5}$	$2.30e^{-5}$ - $2.99e^{-5}$	80

5.4. Discussion

Previous work has highlighted the importance of the statoliths as chronological records of past events in the life of an animal (see Arkhipkin, 2005 for a review). The periodic deposition of incremental bands of material with contrasting light properties not only allows determination of age but can also provide information on the environmental conditions experienced by the animal and on its physiological development (e.g. using the relative width and darkness of the bands). González *et al.* (2010) subsequently expressed concern that this record might be somewhat compromised by the occlusion of early incremental bands in adult statoliths. However, we found no evidence of ontogenetic change in the number of rings in the immediate vicinity of the natal ring, suggesting that rings are not lost.

5.4.1. Growth during the embryonic phase

The variability in the statolith size at hatching was weakly (but significantly) related to salinity and unrelated to temperature. There are several possible interpretations for this: it could be that most variation is not environmentally driven (Villanueva, 2000a), that squid search for environmentally similar grounds on which to lay their eggs (Villanueva *et al*., 2003), that temperature and salinity were not measured at a sufficiently small spatial or temporal scale to be directly relevant to the individual squid, and/or that most variation is due to other environmental factors that were not considered in the GAM models.

Our results showed that lower salinity values tend to produce larger statoliths, similarly to the findings of Sen (2005a) for hatchlings in captivity. There is very limited information concerning the effect of salinity changes on embryos of squids. Nevertheless, within their tolerance limits, marine animals living in lower salinities spend less energy for osmoregulation and therefore have more energy for somatic growth (Boeuf and Payan, 2001).

The interaction between the positive influence of temperature on statolith growth rate (Villanueva, 2000b) and its negative influence on embryonic development duration (e.g. Sen, 2005a), could have balanced the statolith size at hatching under cold vs. warm conditions and thus masked any temperature effects. On the other hand, the low amount of variability in statolith size at hatching in our study can be an indication that most of the squid sampled spent the egg stage at rather constant conditions of temperatures and salinity. Relatively stable sea bed environmental conditions for egg laying are found between 35 and 125 m throughout the year. In this zone, mean monthly bottom temperatures vary between 13.5 and 15.4 ºC (Figure 3) and extreme variations in bottom salinity are episodic (Figure 2). Inshore of the 35 m isobath, salinity may be quite variable and will sometimes reach lethal levels due to rainfall and river runoff. It is therefore plausible that squid search for environmentally similar (and environmentally stable) spawning grounds, as suggested by Villanueva *et al*. (2003).

5.4.2. Growth during the first 3 months and the duration of the paralarvae stage

The western Portuguese coast is in the northernmost part of the Canary Current upwelling system. Here, upwelled cold deep water moves onto the shelf, usually from April to October and more intensely from June to August (Wooster *et al*., 1976). Given this, the seasonality of ocean temperature over the shelf is quite different from the pattern seen in non-upwelling areas at the same latitude. Since temperature has been described as the main abiotic factor influencing loliginid statolith growth during early life stages under experimental conditions in captivity (Villanueva *et al.*, 2007), we modelled effects of temperature, both SST and SBT, on the post-hatching growth. The results of the present study confirm the significant influence of temperature on the early life stages of wild *L. vulgaris*. A higher correlation was detected between statolith growth and SST than between statolith growth and SBT. This is consistent with the fact that the paralarvae are essentially planktonic and thus SST should be a better predictor of early growth. Nevertheless, some influence of SBT was also expected because paralarvae are found near the bottom during part of the day (Moreno *et al.*, 2009). Because SBT decreases during the upwelling season (spring-summer) and increases after its end, while SST increases from winter to summer (although less than expected owing to upwelling) (Figure 28), there are differences in the seasonal patterns of SBT and SST, modulated by upwelling, and thus the influence of temperature on growth is described by a complex model with an almost linear effect of SST and a non-linear effect of SBT.

Inspection of the statolith accretion pattern revealed an inflection point in the increment widths occurring at 60-90 d post-hatching. This age corresponds to the occurrence of important anatomical changes in the mantle muscles in other loliginids (Preuss, 1997; Thompson and Kier, 2001), which mark the end of paralarval swimming characteristics. We therefore tentatively propose that this represents a measure of the duration of the paralarval stage, while recognising that further verification is needed. In relation to the timing of this growth inflection (and regardless of its biological significance), there were differences of the order of 1 month between squids living at $SST_{90d} < 15$ ºC and at $SST_{90d} \geq 15$ ºC. This difference of up to ~1 month is quite significant considering the short (~1 year) life span of the squid (Moreno *et al.*, 2007). We therefore argue that these observations justify viewing the squid population as comprising a cold cohort with slower early growth and a longer planktonic paralarval phase, and a warm cohort with a faster early growth and a shorter planktonic phase.

The western Portuguese coast is characterized by optimum conditions for phytoplankton blooms and consequent high zooplankton abundance (Arístegui *et al.*, 2006). On the northwest coast, the zooplankton biomass is high from April until November (Cunha, 2001). Therefore *L. vulgaris* individuals from the warm cohort, which comprise the second peak of paralarval abundance over the course of the year (in August, Moreno *et al.*, 2009), live in the plankton during a period of very high prey density. This high prey abundance can, in turn, fuel the higher metabolic demands of the warm season. Higher growth rates will allow these individuals to acquire the full flexibility and fine coordination of the locomotory system at a younger age. Thus, the life strategy of this cohort potentially favours its own survival by reducing the duration of the more vulnerable planktonic phase. This of course begs the question of why there is a second annual cohort (the cold cohort). There may also be selective advantages to this alternative life-cycle strategy (assuming that there is some genetic component to the seasonality of spawning) or the separation into two cohorts may be an unavoidable environmentally driven consequence of the life cycle biology – see Boyle *et al.* (1995) for discussion of this theme in relation to *Loligo forbesii.*

Otoliths are frequently used to determine the timing and duration of ontogenetic events in fish (Sponaugle, 2010) but statolith increment widths have seldom been used to determine the duration of a cephalopod life-cycle stage (e.g. Arkhipkin, 2004). We investigated the possibility of using statoliths as an alternative way to estimate the transition from paralarva to juvenile (PLD) because there is still no precise morphological or ecological definition for this transition in loliginids. By using piecewise linear regression (PLR), we found a very clear breakpoint in the growth pattern, with differences at a cohort level.

There is a need to study other morphological indicators in the early life stages of squid, such as changes in the allometric growth of some body parts and modifications in the mantle musculature, to see whether they are related to the timing of the breakpoint in the statolith increment pattern. The estimation of breakpoints in the growth trajectories of morphologic characters in some squid species has proved useful to relate ontogenetic shifts with rapid ecological changes (Shea and Vecchione, 2002).

5.4.2. Life-history strategy and climate change

The significant effect of hatching year on RH and R_{90d} provided an indication of some additional environmental influence not captured by the environmental variables included in the model. This influence may still be related to temperature to some extent, since colder years (1992-1994) could be correlated to larger size at hatching and lower post-hatching growth, in opposition to the effects of the following warmer years.

Nevertheless, inter-annual variation in other environmental/biological conditions (e.g. prey density) may also have an important role in early growth, which may in turn affect mortality rates and consequent cohort survival and recruitment.

Under a scenario of global warming, our results suggest that squid will experience a shorter paralarval stage, undergo faster growth, and mature younger and at a smaller size, similar to the warm cohort life strategy. This would apparently ultimately enhance survival, and favour recruitment and rapid turnover of the local *L. vulgaris* population, as well as of other inshore squid populations. However, while this could be viewed as a beneficial effect of climate change, the situation is probably far more complicated. At an individual level, elevated temperatures pose limitations to individual metabolism, namely concerning oxygen and food requirements (Pecl and Jackson, 2008). At the population level, density may increase to the point that density-dependent effects (e.g. increased intraspecific competition, higher levels of predation) come into play.

Chapter 6

Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia

A. Moreno[1], A. Dos Santos[1], U. Piatkowski[2], A.M.P. Santos[1], H. Cabral[3]

1 IPIMAR, Av. Brasília 1449-006 Lisboa, Portugal
2 Leibniz-Institut fuer Meereswissenschaften (IFM-GEOMAR), Universitaet Kiel, Duesternbrooker weg 20, D-24105 Kiel, Germany
3 Universidade de Lisboa, Faculdade de Ciências, Instituto de Oceanografia, Campo Grande, 1749-016 Lisboa, Portugal

Journal of Plankton Research (2009) 31, 73-91

6. Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia

6.1. Introduction

Cephalopods represent a major fishery resource in Europe, specifically in Portugal and Spain. The most important cephalopod species as fisheries resource, which have planktonic early life stages, thereafter called paralarvae (Young and Harman, 1988), are *Octopus vulgaris*, *Loligo vulgaris*, *L. forbesi*, *Illex coindetii* and *Todaropsis eblanae*. These are neritic species, whose adults and juveniles occur mainly in the middle-shelf (*O. vulgaris* and *Loligo* spp.) or in the outer-shelf region (*I. coindetii* and *T. eblanae*). *O. vulgaris* is presently the fourth most important fishery resource in Portuguese waters with landings of about 7000 tons and a commercial value of 28.7 million € in 2006 (DGPA, 2007), which support many local fishing communities.

The neritic loliginid, octopus and sepiolid females lay their eggs in capsules/clusters attached to hard substratum or branched sessile organisms on the sea bottom, while in ommastrephids and most other oceanic squids the eggs are laid into large masses that drift submerged in the open sea (Jereb and Roper, 2005). Embryonic development lasts from few weeks to few months, depending on water temperature (Villanueva *et al.*, 2003). Soon after hatching cephalopod paralarvae are active predators of other zooplankton and active swimmers by jet propulsion, however during this life stage their distribution is essentially dependent of the oceanic circulation (Hanlon *et al.*, 1985). Information on the distribution of paralarvae and the knowledge on the spawning grounds of the most important European cephalopod resources are scarce. However its knowledge is of major relevance for the understanding of the dispersal behaviour of paralarvae stage with important implications to recruitment success and variability. Some studies have analysed the oceanographic influences on cephalopod paralarvae distribution in the Atlantic (e.g. Diekmann and Piatkowski, 2002, 2004; Roberts and Berg, 2005), but besides studies in Galicia (NW Spain), that relates the distribution of cephalopod paralarvae and the circulation associated to coastal upwelling events (Rocha *et al.* 1999; González *et al.*, 2005), little research has been undertaken in the eastern Atlantic, thus cephalopods early life-history dynamics in coastal upwelling systems are poorly understood. Furthermore, information about the vertical distribution of cephalopod paralarvae are even scarcer, being almost related to pelagic oceanic squid species in non-European waters (e.g. Hatanaka *et al.*, 1985; Roepke *et al.*, 1993; Filippova and Pakhomov, 1994).

The present study aims to explain the cephalopod paralarvae distribution in Portuguese waters based upon the knowledge of the regional oceanography and coastal transition zone processes. Since cephalopod paralarvae are rare in plankton samples (Vechione, 1987), the analysis is based on information from 19 years of plankton sampling (1986 to 2004) off the Portuguese and adjacent Spanish waters. To understand the distribution of paralarvae according to changes in their physical environment, we test the key questions: Do upwelling events promote their advection from the shelf? Does the shelf processes related to upwelling dynamics affect loliginid and octopus paralarvae distribution in the same manner? Does water temperature have influence on their abundance and distribution patterns along the Portuguese coast? Are the differences in the main oceanographic features and processes between the Western Iberia upwelling system and the Gulf of Cadiz reflected on paralarvae abundance and distribution on the western and southern Portuguese waters within each species?

6.2. Material and Methods

6.2.1. Sampling

Cephalopod paralarvae distribution and seasonality was analysed based on plankton samples carried out during 57 survey cruises between October 1986 and December 2004 in the Portuguese waters, and occasionally in adjacent Spanish waters (Figure 31). Table 8 summarise the fieldwork and sampling methodologies used in this study. The sampling area covers latitude 33.23°N to 42.75°N and longitude 6.15°W to 14.37°W in the NE Atlantic. The majority of cruises were performed at a monthly or seasonal basis on board the Portuguese research vessels “Noruega”, “Capricórnio” and

"Mestre Costeiro", under sampling research programmes targeting for fish eggs and larvae. The main gear was a bongo net with a 60 cm mouth aperture diameter fitted with 335 and 500 µm mesh size nets. The net was towed on depth-integrated oblique hauls at ~2 knots from surface to 10 m above the bottom or until 200 m when the bottom was deeper. Filtered water volumes were estimated using calibrated flowmeters mounted on both net mouth apertures.

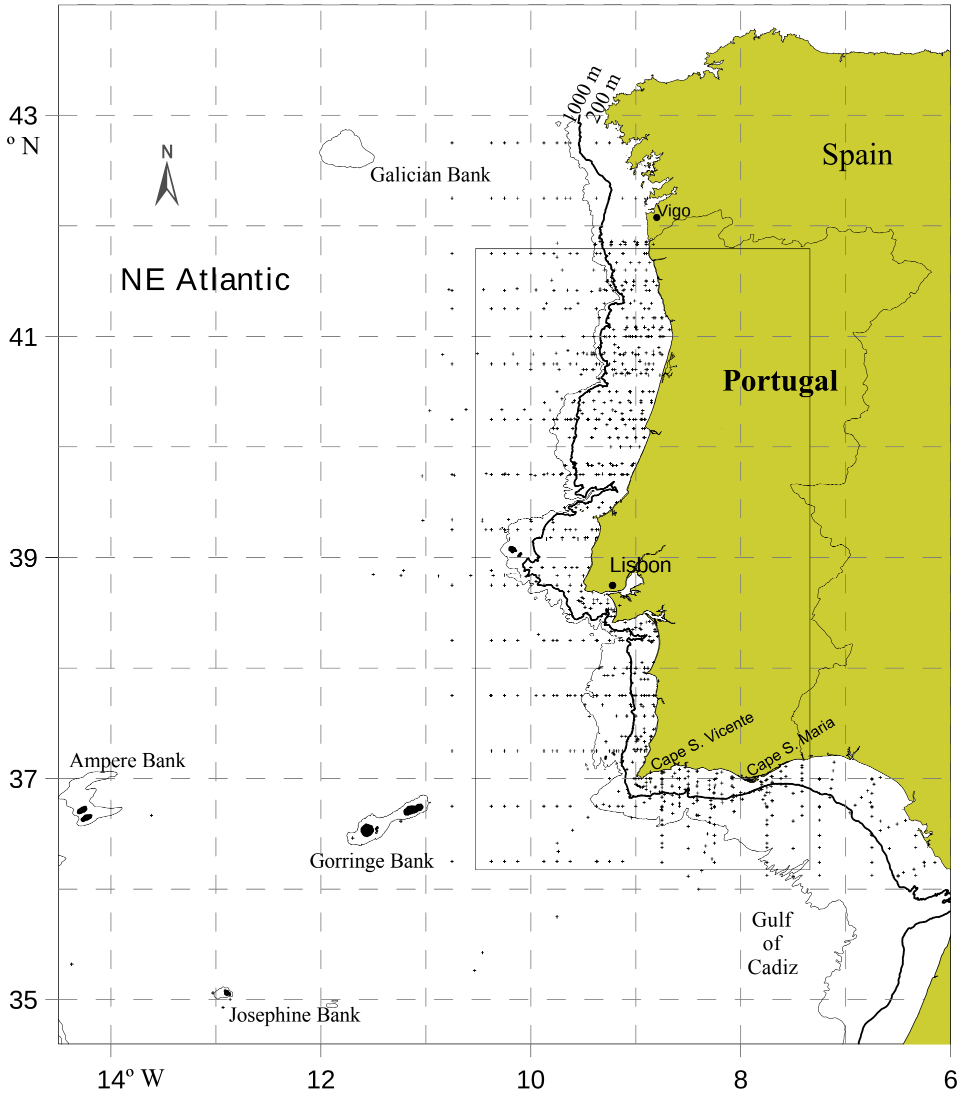

Figure 31 - Sampling stations. Box covers the main study area. Stations outside the box were undertaken only once.

In the cruises targeting for cephalopod paralarvae on board “R/V Mestre Costeiro” and the German “R/V Poseidon” the bongo net was towed horizontally during 10 min near bottom between double oblique tows to surface (Moreno, 1998). Most vertical distribution data was obtained from a survey targeting crustacean larvae, in which a Pro-LHPR system and neuston net were used. The Pro-LHPR system collected samples at approximately 5 m depth intervals in the first 25 m and at 10 m depth intervals down to near bottom (for more complete description of this cruise see dos Santos *et al.*, 2007). Scattered data on vertical distribution were obtained from depth stratified sampling during several cruises, with a WP-2 and a MOCNESS multiple net sampler. Environmental data, when collected, were obtained from CTD casts (see Table 8 for details).

All samples were preserved in 4% borax-buffered formaldehyde, prepared using seawater. A total of 4156 samples were examined and 914 cephalopod paralarvae identified to the lowest taxonomic level possible, and the dorsal mantle length (DML) was measured as defined by Sweeney *et al.* (1992).

6.2.2. Data analysis

Catches of the neritic species were standardised to number per 100 m^{-3} using flowmeter information to study their distribution and seasonality. Latitude, longitude and bottom depth of each sampling station were included in the time series of paralarvae densities to account for spatial variability.

The following oceanographic and climatic factors were included in the dataset, assigned to each sampling station: sea surface temperature (SST), upwelling indices (UI) and the North Atlantic Oscillation (NAO) index. They were used as proxies for the oceanographic processes of the continental shelf as have been pointed out to be key variables to explain distribution and abundance during the life cycle of several marine species (e.g. Bakun, 1996; Santos *et al*., 2007). SST data for most sampling stations were extracted from CTD temperature profiles. SST data (weekly, 1° grid), was also extracted to fill in some gaps in our data matrix, from the integrated Global Ocean Services System – Meteorological Center “IGOSS nmc” database (Reynolds and Smith 1994). Upwelling indices (UI) provided by the NOAA-NMFS-Pacific Fisheries Environmental Laboratory (http://www.pfel.noaa.gov/) were computed from monthly mean pressure fields on a 1° mesh grid.

Table 8 - Plankton sampling summary. Sampling excluded in the horizontal distribution analyses of neritic species is marked with *.

Date	Target	Area	Nº of samples	CTD	Haul type	Net type	Mesh size	Method
Oct 1986 to Jan 1989 (monthly)	Fish eggs and larvae	West coast: 3 transects at 41º05’N, 40º05’N, 38º00’N from shoreline to 9º35’W South coast: 1 transect at 8º35’W, from shoreline to 36º50’N	506	Yes	Oblique	Bongo 60 cm ϕ	335 and 500 µm	Hauls from near bottom to surface, or from 200 m to surface, where the ocean floor > 200 m
Feb/Mar 1992/93;	Fish eggs and larvae	Along the coast: 36º07’ to 42º45’N and 7º30’ to 10º45’W	1756	No				
Jun/Jul 1990/92 Oct/Nov 1990/91/92				No				
Jun/Jul 1993, Oct/Nov 1993				Yes				
May 1994; Mar/Apr 1995;				No				
Jan/Feb/Mar 1998/2001/04				Yes				
Aug 1993	Crustacean larvae	South coast: 33º14’ to 37º17’N and 7º25’ to 14º22’W	62	No				
Jun 1994	Cephalopods			Yes				
Feb 2000	Fish larvae	North coast: 40º24’N to 41º36’N and 8º45’W to 10º00’W	111	No				
Jun 1995	Cephalopods	North coast: 39º45’ to 41º50’N and 8º44’ to 9º45’W	160	Yes	Horizontal and Oblique	Bongo 60 cm ϕ	335 and 500 µm	Hauls towing 10’ near bottom and oblique to surface
Nov 1996; Aug 1996;				No				
Aug/Sep 1995; Nov 1995	Cephalopods	SW and south coast: 36º48’ to 38º38’N and 7º48’ to 9º56’W	97	No				
Dec 2004	Fish eggs			Yes				
Feb/Mar 1996 (WP2)	Fish eggs/larvae	NW coast: 38º17’ to 41º31’N and 8º34’ to 11º30’W	1010	Yes	Oblique	WP2 LHPR or Mocness	200 µm 280 µm or 335 µm	Stratified hauls in 3 to 8 depth layers from near bottom to surface or from 200 m to surface, where the ocean floor > 200 m
May 1996 (WP2)	Cephalopods			No				
May 2002 (LHPR)	Crustacean larvae			Yes				
Jun 1994 (Mocness)	Cephalopods	SW and south coast: 33º14’ to 38º45’ºN and 6º20’ to 14º22’W	314	Yes				
Mar/Apr 1995 (WP2)	Fish larvae			Yes				
Nov 2002/03 (WP2) *	Fish larvae			No				
Nov/Dec 1999/00/01; *	Fish eggs	Along the coast: 36º07’ to 41º52’N and 6º09’ to 10º00’W	140	No	Vertical	Calvet	150 µm	Hauls from near bottom to surfacerface, where the ocean floor > 200 m
Jan 2002 *				No				

The coastal angle needed to compute UI was estimated for each 1º latitude along the Portuguese coast. The Climate Research Unit of the University of East Anglia provided the NAO index, based on the monthly difference of normalized sea level pressures (SLP) between Gibraltar and Reykjavik (http://www.cru.uea.ac.uk/).

The relationships between paralarvae densities and environmental factors were analysed with Generalized Linear Models (GLM) models, using R software (R Development Core Team, 2005). GLM are an extension of linear models allowing the incorporation of non-normal distributions of the response variable and transformations of the dependent variables to linearity (McCullagh and Nelder, 1989). The GLM analysis was conducted in two steps procedures, because of the very high proportion of null samples (e.g. 83% for loliginids) and to account for both the probability of occurrence and the density by sampling station (Sousa *et al.*, 2007): (1) a logistic regression model with the logit link (Hosmer and Lemeshow, 1989) was used to evaluate which factor/factors are important for the presence/absence of paralarvae (data follows a binomial distribution); and (2) the gamma regression model with a log link to assess the factor/factors that influence abundance (paralarvae density) (McCullagh and Nelder, 1989). The occurrence/density was analysed as a response to the following explanatory variables (predictors): year, month, latitude, longitude, depth, NAO, UI, and SST. First order interactions (multiplicative interactions of combinations of predictors, included additively in the models together with the predictors considered) were included in the model whenever considered adequate (based on their contribution to the deviance explained). Although the oceanographic data collected from different sources presented different spatial and temporal resolutions, its effects on the analyses that were performed were presumably low, since GLM were use to explore the relationships between environmental variables and occurrence/abundance of cephalopod paralarvae and not for predictions purposes. These problems were also minimized by choosing the most appropriate scale, both temporal and spatial, to sampling areas and periods established for paralarvae collections. The goodness-of-fit of the models was assessed by comparing their relative contribution to total deviance explained. A significance level of 0.05 was considered in all test procedures.

Knowing that oceanographic features associated to upwelling events are determinant in the distribution of many zooplankton groups off the west Portuguese coast (e.g. Santos *et al.*, 2004; Queiroga *et al.*, 2005; dos Santos *et al.*, 2007), the bathymetric distribution was evaluate in more detail by testing the differences in densities by season and depth range with a factorial ANOVA. For each species, the categorical predictor season grouped samples carried out during two distinct periods (or density peaks), which showed opposite average UI.

All depth-stratified samples were pooled together to provide data on loliginid and octopus vertical distribution and diel vertical migrations.

6.3. Results

6.3.1. Neritic species

The most abundant cephalopod paralarvae off the Portuguese coast belong to the neritic species of the families Loliginidae (40%), Octopodidae (*Octopus vulgaris*) (27.5%), Sepiolidae (11%) and Ommastrephidae (10%). Table 9 presents a summary of the spatial and temporal distribution of the different taxa found, as well as additional information about paralarvae size, total number caught, bottom depth and SST range where they were found.

6.3.1.1. Loliginids

Loliginid paralarvae were found year round over the shelf, with two distinct hatching seasons: a main season between January and April and a secondary season between July and September, with highest densities in March and August, respectively (Figure 32). In general, higher densities were found between the 50 and 125 m isobaths, particularly along the northwest coast off Portugal (between 39.5º N and 41.5º N).

GLM results support the significant role of month, latitude and depth but also the importance of low temperatures and high upwelling in loliginid distribution (Table 10). Moreover, the analysis indicates that there was a significant influence of the interactions month *vs*. latitude (e.g. absence of the summer hatching peak on the south) and SST *vs*. latitude (e.g. occurrences associated to the northern SST < 14 ºC) (Figure 33a,b). On the other hand, the gamma model indicated that the oceanographic conditions alone had the main influence on loliginid density. This model reflects essentially seasonality rather than the spatial distribution, i.e. higher densities associated with lower SST and UI.

Table 9 - List of paralarvae species (taxa) collected in 57 research cruises (1986 -2004) in Portuguese and adjacent waters (* oceanic taxa). Summary of the spatial and temporal distribution, size range and total number caught.

