Universidade de Lisboa

Faculdade de Ciências

Departamento de Biologia Animal

Ecology of the Common Octopus *Octopus vulgaris* (Cuvier, 1797) in the Atlantic Iberian coast: Life cycle strategies under different oceanographic regimes

Sílvia Alexandra Pereira Lourenço

Doutoramento em Biologia

Especialidade de Biologia Marinha e Aquacultura

2014

Universidade de Lisboa

Faculdade de Ciências

Departamento de Biologia Animal

Ecology of the Common Octopus *Octopus vulgaris* (Cuvier, 1797) in the Atlantic Iberian coast: Life cycle strategies under different oceanographic regimes

Sílvia Alexandra Pereira Lourenço

Tese orientada por Prof. Dr. Luís Narciso (FCUL/CO), Dr. Ángel F. González González (IIM-CSIC) e por Dr. João Pereira (IPMA)

2014

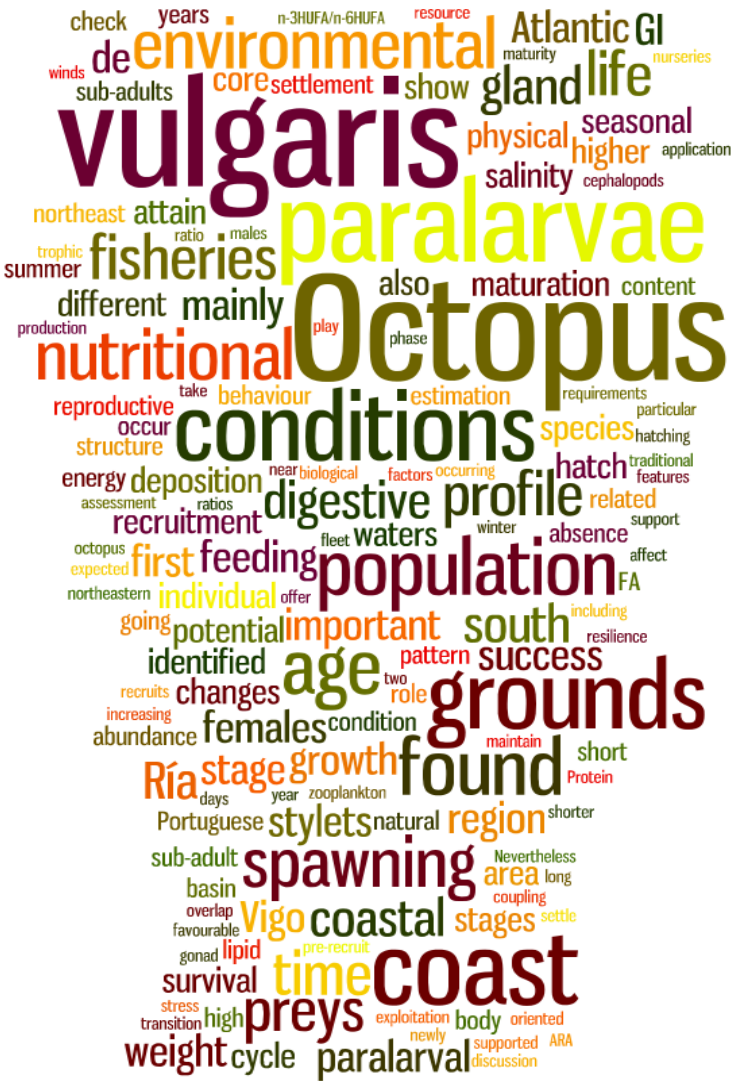
Doctoral dissertation in Biology
(specialization in Marine Biology and Aquaculture)
presented to the University of Lisboa

Dissertação apresentada à Universidade de Lisboa
para a obtenção do grau de doutor

(Especialidade Biologia Marinha e Aquacultura)

Sílvia Alexandra Pereira Lourenço

2014



"Mas não investigues: diverte-te. Crias dificuldades e conceitos para atrasar a tua chegada. Amanhã chegarás no esconderijo onde ainda ontem escondeste a resposta."