Family	**Species**	**Latitude range (ºN)**	**Bottom depth range (m)**	**SST range (ºC)**	**Seasonality (month)**	**ML range (mm)**	**N**
Loliginidae	Loliginidae indet.	36.13 to 42.75	15 to 500	13.0 to 22.7	all	1.5 to 7.5	371
Octopodidae	*Octopus vulgaris*	35.06 to 41.83	14 to 1378	13.9 to 22.7	all	1.2 to 4.2	254
Sepiolidae	Sepiolidae indet.	36.24 to 41.86	21 to 740	13.2 to 20.8	all	1.2 to 9.5	102
	Heteroteuthis dispar *	36.24 to 36.70	587 to 750	17.8 to 20.9	1, 7	10.6	2
Ommastrephidae	Ommastrephidae indet.	33.23 to 42.75	20 to 4142	13.2 to 20.8	all	0.7 to 8.7	91
Pyroteuthidae *	*Pterygioteuthis* sp.	35.75 to 38.00	65 to 2000	16.0 to 17.3	1, 2, 4, 6, 7	1.9 to 13.3	9
	Pyroteuthis margaritifera	36.24 to 36.49	614 to 4788	17.3 to 19.4	1, 2, 3	2.1 to 9.4	5
	Pyroteuthidae indet.	36.25 to 36.87	132 to 4788	17.0 to 19.4	1, 3	1.5 to 4.4	6
Enoploteuthidae *	*Abraliopsis atlantica*	36.12 to 36.69	745 to 3681	17.2 to 17.3	1, 2, 3, 6	2.5 to 7.8	5
	Abralia cf. veranyi	36.12	310	17.7	1	2.1	1
Ancistrocheiridae *	*Ancistrocheirus lesueurii*	36.24 to 37.06	20 to 2400	15.6 to 17.2	1, 3	3.0 to 5.3	2
Enoploteuthid group *	Enoploteuthid group indet.	36.12 to 36.99	63 to 900	17.1 to 17.7	1, 5	1.5 to 2.5	5
Cranchiidae *	*Leachia* sp.	36.24 to 36.75	2043 to 3400	17.0 to 17.3	1	26.8 to 52	4
	Taonius pavo	36.25 to 36.57	800 to 4000	17.2 to 19.5	1, 2	2.5 to 15.6	11
	Helicocranchia pfefferi	36.34 to 36.72	742 to 3681	-	5, 6	2.4 to 8.3	5
	Bathothauma lyromma	36.25 to 37.75	3000 to 4765	16.2 to 18.1	1, 2	7.0 to 24.3	2
	Megalocranchia sp.	36.17 to 36.75	800 to 3000	16.9 to 19.1	2, 3	5.5 to 11.8	5
	Teuthowenia megalops	40.25 to 41.08	45 to 3850	13.8 to 15.0	2, 3	4.4 to 28	5
	Taoninae indet.	34.93 to 37.71	228 to 1290	18.3	3, 6	2.2 to 4.1	5
Mastigoteuthidae *	*Mastigoteuthis* sp.	36.12 to 40.75	310 to 3460	15.6 to 18.8	1, 2, 3, 4	4.9 to 9.9	9
Onychoteuthidae *	*Onychoteuthis banksii*	36.24 to 40.75	230 to 3000	15.0 to 18.8	2, 3, 5, 6, 8	6.8 to 11.2	6
	Onychoteuthidae indet.	39.75 to 40.30	162 to 1030	15.4 to 16.9	2, 5	2.7 to 3.7	2
Chiroteuthidae *	*Chiroteuthis veranyi*	36.25 to 37.25	210 to 3000	16.0 to 19.4	1, 2, 3	4.8 to 16.8	4
Brachioteuthidae *	*Brachioteuthis reesei*	36.24	968	17.8	2	3.2	1
Chtenopterygidae *	*Chtenopterix siculus*	36.00	2000	-	4	2.1	1
Ocythoidae *	*Ocythoe tuberculata*	35.32	4318	-	6	6	1

Nevertheless, the logistic model fitted to presence/absence of loliginids explained only about 8.4% and the gamma model 12.4% of total variability for the whole area. Thus, an additional analysis was carried out for the west coast to highlight the role of oceanography on the loliginid distribution.

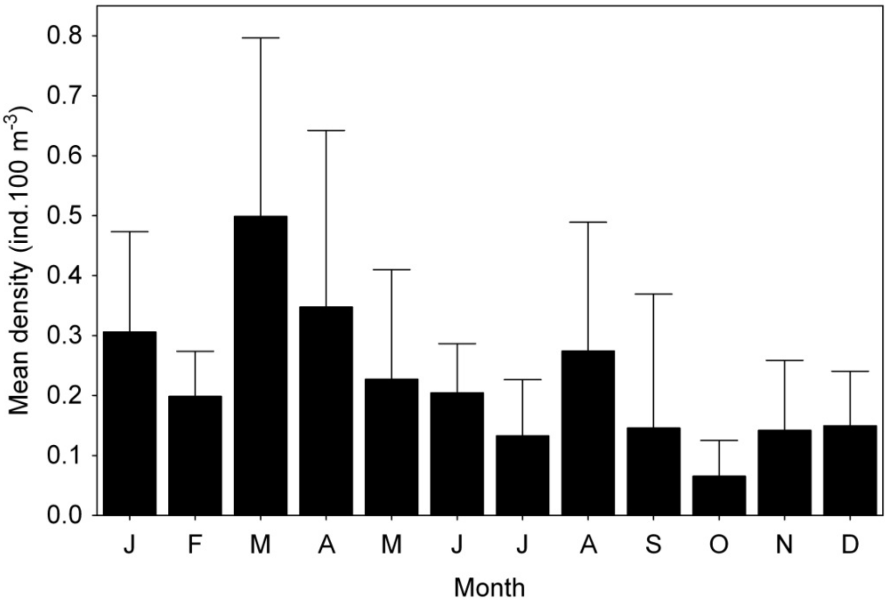

Figure 32 - Monthly mean densities of loliginid paralarvae within their distribution area (bottom depth < 200 m). Vertical bars denote 0.95 confidence intervals.

The spatial distribution on the west coast displayed differences between seasons of contrasting oceanographic regimes (Figure 34). Significantly higher densities were observed offshore (75 m to 125 m isobaths) between December and March, corresponding to the winter relaxation period, and close inshore (15 m to 75 m isobaths) between June and September, during the summer upwelling season (ANOVA season *vs*. depth range effect, F(4, 613) = 2.924, $p < 0.05$). Additionally, during April and May loliginids occurred associated to upwelled waters on the west coast and distributed inshore of the 100 m isobath.

Loliginid paralarvae evidenced a consistent diel vertical migration (Figure 35), being in surface layers at night and deeper during daylight. However, these results were based only in few numbers of paralarvae. No paralarvae were found in the neuston layer at any time.

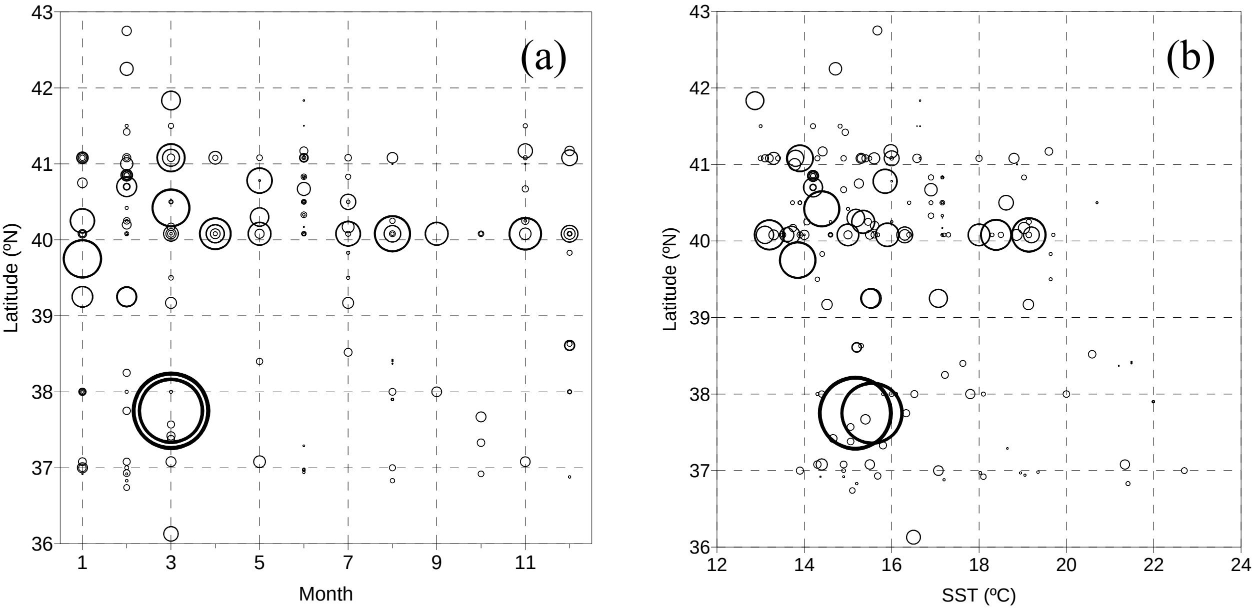

Figure 33 - Interaction of latitude and month (a) and interaction of latitude and SST (b) in loliginid paralarvae abundance. The size of dots represents densities of 1 up to 12 ind 100 m^{-3}.

Table 10 - Deviance tables for loliginid GLM logistic models (probability of positive capture) and gamma models (density in positive captures).

Source of variation	**Deviance %**	**Estimates**		**p-value**	
		βi	**SE**		
Logistic models					
month	0.8	-0.062	0.023	0.006	**
latitude	0.8	0.144	0.050	0.004	**
depth	1.7	-0.006	0.001	0.000	***
UI	1.3	0.007	0.002	0.001	***
SST	0.6	-0.097	0.038	0.012	*
month x latitude	1.4	0.029	0.014	0.041	*
latitude x SST	1.8	0.074	0.027	0.007	**
Total explained	8.4%				
Gamma models					
UI	5.0	-0.004	0.002	0.038	*
SST	7.4	-0.097	0.036	0.008	**
Total explained	12.4%				

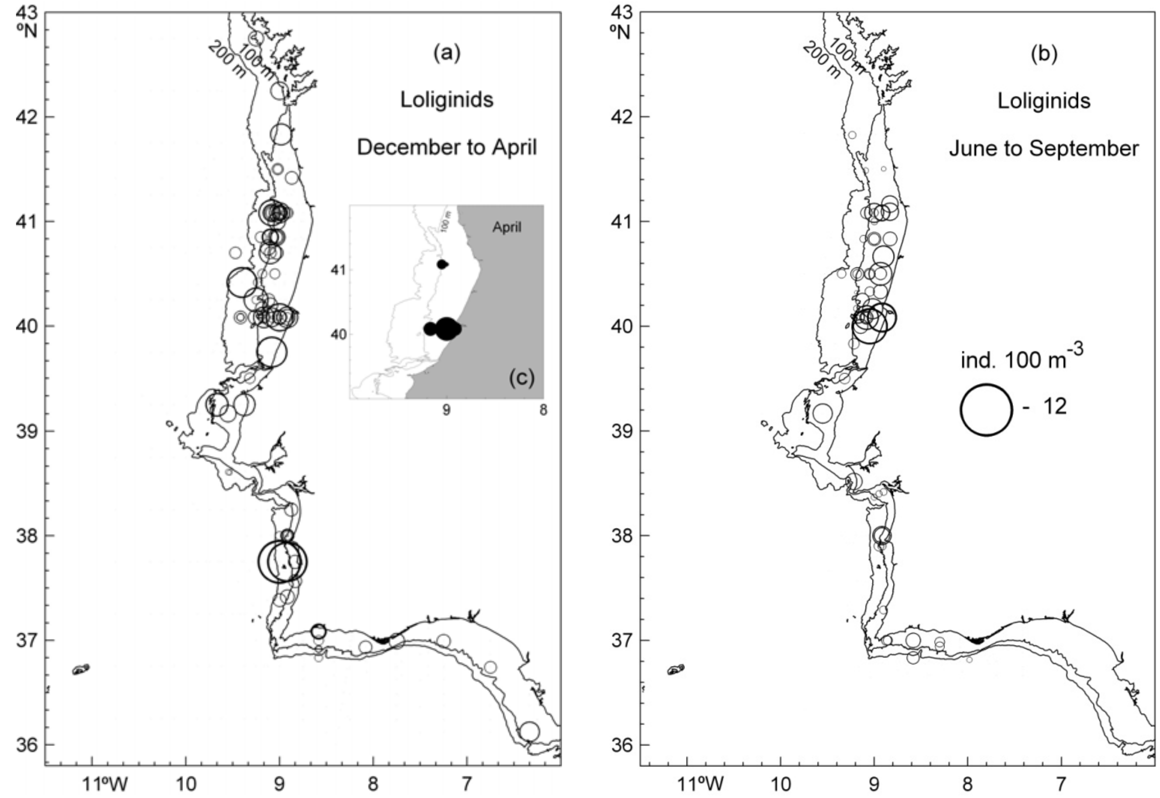

Figure 34 - Horizontal distribution of loliginid paralarvae in Portuguese waters and adjacent Spanish regions during December to March (a) and June to September (b) plankton surveys. Detail of the distribution in April (c).

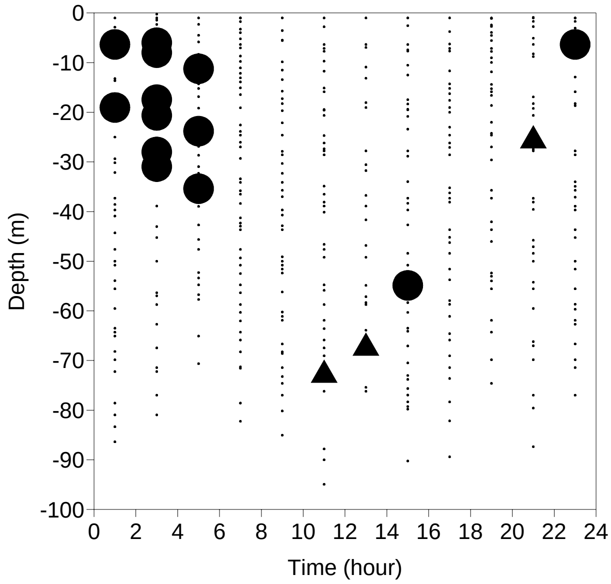

Figure 35 - Diel vertical distribution of loliginid paralarvae. Big dots (LHPR sampling) and triangles (WP2 and MOCNESS sampling) represent paralarvae presence, small dots represent absences. Real depth sampling standardized to 100 m water column. Sampling at -1 m indicates the neuston net sampling.

6.3.1.2. *Octopus vulgaris*

Octopus paralarvae occurred mainly in the second half of the year, with peaks in July and November (Figure 36). These two distinct peaks, a minor peak in summer and a major peak in autumn, were present throughout the sampled area. Paralarvae hatching in spring-summer were distributed over the shelf and slope between the 14 m and 1400 m isobaths and at the Gorringe and Ampere Banks (Figure 37a). During autumn-winter they were found more inshore (Figure 37b).

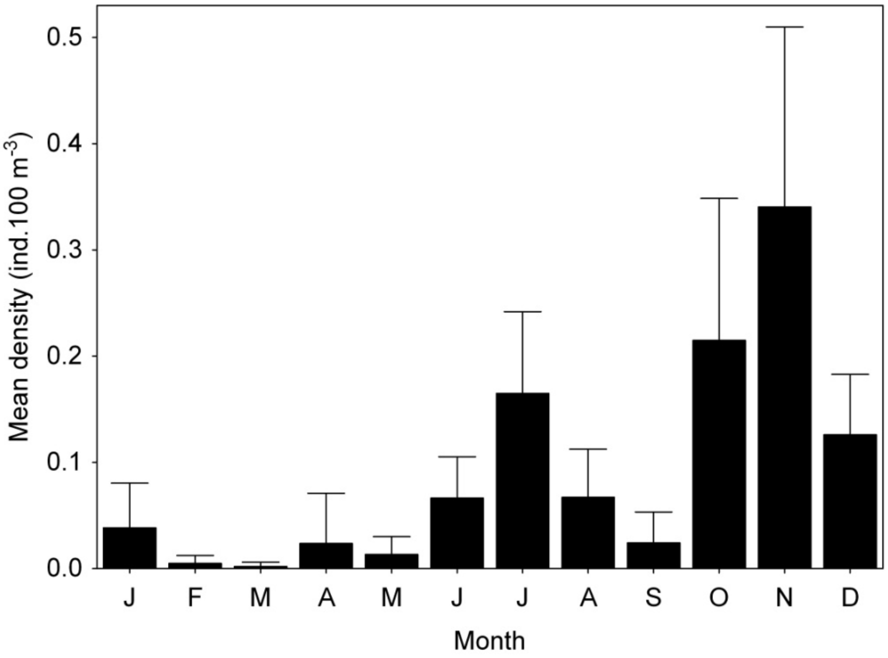

Figure 36 - Monthly mean densities of octopus paralarvae within their distribution area (bottom depth < 1500 m). Vertical bars denote 0.95 confidence intervals.

The GLM analysis revealed an important contribution of several variables and interactions between them to explain the occurrence of octopus paralarvae (total explained = 59.2 %, Table 11). The variables month and its interaction with depth, and SST and its interaction with UI, depth or latitude were the most significant contributors to explain their distribution. Overall, the probability of finding paralarvae increased along the year (higher in autumn), with increasing SST and upwelling, and decreasing latitude (higher in the south) and depth (higher inshore). However, the observed differences between the west coast and the south coast, which is inferred by the significant influence of the association of latitude with depth, UI and SST, could introduce some contradictory influence of some environmental variables in the analysis for the whole coast. For example, the probability of finding paralarvae decreased with depth in the west coast and increased with depth in the south coast. Moreover, the probability of finding paralarvae was positively correlated with stronger upwelling (logistic model), but density inversely correlated (gamma model).

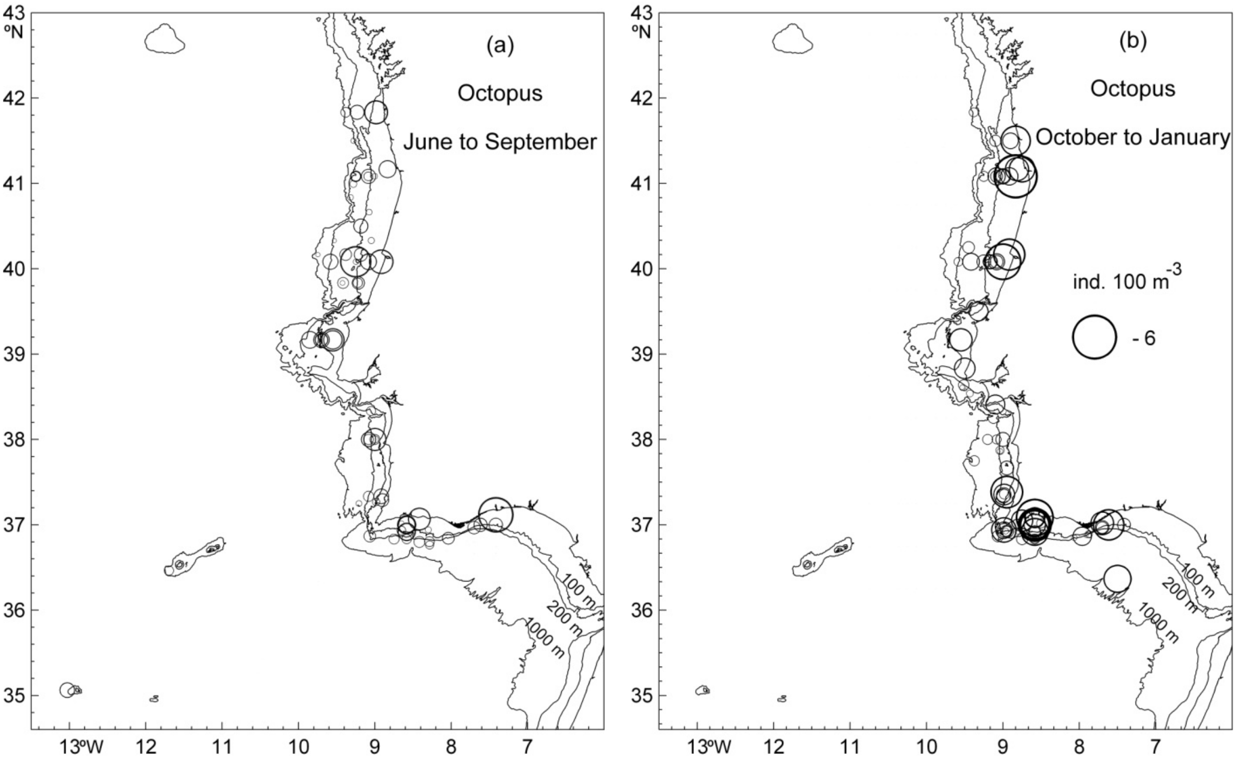

Figure 37 - Horizontal distribution of octopus in Portuguese waters and adjacent Spanish regions from plankton surveys during the two hatching main seasons: June to September (a) and October to January (b).

A more detailed analysis showed that the influence of upwelling was rather different on the west coast, where density was highly correlated with upwelling independently of hatching season (ANOVA, F(1, 1156) = 7.20, $p < 0.05$), in opposition to the south coast, where no relationship was detected between octopus density and UI (ANOVA, F(1, 420) = 2.26, $p > 0.05$). In spite of the gamma model did not show any significant influence for the whole area in relation to the interaction between month and depth, the west coast showed a cross-shore distribution significantly different between seasons (ANOVA season vs. depth range effect, F(3, 769) = 2.852, $p < 0.05$): during the summer upwelling season (June-September) the density was significantly higher offshore (100-200 m) and in autumn/winter, during favourable convergence conditions, higher densities were observed close inshore (15-50 m). In contrast, most paralarvae were found at SST of 18-19 ºC independently of area or even season.

The diel vertical distribution pattern of octopus is not clear from the data available, which were compiled from different stations, seasons and areas. No paralarvae were found in the neuston layer at any time.

Table 11 - Deviance tables for *Octopus vulgaris* GLM logistic models (probability of positive capture) and gamma models (density in positive captures).

Source of variation	**Deviance %**	**Estimates**		**p-value**	
		βi	**SE**		
Logistic models					
month	10.3	0.248	0.026	0.026	***
latitude	1.0	-0.152	0.049	0.049	**
depth	0.6	-0.001	0.000	0.000	*
UI	0.7	0.005	0.002	0.002	**
SST	6.4	0.299	0.038	0.038	***
month x depth	11.0	0.000	0.000	0.000	*
latitude x depth	3.2	0.001	0.000	0.000	***
latitude x UI	2.1	0.003	0.001	0.001	*
latitude x SST	7.3	0.074	0.025	0.025	**
depth x SST	8.2	-0.001	0.000	0.000	**
UI x SST	8.4	-0.005	0.001	0.001	***
Total explained	59.2%				
Gamma models					
year	11.9	-0.060	0.015	0.000	***
longitude	5.2	0.416	0.153	0.007	**
depth	7.6	-0.001	0.000	0.009	**
UI	3.4	-0.005	0.002	0.032	*
year x depth	33.6	0.001	0.000	0.000	***
Total explained	61.6%				

6.3.1.3. Sepiolids

Sepiolids were found mainly over the continental shelf (only 3 specimens deeper than 200 m) in two separate areas (Figure 38): one in the northwestern shelf between 40º and 42º N, mainly inshore of the 100 m isobath and the other one in the southwestern and southern shelves, south to 38º N, mainly offshore of the 100 m isobath.

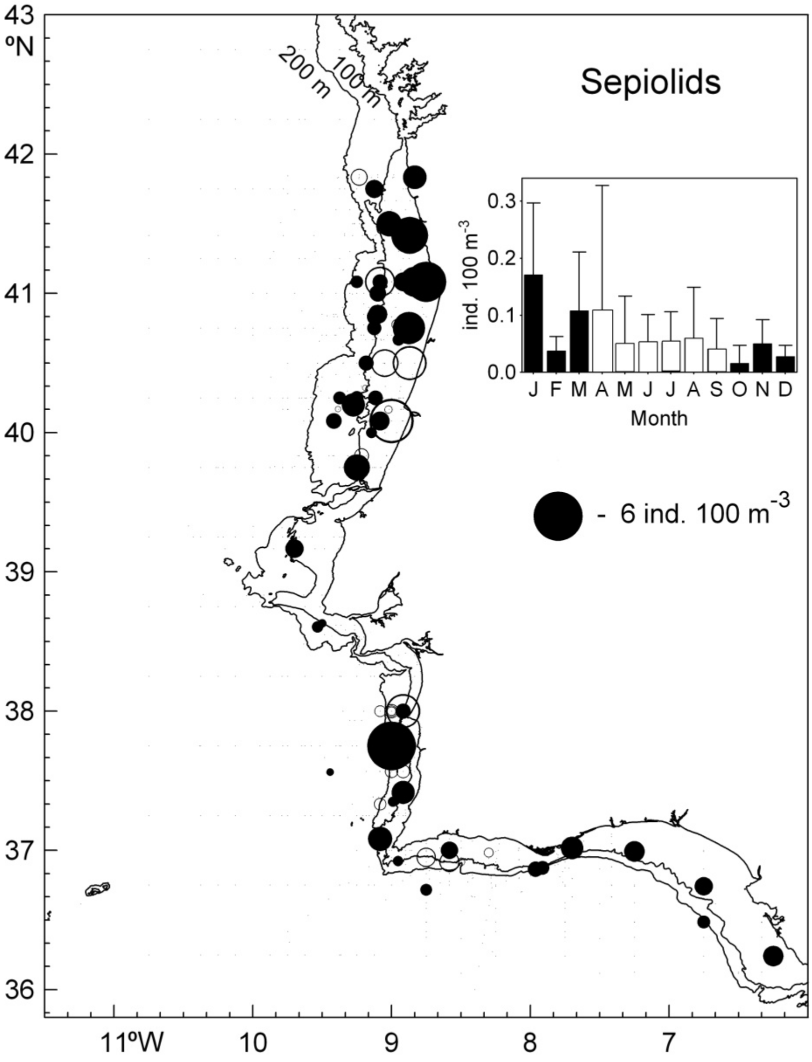

Figure 38 - Horizontal distribution of sepiolid paralarvae in Portuguese waters and adjacent Spanish regions during October to March (black dots) and April to September (open circles) plankton surveys. Monthly mean densities within their distribution area (bottom depth < 200 m), where vertical bars denote 0.95 confidence intervals.

The variables included in the GLM modelling poorly explain the distribution of these paralarvae for the whole area (2.8%), with year and depth being the only significant contributors (Table 12), reflecting mainly the higher occurrences in 2002 and 2004 and generally offshore of the 80 m isobath. At the same time, the gamma model explained 23.7% of the total variance, with month and SST as the major contributors. Abundance was significantly higher during the first part of the year (Figure 38) when the average SST is lower throughout the whole sampling area and this could explain the inverse relationship with this variable. A detailed analysis separately for the northwestern shelf and the southwestern/southern shelf showed no seasonal depth range differences within each area (ANOVA, $F(3, 689) = 0.118$, $p > 0.05$, $F(3, 425) = 0.351$, $p > 0.05$, respectively in the north and south).

Table 12 - Deviance tables for Sepiolids GLM logistic models (probability of positive capture) and gamma models (density in positive captures).

Source of variation	**Deviance %**	**Estimates**		**p-value**	
		βi	**SE**		
Logistic models					
year	1.0	0.051	0.022	0.021	*
depth	1.8	0.008	0.003	0.002	**
Total explained	2.8%				
Gamma models					
month	15.4	-0.103	0.030	0.001	**
SST	8.3	-0.114	0.052	0.031	*
Total explained	23.7%				

6.3.1.4. Ommastrephids

Ommastrephids were mostly in the rhyncoteuthion stage (only 7 specimens presented some proboscis division, DML > 5.3 mm). Paralarvae were found whole year round (Table 9), with three distinct peaks in January, April/May and October and distributed mainly in the northwestern shelf and shelf break north of 40º N (Figure 39), between the 50 m and 300 m isobaths. GLM results (Table 13) significantly highlight the higher probability of capture ommastrephid paralarvae in northern latitudes. The interaction latitude *vs*. month was also significant, reflecting the seasonal variation in the spatial distribution: during spring/summer most paralarvae occurred in the northern shelf and a meridional spread from the centre of distribution was detected in autumn/winter (Figure 39). During winter some specimens occurred in oceanic waters, namely between the continental shelf-break and oceanic seamounts (Galicia and Gorringe Banks).

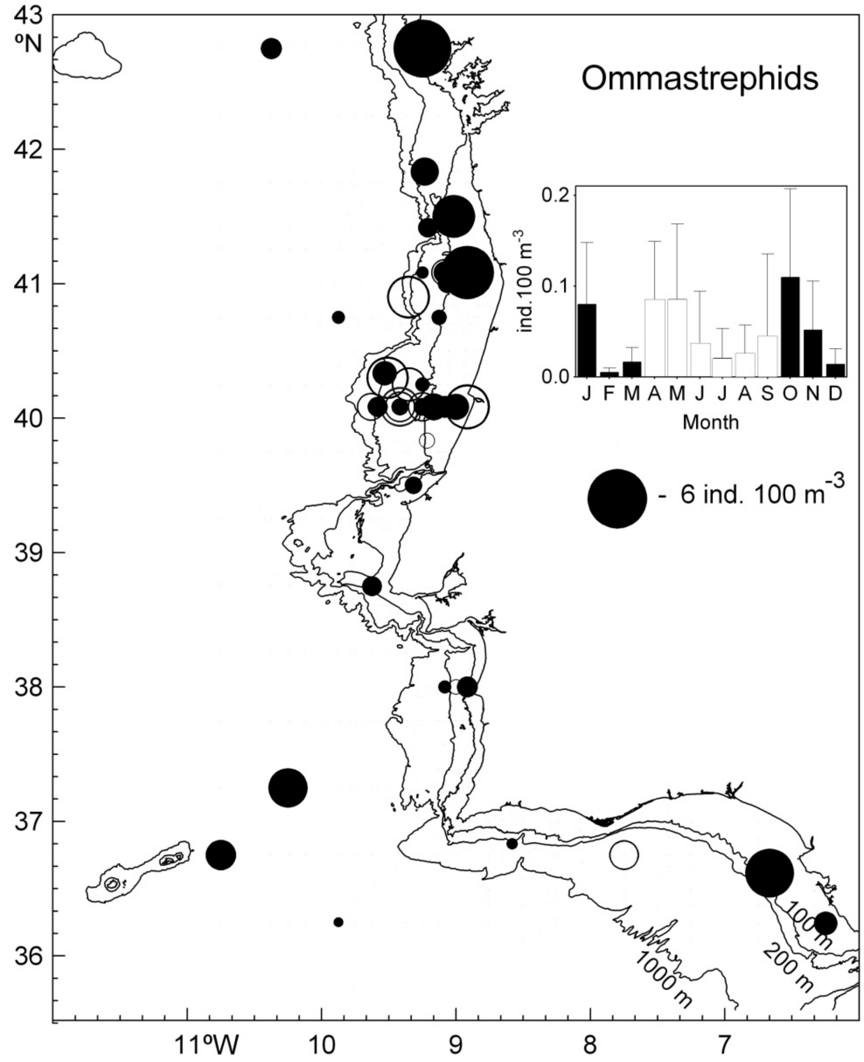

Figure 39 - Horizontal distribution of ommastrephid paralarvae in Portuguese waters and adjacent Spanish regions during October to March (black dots) and April to September (open circles) plankton surveys. Monthly mean densities within their distribution area (bottom depth < 1500 m), where vertical bars denote 0.95 confidence intervals.

There is also a significant role of year and of the interactions year *vs*. month and year *vs*. depth, suggesting high interannual variability. A more detailed analysis revealed higher densities offshore (100-300 m) between April and September under the summer upwelling conditions and close inshore (50-100 m) between October and January, during the autumn/winter convergence (ANOVA, season *vs*. depth range effect, F(5, 868) = 3.9273, $p < 0.05$). The paralarvae distribution was limited to SST between 13 and 20 ºC.

Table 13 - Deviance table for Ommastrephids GLM logistic models (probability of positive capture).

Source of variation	**Deviance %**	**Estimates**		**p-value**	
		βi	**SE**		
Logistic models					
year	4.2	-0.131	0.030	0.000	***
latitude	2.8	0.291	0.079	0.000	***
year x month	5.7	-0.026	0.010	0.008	**
year x depth	5.3	0.000	0.000	0.024	*
month x latitude	5.4	0.068	0.024	0.004	**
Total explained	23.4%				

6.3.2. Oceanic species

Ninety-six paralarvae and early juveniles of 19 different taxa of oceanic cephalopod species occurred in the sampling area. The majority of them belonged to the Enoploteuthid group and the family Cranchiidae. Details concerning distribution, seasonality and size for each of the taxa are summarized in Table 9. The occurrence of oceanic paralarvae peaked during January and decreased until August. Most taxa were found only during winter months (*Chiroteuthis veranyi*, *Pyroteuthis margaritifera*, *Abralia cf. veranyi*, *Ancistrocheirus lesueurii*, *Leachia* sp., *Taonius pavo*, *Bathothauma lyromma*, *Megalocranchia* sp., *Teuthowenia megalops* and *Brachioteuthis reesei*), while others were found during winter and spring (*Abraliopsis atlantica*, *Mastigoteuthis* sp.), only in spring (*Helicocranchia pfefferi*, *Chtenopterix siculus*, *Ocythoe tuberculata*) or throughout winter to summer (*Pterygioteuthis* sp., *Onychoteuthis banksii*, *Heteroteuthis dispar*). No oceanic paralarvae were found between September and December, despite the offshore sampling (over bottom depths > 200 m) undertaken during those months.

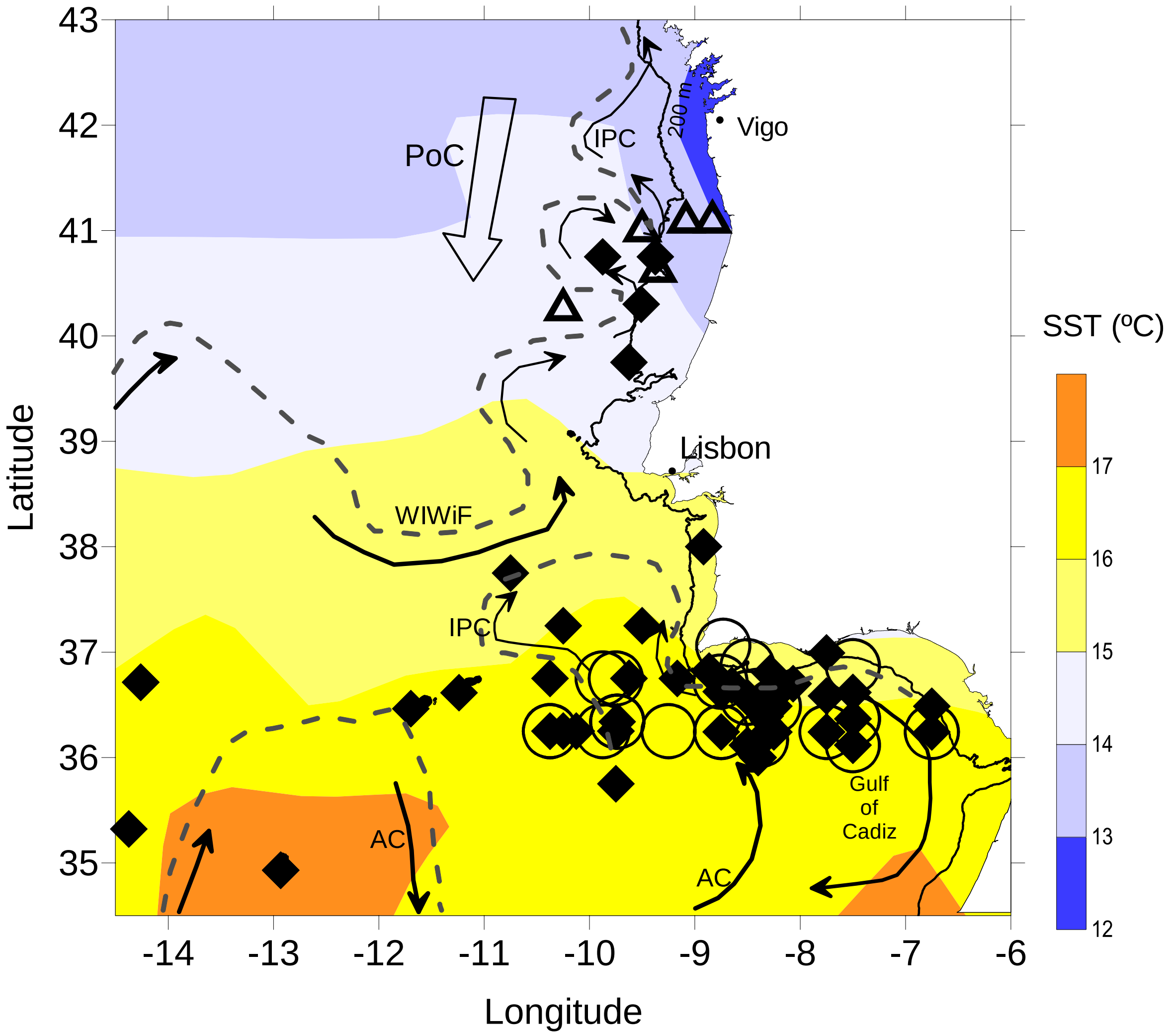

Figure 40 - Distribution of paralarvae of oceanic species. Open triangles are the *Teuthowenia megalops* records, open circles are the tropical/sub-tropical species (*Abralia cf. veranyi*, *Ancistrocheirus lesueurii*, *Pyroteuthis margaritifera*, *Helicocranchia pfefferi*, *Megalocranchia* sp. and *Leachia* sp.), and dots represent the remaining species distribution. Overlay of winter sea surface temperature (SST) and major winter surface circulation features, after Peliz *et al.* (2005): PoC = Portugal Current, IPC = Iberian Poleward Current, AC = Azores current eastern branch (arrows); WIWiF = Western Iberia Winter front, Gulf of Cadiz northern re-circulation and STF/AC frontal systems (thick lines).

Teuthowenia megalops, the only species whose main distribution is in north temperate to sub-artic waters in the Atlantic, was captured on the western Iberia only in winter, north of 40º N and SST below 15 ºC. During this season, only *Mastigoteuthis* sp. and *Onychoteuthis banksii* paralarvae occurred also north of 38º N. Most oceanic paralarvae (91%) were distributed in the southern part of the sampling area, in the warmer oceanic stations and few on the colder continental shelf. Subtropical and tropical species were restricted to the southern area, south of the Gulf of Cadiz northern recirculation front and mostly within SST ~17 ºC (Figure 40).

6.4. Discussion

The Portuguese waters are an important area for the spawning of numerous neritic and oceanic species, which is reflected in the high biodiversity found. This study was based in plankton sampling from several cruises and different programmes, throughout a 19 year period. Sampling methodology bias was minimized by the standardisation of catches, and the adjustment of the analysis to the data available (e.g. high number of zero catches). Despite the net efficiency being different between sampling gears (e.g. Stehle *et al*., 2007), we considered this effect of small relevance on the establishment of distribution and abundance patterns of paralarvae, as most sampling was done with bongo nets with extended spatial and time coverage.

6.4.1. *Loligo vulgaris*

Loliginid paralarvae are very similar in shape and thus difficult to identify to species level, especially when cromatophore pattern is absent, as it is the case of old preserved plankton samples. However, the relatively low abundance of *Loligo forbesi* off the Portuguese coast (Chen *et al.*, 2006); the occasional recoveries of loliginid egg masses, all identified as being *L. vulgaris* (e.g. Cunha *et al.*, 1995; Villa *et al.*, 1997), and the size at hatching measured in reference collections of preserved specimens, lead us to assume that the loliginid paralarvae caught were mainly *L. vulgaris*, with a small percentage of

Alloteuthis subulata. Furthermore, the hatching period inferred from loliginid paralarvae densities matches the spawning season of *L. vulgaris*. The spawning peaks in December/January and June/July (Moreno *et al.*, 2002), followed by an embryonic development of 40-47 days on average (Villanueva *et al.*, 2003), would produce two seasons of higher densities of hatchlings with peaks around March and August, as detected in our analysis.

SST revealed to be the most important environmental factor, enough to be determinant of species seasonality and distribution, in agreement to other studies that put in evidence the role of temperature on *L. vulgaris* life history traits (e.g. Moreno *et al.*, 2005). Therefore, higher densities and a broader distribution were found, when average SST is relatively low throughout the area, whereas in the summer, the higher densities were located in areas with lower temperature (see Figure 33). Therefore, despite the location of an important spawning ground at the southern area (9 to 8º W) in summer (Cunha *et al.*, 1995), we found low density of *L. vulgaris* paralarvae and total absence to the east where summer temperatures are higher. Since *L. vulgaris* southern spawning grounds are located at lower depths during summer months (Villa *et al.*, 1997) and our results found paralarvae in relative low temperatures, we can speculate that hatchlings can be advected by the warm inshore countercurrent. This current extends westwards and turns northwards along the western coast after reaching Cape S. Vincent (Relvas and Barton, 2005; García-Lafuente *et al.*, 2006; Teles-Machado, 2007). Furthermore, this drift is a potential mechanism that allows squid paralarvae to be exposed to colder and more productive upwelled waters of the west coast and thus enhances their survival.

The distribution and seasonality of *L. vulgaris* paralarvae on the northwest shelf were quite distinct and reflect well the different oceanography mesoscale dynamics of the western Iberia. Therefore in summer, although paralarvae are under the influence of upwelling conditions and consequently cross shelf transport in the Ekman layer is expected to advect them towards the open ocean, the higher densities were found mainly in the middle shelf, broadly located in the vicinity of the main northern spawning ground (Cunha *et al.*, 1995). Furthermore, our data suggest that *L. vulgaris* paralarvae could perform diel vertical migrations, similar to other cephalopod paralarvae (Piatkowski *et al.*, 1993). Thus, the cross-shelf net transport associated to upwelling dynamics coupled to larval diel migration patterns could result in favourable conditions for their retention over the spawning grounds during summer as hypothesised for crustacean larvae in the same geographic area using observations and models (Marta-Almeida *et al.*, 2006; dos Santos *et al.*, 2008). The positive influence of upwelling events in the abundance of *L. vulg*aris paralarvae was previously observed during summer in the vicinity of Ria of Vigo (Rocha *et al.*, 1999; González *et al.*, 2005).

In winter, when paralarvae density is higher, a part of the new generation is able to spread offshore but retained over the shelf break by the blocking effect of the IPC which in the long run could promote also a net poleward advection (Santos *et al.*, 2004; Peliz *et al.*, 2005). At the same time, the absence of paralarvae close to the shore in the northwestern coast during winter is likely to avoid the low saline coastal waters derived from intense river runoff (Ribeiro *et al.*, 2005).

6.4.2. *Octopus vulgaris*

O. vulgaris is widely spread over mainland shelf waters, as well as in distant oceanic islands and seamounts. The length of embryonic development has an inverse relationship with temperature and it may take from 1.5 to 4 months until hatching (Mangold and Boletzky, 1973) under the temperature range the eggs may experience within Portuguese waters at depths < 100 m.

Year round spawning is observed in fisheries and survey data off northwestern Portugal with two distinct peaks in April and July/August (Lourenço *et al.*, in press). Giving that mean bottom temperature at depths < 100 m is ~ 14 ºC (IPIMAR cruise data), two main density peaks of paralarvae would be expected ~ 100 days later (Mangold and Boletzky, 1973). These would correspond to higher densities of octopus paralarvae in July and in November, which matches well with our observations. The autumn hatching peak could be magnified as a result of faster development of the eggs laid by the end of the summer spawning season, since with the end of the upwelling season bottom temperatures rise abruptly in shallow depths. Just further north, in Galician waters, a unique peak of early hatched octopus occurs at the end of summer and autumn months, just after the upwelling season (Otero, 2006).

In the north-west coast, the seasonal distribution of octopus seems to agree well with the Ekman dynamics of cross-shelf transport, showing higher densities offshore during the upwelling season and near the shore during the convergence period. Despite it is observed a more offshore distribution of octopus paralarvae in spring/summer in the northwest Portuguese coast; they are still retained over the shelf. A probable explanation for this is the double frontal system on the wide north-west continental shelf described by Peliz *et al*. (2002) that make it a major retention area, preventing massive larvae advection and consequent loss into the open ocean. The retention capabilities of this area minimise the potentially unfavourable conditions induced by the strong upwelling events, allowing a regional spring/summer octopus hatching season. A similar coupling between the spawning strategy and the enrichment/retention shelf processes was described for the Arguin Bank (Mauritania), where *O. vulgaris* also evidences two spawning/hatching peaks (Demarcq and Faure, 2000).

On the other hand, on the northern Gulf of Cadiz the spawning season occurs from February until November with higher intensity from June to September both on the western (Lourenço *et al*., in press) and the eastern areas (Silva *et al*., 2002), and very similar to the reproductive strategy on the western Mediterranean (Rodriguéz-Rúa *et al*., 2005). A single paralarvae peak would be expected on the southern Portuguese waters ~80 days later (Mangold and Boletzky, 1973), taking into account the mean local bottom temperature (~ 15 ºC) that follows the main spawning season (IPIMAR cruise data). This is in agreement to the observed dominant autumn peak in paralarvae density. However, it does not explain well the distribution of paralarvae during summer in this area.

A plausible explanation is a drift of the newly hatchlings, equatorward within the western upwelling-derived flow, and eastwards around Cape S. Vincent (Relvas and Barton, 2002; 2005) within the Gulf of Cadiz slope Current (GCC) along the southern Portuguese coast, which in summer is connected upstream with the upwelling current (Peliz *et al*., 2007a). This water mass with SST below 19 ºC (Relvas *et al*., 2007) coincides with the distribution of paralarvae along the southern shelf break until ~ 8º W and over the shelf east of Cape Santa Maria (see fig. 7a). As the swimming capacity of newly hatchlings (< 3 cm s^{-1}) is very weak, (Villanueva *et al*., 1995), some may be carried as far as ~190 km within the upwelling jet (~16-22 cm s^{-1}) (Sanchez and Relvas, 2003) and the GCC (~ 20 cm s^{-1}) (Peliz *et al*., 2007a), from the west to the south coast in 10 days. This should be enough to reveal a paralarvae density peak in July in the south coast.

Following this oceanic circulation a few specimens could also be advected offshore by the long upwelling filament that protrudes close to Cape S. Vincent (Relvas *et al*., 2007), and be caught in June at the Gorringe and Ampere seamounts. Nevertheless, we do not exclude the possibility that the paralarvae on the banks result from resident populations.

The drift hypothesis from the west to the south coast is supported by the significant influence of SST and upwelling on *O. vulgaris* paralarvae distribution that was demonstrated by our study, which denotes a strong association of these paralarvae and water masses properties, namely the 17 - 18 ºC upwelled waters. Additionally, the distribution of paralarvae during summer on the southern coast may also be an indication that a significant part of the summer spawning would occur in shallow waters, at areas with high bottom temperatures, producing significant offspring within a month time.

The different distribution of *L. vulgaris* and *O. vulgaris* paralarvae on the west coast during summer may be related to distinct diel vertical migration patterns. However, the knowledge about the vertical distribution and behaviour of those paralarvae is still very scarce.

6.4.3. Sepiolids

The poor correlation observed between paralarval sepiolid distribution and environmental variables is caused by the occurrence of several species with different distributions and spawning seasonality. Indeed, the paralarvae found, which were not identified to the species level, may include a mixture of *Rossia macrosoma*, *Sepietta oweniana*, and *Rondeletiola minor* that are the most abundant species within the surveyed area (Guerra, 1992). Some paralarvae caught could belong also to the less abundant species: *Sepietta neglecta, Sepiola atlantica*, and *Sepiola rondeleti*. Nevertheless, our observations are consistent with the knowledge that all those species undergo spawning all year round (Jereb and Roper, 2005). The most interesting finding was that paralarvae were retained on the continental shelf, in spite of adult distribution range spreads far offshore (until 500 m or even 1000 m depth). This could be an indication that these species migrate inshore for spawning in Atlantic waters, as observed for many species in the Mediterranean Sea (Jereb and Roper, 2005). Unlike the other cephalopod paralarvae, no seasonal oceanographic effects on paralarvae dispersal were observed. Thus, the distinct depth distribution detected between the northern and southern areas may be more related to the actual geographic differences between those areas. Namely, in the northern area the shelf is much wider (~ 60 km) than in the southern area (~ 25 km) (Peliz *et al.*, 2005), and distinct depth range distributions suggest similar distances from the coast.

6.4.4. Ommastrephids

Some morphological differences were detected among ommastrephids, however, as there were no paralarvae descriptions available yet, all specimens were analysed as a single group. Although the most abundant species, *Illex coindetii* and *Todaropsis eblanae* spawn all year round throughout Atlantic European waters, *I. coindetii* have a main spawning season between spring and summer (González and Guerra, 1996; Arvanitidis *et al.,* 2002; Hernández-García, 2002) and *T. eblanae* spawns mainly during summer in northern waters (Hastie *et al*., 1994; Robin *et al*., 2002; Zumholtz and Piatkowsky, 2005) and south of 44º N have two spawning seasons, in early spring and early autumn (González *et al*., 1994; Arkipkin and Laptikovsky, 2000). The high paralarvae density in spring matches well the spawning peak of both species, giving ~ 15 days for egg development (Sakai *et al*., 1998). On the other hand, the paralarvae of the October peak may represent mainly the *T. eblanae* second spawning season.

Paralarvae of *Todarodes sagittatus* could be considered negligible as a component of this group since the spawning females that approach the Portuguese continental waters are in few numbers from May until December (IPIMAR cruise data). Nevertheless, *T. sagittatus*, in spite of having an extended spawning season, show a well-pronounced winter peak in the northwestern African waters (Arkhipkin *et al*., 1999) and thus the January peak of ommastrephids in our samples could correspond to *T. sagittatus* paralarvae, including those specimens found between the continental shelf-break and oceanic seamounts.

Ommastrephid spawning grounds in the northeast Atlantic are unknown but it is possible to suggest their location based on the occurrence of females in spawning condition, namely regarding *T. eblanae*, which is less migratory than the sympatrics *I. coindetii* and *T. sagitattus* (Lordan, 2001). *I. coindetii* and *T. eblanae* spawning females may be found mainly in the southwest and southern areas during spring and summer months (IPIMAR cruise data). During this season there is a clear mismatch between the spatial distribution of spawning females and paralarvae, which were found mainly confined to the continental shelf between 42º and 40º N. Although paralarvae may be rapidly transported within water masses (Trites, 1983), it is more likely that the mature fast swim ommastrephids migrate northwards, namely by taking advantage of the subsurface Iberian Poleward Current (IPC) in the vicinity of the shelf-break. This northwards migration in spring summer months allow females to meet favourable environments for spawning in more productive areas with moderate temperatures, because eggs and paralarvae fail to develop at temperatures >20 ºC (Boletzky *et al.*, 1973).

The area of higher density of ommastrephid paralarvae was at all seasons within the one already described as retentive for biogenic material in northwestern Iberia. These results emphasises the importance of the productive upwelling areas for the reproduction and early growth of migratory pelagic species, thus the lack of significant influence of temperature or upwelling in the statistical analysis of distribution and abundance was hide by the mixture of species.

6.4.5. Oceanic species

The oceanic species composition in the Portuguese waters was similar to other sub-tropical eastern Atlantic areas. In spite of histioteuthids being fairly more common in this region (Nixon and Young, 2003; Clarke, 2006), their early young stages were not found, and the mesopelagic enoploteuthids and cranchiids, whose hatchlings occur in near-surface waters (e.g. Gibbs and Roper, 1970), dominated the catches. The adults of most of the paralarvae identified may be found in the northwest Africa (Adam, 1983) and in the Madeiran waters (Rees and Maul, 1956; Clarke and Lu, 1995) towards the mid-Atlantic ridge, in the vicinity of seamounts and in the Azores waters (Dieckmann and Piatkowski, 2004; Clarke, 2006). Those species have their northernmost range of distribution in Portuguese waters, when approaching for spawning, between January and June. They take advantage of the major circulation regimes, illustrated in figure 10, such as the eastern branch of the Azores current (AC) and the Gulf of Cadiz northern recirculation, which transports warm sub-tropical water, as well as the flow associated to the Western Iberia Winter Front (WIWiF) formed around 39-40º N in winter.

On the contrary, the cranchiid *Teuthowenia megalops*, which is a common species west of British Islands (Collins *et al.*, 2002), extends its spawning ground further south in winter and its paralarvae will reach the northwest Portuguese coastal waters. For the northern species, warmer temperatures are likely to be a major boundary to equatorward dispersal. *T. megalops* specimens were all found in the same geographic area, and are a good example of adult or larval drift equatorwards by the broad southward-flowing Portugal Current (Pérez *et al.*, 2001), and inshoreward advection by the prevailing winter convergence in northwest Portuguese waters (Peliz *et al*. 2007b).

Despite the wide geographic sampling coverage, the oceanic paralarvae were mainly concentrated in the Gulf of Cadiz system, including many cosmopolitan species as *Brachioteuthis reesei*, *Chiroteuthis veranyi*, *Ctenopterix siculus*, *Taonios pavo* and *Ocythoe tuberculata*. The persistent fronts between the shelf and deep waters close to Cape S. Vicente (Sanchez and Relvas, 2003; Peliz *et al.*, 2005), together with water temperature, may represent a boundary to poleward dispersal, which would result in the concentration of oceanic cephalopod paralarvae in the southwestern Iberian Peninsula, within the transition from subtropical to temperate ecosystems.

Chapter 7

Nursery grounds of *Octopus vulgaris* along the Portuguese coast in autumn and winter

7. Seasonal nursery grounds of *Octopus vulgaris* along the Portuguese coast

7.1. Introduction

The common octopus, *Octopus vulgaris* Cuvier 1797, is one of the most commercially important cephalopods worldwide. It is exploited as a target species by demersal trawl fleets and several local fisheries, using hand jigs, pots, traps and trammel nets operating in southern Europe and northwestern Africa (Hastie *et al*., 2009). In Portugal it is also one of the most important fisheries resources, often the most important species in first sale value, with average landings of 8500 tonnes per year (1986 - 2010). The exploitation of octopus in Portugal increased 50% in the last 20 years. It is captured mainly with traps (~90%), thus having a major importance in the Portuguese artisanal fishery, namely on the south region (Algarve), where it accounts for more than 20% of the fishing activity income (DGPA, 2011). On the northwest and south regions there is also a minor directed trawl fishery to the common octopus (Fonseca *et al*., 2008).