Gonçalo M. Tavares, *Breves notas sobre ciência*, 2006

TABLE OF CONTENTS

SUMMARY

Octopus vulgaris is one of the most important fishery resources in the coastal area of the Atlantic Iberian basin. One to two years lifespan, terminal semelparity with numerous eggs and the absence of generation overlap are the reasons for high variability in the species abundance between years strongly connected with environmental conditions. The overall aim of this thesis is to identify which factors, physical, environmental and biologic influencing the *Octopus vulgaris* life cycle. First of all, we look at the reproductive season of the species in the northwest and south Portuguese coasts and conclude that spawning depends on the local oceanographic regime but also partially on the nutritional quality of the food. The study was followed by an analysis of the local factors that influence abundance in the nursery of Ría de Vigo (Southwest Galicia). Here, the newly-hatched paralarvae concentrate in shallow waters and protected areas under the influence of the Cies islands and feed on the meso-zooplanktonic community that supply these paralarvae with the adequate nutritional requirements balanced to protein but also rich in n-3HUFA fatty acids essential for growth and survival at this stage. After settlement, the physical and environmental characteristics of the feeding grounds are essential for recruitment success. Here we identified five areas of pre-recruit aggregation along the Portuguese Coast. Finally, the age and growth were determined for juveniles of the Northwest Portuguese coast. Age estimates are fundamental tools to chronologically follow the effect of the environmental factors through the life cycle. Here, ageing methodologies using the stylets as age recorders were improved to achieve precision and accuracy in ageing estimates in this species. The results and conclusions of the thesis present important data to incorporate in the future ecosystem approach models for the assessment and management of the octopus fisheries in the Portuguese coast.

Key words: *Octopus vulgaris*, adults, paralarvae, juvenile, reproduction, feeding requirements, essential habitats, age, growth, stylets.

RESUMO

Octopus vulgaris é um dos recursos marinhos mais capturados na área costeira da costa Ibérica Atlântica. A longevidade de um a dois anos, semelparidade com descendência numerosa e ausência de sobreposição de gerações faz com que a abundância da espécie varie muito de um ano para o outro em função de condições ambientais. O objetivo geral desta tese é identificar quais os fatores físicos, ambientais e biológicos que condicionam o ciclo de vida das populações de *O. vulgaris* da costa continental portuguesa. Para desenvolver esta tese observámos, numa primeira fase, a época reprodutiva das duas populações da costa noroeste e sul portuguesas concluindo que esta depende do regime oceanográfico local mas também é parcialmente influenciada pela disponibilidade nutricional no momento. De seguida, analisaram-se os fatores locais que fazem com que a Ría de Vigo (Galiza) seja área de berçário importante para esta espécie. Aqui as paralarvas recém-eclodidas concentram-se em águas pouco profundas do interior da ria junto às Ilhas Cies, onde se alimentam da comunidade de meso-zooplâncton que lhes fornece o equilíbrio nutricional adequado de proteínas e lípidos e o suficiente em ácidos gordos poli-insaturados da família n-3 de que necessitam para a fase de crescimento exponencial que ocorre até ao assentamento. Após o assentamento, as características físicas e ambientais e locais são essenciais para o sucesso do recrutamento à pesca. Com este trabalho, identificaram-se e caracterizaram-se cinco áreas de agregação de juvenis ao longo da costa Portuguesa. Finalmente, foram abordados a idade e o crescimento. A estimação da idade é uma ferramenta essencial para acompanhar cronologicamente o efeito dos fatores ambientais ao longo do ciclo de vida. Neste trabalho foram desenvolvidas metodologias que permitiram melhorar a precisão e a exatidão das estimativas diretas da idade dos polvos a partir da deposição de anéis de crescimento na concha vestigial. Os resultados e conclusões obtidos ao longo deste documento oferecem dados fundamentais para os modelos de abordagem ecossistémica que serão usados na futura avaliação e gestão efetiva do polvo-comum na costa continental portuguesa.

Palavras chave: *Octopus vulgaris*, adultos, paralarvas, juvenis, reprodução, requisitos alimentares, habitats essenciais, idade, crescimento, bastonetes.