The biology of *O. vulgaris* has been studied thoroughly in the last decades in the Mediterranean and the central eastern Atlantic (e.g. Mangold-Wirz, 1963; Quetglas *et al*., 1998; Hernández-García *et al*., 2002), and more recently in the NE Atlantic (e.g. Silva *et al*., 2002; Rodríguez-Rúa *et al*., 2005; Otero *et al*., 2007; Lourenço *et al*. in press). *O. vulgaris* has a short life cycle of 12-14 months (Domain *et al*., 2000; Iglesias *et al*., 2004) and terminal spawning with egg care by the female. The incubation of eggs lasts 60-120 days (Mangold and Boletzky, 1973), and paralarvae are planktonic for one to three months, depending on temperature that affects growth rates and thus the time to reach the critical size for settlement (> 7.5 mm ML) in adults benthic habitat (Villanueva, 1995). Paralarva mortality on the phase is sought to be very high and environmental dependent thus inducing high variability on recruitment success/biomass (Faure *et al*., 2002; Otero *et al*., 2008; Otero *et al*., 2009). The growth is very rapid with extreme individual variation in growth rates being observed in culture experiments (Iglesias *et al*., 2004) and in wild populations (Domain *et al*., 2000). *O. vulgaris* like other cephalopods is ecologically opportunistic and capable of a highly labile life history, which is well correlated to the local environmental dynamics. The spawning season extends, in general, throughout the year with one or two peaks geographically variable in timing (Hastie *et al.*, 2009). Over the NW Portuguese shelf spawning occurs in two main peaks (April and August) and at the south shelf a single peak is generally detected in summer (Lourenço *et al.*, in press). However, *O. vulgaris* paralarvae present the highest abundances in autumn, both at the west and south regions, but high abundances are also observed during spring (Moreno *et al.*, 2009). The differences between the western and the southern Portuguese waters are related with the different oceanographic regimes, leading to differences in the structure of the respective populations (Lourenço *et al.*, in press).

A connection between populations through paralarvae drift from the northwest shelf towards southern nursery grounds was hypothesized by Moreno *et al.* (2009), but it is still a subject of debate. Despite the great commercial value of the *O. vulgaris* fisheries for Portugal and Spain, studies on the identity and distribution of Iberian stock(s) are scarce. Analysis of microsatellites DNA markers showed an isolation-by-distance model for Atlantic populations (Cabranes *et al.*, 2008), with significant genetic variation between pairs of distant samples (e.g. Asturias-Galicia *vs.* South Portugal-Cadiz), but not within neighbouring pairs. There are no studies on the genetic sub-structuring between the Portuguese western and southern octopus populations, but for fisheries management purposes these populations may be viewed as two distinct stocks, to be managed according to their respective life history traits. Given the depletion of many marine resources, there is also an increasing need for studies on the distribution of the several key life stages, e.g. juveniles, to ensure sustainability and aid in the conservation of the most heavily exploited species, such as *O. vulgaris* in Portugal. Many studies have analyzed the distribution and abundance of *O. vulgaris* by trawl surveys (Guerra, 1981; Sánchez *et al.*, 1998; Quetglas *et al.*, 2000; Belcari *et al.*, 2002; Faraj and Bez, 2007), despite its recognized biased catchability (Katsanevakis and Verriopolus, 2004a), but this is most of the times, the only practical way to cover their extended distribution depth range (up to 500 m).

The aims of this study are to identify seasonal nursery grounds of *O. vulgaris* along the Portuguese coast using georeferenced fishery independent data, to analyse the relationships between juvenile abundance and several environmental variables, as indicators of possible juvenile essential habitats, and identify critical areas to be protected, if needed, in future management options.

7.2. Material and Methods

7.2.1. Fisheries sampling to determine recruitment seasonality

Biological data on *O. vulgaris* was obtained from fishery sampling in two areas of the Portuguese coast: the northwest coast (NW) grouping catches at latitudes 38.5 ºN to 41 ºN and the southern coast (South) grouping catches at longitudes 7 to 8 ºW. Monthly samples from NW were obtained from the trap fishery landings in Peniche and neighbouring ports, between February 1997 and January 2008. Samples from the South region were obtained monthly from the trap artisanal fishery landings in Stª. Luzia, between November 2000 and November 2001 and landings in Olhão, between December 2006 and January 2008. From each specimen dorsal mantle length (ML) and body weight (BW) were measured.

7.2.2. Survey sampling to determine nursery grounds

Two sets of bottom trawl surveys (BTS) carried out on the Portuguese continental coast on board R/V Noruega and R/V Capricórnio were selected for the analysis of *Octopus vulgaris* nursery areas (autumn and the winter surveys). The sampling area covers latitudes 36.7º to 41.8º N and longitudes 7.47º to 10.0º W in the NE Atlantic. The main objective of these research surveys was to estimate indices of abundance and biomass of the most commercially important fish and crustacean species. Autumn BTS generally employ a Norwegian Campbell Trawl type with bobbins, not suitable to sample benthic species (*Octopus vulgaris* is not a target species). Therefore, for our study we selected only the autumn BTS conducted with R/V Capricórnio, which used an FGAV019 bottom trawling net, with a cod end of 20 mm mesh size, a mean vertical opening of 2.5 m and a mean horizontal opening between wings of 25 m. The winter cruises were carried out in the R/V Noruega using the same trawl net. Both datasets were obtained following a similar depth stratified sampling design, with ca. 70-80 hauls distributed along the Portuguese continental shelf and slope (Figure 41). The tow duration varied between 20 and 60 min. No significant differences were previously found in the mean abundance and length distribution for several species due to different tow duration (Cardador, com.pers.). The number of hauls by research cruise and other characteristics of the sampling procedure are detailed in Table 14. In each sampling station, all of the O. *vulgaris* captured were weighted (individual body weight, BW, to the nearest g) and measured (mantle length, ML, to the nearest 0.5 mm).

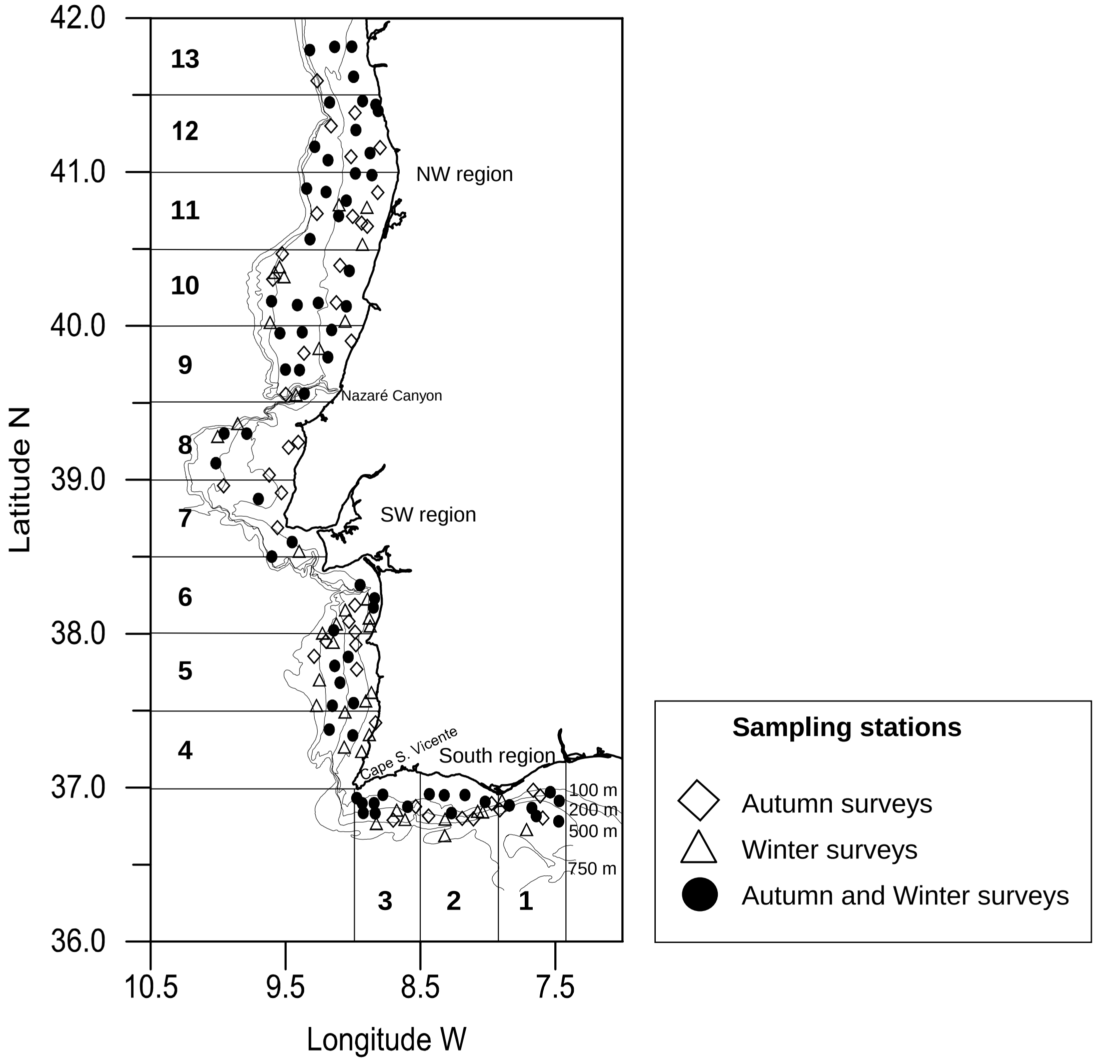

Figure 41 - Sampling stations in autumn and winter surveys. Location of sampling areas (1 to 13) and regions (NW, SW, South) and depth strata.

7.2.3. Environmental data

The following environmental variables were selected and assigned to each sampling station: Sea Surface Temperature data (SST), Sea Bottom temperature (SBT), Sea Surface Salinity (SSS), Sea Bottom Salinity (SBS), Bottom sediment type (BS); or to each area for each survey cruise: Rainfall (RF) and River runoff (RR).

Satellite-derived SST data were obtained for each sampling station from the AVHRR Pathfinder V.5 provided by NASA-JPL-Physical Oceanography Distributed Active Archive Center (PO.DAAC). SBT, SSS and SBS were extracted from CTD temperature profiles undertaken during the survey cruises at the end of the fishing stations. CTD data were not available for a few stations and for the Winter2005 cruise. SBT data to fill in these gaps were obtained from CTD casts extracted from NODC and ICES databases.

Table 14 - *Octopus vulgaris* sampling details of in each research survey: number of fishing hauls, number of fishing hauls with *O. vulgaris* catches (+), number of individuals captured and sampled, percentage of juveniles and mean body weight of the juveniles (BWJ).

Cruise	**Start date**	**End date**	**Fishing hauls**	**Fishing hauls +**	**CTD**	***O. vulgaris***	**% juveniles**	**mean BWJ**
Autumn96	11/10/1996	09/11/1996	82	39	+	397	69.8	373.3
Autumn99	29/10/1999	22/11/1999	82	27	+	103	55.3	403.8
Autumn03	07/10/2003	08/11/2003	83	33	+	86	55.8	397.4
Autumn04	23/10/2004	18/11/2004	79	34	+	140	75.0	311.7
Autumn			326	133		726	64.0	371.5
Winter05	03/03/2005	31/03/2005	72	36	NA	368	83.4	350.2
Winter06	08/03/2006	03/04/2006	68	28	+	214	78.5	444.2
Winter07	10/03/2007	03/04/2007	68	41	+	187	78.6	307.7
Winter08	26/02/2008	18/03/2008	69	43	+	372	70.2	390.5
Winter			276	148		1141	77.7	373.2

Bottom sediment type in each sampling station was classified based on the fishery charts from the Instituto Hidrográfico, as gravel (gv, >50% of grain bigger than 2 mm), coarse sand (cs, >15% of grain bigger than 2 mm), sand (s, <15% of grain bigger than 2 mm and < 25% of mud), mud (md, > 25% mud) and rocky mud (rm, mud with rocky outcrops).

A measure of rainfall by cruise and area was calculated as the total rainfall for the period of each survey cruise measured in the main coastal city in each of the 13 Areas. A measure of river runoff by cruise and area was calculated as the total runoff from the main rivers in each of the 13 Areas. These values were assigned to each sampling station to test their effects on juvenile abundance. Additionally, annual means of the total rainfall for the autumn months (October to December) and winter months (January to March) in areas 1 to 13 were estimated for the period between 1991 and 2009. Rainfall and river runoff data were extracted from the SNIRH - Sistema Nacional de Informação de Recursos Hídricos (http://snirh.pt).

7.2.4. Data analysis

Fishery sample sizes were standardised to 100 specimens by sampled month. The recruitment seasons in the NW and South regions were determined by calculating a Recruitment Index as,

$$RI = Rm/R$$

where R*m* is the number of recruits observed in month *m* and R represents all the recruits observed in each year. The computation of the RI assumes that recruits are animals with BW $\leq$ T. The species threshold T was defined as the modal BW of the body weight frequency distribution, computed pulling together all animals sampled from the fishery in both geographical regions. The recruitment intensity and its seasonality were compared between areas by factorial ANOVA.

The octopus with BW below the fisheries minimum landing weight (750 g) captured on the survey cruises listed in Table 14, were classified as juveniles (pre-recruits) and their distribution and abundance used to identify *O. vulgaris* nursery grounds. Catches were converted into an abundance index as number of juveniles per hour trawling.

To analyze the spatial distribution the sampled area was divided into 3 regions, which are environmentally different (see chapter 2) or 13 areas: 3 areas on the south region (South), 4 areas (0.5° latitude each) on the southwest region (SW), and 6 areas (0.5°

latitude each) on the northwest region (NW). To analyze distribution with depth (Dzone), the sampling stations were also assigned to the inner-shelf (<40 m depth, is), middle-shelf (40-90 m depth, ms), outer-shelf (90-200 m depth, os), and slope (> 200 m depth, s). The distance of each sampling station to the coast line (Dcoast) was estimated and data grouped in 5km classes.

Mean juvenile abundance and standard deviation (SD) were calculated for several temporal, spatial and environmental variables: Year (1996, 1999, 2003 to 2008) and Season (Autumn and Winter); Region (NW, SW and South), Area (a1 to a13), Dzone (i, m, o, s), and Dcoast (5km to 60km, 5 km classes); Sediment (g, cs, s, rm, m), SST (st13 to st20 ºC, 1 ºC classes), SBT (bt12 to bt16 ºC, 1 ºC classes), SSS(ss34.5 to ss36.0, 0.5 classes), SBS (bs35.0 to bs36.0, 0.5 classes), River runoff (r1<1000, r10=1000 to 10000, r100=10000 to 100000, r1000>100000 dam^3) and Rainfall (ra1<100, ra2=100 to 200, ra3=200 to 300, ra4=300 to 400, ra5>400 mm). The effects of temporal, spatial and physical variables in juvenile abundances (J) were investigated using one-way ANOVA, after checking for normality in the sample distributions (Shapiro test), and for homogeneity of variances (Bartlett test). The interactions between season (cruise) and the other variables were also tested by two-way ANOVA.

The seasonal spatial distribution of juveniles was analyzed and mapped with the geostatistics interpolation method, Krigging (Cressie, 1991), implemented in the software SURFER 9.0. The seasonal mean of juvenile abundance was estimated for each sampling station. Mean abundance data for autumn and winter cruises was interpolated separately by Krigging, using a linear variogram with no nugget effect. Discrete juvenile abundance areas were visually identified and classified as distinct nursery grounds. To verify whether those grounds were located in the same area consistently through time each season, a persistence index PI (adapted from Garofalo *et al.*, 2010) was estimated as the percentage of cruises a given area was classified as a nursery ground. Nursery grounds were superimposed to physical variable maps for a better visualization of relationships. The means, SD and range of several environmental variables were estimated for each identified nursery ground as proxies of juvenile essential habitat.

7.3. Results

7.3.1. Recruitment season

All specimens ≤ 1000 g may be considered recruits to the trap fishery. The modal body weight class is the 750 g BW class (750 to 1000 g), which corresponds to the modal size class of 140 mm ML (Figure 42a). Recruits appear in trap fisheries throughout the year, with maximum numbers in autumn on both NW and South regions (Figure 42b). However, each geographic region shows a significantly distinct recruitment seasonality (ANOVA, region x trimester effect: $F(3, 37) = 3.15$, $p < 0.05$). Recruitment on the NW region shows an extended season from June until December, despite the evidence of two peaks, one in late summer (September) and another in late autumn (December). On the other hand, two marked recruitment seasons are present on the South region; the most important season beginning in August and lasting until January of the next year, with a maximum RI in November. A secondary recruitment season starts in March and ends in June, with a maximum RI in April.

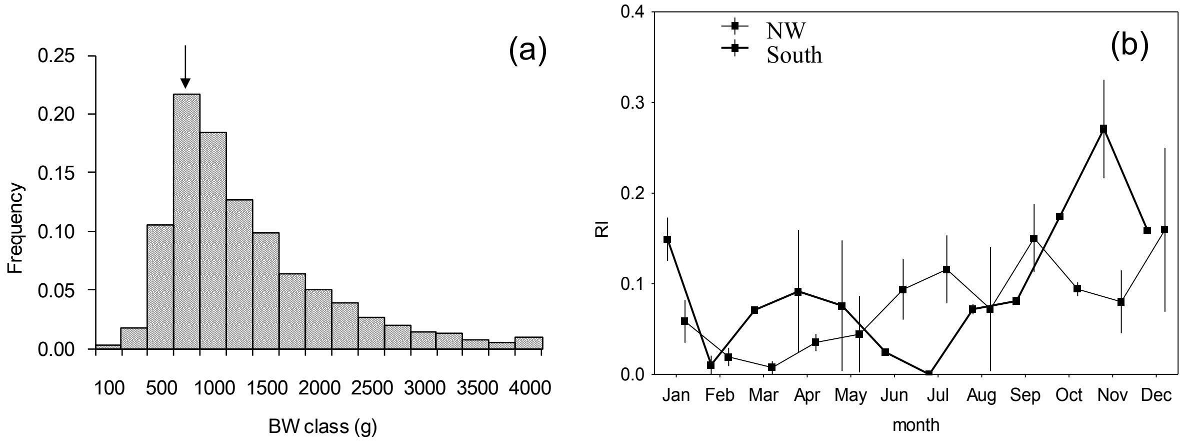

Figure 42 - Overall length frequency and body weight (BW) frequency of *O. vulgaris* from trap fisheries (a) and monthly recruitment indices (RI) on NW and South regions for the sampling periods November 2000 to November 2001 and December 2006 to January 2008. Vertical bars denote monthly standard error (b).

7.3.2. Environmental variation along the Portuguese coast

On the Portuguese coast SST is much higher in autumn than in winter (Figure 43). In autumn SST ranges between 14.0 in the north and 21.7 ºC in the south. In winter

SST ranges between 11.5 in the north and 16.6 ºC in the south. Autumn SST decrease sharply from area 1 in the South region towards area 13 in the NW region. During winter areas 1 to 6 show similar SST (~15ºC) and a decrease is observed from area 6 towards the north. SBT is also warmer in the autumn than in winter time, with a similar pattern of decreasing from area 1 towards area 13. In autumn the water column is stratified and important differences exist between SST and SBT. On the contrary SST is only slightly higher than SBT during the winter period of water column mixing.

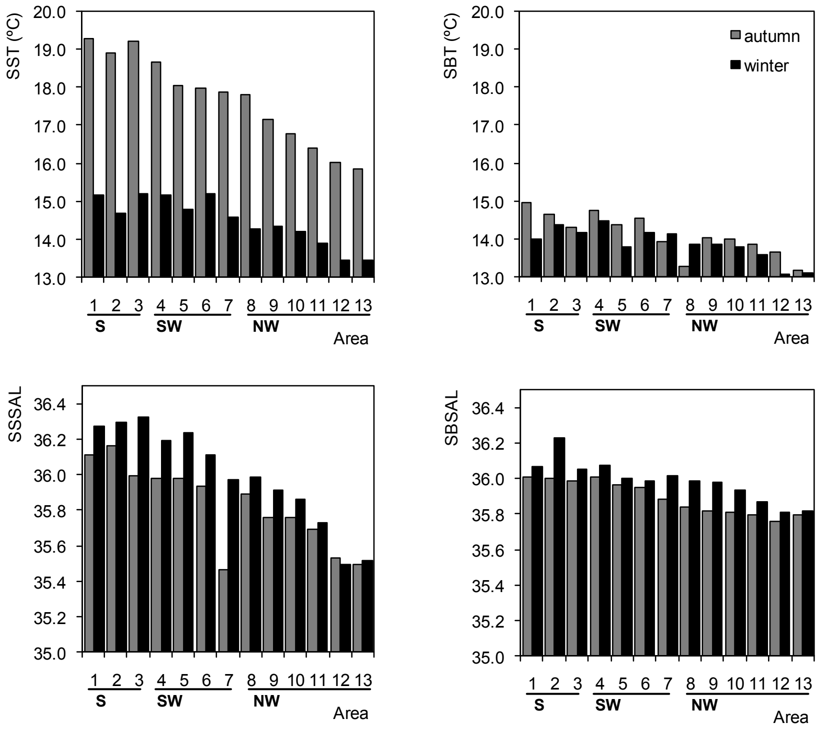

Figure 43 - Spatial variation of temperature and salinity during the autumn and winter surveys in areas 1 to 13 within the northwest (NW), southwest (SW) and south (S) regions.

The expected positive correlation between temperature and salinity may be observed by the spatial variation of salinity similar to that of temperature, decreasing with latitude and also away from the influence of the Mediterranean salty waters. Nevertheless, salinity is lower in autumn than in winter along the Portuguese shelf, both SSS and SBS, with the exception of area 13. This is most likely derived from the higher levels of autumn rainfall (Figure 44), which contribute to a decrease in salinity of coastal waters more significant in autumn than in winter. Rainfall levels during autumn and winter are similar between areas 1 and 10, but towards the north rainfall increases substantially. On the other hand, river runoff from the main rivers is higher in winter. The higher runoff is located at areas 7, 10, 12 and 13, where the main rivers are located. The low saline waters from these river discharges spread along the west coast with a poleward direction, therefore with no influence in the areas south of 7.

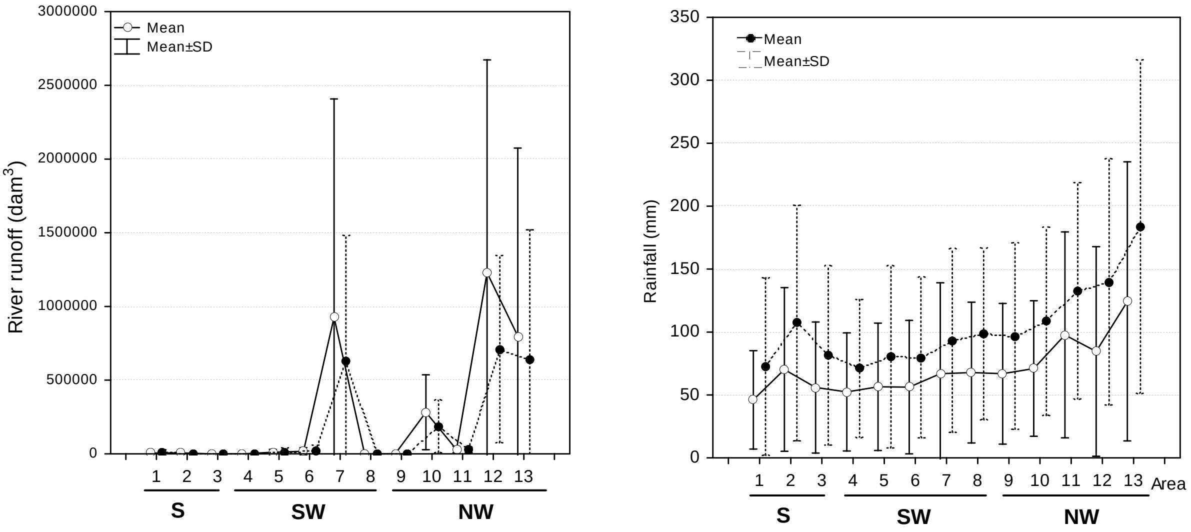

Figure 44 - Spatial variation of river runoff and rainfall in autumn (black circles) and winter (open circles), estimated as the mean (and standard deviation, SD) of the total annual rainfall for the autumn months (October to December) and winter months (January to March) registered in areas 1 to 13 within the northwest (NW), southwest (SW) and south (S) regions, between 1991 and 2009.

7.3.3. Distribution and abundance of the juveniles

The effects on juvenile abundance of all single temporal and spatial variables were significant, namely region and depth zone (Table 15). Juveniles were widely distributed along the Portuguese coast (Figure 45). They were mainly found in the middle-shelf at a mean depth of 79 m (±45 SD) in autumn and at a mean depth of 85 m (±41 SD) in winter. Their distribution extended slightly deeper in winter (360 m) than in autumn

(332 m), but abundances on the slope, observed as far as 60 km from the coastline (Figure 46), were always rather low (mean<0.3 J/h).

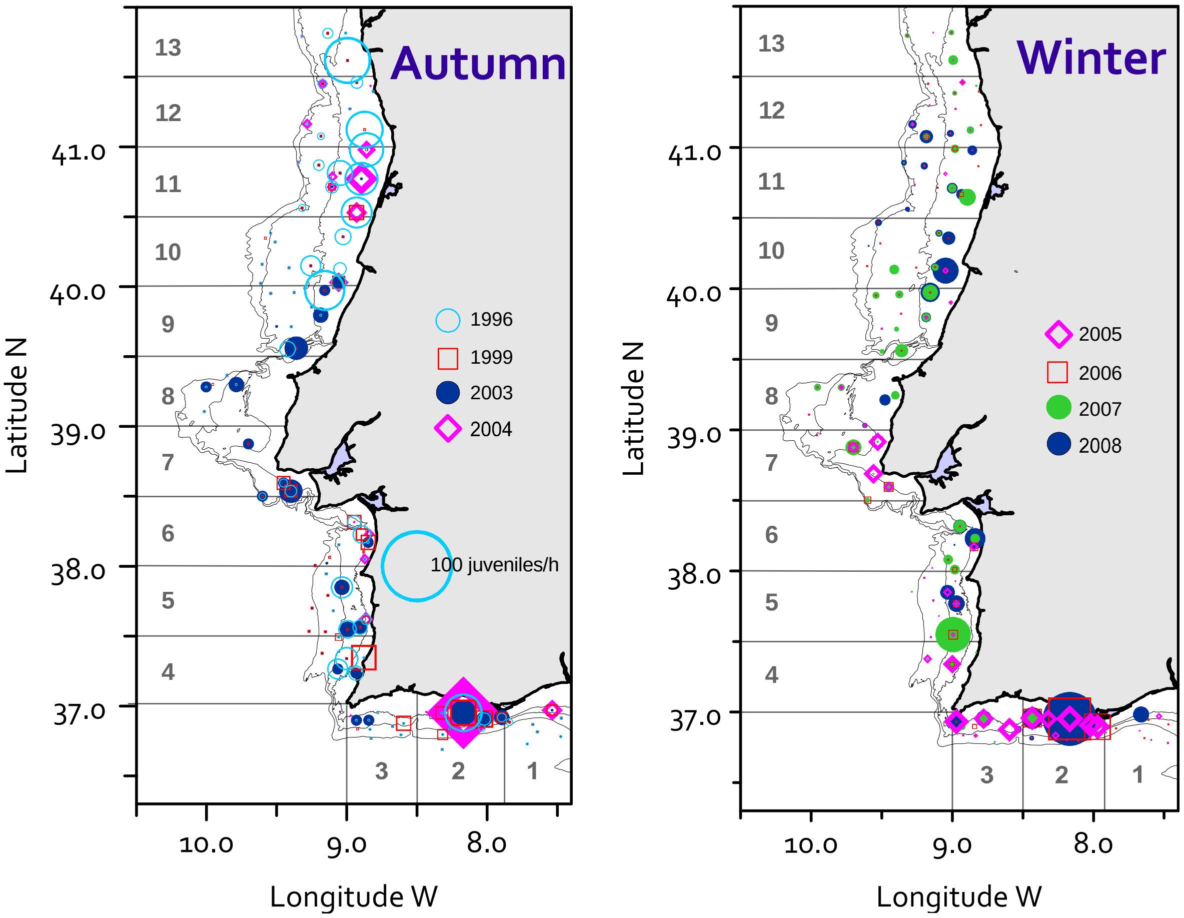

Figure 45 - Distribution and abundance of *O. vulgaris* juveniles in each survey cruise.

Juveniles were found at the inner-shelf mainly in autumn, but higher abundance was generally observed more inshore in winter (mainly from 15 km offshore to the coastline) than in autumn (mainly between 10 to 20 km to the coastline). In winter abundance was ~ 6 juveniles per hour trawling, twice as much as in autumn (3 J/h), in particular during the 2005 and 2008 cruises (Figure 46). However, in the NW region, in areas 9, 11, 12 and 13, juvenile abundance was higher in autumn than in winter. In each season abundance was much higher in the south region, especially in area 2, where the concentration of juveniles was recurrent each year. Nevertheless, the significant effects of the interactions between season and region or between season and area highlight important seasonal differences in the spatial distribution of juveniles.

Table 15 - Effects of temporal, spatial and environmental variables on juvenile abundances. Summary of ANOVA results (‘***’<0.001, ‘**’<0.01, ‘*’<0.05, ‘*ns*’>0.1).

Juveniles ~	**Mean Square**	**df**	**Residuals Mean Square**	**Residuals df**	**F value**	**p**	
Year	1.078	7	0.204	378	5.293	0.0000	***
Season	1.884	1	0.215	384	8.753	0.0033	**
Region	4.747	2	0.196	383	24.230	0.0000	***
Area	1.192	12	0.188	373	6.328	0.0000	***
DepthZ	2.109	2	0.21	383	10.058	0.0001	***
Dcoast	1.242	10	0.192	375	6.459	0.0000	***
BS	1.649	4	0.205	381	8.064	0.0000	***
SST	0.582	7	0.213	378	2.734	0.0089	**
SSS	1.136	3	0.19	324	5.980	0.0006	***
SBT	0.967	4	0.212	380	4.563	0.0013	**
SBS	3.543	2	0.177	323	19.994	0.0000	***
River runoff	0.965	3	0.215	377	4.498	0.0041	**
Rainfall	1.125	4	0.21	381	5.357	0.0003	***
Season*Region	3.148	2	0.174	380	18.134	0.0000	***
Season*Area	0.612	11	0.168	361	3.655	0.0001	***
Season*DepthZ	0.302	2	0.205	380	1.471	0.2310	*ns*
Season*Dcoast	0.153	10	0.190	364	0.808	0.6216	*ns*
Season*BS	0.038	4	0.202	376	0.186	0.9454	*ns*
Season*SST	1.241	2	0.202	375	6.134	0.0024	**
Season*SBT	0.016	2	0.204	375	0.077	0.9725	*ns*
Season*SSS	0.414	3	0.187	320	2.212	0.0866	*ns*
Season*SBS	0.208	2	0.177	320	1.172	0.3110	*ns*
Season*River runoff	0.531	3	0.208	373	2.556	0.0551	*ns*
Season*Rainfall	0.223	4	0.209	376	1.068	0.3722	*ns*

The influence of the environmental variables analysed was also significant, in particular sediment, salinity (both SSS and SBS) and rainfall (Table 15). The effect of all of these variables is independent of seasonal effects, and the variable which showed a higher influence on juvenile abundance was SBS.

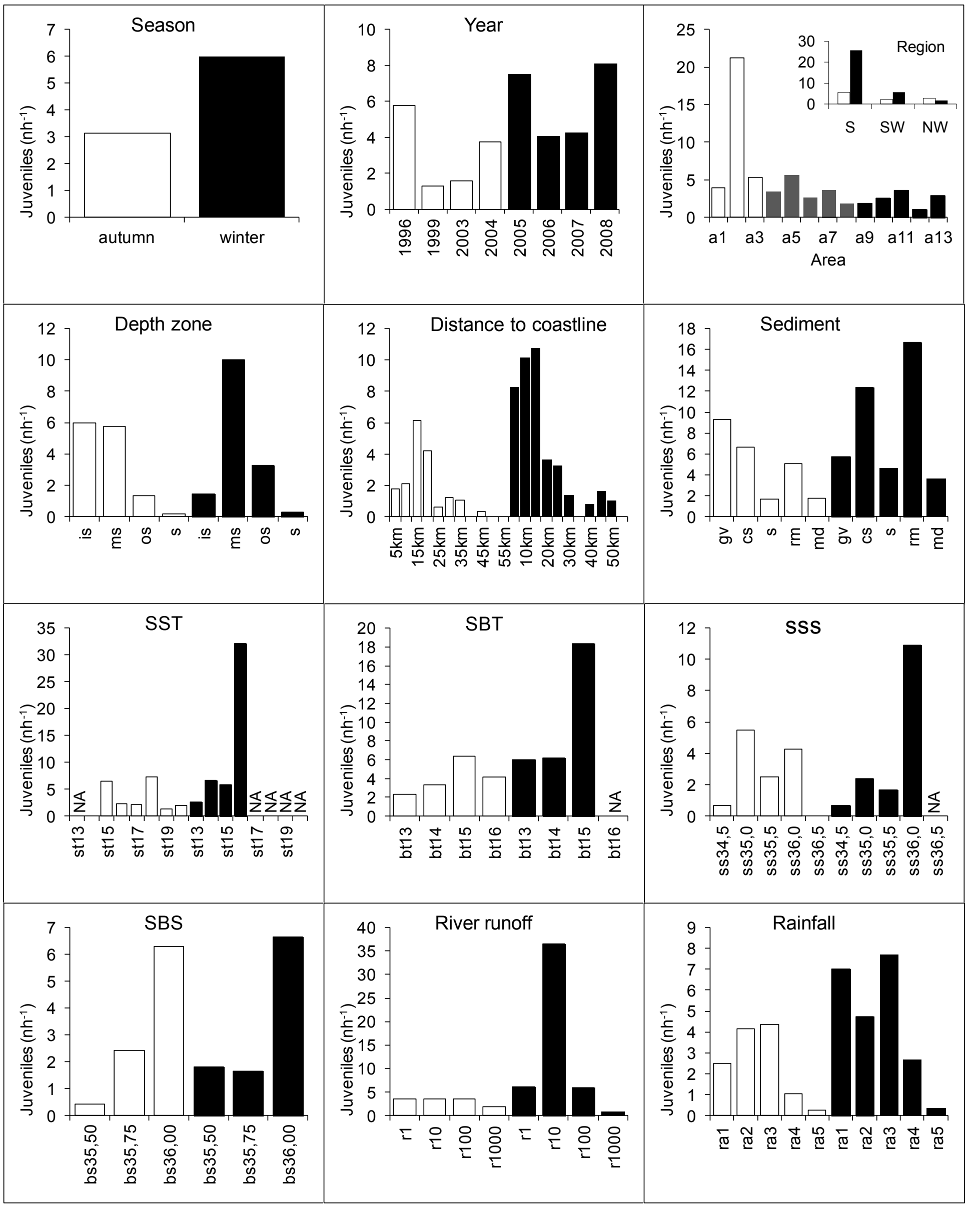

Figure 46 - Mean abundance of *O. vulgaris* juveniles in relation to temporal, spatial, and environmental variables. White bars correspond to the autumn cruises and the black bars to de winter cruises.

Juveniles were found over a variety of bottom sediments, but they seemed to concentrate preferably over large grain sediments. Their abundance was higher over gravel, coarse sand and rocky mud than over fine sand or mud. Juvenile abundance was also higher in the more saline waters of the south coast, with salinity ~36.0. Nevertheless, no juveniles were found at SSS or SBS >36.5 (Figure 46). Lower salinity limit for juvenile distribution was 28.8. Higher concentrations were also related to areas with lower rainfall (<300 mm).

The effect of temperature (SST and SBT), on the other hand, showed some seasonal dependence. Juveniles were found associated to two discrete SST classes either in autumn (15 and 18 ºC) or in winter (14 and 16 ºC), each of the two classes corresponding to their distribution on the NW (colder) and South (warmer) regions. They were also found associated preferably with SBT between 15 and 16 ºC. In general juveniles seemed to concentrate more under warmer temperatures, and their distribution was limited above 12 ºC and below 21.7 ºC. The highest concentrations of juveniles were found in the south region, where there is less river runoff.

7.3.4. Nurseries

Juvenile concentration areas with seasonal mean abundance over 5 J/h, were spatially identified and classified in nine distinct nursery grounds, A to I (Figure 47). During autumn, 3 nursery grounds were located on the NW region inshore of the 100 m isobath: nursery ground A at area 13, nursery area B spreading along areas 12 and 11 in the vicinity of the lagoon system of Ria de Aveiro; and nursery ground C located at the northern part of area 9. A fourth nursery ground, with the highest concentration of juveniles (up to 35 J/h), was located on the South region at area 2, west of the lagoon system of Ria Formosa.

In winter, octopus juveniles were more spread and shifted towards the south, and eight nursery grounds were identified, B to I. On the NW region, nursery ground A present in autumn, disappeared and nursery area B shifted to the southern part of area 11, partially merging with nursery ground C. Three nursery grounds were identified on the SW region: nursery ground D at area 7 and nursery ground E at area 6, close to the estuaries of the rivers Tagus and Sado, respectively; and nursery ground F at areas 5 and 4, extending considerably offshore.

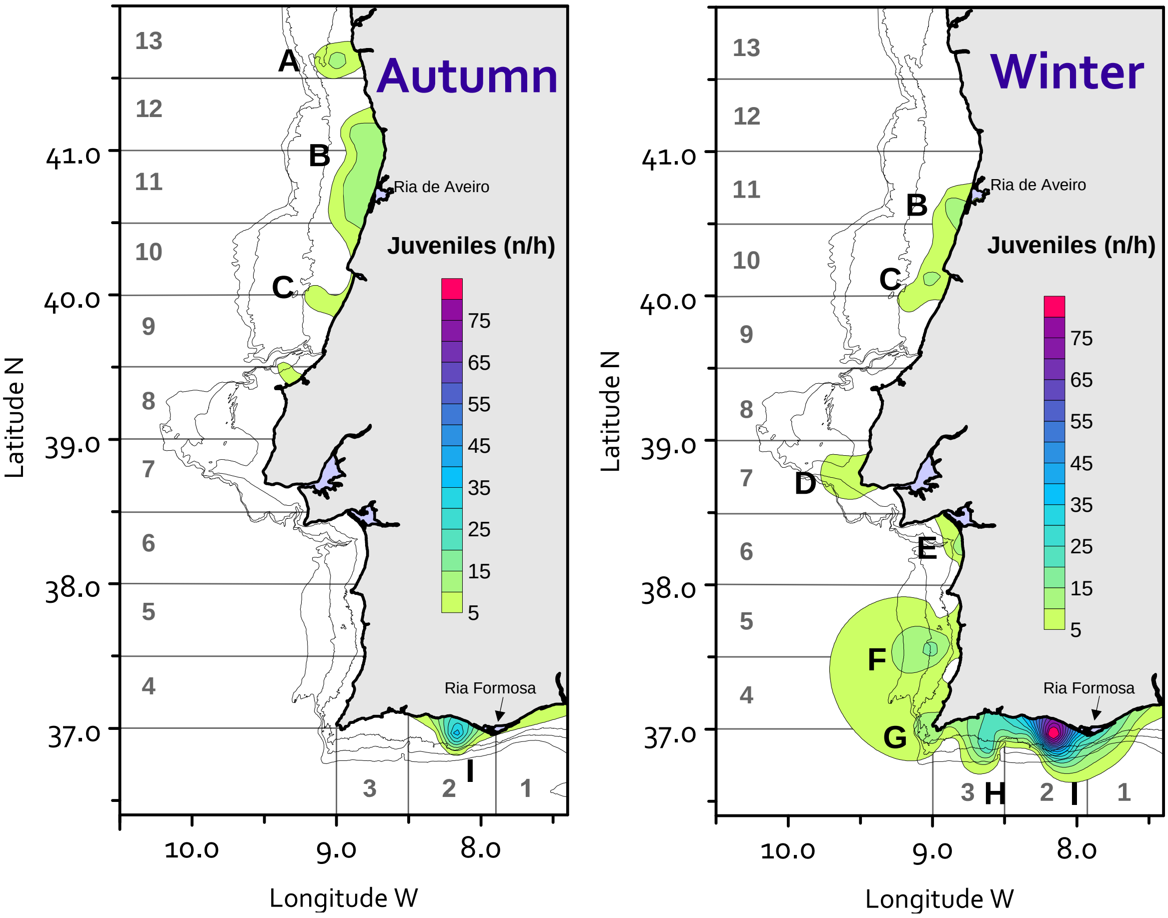

Figure 47 - Nursery grounds of *O. vulgaris* identified by mapping of juvenile mean abundances in autumn and winter survey cruises.

The whole South region could be considered a single nursery ground during winter. Despite this, based on spatial differences in abundance within this region, we identified 3 distinct areas of concentration of juveniles: nursery ground G with the lowest abundance of juveniles (mean < 10 J/h) between the three, located at the vicinity of Cape S. Vicente, in the western part of area 3, and possibly extending partially to the west coast into area 4; nursery ground H with higher abundance of juveniles (mean < 20 J/h), located at area 3; and nursery ground I with the highest concentration of juveniles (up to 75 J/h), located at area 2 west of the lagoon system of Ria Formosa, as in autumn.

The overlay of the nine nursery grounds on the bottom sediments mapping

(Figure 48) highlights the preference of octopus juveniles for larger grain sediments (gravel, coarse sand and rocky mud). Additionally, it highlights the location of nursery grounds preferably in the vicinity of rocky outcrops, which is particularly evident on the NW region.

The overlay of the nursery grounds over temperature and salinity maps also provided additional information on the relationship between these environmental variables and the concentration of juveniles (Figure 49).

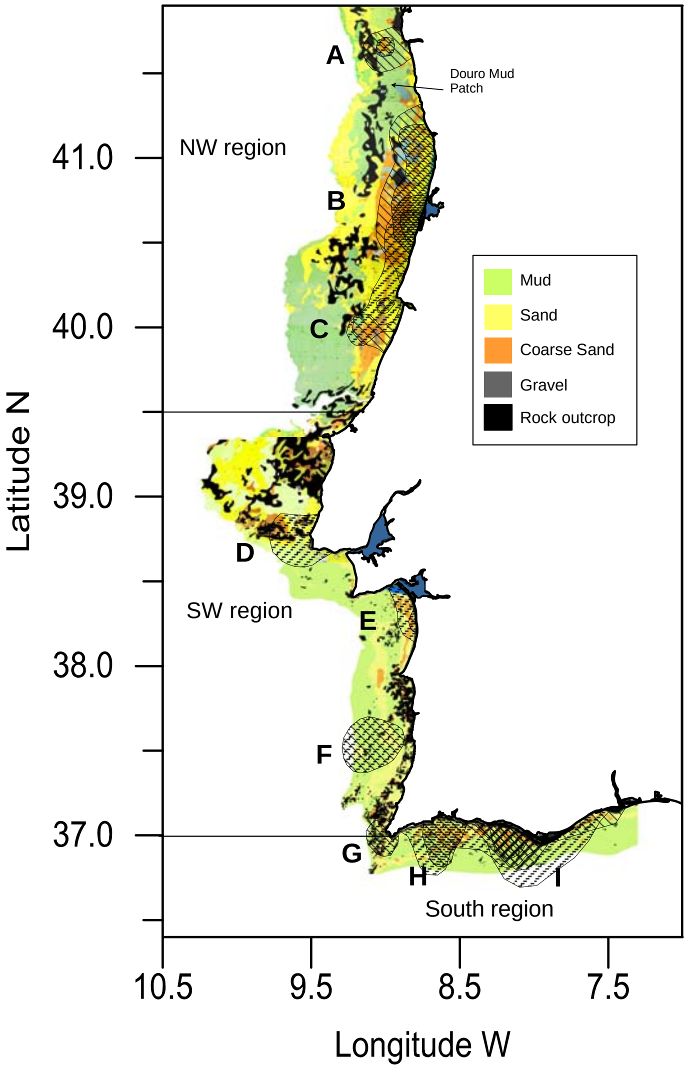

Figure 48 – Overlay of autumn and winter nursery grounds (A to I, dashed polygons) of *O. vulgaris* on the distribution of bottom sediments.

SBT seemed to constrain the location of nursery grounds more clearly than SST, as expected for benthic animals. The main nursery grounds are limited to SBT > 13ºC and the southward shift of the NW nursery grounds from autumn to winter is related to the decrease in temperature, namely below the 12 ºC. Moreover, the southward shift is also related to the decrease in salinity in the coastal waters on the NW region (SSS < 35) due to the intense river runoff that occurs in winter. An area with SSS above 36.5 on the South region in autumn seemed also to limit westwards the size of area I.

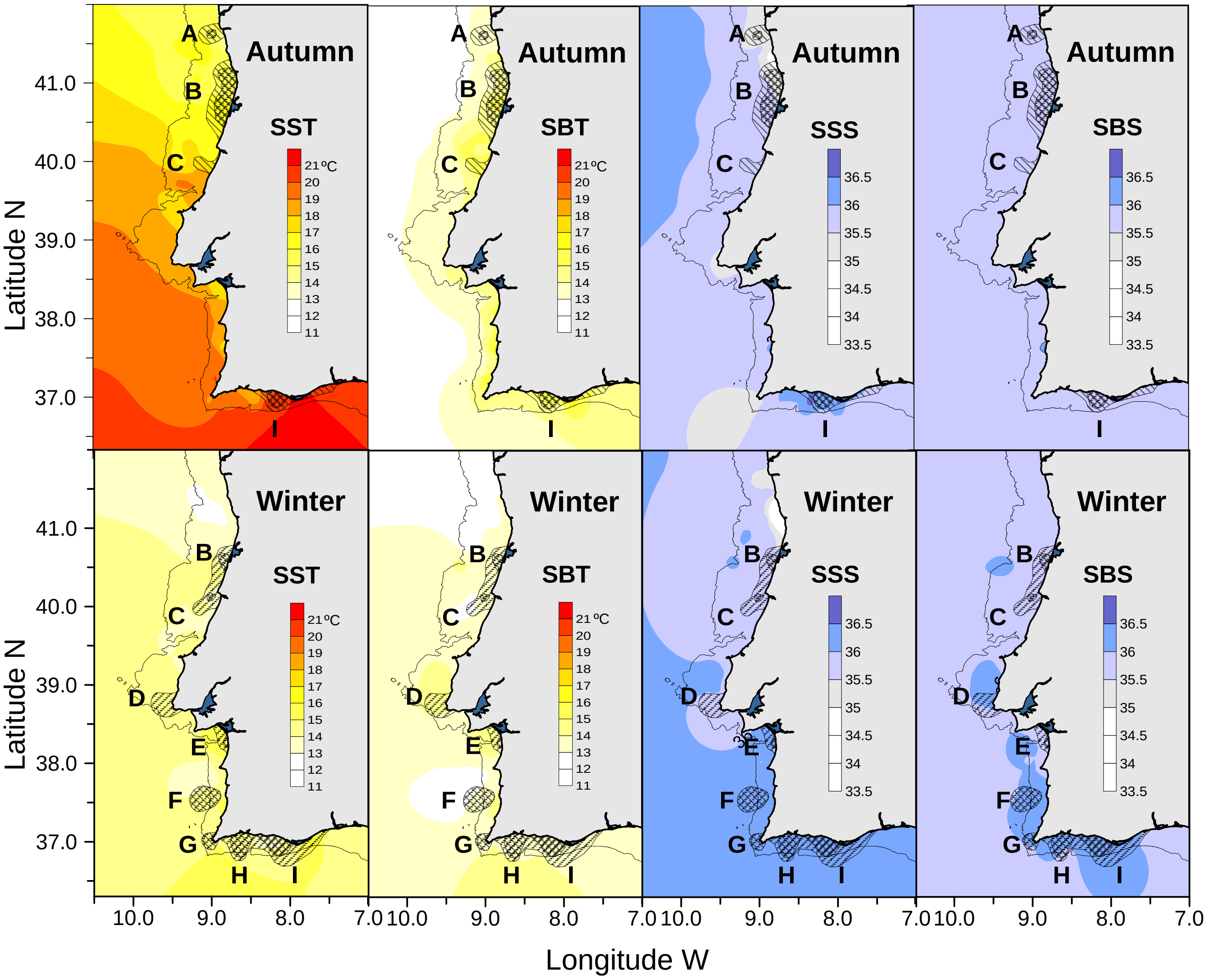

Figure 49 - Overlay of autumn and winter nursery grounds *O. vulgaris* (A to I, dashed polygons) on the distribution of sea surface temperature (SST), sea bottom temperature (SBT), sea surface salinity (SSS) and sea bottom salinity (SBS).

The location and the environmental characterization of each nursery ground are summarized in Table 16. The most persistent nursery grounds were the winter nurseries F and H both with PI = 75% and the nursery ground I with PI = 100% both in autumn and in winter. Due to the high abundance and its persistence over the time–series, nursery ground I is probably the most important nursery of *O. vulgaris* along the Portuguese coast.

7.4. Discussion

Common octopus recruits to the artisanal trap fishery at a very small size, however most animals smaller than 750 g are discarded due a mandatory legal minimum landing weight. Therefore, the recruits in landings from the trap fishery weight on average between 750 and 1000g. These recruits were more abundant in September and December on the NW Portuguese shelf, and in April and November on the South Portuguese shelf. Winter and autumn survey cruises analysed were carried out approximately 1 month before the spring and autumn recruitment peaks of the south region, respectively. Autumn cruises were carried out approximately 1-2 months before the autumn recruitment peak of the NW region. Therefore, the distribution of the pre-recruit juveniles analysed with those survey data (mean body weight ~ 370 - 390g, Table 14) could be used to obtain an accurate picture of the following recruitment to the fishery in those grounds. On the other hand, the winter cruises completely mismatch the first recruitment season on the NW region and may be less suitable for the evaluation of the main nursery grounds in that region.

Juveniles were widely distributed along the Portuguese coast. Their distribution was patchy, with juveniles aggregated in several distinct nursery grounds. Juvenile abundance and the location of these nursery grounds were significantly influenced by all of the variables analysed.

The abundance of juveniles was substantially higher in the south region than on the west coast, considering that the general distribution of *O. vulgaris* in the Eastern Atlantic have the highest densities towards the south, namely on the coasts of NW Africa (Balguerias *et al.*, 2000).

Table 16 - Environmental characterization of *O. vulgaris* nursery grounds A to I. SD = standard deviation.