RESUMO ALARGADO

O polvo-comum *Octopus vulgaris* (Cuvier 1797) é um dos mais importantes recursos explorados pela frota pesqueira em Portugal e na Galiza. Com desembarques em Portugal na ordem das 7 500 toneladas (média dos desembarques registados entre 1982 e 2012) dos quais 90% são da responsabilidade da frota artesanal e costeira, este é o recurso que permite maior rendimento ao pescador no leilão de primeira venda uma vez que associa frequentemente a espécie com maior peso desembarcado e o valor de primeira venda mais elevado (em média, no mesmo período, 4€/kilo). Estes dois fatores fazem com que o polvo-comum seja considerado atualmente um recurso de rendimento garantido para várias frotas artesanais e de pequena escala. Este rendimento garantido não passa despercebido à frota industrial de arrasto de fundo que é hoje em dia responsável por 10 % dos desembarques totais de Polvo-comum em águas portuguesas. Porém, o interesse do Polvo-comum como recurso pesqueiro é partilhado pelas comunidades costeiras de Espanha, Itália e outros países mediterrânicos. Em particular, a comunidade autónoma da Galiza é responsável por desembarcar em média 5, 700 ton de polvo por ano.

Ao nível ecológico e biológico, *O. vulgaris* é uma das espécies que melhor se adapta a diferentes condições ambientais como mostra a sua extensa distribuição geográfica desde o Mediterrâneo, passando pelo Atlântico Sul, Sul do Índico e Noroeste do Pacífico. Hoje em dia considera-se mesmo que *O. vulgaris* é um complexo de espécies que engloba *O. oculifer*, *O. tetricus* e *O. insularis*. A pressão evolutiva sobre a espécie favoreceu a redução da concha vestigial a dois bastonetes com função de suporte do funil, melhorando a mobilidade e passagem para águas mais profundas, a adoção do estilo de vida bento-pelágico com uma recente fase paralarvar pelágica, a estratégia reprodutiva de semelparidade com a produção de milhares de ovos numa única desova terminal e proteção materna dos ovos durante o desenvolvimento embrionário. *O. vulgaris* é também um predador do nível superior da cadeia trófica que se alimenta de forma oportunistica de peixes, crustáceos e outros moluscos. Por isto, podemos dizer que *O. vulgaris* se especializou em ser um generalista.

Para espécies como esta que combinam uma fase paralarvar pelágica relativamente longa com uma fase bentónica juvenil e adulta sedentária (não são conhecidos padrões migratórios significativos) as condições ambientais que uma e outra experimentam são particularmente importantes para a sobrevivência e o sucesso do recrutamento à pesca.

Neste aspeto, a costa Ibérica Atlântica apresenta condições ideais para avaliar como a espécie se adapta a diferentes ambientes. Aqui podemos encontrar dois regimes oceanográficos diferentes cujas características costeiras influenciam diretamente os ciclos biológicos. Na costa noroeste, a circulação costeira (até aos 200 m) é dominada pelo Sistema de afloramento costeiro Ibérico ocidental (Western Iberian Upwelling System, WIUS). Aqui os ciclos biológicos da maioria das espécies são influenciados pela sazonalidade e intensidade do afloramento costeiro provocado pelo padrão de ventos predominantes de norte da primavera e verão, alternando com ventos de sudoeste predominantes no Inverno que promovem uma contra circulação de água mais quente e menos salina junto à costa em direcção ao pólo. Na costa sul, o afloramento costeiro sazonal não é tão intenso devido ao efeito sombra provocado pela orientação a sul da costa, onde a circulação depende do padrão de ventos este-oeste. Aqui os ciclos biológicos estão sincronizados com a época de maior temperatura da água do mar e com os regimes de *input* de nutrientes provenientes das bacias hidrográficas do Guadiana e do Guadalquivir e a sua área de influência que depende da orografia local.

Esta tese desenvolve-se em cinco capítulos de forma a explicar o condicionamento das condições ambientais sobre o ciclo do *O. vulgaris*, focando-se no ciclo reprodutivo da espécie nas duas áreas da costa continental portuguesa que apresentam condições oceanográficas diferentes; nas características físicas, ambientais e nutricionais que habitats reconhecidos como importantes berçários ou áreas de alimentação apresentam e que fazem deles habitats essenciais (*Essential Fish Habitats*, EFH) e no desenvolvimento de ferramentas de estimação da idade e do crescimento da espécie. O capítulo 1 faz o enquadramento da tese no que diz respeito à exploração do recurso no mundo e em especial em Espanha e Portugal, sobre os eventos evolutivos relevantes para a plasticidade ambiental da espécies, sobre as características e regime oceanográfico da área costeira menos profunda (< 200 m) da costa Ibérica Atlântica e por último sobre a metodologia escolhida para estudar o ciclo de vida da espécie.