Nursery ground		**A**	**B**	**B**	**C**	**C**	**D**
Season		**autumn**	**autumn**	**winter**	**autumn**	**winter**	**winter**
Region		NW	NW	NW	NW	NW	SW
Area		13	11, 12	11	9	10	7
Year		1996	1996, 2004	2007, 2008	1996	2007, 2008	2005, 2007
Depth (m)	**mean** ± SD	**80** ± 0.00	**46** ± 4.37	**40** ± 0.00	**80** ± 0.00	**68** ± 13.69	**110** ± 15.49
Depth (m)	range	80	?-50	?-40	?-80	55-80	?-120
Dcoast (km)	**mean** ± SD	**13.9** ± 0.00	**14.3** ± 2.25	**12.0** ± 0.00	**18.3** ± 0.14	**15.5** ± 3.12	**19.3** ± 6.23
Dcoast (km)	range	13.9	?-16.7	?-12	?-18.5	12.1-18.7	?-26.1
SST (ºC)	**mean** ± SD	**15.8** ± 0.73	**16.3** ± 0.86	**13.5** ± 0.00	**17.0** ± 0.93	**14.2** ± 0.43	**14.5** ± 0.40
SST (ºC)	range	15.2-16.7	15.0-17.9	13.5	16.0-17.8	13.6-14.9	14.0-15.1
SBT (ºC)	**mean** ± SD	**13.6** ± 0.38	**15.1** ± 1.46	**13.7** ± 0.00	**14.6** ± 0.49	**13.8** ± 0.74	**14.3** ± 0.74
SBT (ºC)	range	13.2-13.9	13.9-17.3	13.7	14.1-15.3	12.8-14.8	13.4-15.2
SSS	**mean** ± SD	**35.0** ± 0.40	**35.6** ± 0.14	**35.7** ± 0.00	**35.7** ± 0.08	**35.9** ± 0.23	**36.0** ± 0.10
SSS	range	34.6-35.4	35.3-35.7	35.7	35.6-35.8	35.6-36.1	35.9-36.1
SBS	**mean** ± SD	**35.8** ± 0.15	**35.8** ± 0.16	**35.7** ± 0.00	**35.9** ± 0.06	**34.2** ±3.61	**36.0** ± 0.15
SBS	range	35.7-36.0	35.5-36.0	35.7	35.8-35.9	28.8-36.1	35.9-36.2
River runoff (1000dam^3)	**mean** ± SD	**1149** ± 1173	**850** ± 300	**64** ± 22	**0**	**322** ± 165	**945** ± 558
River runoff (1000dam^3)	range	392-3201	27-2693	42-91	0	53-540	477-1769
Rainfall (mm)	**mean** ± SD	**382** ± 234	**300** ± 57	**218** ± 79	**253** ± 106	**208** ± 64	**166** ± 88
Rainfall (mm)	range	161-730	213-401	90-319	141-368	83-275	67-314

Nursery ground		**E**	**F**	**G**	**H**	**I**	**I**
Season		**winter**	**winter**	**winter**	**winter**	**autumn**	**winter**
Region		SW	SW	South	South	South	South
Area		6	5	3	3	2	2
Year		2007, 2008	2005, 07, 08	2005, 2008	2005, 07, 08	all	all
Depth (m)	**mean** ± SD	**60** ± 0.00	**168** ± 5.42	**95** ± 0.00	**110** ± 0.00	**62** ± 18.98	**72** ± 16.92
Depth (m)	range	?-60	165-175	?-95	?-110	40-80	45-102
Dcoast (km)	**mean** ± SD	**12.0** ± 9.01	**15.0** ± 6.27	**13.0** ± 7.73	**22.4** ± 0.00	**12.0** ± 1.68	**12.1** ± 2.72
Dcoast (km)	range	?-18.4	2.3-18.5	?-28.4	?-22.4	10.3-14.1	7.8-15.2
SST (ºC)	**mean** ± SD	**15.3** ± 0.07	**15.0** ± 0.88	**15.3** ± 0.81	**14.5** ± 0.00	**18.9** ± 1.28	**14.9** ± 0.91
SST (ºC)	range	15.2-15.3	13.4-15.8	14.3-16.2	14.5	17.6-20.8	13.7-16.6
SBT (ºC)	**mean** ± SD	**13.9** ± 0.07	**13.7** ± 0.60	**14.1** ±0.84	**13.8** ± 0.00	**15.1** ± 1.06	**14.3** ± 0.80
SBT (ºC)	range	13.8-13.9	12.6-14.3	13.1-15.2	13.8	14.0-17.0	13.3-15.8
SSS	**mean** ± SD	**36.0** ± 0.00	**36.3** ± 0.00	**36.4** ± 0.08	**36.4** ± 0.00	**36.1** ± 0.22	**36.3** ± 0.13
SSS	range	36.0	36.3	36.3	36.4	35.9-36.5	36.1-36.4
SBS	**mean** ± SD	**35.9** ± 0.00	**36.0** ± 0.00	**36.1** ± 0.10	**35.9** ± 0.00	**36.1** ± 0.15	**36.2** ± 0.11
SBS	range	35.9	36.0	36.0-36.2	35.9	35.8-36.2	36.0-36.3
River runoff (1000dam^3)	**mean** ± SD	**39** ± 22	**18** ± 18	**0**	**0**	**2** ± 2	**3** ± 5
River runoff (1000dam^3)	range	2-58	0-46	0	0	2-4	0-13
Rainfall (mm)	**mean** ± SD	**139** ± 51	**112** ± 74	**106** ± 54	**106** ± 54	**172** ± 47	**119** ± 93
Rainfall (mm)	range	43-177	42-241	47-166	47-166	120-229	40-242

Along the surveyed area, *O. vulgaris* juveniles were concentrated at mean depths of ~80 m, and approximately at 10-15 km from the coast, showing little seasonal variation in the depth range distribution. The common octopus is known to be a coastal benthic species, living from 0 to 510 m in the Atlantic coasts (unpublished data), but mostly restricted to depths below 100 m, both in the Eastern Atlantic and in the Mediterranean (Guerra, 1981; Belcari *et al*., 2002). In the Mediterranean, the highest abundances of *O.vulgaris* captured by bottom trawl surveys (mostly juveniles) were found within the depth range of 10-50 m (Belcari *et al*., 2002). Similarly, juvenile densities are higher in shallow waters (20-40 m) than further offshore in the NW African coasts (Faraj and Bez, 2007). Therefore, these authors locate the preferred distribution of octopus juveniles inshore of the main nursery grounds identified along the Portuguese coast. This difference may have several explanations, but among them could be considered a sampling constrain. As a matter of fact, the surveyed area of the cruises analysed, have a poor coverage of the inner-shelf on the south region (see Figure 41), the region where the abundance is the highest, which may have contributed to an overestimation of the juvenile mean depth distribution. However, Garofalo *et al*. (2010) identified the preferred distribution of octopus juveniles in the Strait of Sicilly at mean depths more similar to our findings (~60 m). In any case, juveniles generally aggregate in coastal waters at a relatively short distance from the coastline.

O. vulgaris adults are equally abundant over a variety of sediment types (Mangold, 1983). Nevertheless, we observed that juveniles have a preference for large grain sediments, namely gravel and coarse sand. Their distribution was also closely related to the vicinity of rocky bottoms and abundance over mud with rock outcrops was significantly higher than over soft mud. This positive relationship between small size octopus density and sediment grain size was also documented by Katsanevakis and Verriopoulos (2004a) in Greek waters. This sediment type preference is, to some extent, related to their necessity of sheltering to avoid predation, and octopuses do it either selecting or building the shelters (“dens”) in the substratum (Mather, 1988). Besides the natural dens (rocks, shells, etc.), a variety of materials of human origin (empty bottles, buckets, pots, pipes, etc.) could be used as dens, and on soft sediment the availability of material that can be utilized for den construction could be a limiting factor for octopus distribution (Katsanevakis and Verriopoulos, 2004b). Small octopus generally digs and build wells for shelter and this type of dens are also the more common in areas with large grain sediments (Katsanevakis and Verriopoulos, 2004b). The anthropogenic impact on octopus distribution, as a result of the increasing number of dens of human origin in the ocean, is difficult to assess and not well documented, thus was considered here.

Using presence-absence data, Hermosilla *et al*. (2011) modelled the distribution and habitat preferences of *O. vulgaris* in the Eastern Atlantic and Mediterranean and concluded that the area used by octopus differs from the average available environmental conditions in terms of bottom temperature and bottom salinity. These authors concluded that octopus prefers habitats with bottom salinity between 30 and 45. On the Portuguese coast distribution of octopus juveniles was limited to a minimum salinity of 28.8 and a maximum salinity of 36.5, with higher abundance in the more saline waters. This species is abundant under salinities at least up to 39 (e.g. in the Greek Seas, Katsanevakis and Verriopoulos, 2004a), therefore the reason why no animals were found in salinities above 36.5 in Portuguese waters was probably related to the low availability of more saline waters in the study area, as well as other factors not related to this particular variable. At the same time, low salinity is a limitation for octopus survival (Vaz-Pires *et al*., 2004; Chapela *et al*., 2006) and therefore constrains its distribution. Nevertheless, several nursery grounds were identified in the vicinity of large rivers and lagunar systems (although this does not equate to low salinity water). The proximity to estuarine systems provides an increased availability of prey, namely bivalves, but greatly enhances the risk of low salinity episodes. In fact, mass mortality episods of *O. vulgaris* have sometimes been reported on the Portuguese coast and have been related to sudden falls in water salinity due to intense river discharges, following heavy rainfall periods (Ruano, 2011).

The influences of sediment, salinity and rainfall on juvenile spatial distribution were significant and independent of seasonal effects. However, the spatial distribution and abundance of juveniles evidenced significant seasonality, which is derived from the different population dynamics between the octopus of the west and the south regions (Lourenço *et al*., in press). Juvenile abundance was much higher in the winter cruises on the SW and South regions, and in the autumn cruises on the NW region. The autumn and winter cruises catch juveniles originated from previous paralarvae summer peaks or autumn peaks, respectively, and the relative magnitudes of seasonal paralarvae abundance peaks (Moreno *et al*., 2009) explain well the highest juvenile abundance in the South region during winter. However, given that paralarvae abundance on the NW region is also generally higher during autumn, it was also expected an increased abundance of juveniles in the following season, which is not observed. This decoupling is not well understood, but we can hypothesised that a higher early life stages natural mortality caused by more severe environmental conditions is expected between autumn and winter than between summer and autumn. Additionally, autumn paralarvae will experience lower temperature and stay longer in the plankton than summer paralarvae and will also grow slowly after settlement. Thus, juveniles will be available on the NW nursery grounds later in the year and be missed by the winter cruises sampling.

It was observed that SST affects significantly the distribution and abundance of octopus paralarvae in their zooplanktonic habitat (González *et al*., 2005; Moreno *et al*., 2009). However, it is expected that the subsequent life stages, living strongly associated with the ocean floor, suffer a more intense (or direct) influence of the bottom environmental variables, such as SBS and SBT. In that sense, juveniles were found associated preferably with SBT between 15 and 16 ºC and the main nursery grounds were limited to mean SBT > 13.6 ºC. In general, juveniles were more concentrated under the warmest temperatures and cold water (SBT < 12 ºC) seemed to be a limitation for their distribution.

Nine different nursery grounds were detected along the Portuguese shelf based on the concentration of juveniles. On the NW region juveniles were concentrated into 3 nursery grounds in autumn and only in two during winter. However, the location of these nurseries was variable and the reasons for their inter-annual variation need to be further investigated. Nevertheless, the area surrounding Ria de Aveiro seems to be an important nursery for *O. vulgaris*. In the SW region juveniles aggregated in nursery grounds only during winter and close to Tagus and Sado estuaries, as well as offshore of an extensive rocky area further south. The location of this later nursery ground was persistent throughout the time-series. In the South region one nursery ground was identified west of Ria Formosa during autumn. In winter, 3 nursery grounds were identified, being one of them in the west of Ria Formosa referred previously. Estuaries are highly productive ecosystems with high abundance and diversity of marine fish and invertebrate species, providing nursery conditions to the juvenile stages of many species (Cabral *et al*. 2007). Species such as *O. vulgaris*, which are stenohaline in all life stages, are not estuarine inhabitants, but can take advantage of these productive ecosystems by having nursery grounds in their vicinity.

Ria Formosa nursery ground is probably the most important nursery of *O.*

vulgaris along the Portuguese coast because of the presence of high juvenile abundances and its persistence over the time-series.

The coastal areas adjacent to estuaries are also the feeding grounds of many fish and invertebrates. In particular, the octopus nursery areas located in the proximity of Ria de Aveiro and close to Ria Formosa are areas subject to intense fishing activity: the artisanal fishery (traps and pots) exploits the inshore part and the bottom trawl fishery exploits the offshore part of those nursery grounds (Fonseca *et al*., 2008). However, Portuguese fishery regulations determine that small animals (<750g) are to be returned to the water if caught, which if respected would suffice to maintain the safety of octopus nursery grounds, regardless of the type of activity in the area. Nevertheless, the identification of a spatially restricted critical area could be useful to the legislation reinforcement.

This study was an essential first step towards the understanding of *O. vulgaris* distribution and associated environmental variables. Nevertheless, we recognize that further information should be obtained in future studies to complement and refine our research. First of all, abundance based on trawl data should be complemented with data from other sources because the efficiency of a fishing trawl depends on the type of dens that the octopuses use in the surveyed area, which varies in relation to depth, octopus size, and sediment type (Katsanevakis and Verriopolus, 2004b). This could have compromised in some extent the reliability of octopus abundance estimates. Additionally, it would be desirable to search other sources of abundance data to have a better coverage of octopus distribution over the inner-shelf, and in areas with extensive deployment of artisanal fishing gears, where survey sampling is often compromised.

Chapter 8

Concluding remarks and future perspectives

8. Concluding remarks and future perspectives

8.1. Concluding remarks

Age-at-maturity, size-at-maturity, reproductive investment, growth rates, growth models, and planktonic stage duration of *Loligo vulgaris* were studied in order to explain its complex population structure strongly influenced by continuous spawning in Portuguese waters. Age and growth of paralarvae, juveniles and adults were estimated by increment counting and measurement in the statoliths in two groups of squid, the cold cohort and the warm cohort, hatched under distinct environmental conditions, namely under the influence of temperature during the first 3 months of life,. Size-at-age was extremely variable in this species and age information proved to be mandatory for the understanding of its life history strategies modulated by the seasonal environmental variation.

From the age readings in statoliths one may conclude that the commercial trawl fishery catches squid with ages between 5 and 14 months, and mainly 6 to 10 months old animals. Therefore, the common squid population on the NW Portuguese area spends less than half of its life vulnerable to fishery exploitation. Males mature one month earlier and in smaller sizes than females. In both sexes, maturity was ultimately found to be primarily dependent on size rather than age.

Significant differences were found between cohorts on age-at-maturity, size-at-maturity and reproductive investment: the cold cohort squid matured ca. 2 months later in life than the warm cohort squid and at a significantly larger size. The reproductive investment was higher in the warm cohort. Much of the variability in age and size-at-maturity of females in the population was due to differences between cohorts. However, this was not observed in males; their reproductive strategies are mainly from endogenous origin..

Male and female growth followed different models, males attaining a higher length-at-age than females. Males displayed increasing growth rates irrespective of the hatching season, but the length-at-age was higher in animals hatched during the warm season. Females may exhibit asymptotic growth or not, depending on the environmental conditions to which they are exposed through their life cycle. Although growth rates after hatching were lower in females hatched during the cold season, favourable feeding and temperature conditions during the following spring and summer months contributed not only to increase growth rates but also to delay in sexual maturation. The higher length-at-age of squid hatching in the warm season, observed in both genders, provides evidence that the temperature close to hatching has a significant impact on the size of juveniles and subadults. However, there is also strong evidence that throughout their life, environmental conditions continue to play an important role in growth rates and in defining the shape of growth.

The effect temperature and salinity, as proxies of environmental variability, in the growth during the embryonic and post-hatching phase, as well as the duration of the planktonic phase were analyzed based on measurements of growth increments in statoliths of juveniles and adults. The variability in the statolith size at hatching was weakly explained by the variables in the model, suggesting that winter and summer spawning grounds could occur in areas with similar temperature and salinity. On the other hand, the effects of both sea surface temperature and sea bottom temperature on statolith growth were significant during the early life. Thicker increments are deposited in the statoliths of squid living under high temperatures which result in summer hatchers with larger statoliths at the age of 90 days.

The inspection of the statolith accretion pattern revealed an ontogenetic shift in increment widths, enabling the estimation of the age of transition from paralarva to juvenile. The planktonic stage lasts 60 or 90 days, depending on whether the paralarvae inhabited at higher (> 15 ºC) or lower (< 15 ºC) sea surface temperatures. The life strategy under warmer conditions potentially favours its own survival by reducing the duration of the vulnerable planktonic phase.

From the analyses of paralarvae distributions one can conclude that temperature along with oceanographic mesoscale features, including horizontal currents, thermal fronts, coastal upwelling and related features play a crucial role in modulating seasonality and distribution of cephalopod planktonic paralarvae, as observed for other plankton communities (e.g. Bakun, 1996; dos Santos *et al.*, 2007, 2008; Santos *et al.*, 2007).

L. vulgaris paralarvae are more abundant and occur over a large part of the year on the western than on the southern shelf. The western Iberia upwelling area provides more favourable environmental conditions for these paralarvae, with relatively low temperatures throughout the year, even in summer, due to the presence of cold upwelled waters over the shelf. The eventual advection from the shelf during upwelling events is avoided by their diel migration behaviour. The summer inshore countercurrent is proposed to advect *L. vulgaris* paralarvae from the warm summer spawning grounds on the northern Gulf of Cadiz to the western cold upwelled waters around Cape S. Vicente.

On the other hand, *O. vulgaris* paralarvae occurred in the plankton during a more restricted period of the year, avoiding the lower temperatures registered in winter months. In the west coast, higher densities were located on the outer-shelf during spring and summer and near the shore during autumn, following the Ekman dynamics of cross-shelf transport. The abundance was higher after the upwelling season, although a summer peak was also observed on the northwest shelf. The eventual advection from the outer-shelf during the upwelling events is avoided by the retention characteristics of this area. Two hatching peaks also occurred on the southern coast, though preceded by a single regional spawning peak. A drift of the newly hatchlings from the west to the south within the upwelling and other mesoscale currents is proposed.

The poor correlation observed between paralarval sepiolidae and ommastrephidae distribution and environmental variables is considered to be related to the occurrence of several unidentified species within each family, with different distributions and spawning seasonality. Nevertheless, data showed that their spawning areas occurred over the continental shelf despite the adult distribution range spreads far offshore. In the case of ommastrephids the distribution of paralarvae revealed that the spawning area in spring and summer was restricted to the northwest continental shelf, where water temperature was not a limiting factor for embryonic development.

Off Portuguese coast, paralarvae of the neritic species occurred during a considerably extended period of the year with two or three density peaks, namely within the highly productive upwelling system of the western Portuguese coast and contrasting to the Gulf of Cadiz area. Important differences were noted in the reproductive strategies between species (or groups of species), translated in the seasonality and/or the distribution of paralarvae.

The relationship between environmental factors and paralarvae distribution or juvenile distribution was particularly significant in *O. vulgaris*, providing further evidence that the reproductive strategy of this species follows the seasonal dynamics of the local environmental processes. This species, among the studied ones, showed the most dramatic difference between the planktonic phase and the adult mode of life, and that exceptional plasticity to the physical environment during the vulnerable paralarvae phase was therefore to be expected.

O. vulgaris juveniles were widely distributed along the Portuguese coast. Their distribution was patchy, with juveniles aggregated in several distinct nursery grounds at mean depths ~ 80 m (possibly more shallow), approximately at 10-15 km from the coastline, mainly located in areas adjacent to large rivers and lagunar systems. Juvenile abundance and the location of these nursery grounds were significantly influenced by all of the temporal, spatial, and environmental variables analysed. Namely, the influences of sediment, salinity (both SSS and SBS) and rainfall on juvenile spatial distribution were significant and independent of seasonal effects.

Juvenile abundance was substantially higher in the south region than on the west coast, associated to warmer and more saline waters. This age group also showed a preference for large grain sediments, namely gravel, coarse sand and mud with rock outcrops, which are easier substrates for ‘well’ (vertical hole) construction, providing more natural shelters to avoid predation.

The spatial distribution and abundance of juveniles evidenced significant seasonality, derived from the different environmental conditions experienced by animals of the west and the south regions. Juvenile abundance was much higher in winter on the southwest and south regions and in autumn on the northwest region. Slower growth and higher mortality of late autumn paralarvae in the northwest coast may explain the delay in settlement and the lower juvenile abundance observed in late winter.

The area adjacent to the Ria de Aveiro seems to be the most important nursery for *O. vulgaris* on the northwest coast. In the southwest region juveniles aggregated in nursery grounds only during winter. Due to the high juvenile abundance and its persistence over the time–series, the Ria Formosa nursery ground is probably the most important one for *O. vulgaris* along the Portuguese coast. However, this area is subject to intense fishing activity both by the artisanal and trawling fisheries, a feature that should be taken into account in future management decisions and planning.

8.2. Perspectives for future research

A better knowledge of the spawning areas and of the vertical distribution of the planktonic stage for each cephalopod species is still required to fully understand and predict dispersion/retention patterns in the Portuguese coast. New technologies for tag and track paralarval cephalopods may be one of the most promising approaches to address this question as pointed out by Semmens *et al.* (2007). The drift of *O. vulgaris* newly hatchlings from the west Portuguese shelf towards the south shelf within the upwelling and other mesoscale currents was proposed in the present study and such new technologies would greatly help to resolve it.

An accurate paralarvae identification key is an essential tool to describe the distribution patterns of these early life stages. Difficulties still exist in the distinction among loliginids and among ommastrephid species. Future efforts should be made to attempt the description of these species both by artificial fertilization and genetic identification. Some of this work is already underway.

The study of the octopus nursery grounds was an essential first step towards the understanding of *O. vulgaris* distribution and of the associated environmental variables. However, more information should be gathered in future studies to further improve the reliability of octopus abundance estimates and to obtain a better coverage of octopus distribution over the inner-shelf and in areas with extensive deployment of artisanal fishing gears where survey sampling is often compromised. Abundance data obtained on board of commercial fishing vessels should be considered in the future to acquire additional data for nursery grounds studies. Moreover, visual census by scuba-diving or by remote vehicles focused on the main nursery grounds identified herein would greatly help to improve density estimates and the spatial delineation of each nursery ground.

Literature cited

Adam, W. (1983) Cephalopoda from West and South Africa. *Atlantide Report* 13, 51 - 180.

Agnew, D.J., Beddington, J.R., Hill, S.L. (2002) The potential use of environmental information to manage squid stocks. *Canadian Journal of Fisheries and Aquatic Sciences* 59, 1851 - 1857.

Álvarez-Salgado, X.A., Figueiras, F.G., Pérez, F.F., Groom, S., Nogueira, E., Borges, A.V., Chou, L., Castro, C.G., Moncoiffé, G., Ríos, A.F., Miller, A.E.J., Frankignoulle, M., Savidge, G., Wollast, R. (2003) The Portugal coastal counter current off NW Spain: new insights on its biogeochemical variability. *Progress in Oceanography* 56, 281 – 321.

Anon (2004) Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH). ICES CM 2004/G:02, Ref. ACFM, ACE.

Arístegui, J., Álvarez-Salgado, X., Barton, E., Figueiras, F., Hernández-León, S., Roy, C., Santos, A. (2006) Oceanography and fisheries of the Canary Current/Iberian region of the eastern North Atlantic. In: Brink, K.H., Robinson, A.R. (Eds), *The Sea*. Harvard University Press, 23, p. 877 - 931.

Arkhipkin, A.I. (1995) Age, growth and maturation of the European squid *Loligo vulgaris* (Myopsidae, Loliginidae) on the west Saharan Shelf. *Journal of the Marine Biological Association of United Kingdom* 75, 593 - 604.

Arkhipkin, A.I., Laptikhovsky, V.V., Golub, A. (1999) Population structure and growth of the squid *Todarodes sagittatus* (Cephalopoda: Ommastrephidade) in the north-west African waters. *Journal of the Marine Biological Association of United Kingdom* 79, 467 - 477.

Arkhipkin, A.I., Jereb, P., Ragonese, S. (2000) Growth and maturation in two successive seasonal groups of the short-finned squid, *Illex coindetii* from the Strait of Sicily (central Mediterranean). *ICES Journal of Marine Science* 57, 31 - 41.

Arkhipkin, A.I., Laptikhovsky, V.V. (2000) Age and growth of the squid *Todaropsis eblanae* (Cephalopoda: Ommastrephidae) on the north-west African shelf. *Journal of the Marine Biological Association of United Kingdom* 80, 747 - 748.

Arkhipkin, A.I. (2004) Diversity in growth and longevity in short-lived animals: squid of the suborder Oegopsina. *Marine and Freshwater Research* 55, 341 - 355.

Arkhipkin, A.I. (2005) Statoliths as 'black boxes' (life recorders) in squid. *Marine and Freshwater Research* 56, 573 - 583.

Arvanitidis, C., Koutsoubas, D., Robin, J.P., Pereira, J., Moreno, A., Cunha, M.M., Valavanis, V.E., Eleftheriou, A. (2002) A comparison of the fishery biology of three *Illex coindetii* vérany, 1839 (Cephalopoda: Ommastrephidae) populations from the European Atlantic and Mediterranean waters. *Bulletin of Marine Science* 71, 129 - 146.

Baddyr, M. (1991) Biology of the squid *Loligo vulgaris* in relation to the artisanal fishing site of Tifnit, Morocco. *Bulletin of Marine Science* 49, 661 (Abstract).

Bakun, A. (1996) *Patterns in the Ocean: Ocean Processes and. Marine Population Dynamics*. Univiversity of California, La Jolla: Calif. Sea Grant College System.

Balguerías, E., Quintero, M.E., Hernández-González, C.L. (2000) The origin of the Saharan Bank cephalopod fishery. *ICES Journal of Marine Science* 57, 15 - 23.

Barton, E. (1998) Eastern boundary of the North Atlantic–northwest Africa and Iberia. In: Robinson, A., Brink, K.H. (Eds). *The Sea: The Global Coastal Ocean. Regional Studies and Syntheses*, John Willey & Sons, New York, 11, 633 - 658.

Belcari, P., Cuccu, D., González, M., Srairi, A., Vidoris, P. (2002) Distribution and abundance of *Octopus vulgaris* Cuvier, 1797 (Cephalopoda: Octopoda) in the Mediterranean Sea. *Scientia Marina* 66, 157 - 166.

Bertalanffy, L.v. (1938) A quantitative theory of organic growth. *Human Biology* 10, 181 - 213.

Bettencourt, V., Coelho, M.L., Andrade, J.P., Guerra, A. (1996) Age and growth of the squid *Loligo vulgaris* off the south coast of Portugal, using statolith analysis. *Journal of Molluscan Studies* 62, 359 - 366.

Boavida-Portugal, J., Moreno, A., Gordo, L., Pereira, J. (2010) Environmentally adjusted reproductive strategies in females of the commercially exploited common squid *Loligo vulgaris*. *Fisheries Research* 106, 193 - 198.

Boeuf, G., Payan, P. (2001) How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C* 130, 411 - 423.

Boletzky, S.v., Rowe, L., Aroles, D.L. (1973) Spawning and development of the eggs, in the laboratory of *Illex coindetii* (Mollusca:Cephalopoda). *Veliger* 15, 257 - 258.

Boletzky, S.v. (2003) Biology of early life stages in cephalopod molluscs. *Advances in Marine Biology* 44, 143 - 203.

Borges, M., Santos, A., Crato, N., Mendes, H., Mota, B. (2003) Sardine regime shifts off Portugal: a time series analysis of catches and wind conditions. *Scientia Marina* 67, 235 - 244.

Boyle, P.R., Ngoile, M.A.K. (1993) Assessment of maturity state and seasonality of reproduction in *Loligo forbesi* (Cephalopoda: Loliginidae) from Scottish waters. In: Okutani, T., O'Dor, R.K., Kubodera, T. (Eds.) Recent advances in fishery biology. Tokai University Press, Tokyo, p. 37 - 48.

Boyle, P.R., Pierce, G.J., Hastie, L.C. (1995) Flexible reproductive strategies in the squid *Loligo forbesi*. *Marine Biology* 121, 501 - 508.

Boyle, P.R., Boletzky, S.v. (1996) Cephalopod populations: definition and dynamics. *Philosophical Transactions of the Royal Society B: Biological Sciences* 985, 1002.

Brodziak, J.K.T., Macy, W.K. (1996) Growth of long-finned squid, *Loligo pealei*, in the Northwest Atlantic. *Fisheries Bulletin* 94, 212 - 236.

Cabral, H.N., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos P., Lopes, M., Ruano, M., Campos, J., Freitas, V., Santos, P.T., Costa, M.J. (2007) Relative importance of estuarine flatfish nurseries along the Portuguese coast. *Journal of Sea Research* 57, 209 - 217.

Cabranes, C., Fernandez-Rueda, P., Martínez, J.L. (2008) Genetic structure of *Octopus vulgaris* around the Iberian Peninsula and Canary Islands as indicated by microsatellite DNA variation. *ICES Journal of Marine Science* 65, 12 - 16.

Caddy, J.F., Rodhouse, P.G. (1998) Cephalopod and groundfish landings: evidence for ecological change in global fisheries? *Reviews in Fish Biology and Fisheries* 8, 431 - 444.

Campana, S.E., Neilson, J.D. (1985) Microstructure of fish otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 42, 1014 - 1032.

Challier, L., Orr, P., Robin, J-P. (2006a) Introducing inter-individual growth variability in the assessment of a cephalopod population: application to the English Channel squid *Loligo forbesi*. *Oecologia* 150, 17 - 28.

Challier, L., Pierce, G.J., Robin, J-P. (2006b) Spatial and temporal variation in age and growth in juvenile *Loligo forbesi* and relationships with recruitment in the English Channel and Scottish waters. *Journal of Sea Research* 55, 217 - 229.

Chapela, A., González, A.F., Dawe, E.G., Rocha, F.J., Guerra, A. (2006) Growth of common octopus (*Octopus vulgaris*) in cages suspended from rafts. *Scientia Marina* 70, 121 - 129.

Chen, C., Chiu, T. (2003) Variations on life history parameters in two geographical groups of neon flying squid, *Ommastrephes bartramii*, from the North Pacific. *Fisheries Research* 63, 349- 366.

Chen, C.S., Pierce, G.J., Wang, J., Robin, J-P., Poulard, J.C., Pereira, J., Zuur, A.F., Boyle, P.R., Bailey, N., Beare, J., Jereb, P., Ragonese, S., Mannini, A., Orsi-Relini, L. (2006) The apparent disappearance of *Loligo forbesi* from the south of its range in the 1990s: Trends in *Loligo* spp. abundance in the northeast Atlantic and possible environmental influences. *Fisheries Research* 78, 44 - 54.

Cherel, Y., Klages, N. (1998) A review of the food of albatrosses. In: Robertson, G., Gales, R. (Eds.) *Albatross Biology and Conservation*. Surrey Beatty, Sydney, p. 113 - 136.

Cinti, A., Barón, P.J., Rivas, A.L. (2004) The effects of environmental factors on the embryonic survival of the Patagonian squid *Loligo gahi*. *Journal of Experimental Marine Biology and Ecology* 313, 225 - 240.

Clarke, M.R. (1980) Cephalopoda in the diet of sperm whales of the southern hemisphere and their bearing on sperm whales biology. *Discovery Reports* 37, 1 – 324.

Clarke, M.R., Lu, C.C. (1995) Cephalopoda of Madeiran waters. *Boletim do Museu.*

Municipal do Funchal 4, 181 - 200.

Clarke, M.R. (1996) The Role of Cephalopods in the World's Oceans: An Introduction *Philosophical Transactions of the Royal Society B: Biological Sciences* 351, 979 – 983.

Clarke, M.R. (2006) Oceanic cephalopod distribution and species diversity in the eastern north Atlantic. *Arquipélago. Life and Marine Sciences* 23A, 27 - 46.

Clarke, M.R. (2007) Seamounts and cephalopods. In: Pitcher, T.J. *et al*. (Eds.) *Seamounts: ecology, fisheries and conservation. Fish and Aquatic Resources Series*. Blackwell Publishing Ltd., UK, 12, 207 - 229.

Coelho, M.L., Quintela, J., Bettencourt, V., Olavo, G., Villa, H. (1994) Population structure, maturation and fecundity of the squid *Loligo vulgaris* from southern Portugal. *Fisheries Research* 21, 87 - 102.

Collins, M.A., Pierce, G.J., Boyle, P.R. (1997) Population indices of reproduction and recruitment in *Loligo forbesi* (Cephalopoda: Loliginidae) in Scottish and Irish waters. *Journal of Applied Ecology* 34, 778 - 786.

Collins, M.A., Yau, C., Boyle, P.R., Friese, D., Piatkowski, U. (2002) Distribution of cephalopods from plankton surveys around the British Isles. *Bulletin of Marine Science* 71, 239 - 254.

Costa, M.D.S. (1992/3/4) Agitação marítima na costa portuguesa. *Anais do Instituto Hidrográfico* 13, 35 - 40.

Cressie, N.A.C. (1991) *Statistics for Spatial Data*. John Wiley and Sons, Inc., New York.

Cunha, M.E. (1993) Variabilidade estacional do zooplâncton na plataforma continental Portuguesa [Seasonal variability of zooplankton in the Portuguese Mainland coast]. *Boletim UCA* 1, 229 - 241.

Cunha, M.E. (2001) *Physical control of biological processes in a coastal upwelling system: comparison of the effects of coastal topography, river run-off and physical oceanography in the northern and southern parts of western Portuguese coastal waters.* PhD thesis. Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal.

Cunha, M.M., Moreno, A., Pereira, J. (1995) Spatial and temporal occurrences of *Loligo* spp. in Portuguese waters. *ICES CM 1995*/K:33.

Dawe, E.G., Beck, P.C. (1997) Population structure, growth and sexual maturation of short-finned squid (*Illex illecbrosus*) at Newfoundland. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 137 - 146.

Demarcq, H., Faure, V. (2000) Coastal upwelling and associated retention indices derived from satellite SST. Application to *Octopus vulgaris* recruitment. *Oceanologica Acta* 23, 391 - 408.

DGPA (2007) Recursos da Pesca. *Direcção Geral das Pescas e Aquicultura. Série Estatística*, 20.

DGPA (2011) Recursos da Pesca. *Direcção Geral das Pescas e Aquicultura. Série Estatística*, 24A-B.

Dias, J.M.A., Monteiro, J.H., Gaspar, L.C. (1980) Potencialidades em cascalhos e areias da plataforma continental portuguesa. *Comunicações dos Serviços Geológicos de Portugal* 66, 227 - 240.

Dias, J.M.A., Nittrouer, C.A. (1984). Continental shelf sediments of northern Portugal. *Continental Shelf Research* 3, 147 - 165.

Dias, J.M.A. (1987) *Dinâmica sedimentar e evolução recente da plataforma continental portuguesa setentrional*. PhD. Thesis, Lisbon University.

Diekmann, O.E., Coyer, J.A., Ferreira, J., Olsen, J.L., Stam, W.T., Pearson, G.A., Serrão, E.A. (2005) Population genetics of *Zostera noltii* along the west Iberian coast: consequences of small population size, habitat discontinuity and near-shore currents. Marine Ecology Progress Series 290, 89 - 96.

Diekmann, R., Piatkowski, U. (2002) Early life stages of cephalopods in the Sargasso Sea: distribution and diversity relative to hydrographic conditions. *Marine Biology* 141, 123 - 130.

Diekmann, R., Piatkowski, U. (2004) Species composition and distribution patterns of early life stages of cephalopods at Great Meteor Seamount (subtropical North-east Atlantic). *Archive Of Fishery And Marine Research* 51, 115 - 131.

Dietrich G., Kalle, K., Krauss, W., Siedler, G. (1980) *General Oceanography*. 2nd ed. John Wiley and Sons, New York.

Domain, F., Jouffre, D., Caveriviere, A. (2000) Growth of *Octopus vulgaris* from tagging in Senegalese waters. *Journal of Marine Biological Association of the United Kingdom* 80, 699-705.

Dos Santos, A., Santos, A.M.P., Conway, D.V.P. (2007) Horizontal and vertical distribution of cirripede cyprid larvae in an upwelling system off the Portuguese coast. *Marine Ecology Progress Series* 329, 145 - 155.

Dos Santos, A., Santos, A.M.P., Conway, D.V.P., Bartilotti, C., Lourenço, P., Queiroga, H. (2008) Diel vertical migration of decapod larvae in the Portuguese coastal upwelling ecosystem: implications for offshore transport. *Marine Ecology Progress Series* 359, 171 - 183.

Durholtz, M.D., Lipinski, M.R., Field, J.G. (2002) Laboratory validation of periodicity of incrementation in statoliths of the South African chokka squid *Loligo vulgaris reynaudii* (d’Orbigny, 1845): a re-evaluation. *Journal of Experimental Marine Biology and Ecology* 279, 41 - 59.

FAO (2010) The state of world fisheries and aquaculture 2010. FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the Inited Nations, Rome.

Faraj, A., Bez, N. (2007) Spatial considerations for the Dakhla stock of *Octopus vulgaris*: indicators, patterns, and fisheries interactions. *ICES Journal of Marine Science* 64, 1820 - 1828.

Faure, V., Inejih, A.C., Demarcq, H., Cury, P. (2000) The importance of retention processes in upwelling areas for recruitment of *Octopus vulgaris*: the example of the Arguin bank (Mauritania). *Fisheries Oceanography* 9, 343 - 355.

Fernández, E., Pingree, R.D. (1996) Coupling between physical and biological fields in the North Atlantic subtropical front southeast of the Azores. *Deep-Sea Research I* 43, 1369 - 1393.

Filippova, J.A., Pakhomov, E.A. (1994) Young squid in the plankton of Prydz Bay, Antarctica. *Antarctic Science* 6, 171 - 173.

Fiúza, A.F.G., de Macedo, M.E., Guerreiro, M.R. (1982) Climatological space and time variation of the Portuguese coastal upwelling. *Oceanologica Acta* 5, 31 - 40.

Fonseca, T., Campos, A., Afonso-Dias, M., Fonseca, P., Pereira, J. (2008) Trawling for cephalopods off the Portuguese coast – Fleet dynamics and landings composition. *Fisheries Research* 92, 180 - 188.

Forsythe, J.W., Van Heukelem, W.F. (1987) Growth. In: Boyle, P.R. (Ed.) *Cephalopod Life Cycles. Vol.II: Comparative reviews.* Academic Press, London, p 135 - 156.

Forsythe J.W. (1993) A working hypothesis of how seasonal temperature change may impact the field growth of young cephalopods. In: Okutani T., O'Dor R.K., Kubodera, T. (Eds.) *Recent Advances in Fishery Biology*. Tokai University Press, Tokyo, p. 133 - 143.

Forsythe, J.W. (2004) Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice. *Marine and Freshwater Research* 55, 331 - 339.

Frouin, R., Fiúza, A., Ambar, I., Boyd, T. (1990) Observations of a poleward surface current off the coasts of Portugal and Spain during winter. *Journal of Geophysical Research* 95, 679 - 691.

García, C.M., Prieto, L., Vargas, M., Echevarría, F., García-Lafuente, J., Ruiz, J., Rubín, J.P. (2002) Hydrodynamics and spatial distribution of plankton and TEP in the Gulf of Cádiz (SW Iberian Peninsula). *Journal of Plankton Research* 24, 817 - 833.

García-Lafuente, J., Delgado, J., Criado-Aldeanueva, F., Bruno, M., del-Río, J., Vargas, J. M. (2006) Water mass circulation on the continental shelf of the Gulf of Cádiz. *Deep-Sea Research II* 53, 1182 - 1197.

García-Lafuente, J.G., Ruiz, J. (2007) The Gulf of Cádiz pelagic ecosystem: A review. *Progress in Oceanography* 74, 228 - 251.

Garofalo, G., Ceriola, L., Gristina, M., Fiorentino, F., Pace, R. (2010) Nurseries, spawning grounds and recruitment of *Octopus vulgaris* in the Strait of Sicily, central Mediterranean Sea. *ICES Journal of Marine Science* 67, 1363 - 1371.

Gibbs, R.H., Roper, C.F.E. (1970) Ocean Acre preliminary report on vertical distribution of fishes and cephalopods. In: Farquhar, G.B. (Ed.) *Proceedings of an international symposium on biological sound scattering in the ocean.* Washington DC: Department of Navy, Maury Center Report 5, 119 - 133.

González, A. F., Rasero, M., Guerra, A. (1994) Preliminary study of *Illex coindetii* and *Todaropsis eblanae* (Cephalopoda: Ommastrephidae) in northern Spanish Atlantic waters. *Fisheries Research* 21, 115 - 126.

González, A.F., Guerra, A. (1996) Reproductive biology of the short-finned squid *Illex coindetii* (Cephalopoda, Ommastrephidae) of the Northeastern Atlantic. *Sarsia*, 81, 107 - 118.

González, A.F., Otero, J., Guerra, A., Prego, R., Rocha, F.J., Dale, A.W. (2005) Distribution of common octopus and common squid paralarvae in a wind-driven upwelling area (Ria of Vigo, northwestern Spain). *Journal of Plankton Research* 27, 271 - 277.

González, A.F., Otero, J., Pierce, G.J., Guerra, A. (2010) Age, growth and mortality of *Loligo vulgaris* wild paralarvae: implications for understanding of the life cycle and longevity. *ICES Journal of Marine Science* 67, 1119 - 1127.

Grist, E.P.M., des Clers, S. (1999) Seasonal and genotypic influences on life cycle synchronisation: further insights from annual squid. *Ecological Modelling* 115, 149 - 163.

Gu, C., Wahba, G. (1991) Minimizing GCV/GML scores with multiple smoothing parameters via the Newton method. *SIAM Journal on Scientific and Statistical Computing* 12, 383 - 398.

Guerra, A. (1981) Spatial distribution pattern of *Octopus vulgaris*. *Journal of Zoology* 195, 133 - 146.

Guerra, A. (1992) Mollusca, Cephalopoda. In: Ramos, M. A. *et al.* (Eds.) *Fauna Ibérica*. Vol. 1. Museo Nacional de Ciencias Naturales, CSIC, Madrid.

Guerra, A., Simon, F., González, A.F. (1993) Cephalopods in the diet of the swordfish, *Xiphias gladius*, from the northeastern Atlantic Ocean. In: Okutani, T., O'Dor, R.K., Kubodera, T. (Eds) Recent advances in fishery biology. Tokai University Press, Tokyo, p. 159-164.

Guerra, A., Rocha, F. (1994) The life history of *Loligo vulgaris* and *Loligo forbesi* (Cephalopoda: Loliginidae) in Galician waters (NW Spain). *Fisheries Research* 21, 43 - 69.

Hanlon, R.T., Hixon, R.F., Turk, P.E., Lee, P.G., Yang, W.T. (1985) Behaviour, feeding and growth of young *Loligo forbesi* (Cephalopoda : Myopsida) reared in the laboratory. *Vie Milieu* 35, 1 - 247.

Hastie, L.C., Joy, J.B., Pierce, G.J., Yau, C. (1994) Reproductive biology of *Todaropsis eblanae* (Cephalopoda: Ommastrephidae) in Scottish waters. *Journal of Marine Biological Association of the United Kingdom* 74, 367 - 382.

Hastie, L.C., Pierce, G.J., Wang, J., Bruno, I., Moreno, A., Piatkowski, U., Robin, J.P. (2009) Cephalopods in the north-east Atlantic: species, biogeography, ecology, exploitation and conservation. *Oceanography and Marine Biology: Annual Review* 47, 119 - 190.

Hastie, T., Tibshirani, R. (1990) *Generalized additive models*. London: Chapman & Hall.

Hatanaka, H., Lange, A.M.T., Amaratunga, T. (1985) Geographical and vertical distribution of shortfinned squid (*Illex illecebrosus*) larvae in the northwest Atlantic. *NAFO Scientific Council Studies* 9, 93 - 99.

Hatfield, E.M.C., Rodhouse, P.G., Barber, D.L. (1992) Production of soma and gonad in maturing female *Illex argentinus* (Mollusca: Cephalopoda). *Journal of Marine Biological Association of the United Kingdom* 72, 281 - 291.

Hatfield, E. (2000) Do some like it hot? Temperature as a possible determinant of variability in the growth of the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae). *Fisheries Research* 47, 27 - 40.

Haynes, R., Barton, E. (1990) A poleward flow along the Atlantic coast of the Iberian peninsula. *Journal of Geophysical Research* 95, 11425 - 11441.

Hendrickson, L.C. (2004) Population biology of northern shortfin squid (*Illex illecebrosus*) in the northwest Atlantic Ocean and initial documentation of a spawning area *ICES Journal of Marine Science* 61, 252 - 266.

Hermosilla, C., Rocha, F., Valavanis, V.D. (2011) Assessing *Octopus vulgaris* distribution using presence-only model methods. *Hydrobiologia* 670, 35 - 47.

Hernández-Garcia, V., Hernández-Lopez, J.L., Castro, J.J. (1997) The octopus (*Octopus vulgaris*) in the small-scale trap fishery off the Canary Islands (Central-East Atlantic). *Fisheries Research* 35, 183 - 189.

Hernández-García, V. (2002) Reproductive biology of *Illex coindetii* and *Todaropsis eblanae* (Cephalopoda: ommastrephidae) off northwest Africa (4°N, 35°N). *Bulletin of Marine Science* 71, 347 - 366.

Ho, J.D., Moltschaniwskyj, N.A., Carter, C.G. (2004) The effect of variability in growth on somatic condition and reproductive status in the southern calamary *Sepioteuthis australis*. *Marine and Freshwater Research* 55, 423 - 428.

Hosmer, D.W., Lemeshow, S. (1989) *Applied Logistic Regression*. John Wiley, New York.

Hunsicker, M.E., Essington, T.E., Watson, R., Sumaila, U.R. (2010) The contribution of cephalopods to global marine fisheries: can we have our squid and eat them too? *Fish and Fisheries* 11, 421 - 438.

Iglesias, J., Otero, J.J., Moxica, C., Fuentes, L., Sánchez, F.J. (2004) The completed life cycle of the octopus (*Octopus vulgaris*, Cuvier) under culture conditions: paralarval rearing using Artemia and zoeae, and first data on juvenile growth up to 8 months of age. *Aquaculture International* 12, 481 - 487.

Jackson, G.D. (1993) Seasonal variation in reproductive investment in the tropical loliginid squid *Loligo chinensis* and the small tropical sepioid *Idiosepius pygmaeus*. *Fishery Bulletin* 91, 260 - 270.

Jackson, G.D., Arkhipkin, A.I., Bizikov, V.A., Hanlon, R.T. (1993) Laboratory and field corroboration of age and growth from statoliths and gladii of the loliginid squid *Sepioteuthis lessoniana* (Mollusca: Cephalopoda). In: Okutani, T., O'Dor, R.K., Kubodera, T. (Eds) *Recent advances in fishery biology*. Tokai University Press, Tokyo, p. 189 - 199.

Jackson, G.D. (1994) Application and future potential of statolith increment analysis in squids and sepiods. *Canadian Journal of Fisheries and Aquatic Sciences* 51, 2612 - 2625.

Jackson, G.D., Forsythe, J.W., Hixon, R.F., Hanlon, R.T. (1997) Age, growth, and maturation of *Lolliguncula brevis* (Cephalapoda: Loliginidae) in the northwestern Gulf of Mexico with a comparison of length-frequency versus statolith age analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 54, 2907 - 2919.

Jackson, G.D., Moltschaniwskyj, N.A. (2002) Spatial and temporal variation in growth rates and maturity in the Indo-Pacific squid *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae). *Marine Biology* 140, 747 - 754.

Jackson, G.D., Domeier, M.L. (2003) The effects of an extraordinary El Niño / La Niña event on the size and growth of the squid *Loligo opalescens* off Southern California. *Marine Biology* 142, 925 - 935.

Jereb, P., Roper, C.F.E. (2005) *Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Chambered nautiluses and sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and Spirulidae).* FAO Species Catalogue for Fishery Purposes, Rome, 4.

Jereb, P., Roper, C.F.E., Vechionne, M. (2005) Introduction. In: Jereb, P., Roper, C.F.E (Eds). *Cephalopods of the world. An annotated and illustrated catalogue of species known to date. Volume 1. Chambered nautiluses and sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae, and Spirulidae).* FAO Species Catalogue for Fishery Purposes, Rome, 4, p. 1 - 19.

Jouanneau, J.M., Garcia, C., Oliveira, A., Rodrigues, A., Dias, J.A., Weber, O. (1998) Dispersal and deposition of suspended sediment on the shelf off the Tagus and Sado estuaries, SW Portugal. *Progress in Oceanography* 42, 233 - 257.

Jouanneau, J.M., Weber, O., Drago, T., Rodrigues, A., Oliveira, A., Dias, J. A., Garcia, C., Schmidt, S., Reyss, J.L. (2002) Present day sedimentation and sedimentary budgets on the Northern Iberian Shelf. *Progress in Oceanography* 52, 261 - 275.

Katsanevakis, S., Verriopoulos, G. (2004a) Abundance of *Octopus vulgaris* on soft sediment. *Scientia Marina* 68, 553 - 560.

Katsanevakis, S., Verriopoulos, G. (2004b) Den ecology of Octopus vulgaris Cuvier, 1797, on soft sediment: availability and types of shelter. *Scientia Marina* 68, 147 - 157.

Katsanevakis, S., Verriopoulos, G. (2006a) Modelling the effect of temperature on hatching and settlement patterns of meroplanktonic organisms: the case of octopus. *Scientia Marina* 70, 699 - 708.

Katsanevakis, S., Verriopoulos, G. (2006b) Seasonal population dynamics of *Octopus vulgaris* in the eastern Mediterranean. *ICES Journal of Marine Science* 63: 151 - 160.

Krauss, W. (1986) The North Atlantic Current. *Journal of Geophysical Research* 91(C4), 5061 - 5074.

Lamas, L., Peliz, A., Ambar, I., Barbosa-Aguiar, A., Maximenko, N., Teles-Machado A. (2010) Evidence of time-mean cyclonic cell southwest of Iberian Peninsula: The Mediterranean Outflow-driven b-plume?, *Geophysical Research Letters* 37, L12606.

Lipinski, M.R., Durholtz, M.D., Underhill, L.G. (1998) Field validation of age readings from the statoliths of chokka squid (*Loligo vulgaris reynaudii* d'Orbigny 1845) and an assessment of associated errors. *ICES Journal of Marine Science* 55, 240 - 257.

Lordan, C. (2001) *Investigations into the fisheries and biology of ommastrephid squid species in Irish waters*. PhD thesis, Aquaculture Development Centre, Department of Zoology and Animal Ecology, Cork, National University of Ireland.

Lourenço, S., Moreno, A., Narciso, L., González, A.F., Pereira, J. (in press) Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. *Fisheries Research*.

Macy, K.W., Brodziak, J.K.T. (2001) Seasonal maturity and size at age of *Loligo pealeii* in waters of southern New England. *ICES Journal of Marine Science* 58, 852 - 864.

Mangold, K., Boletzky, S.v. (1973) New data on reproductive biology and growth of *Octopus vulgaris*. *Marine Bioogy* 19, 7 - 12.

Mangold, K. (1983) *Octopus vulgaris*. In: Boyle, P.R. (Ed.), *Cephalopod Life Cycles Volume I: Species Accounts*. Academic Press, New York, p. 335 – 364.

Mangold, K. (1987) Reproduction. In: Boyle, P.R. (Ed.) *Cephalopod Life Cycles. Comparative reviews*. Vol. II. Academic Press, London, p. 157 - 200.

Mangold-Wirz, K. (1963) Biologie des cephalopods bentiques et nectoniques de la mer Catalone. *Vie Milieu* 13, 1 - 285.

Markaida, U., Quiñónez-Velasquez, C., Sosa-Nishizaki, O. (2004) Age, growth and maturation of jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae) from the Gulf of California, Mexico. *Fisheries Research* 66, 31 - 47.

Marta-Almeida, M., Dubert, J., Peliz, A., Queiroga, H. (2006) Influence of vertical migration pattern on retention of crab larvae in the shelf in a seasonal upwelling system. *Marine Ecology Progress Series* 307, 1 - 19.

Martins, C.S., Hamann, M., Fiúza, A.F.G. (2002) Surface circulation in the eastern North Atlantic, from drifters and altimetry. *Journal of Geophysical Research* 107, 3217.

Mather, J.A. (1988) Daytime activity of juvenile *Octopus vulgaris* in Bermuda. *Malacologia* 29, 69 - 76.

McCullagh, P., Nelder, J.A. (1989) *Generalized Linear Models*. Chapman and Hall, London.

Melo, Y.C., Sauer, W.H.H. (1999) Confirmation of serial spawning in the chokka squid *Loligo vulgaris reynaudii* off the coast of South Africa. *Marine Biology* 135, 307-313.

Moltschaniwskyj, N.A. (1994) Muscle tissue and muscle fiber dynamics in the tropical Loliginid squid *Photololigo* sp. (Cephalopoda: Loliginidade). *Canadian Journal of Fisheries and Aquatic Sciences* 51, 830 - 835.

Moltschaniwskyj, N.A. (1995) Multiple spawning in the tropical squid *Photololigo sp.*: What is the cost in somatic growth? *Marine Biology* 124, 127 - 135.

Moltschaniwskyj, N.A., Semmens, J.M. (2000) Limited use of stored energy reserves for reproduction by the tropical loliginid squid *Photololigo sp*. Journal of Zoology 251, 307 - 313.

Moreno, A., Cunha, M.M., Pereira, J.M.F. (1994) Population biology of the veined squid (*Loligo forbesi*) and European squid (*Loligo vulgaris*) from the Portuguese coast. *Fishery Research* 21, 71 - 86.

Moreno, A. (1998) Variação sazonal na distribuição e abundância dos cefalópodes da plataforma continental entre Espinho e Nazaré: resultados dos cruzeiros de Agosto e Novembro de 1996 no N/I “Mestre Costeiro”, *Relatórios Científicos e Técnicos do Instituto de Investigação das Pescas e do Mar* 29, 1 - 23.

Moreno, A. (2002) Morfologia e micro-estrutura dos estatólitos de lula, *Loligo vulgaris*: Metodologias de determinação de idades. *Relatórios Científicos e Técnicos do Instituto de Investigação das Pescas e do Mar* 86, 1 - 46.

Moreno, A., Pereira, J., Arvanitidis, C., Robin, J-P., Koutsoubas, D., Perales-Raya, C., Cunha, M.M., Balguerías, E., Denis, V. (2002) Biological variation of *Loligo vulgaris* (Cephalopoda: Loliginidae) in the eastern Atlantic and Mediterranean. *Bulletin of Marine Science* 71, 515 - 534.

Moreno, A., Pereira, J., Cunha, M.M. (2005) The effect of time of hatching in age and size at maturity of *Loligo vulgaris*. *Aquatic Living Resources* 18, 377 - 384.

Moreno, A., Azevedo, M., Pereira, J., Pierce, G.J. (2007) Growth strategies in the squid *Loligo vulgaris* from Portuguese waters. *Marine Biology Research* 3, 49 - 59.

Moreno, A., dos Santos, A., Piatkowski, U., Santos, A.M.P., Cabral, H. (2009) Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia. *Journal of Plankton Research* 31, 73 - 91.

Muggeo, V.M.R. (2003) Estimating regression models with unknown break-points. *Statistics in Medicine* 22, 3055 - 3071.

Muggeo, V.M.R. (2008) Segmented: an R Software to Fit Regression Models with Broken-Line Relationships. *RNews* 8/1, 20 - 25.

Murphy, E.J., Rodhouse, P.G. (1999) Rapid selection effects in a short-lived semelparous squid species exposed to exploitation: inferences from the optimisation of life-history function. *Evolutionary Ecology* 13, 517 - 537.

Natsurari, Y., Nakanose, T., Oda, K. (1988) Age and growth of the loliginid squid *Photololigo edulis* (Hoyle 1885). *Jounal of Experimental Marine Biology and Ecology* 116, 177 - 190.

Natsukari, Y., Komine, N. (1992) Age and growth estimation of the European squid *Loligo vulgaris*, based on statolith microstructure. *Journal of the Marine Biological Association of United Kingdom* 72, 271 - 280.

Nixon, M., Young, J.Z. (2003) *The brains and lives of cephalopods*. Oxford University Press, Oxford, UK.

O'Dor, R.K., Webber, D.M. (1986) The constraints on cephalopods: why squid aren't fish. *Canadian Journal of Zoology* 64, 1591 - 1605.

O’Dor, R.K. (1998) Can understanding squid life history strategies and recruitment improve management? *South African Journal of Marine Science* 20, 193 - 206.

Oliveira, A., Vitorino, J., Rodrigues, A., Jouanneau, J. M., Dias, J. M. A., Weber, A. (2002). Nepheloid layer dynamics of the northern Portuguese shelf. *Progress in Oceanography* 52, 195 - 213.

Otero, J. (2006) *Ecología del pulpo común (Octopus vulgaris Cuvier, 1797) en un área de afloramiento costero (Galicia, NE Atlántico)*. Ph. D. Thesis. University of Vigo.

Otero, J., González, A.F., Sieiro, M.P., Guerra, A. (2007) Reproductive cycle and energy allocation of Octopus vulgaris in Galician waters, NE Atlantic. Fisheries Research 85, 122 - 129.

Otero, J., Álvarez-Salgado, X.A., González, A.F., Miranda, A., Groom, S.B., Cabanas, J.M., Casas, G., Wheatley, B., Guerra, A. (2008) Bottom-up control of common octopus *Octopus vulgaris* in the Galician upwelling system, northeast Atlantic Ocean. *Marine Ecology Progress Series* 362, 181 - 192.

Otero, J., Álvarez-Salgado, X.A., González, A.F., Gilcoto, M., Guerra, A. (2009) High frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. *Marine Ecology Progress Series* 386, 123 - 132.

Pecl, G.T. (2004) The in situ relationships between season of hatching, growth and condition in the southern calamary, *Sepioteuthis australis*. *Marine and Freshwater Research* 55, 429 - 438.

Pecl, G.T., Moltschaniwskyj, N.A., Tracey, S., Jordan, A. (2004) Inter-annual plasticity of squid life-history and population struture: ecological and management implications. *Oecologia* 139, 515 - 524.

Pecl, G.T., Jackson, G.D. (2008) The potential impacts of climate change on inshore squid: biology, ecology and fisheries. *Reviews in Fisheries Biology and Fisheries* 18, 373 - 385.

Peliz, A., Rosa, T., Santos, A.M.P., Pissarra, J. (2002) Jets, Eddies, and Counterflows in the Western Iberia Upwelling System. *Journal of Marine Systems* 35, 61 - 77.

Peliz, A., Santos, A.M., Oliveira, P.B., Dubert, J.D. (2004) Extreme cross-shelf transport induced by eddy interactions southwest of Iberia in winter 2001. *Geophysical Research Letters* 31, L08301.

Peliz, A., Dubert, J., Santos, A.M.P., Oliveira, P.B., Le Cann, B. (2005) Winter upper ocean circulation in the Western Iberian Basin—Fronts, Eddies and Poleward Flows: an overview. *Deep-Sea Research I* 52, 621 - 646.

Peliz, A., Dubert, J., Marchesiello, P., Teles-Machado, A. (2007a) Surface circulation in the Gulf of Cadiz: Model and mean flow structure. *Journal of Geophysical Research* 112, C11, C11015.

Peliz, A., Marchesiello, P., Dubert, J., Marta-Almeida, M., Roy, C., Queiroga, H. (2007b) A study of crab larvae dispersal on the Western Iberian Shelf: Physical processes. *Journal of Marine Systems* 68, 215 - 236.

Peliz, A., Marchesiello, P., Dubert, J., Teles-Machado, A., Marta-Almeida, M., Le Cann B.

(2009) Surface Circulation in the Gulf of Cadiz. Part 2: Inflow/outflow coupling and the Gulf of Cadiz Slope Current. *Journal of Geophysical Research* 114, C03011.

Pérez, F.F., Castro, C.G., Álvarez-Salgado, X.A., Ríos, A. (2001) Coupling between the Iberian Basin-scale circulation and the Portugal boundary current system: a chemical study. *Deep Sea Research I* 48, 1519 - 1533.

Piatkowski, U., Welsch, W., Röpke, A. (1993) Distribution patterns of the early life stages of pelagic cephalopods in three geographically different regions of the Arabian Sea. In: Okutani, T., O'Dor, R.K., Kubodera, T. (Eds.) *Recent advances in cephalopod fisheries biology*. Tokai University Press, Tokyo, p. 417 - 431.

Pierce, G.J., Guerra, A. (1994). Stock assessment methods used for cephalopod fisheries. *Fisheries Research* 21, 255 - 285.

Pierce, G.J., Boyle, P.R., Hastie, L.C., Key, L. (1994a). The life history of *Loligo forbesi* (Cephalopoda: Loliginidae) in Scottish waters. *Fishery Research* 21, 17 - 41.

Pierce, G.J., Hastie, L.C., Guerra, A., Thorpe, R.S., Howard, F.G., Boyle, P.R. (1994b) Morphometric variation in *Loligo forbesi* and *Loligo vulgaris*: regional, seasonal, sex, maturity and worker differences. *Fisheries Research* 21, 117 - 148.

Pierce, G.J., Boyle, P.R., Hastie, L.C., Santos, M.B. (1994c). Diets of squid *Loligo forbesi* and *Loligo vulgaris* in the Northeast Atlantic. *Fisheries Research* 21, 149 - 163.

Pierce, G.J., Boyle, P.R. (2003) Empirical modelling of interannual trends in abundance of squid (*Loligo forbesi*) in Scottish waters. *Fisheries Research* 59, 305 - 326.

Pierce, G.P., Zuur, A.F., Smith, J.M., Santos, M.B., Bailey, N., Chen, C-S., Boyle, P,R. (2005) Interannual variation in life-cycle characteristics of the veined squid (*Loligo forbesi*) in Scottish waters. *Aquatic Living Resources* 18, 327 - 340.

Pierce, G. J., Allcock, L., Bruno, I., Bustamante, P., González, Á., Guerra, Á., Jereb, P., Lefkaditou, E., Malham, S., Moreno, A., Pereira, P., Piatkowski, U, Rasero, M., Sánchez, P., Santos, M.B., Santurtún, M., Seixas, S., Sobrino, I., Villanueva, R. (2010) Cephalopod biology and fisheries in Europe. *ICES Cooperative Research Report* 303, 1 - 175.

Pilar-Fonseca, T., Campos, A., Afonso-Dias, M., Fonseca, P., Mendes, B. (2009) Fleet segmentation of the Portuguese coastal trawl fishery: a contribution to fisheries management, *International Council for the Exploration of the Sea (CM Papers and Reports)*, CM 2009/O:29.

Preuss, T., Lebaric, Z.N., Gilly W.F. (1997) Post-hatching development of circular mantle muscles in the squid *Loligo opalescens*. *Biology Bulletin* 192, 375 - 387.

Queiroga, H., Silva, C., Sorbe, J-C., Morgado, F. (2005) Composition and distribution of zooplankton across an upwelling front on the northern Portuguese coast during summer. *Hydrobiologia* 545, 195 - 207.

Quetglas, A., Alemany, F., Carbonell, A., Merella, P., Sanchez, P. (1998) Biology and fishery of *Octopus vulgaris* Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, Western Mediterranean). *Fisheries Research* 36, 237 - 249.

Quetglas, A., Carbonell, A., Sanchez, P. (2000) Demersal continental shelf and upper slope cephalopod assemblages from the Balearic Sea (North-Western Mediterranean): biological aspects of some deep-sea species. *Estuarine, Coastal and Shelf Science* 50, 739 - 749.

R development core team (2006) R: A language and environment for statistical computing. R Foundation for Statistical Computing, ISBN 3-900051-07-0, URL http:\\www.r-project.org.

Raya, C.P., Balguerias, E., Fernandez-Nunez, M.M., Pierce, G.J. (1999) On reproduction and age of the squid *Loligo vulgaris* from the Saharan Bank (north-west African coast). *Journal of the Marine Biological Association of United Kingdom* 79, 111 - 120.

Rees, W.J., Maul, G.E. (1956) The cephalopoda of Madeira, records and distribution. *Bulletin of the British Museum of Natural History (Zoology)* 3, 257 - 281.

Relvas, P., Barton, E.D. (2002) Mesoscale patterns in the Cape São Vicente (Iberian Peninsula) upwelling region. *Journal of Geophysical Research* 107(C10), 3164.

Relvas, P., Barton, E.D. (2005) A separated jet and coastal counterflow during upwelling relaxation off Cape São Vicente (Iberian Peninsula). *Continental Shelf Research* 25, 29 - 49.

Relvas, P., Barton, E.D., Dubert, J., Oliveira, P.B., Peliz, A., Silva, J.C.B., Santos, A.M.P. (2007) Physical oceanography of the western Iberia ecosystem: Latest views and challenges, *Progress in Oceanography* 74, 149 - 173.

Reynolds, R.W., Smith, T.M. (1994) Improved global sea surface temperature analyses using optimum interpolation. *Journal of Climate* 7, 929 - 948.

Ribeiro, A.C., Peliz, A., Santos, A.M.P. (2005) A study of the response of chlorophyll-a biomass to a winter upwelling event off western Iberia using SeaWiFS and in situ data. *Journal of Marine Systems* 53, 87 - 107.

Richard, A. (1966) La temperature facteur externe essentiel de croissance pour le cephalopod *Sepia officinalis*. *Les Comptes Rendus de l'Academie des Sciences, Série D Paris* 263, 1138 - 1141.

Ricker, W.E. (1979) Growth rates and models. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.) *Fish Physiology, Vol. III: Biogenics and growth*. Academic Press, Orlando, p 677 - 743.

Roberts, M.J., Berg, M.v. (2005) Currents along the Tsitsikamma coast, South Africa, and potential transport of squid paralarvae and ichthyoplankton. *African Journal of Marine Science* 27, 375 - 388.

Robin, J-P., Denis, V. (1999) Squid stock fluctuations and water temperature: temporal analysis of English Channel Loliginidae. *Journal of Applied Ecology* 36, 101 - 110.

Robin, J-P., Denis, V., Royer, J., Challier, L. (2002) Recruitment, growth and reproduction in *Todaropsis eblanae* (Ball, 1841), in the area fished by French Atlantic trawlers. *Bulletin of Marine Science* 71, 711 - 724.

Rocha, F., Guerra, A. (1999) Age and growth of two sympatric squids *Loligo vulgaris* and *Loligo forbesi*, in Galician waters (NW Spain). *Journal of Marine Biological Association of the United Kingdom* 79, 697 - 707.

Rocha, F., Guerra, A., Prego, R., Piatkowski, U. (1999) Cephalopod paralarvae and upwelling conditions off Galician waters (NW Spain). *Journal of Plankton Research* 21, 21-33.

Rodhouse, P.G., Hatfield, E.M.C. (1990) Age determination in squid using statolith growth increments. *Fisheries Research* 8, 323 - 334.

Rodriguez-Rúa, A., Pozuelo, I., Prado, M.A., Gomez, M.J., Bruzon, M.A. (2005) The gametogenic cycle of *Octopus vulgaris* (Mollusca: Cephalopoda) as observed on the Atlantic coast of Andalusia (south of Spain). *Marine Biology* 147, 927 - 933.

Roepke, A., Nellen, W., Piatkowski, U. (1993) A comparative study on the influence of the pycnocline on the vertical distribution of fish larvae and cephalopod paralarvae in three ecologically different areas of the Arabian Sea. *Deep-Sea Research II* 40, 801 - 819.

Royer, J., Peries, P., Robin, J-P. (2002) Stock assessments of English Channel Loliginid squid: updated depletion methods and new analytical methods. *ICES Journal of Marine Science* 59, 445 - 457.

Ruano, F. (2011) Mortalidade massiva de polvos (*Octopus vulgaris*) na costa norte de Portugal. *V Congresso da Sociedade Portuguesa de Ciências Veterinárias, Livro de resumos*, 113 (Abstract).

Sakai, M., Brunetti, N.E., Elena, B., Sakurai, Y. (1998) Embryonic development and hatchlings of *Illex argentinus* derived from artificial fertilization. *South African Journal of Marine Science* 20, 255 - 265.

Sánchez, R., Relvas, P. (2003) Spring-Summer climatological circulation in the upper layer in the region of Cape St. Vincent, SW Portugal. *ICES Journal of Marine Science* 60, 1232 - 1250.

Sánchez, R., Mason, E., Relvas, P., da Silva, A.J., Peliz, A. (2006). On the inshore circulation in the northern Gulf of Cadiz, southern Portuguese Shelf. *Deep-Sea Research II* 53, 1198 - 1218.

Santos, A.M.P., Borges, M.F., Groom, S. (2001) Sardine and horse mackerel recruitment and upwelling off Portugal. *ICES Journal of Marine Science* 58, 589 - 596.

Santos, A.M.P., Peliz, A., Dubert, J., Oliveira, P.B., Angélico, M.M., Ré, P. (2004) Impact of a Winter upwelling event on the distribution and transport of sardine eggs and larvae off western Iberia: a retention mechanism. *Continental Shelf Research* 24, 149 - 165.

Santos, A.M.P, Chicharo, A., dos Santos, A., Moita, T., Oliveira, P., Peliz, A., Ré, P. (2007) Physical-biological interactions in the life history of small pelagic fish in the Western Iberia Upwelling Ecosystem. *Progress in Oceanography* 74, 192 - 209.

Santos, M.B., Clarke, M.R., Pierce, G.J. (2001) Assessing the importance of cephalopods in the dieats of marine mammals and other top predators: problems and solutions.

Fisheries Research 52, 121 - 129.

Semmens, J.M., Pecl, G.T., Gillanders, B.M., Waluda, C.M., Shea, E.K., Jouffre, D., Ichii, T., Zumholz, K., Katugin, O.N., Leporati, S.C., Shaw, P.W. (2007) Approaches to resolving cephalopod movement and migration patterns. *Reviews in Fisheries Biology and Fisheries* 17, 401 - 423.

Sen, H. (2005a) Temperature tolerance of loliginid squid (*Loligo vulgaris* Lamarck, 1798) eggs in controlled conditions. *Turkish Journal of Fisheries and Aquatic Sciences* 5, 53 - 56.

Sen, H. (2005b) Incubation of European Squid (*Loligo vulgaris* Lamarck, 1798) eggs at different salinities. *Aquaculture Research* 36, 876 - 881.

Shaw, R. (1994) An economic description and assessment of the squid catching industry in the UK, Portugal and Spain. *Fisheries Research* 21, 287 - 303.

Shea, E.K., Vechione, M. (2002) Quantification of ontogenetic discontinuities in three species of oegopsid squids using model II picewise linear regression. *Marine Biology* 140, 971 - 979.

Silva, L., Sobrino, I., Ramos, F. (2002) Reproductive biology of the common octopus *Octopus vulgaris* Cuvier, 1797 (Cephalopoda: Octopodidae) in the Gulf of Cadiz (SW Spain). *Bulletin of Marine Science* 71, 837 - 850.

Smith, J.M., Pierce, G.J., Zuur, A.F., Boyle, P.R. (2005) Seasonal patterns of investment in reproductive and somatic tissues in the squid *Loligo forbesi*. Aquatic *Living Resources* 18, 341 - 351.

Sousa, P., Lemos, R.T., Gomes, M.C., Azevedo, M. (2007) Analysis of horse mackerel, blue whiting, and hake catch data from Portuguese surveys (1989–1999) using an integrated GLM approach. *Aquatic Living Resources* 20, 105 - 116.

Sponaugle, S. (2010) Otolith microstructure reveals ecological and oceanographic processes important to ecosystem-based management. *Environmental Biology of Fishes* 89, 221 - 238.

Stearns, S.C. (1992) The evolution of life histories. Oxford University Press, New York.

Steer, B.L.M., Jackson, G.D. (2004) Temporal shifts in the allocation of energy in the arrow squid, *Nototodarus gouldi*: sex-specific responses. *Marine Biology* 144, 1141 - 1149.

Steer, M.A., Pecl, G., Moltschaniwskyj, N.A. (2003) Are bigger calamary *Sepioteuthis australis* hatchlings more likely to survive? A study based on statolith dimensions. *Marine Ecology Progress Series* 261, 175 - 182.

Stehle, M., dos Santos, A., Queiroga, H. (2007) Comparison of zooplankton sampling performance of Longhurst–Hardy Plankton Recorder and Bongo nets. *Journal of Plankton Research* 29, 169 - 177.

Sweeney, M.J., Roper, C.F.E., Mangold, K.M., Clarke, M.R., Boletzky, S.v. (Eds.) (1992) *„Larval“ and juvenile cephalopods: A manual for their identification* 513, Smithsonian Contributions to Zoology, Washington.

Teles-Machado, A., Peliz, A., Dubert, J., Sanchez, R. (2007) On the onset of the Gulf of Cadiz Coastal Countercurrent. *Geophysical Research Letters* 34, L12601.

Thompson, J.T., Kier, W.M. (2001) Ontogenetic changes in fibrous connective tissue organization in the oval squid, *Sepioteuthis lessoniana* Lesson, 1830. *Biology Bulletin* 201, 136 - 153.

Triantafillos, L. (2004) Effects of genetic and environmental factors on growth of southern calamary, *Sepioteuthis australis*, from southern Australia and northern New Zealand. *Marine and Freshwater Research* 55, 439 - 446.

Trites, R.W. (1983) Physical oceanographic factors and processes relevant to *Illex illexebrosus* spawning in the western North Atlantic and subsequent larval distribution. *NAFO Scientific Council Studies* 6, 39 - 55.

Turk, P.E., Hanlon, R.T., Bradford, L.A., Yang, W.T. (1986) Aspects of feeding, growth and survival of the European squid *Loligo vulgaris* Lamarck 1799, reared through the early growth stages. *Vie et Milieu* 36, 9 - 13.

Valdés, L., López-Urrutia, A., Cabal, J., Alvarez-Ossorio, M., Bode, A., Miranda, A., Cabanas, M., Huskin, I., Anadón, R., Alvarez-Marqués, F., Llope, M., Rodríguez, N. (2007) A decade of sampling in the Bay of Biscay. What are the zooplankton time series telling us? *Progress in Oceanography* 74, 98 - 114.

Vanney, J.R., Mougenot, D. (1981) La plateforme continentale du Portugal et les provinces adjacentes: analyse géomorphologique. *Memórias dos Serviços Geológicos Portugal* 28, 145.

Vaz-Pires, P., Seixas, P., Barbosa, A. (2004) Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. *Aquaculture* 238, 221 - 238.

Vechione, M. (1987) Juvenile ecology. In Boyle, P.R. (Ed.), *Cephalopod life cycles.* Vol. 2. Academic Press, London, p. 61 - 84.

Vidal, E.A.G., DiMarco, F.P., Wormuth, J.H. (2002) Optimizing rearing conditions of hatching loliginid squid. *Marine Biology* 140, 117 - 127.

Villa, H., Quintela, J., Coelho, M.L., Icely, J.D., Andrade, J.P. (1997) Phytoplankton biomass and zooplankton abundance on the south coast of Portugal (Sagres), with special reference to spawning of *Loligo vulgaris*. *Scientia Marina*, 61, 123 - 129.

Villanueva, R. (1995) Experimental rearing and growth of planktonic Octopus vulgaris from hatching to settlement. *Canadian Journal of Fisheries and Aquatic Sciences* 52, 2639 - 2650.

Villanueva, R. (2000a) Differential increment-deposition in embryonic statoliths of loliginid squid *Loligo vulgaris*. *Marine Biology* 137, 161 - 168.

Villanueva, R. (2000b) Effect of temperature on statolith growth of the European squid *Loligo vulgaris* during early life. *Marine Biology* 136, 449 - 460.

Villanueva, R., Arkhipkin, A., Jereb, P., Lefkaditou, E., Lipinski, M.R., Perales-Raya, C., Riba, C., Rocha, F. (2003) Embryonic life of the loliginid squid *Loligo vulgaris*: comparison between statoliths of Atlantic and Mediterranean populations. *Marine Ecology Progress Series* 253, 197 - 208.

Villanueva, R., Moltschaniwskyj, N.A., Bozzano, A. (2007) Abiotic influences on embryo growth: statoliths as experimental tools in the squid early life history. *Reviews in Fisheries Biology and Fisheries* 17, 101 - 110.

Villanueva, R., Nozais, C., Boletzky, S. v. (1995) The planktonic life of octopuses. *Nature* 377, 107.

Villegas, P. (2001) Growth, life cycle and fisheries biology of *Loligo gahi* (d'Orbigny, 1835) off the Peruvian coast. *Fisheries Research* 54, 123 - 131.

Vitorino, J., Oliveira, A., Jouanneau, J., Drago, T. (2002) Winter dynamics on the northern Portuguese shelf. Part 1: physical processes. *Progress in Oceanography* 52, 129 - 153.

Wood, S.N. (2000) Modelling and Smoothing Parameter Estimation with Multiple Quadratic Penalties. *Journal of the Royal Statistical Society - Series B: Statistical Methodology* 62, 413 - 428.

Wooster, W., Bakun, A., McLain, D. (1976) The seasonal upwelling cycle along the eastern boundary of the North Atlantic. *Journal of Marine Research* 34, 131 - 141.

Young, I.A.G., Pierce, G.J., Dali, H.I., Santos, M.B., Key, L.N., Bailey, N., Robin, J-P., Bishop, A.J., Stowasser, G., Nyegaard, M., Cho, S.K., Rasero, M., Pereira, J.M.F. (2004) Aplication of depletion methods to estimate stock size in the squid *Loligo forbesi* in Scottish waters (UK). *Fisheries Research* 69, 211 - 227.

Young, R.E., Harman, R.F. (1988) “Larva”, “paralarva”, and “subadult” in cephalopod terminology. *Malacologia* 29, 201 - 207.

Zumholz, K., Piatkowski, U. (2005) Research cruise data on the biology of the lesser flying squid, *Todaropsis eblanae*, in the North Sea. *Aquatic Living Resources* 18, 373 - 376.

Zuur, A.F., Ieno, E.N., Elphick, C.S. (2010) A protocol for data exploration to avoid common statistical problems. *Methods in ecology and evolution* 1, 3 - 14.

Agradecimentos

Por me terem ajudado na realização deste trabalho quero agradecer:

Ao Prof. Dr Henrique Cabral da Faculdade de Ciências da Universidade de Lisboa, por ter aceitado orientar esta tese, pela confiança que sempre depositou em mim e no meu trabalho, pelos seus ensinamentos e sugestões e pela sua amizade e estímulo ao longo destes anos.

Ao Dr. António Miguel Santos meu orientador no IPIMAR, estou muito grata pelo seu incentivo, disponibilidade e apoio e também pelos seus ensinamentos que muito ajudaram a melhorar esta dissertação e contribuíram para o alargamento dos meus conhecimentos na área da oceanografia. Foi um prazer trabalharmos juntos.

Agradeço especialmente ao Dr. Graham Pierce da Universidade de Aberdeen, que co-orientou esta tese, com quem partilhei longas discussões científicas e que tem sido o meu verdadeiro mentor na investigação dos cefalópodes desde há longos anos.

Aos meus colegas da equipa de investigação de cefalópodes do IPIMAR, João Pereira, Manuela Cunha, Sílvia Lourenço e Pedro da Conceição com quem partilho diariamente os desafios de estudar estes animais tão fascinantes, vão os meus maiores agradecimentos, assim como à minha amiga e colega Antonina dos Santos que me ensinou tudo o que sei sobre plâncton e que sempre me incentivou a publicar os meus trabalhos e a realizar este grau académico. Estou ainda grata à Manuela Azevedo, co-autora em dois artigos, pela colaboração e pela sua disponibilidade para animadas discussões científicas que muito beneficiaram este trabalho.

O meu sincero agradecimento vai ainda para muitos outros colegas do IPIMAR que também me incentivaram e escutaram os meus desabafos e incertezas, ou de algum modo me ajudaram durante os últimos anos. Em especial ao João Pastor, à Teresa Cabrita e ao Hugo Mendes obrigada pela vossa amizade e confiança.

Agradeço ainda a todos os meus colegas aficionados dos cefalópodes que participaram nos projectos europeus Eurosquid II e CEPHVAR, nos quais parte deste trabalho se integrou. Em particular, agradeço ao meu colega e amigo Hervé Troadec, por toda a ajuda prestada na utilização do programa TNPC e por todo o trabalho nas técnicas de leitura de estatólitos que desenvolvemos em conjunto.

Agradeço às equipas de investigação dos 57 cruzeiros de plâncton e 8 cruzeiros demersais aqui analisados, cujo contributo foi indispensável para este trabalho e aos colegas do laboratório de zooplâncton que recolheram as amostras, triaram as paralarvas de cefalópodes e disponibilizaram os dados associados. À colega Corina Chaves pela sua amizade e pela sua disponibilidade relativamente à utilização da base de dados de cruzeiros CRUZDEM e aos colegas Beatriz Mendes e Victor Henriques pela disponibilização dos dados de sedimentologia.

Por fim agradeço aos meus pais que sempre acreditaram em mim, à minha mãe, que onde quer que esteja verá um sonho realizado e ao meu pai pelo seu apoio incondicional. Ao Pedro, à Catarina e à Raquel por estarem comigo todos os dias.

Este trabalho foi em parte financiado ao abrigo de vários projectos nacionais e europeus, nomeadamente: Eurosquid II (AIR-CT92-0573), CEPHVAR (FAIR-CT96-1520), CEPHSTOCK (Q5CA-2002-00962) e "Desarrollo Sostenible de las Pesquerías Artesanales del Arco Atlántico - PRESPO" (Programme INTERREG IV B, co-financed by European Union, ERDF funds). Algumas das amostras biológicas utilizadas foram financiadas pelo programa "Data Collection For Fisheries Sector Programme" (DCF - EC/DG Maritime Affairs & Fisheries/Joint Research Center).