No capítulo dois são analisados os ciclos de reprodução de duas populações da costa continental portuguesa e que estão sujeitas aos dois regimes oceanográficos da costa continental portuguesa. Inicialmente foi estudada uma série temporal de dados biológicos de peso, sexo, estado de maturação e índice gonadossomático que permitiu concluir que apesar das épocas em que podemos encontrar mais indivíduos em estado reprodutivo serem coincidentes no Verão, na costa noroeste a época reprodutiva prolonga-se por toda a

Primavera e Verão e coincide com o afloramento costeiro sazonal; enquanto que na costa sul, a época reprodutiva (a época com a maior proporção de indivíduos maduros) ocorre no fim do Verão em Agosto/Setembro e coincide com a época do ano em que temperatura da água do mar atinge o seu valor máximo. Conhecendo esta diferença espacial da época de reprodução e sabendo que à partida podemos encontrar indivíduos maduros ao longo de todo ano, investigou-se se as condições nutricionais encontradas fora e durante a época reprodutiva variam e se esta variação poderia afectar a condição nutricional dos ovos. Analisaram-se classes de lípidos e ácidos gordos na glândula digestiva e nas gónadas de fêmeas imaturas e maduras das duas áreas de estudo. Os resultados mostram que apesar de existirem diferenças significativas na qualidade da alimentação que o polvo encontra numa e noutra costa, estas diferenças não afectam a qualidade dos ovos, indicando que os factores que despoletam a reprodução são fundamentalmente intrínsecos ao organismo e não à quantidade ou qualidade do alimento encontrado.

O capítulo três lida com os fatores e áreas que potenciam a sobrevivência de paralarvas e juvenis. Numa espécie cujos adultos têm poucos predadores (o seu maior predador é o homem) e que são bastante resistentes às condições ambientais adversas, a maior parte da pressão sobre os indivíduos ocorre antes (na fase de paralarva) e durante o assentamento (quando chegam a sub adulto). A Ría de Vigo é protegida na sua embocadura pelas Ilhas Ciés que funcionam como barreira à ondulação de oeste e sudoeste, predominante na costa nordeste Atlântica e formam a fronteira exterior da Ría de Vigo. Aqui, monitoriza-se regularmente a comunidade de zooplancton da Ría em transectos longitudinais às Ilhas Cies localizados a oeste (fora da Ría) e a este destas (dentro da Ría). Os resultados mostram que as maiores abundâncias de zooplankton se correlacionam com as áreas de menor profundidade e mais próximas das ilhas. Estas condições facilitam a análise do teor nutricional das potenciais presas das paralarvas nesta área de berçário e como o perfil nutricional varia entre presas e predadores. As maiores diferenças são quantitativas com variações em alguns rácios nutricionais como o rácio n-3HUFA/n-6HUFA, nas razões DHA/ARA e DHA/EPA mas em especial as paralarvas de *O. vulgaris* apresentam um teor significativamente maior em ARA do que as suas presas. Os resultados que obtivemos neste capítulo permitem confirmar a importância da manutenção da razão Proteina: Lípidos entre presas e predadores mas também evidenciam a importância de disponibilidade de uma fonte de n-3 HUFA para suprir os requisitos em ARA essencial para o crescimento. O capítulo três descreve ainda as áreas de alimentação e crescimento

dos juvenis. Aqui, definiram-se ao longo da costa portuguesa 8 áreas de maior abundância de pré-recrutas. Estas áreas localizam-se em zonas de pouca profundidade (aproximadamente 80 m) sob a influência dos grandes estuários onde a abundância de potenciais presas é maior. Este estudo permitiu também concluir que as condições de salinidade local são muito mais determinantes para a agregação dos juvenis do que a temperatura local.

O capítulo quatro lida com o desenvolvimento de metodologias de estimação direta de idades que permitam a estimação mais precisa e exacta da idade individualmente. A maioria dos modelos de avaliação de mananciais de pesca requer a inclusão de informação relativa à estrutura etária das populações exploradas. Porém, a impossibilidade de observar incrementos de crescimento diário nos estatólitos, a erosão a que as mandíbulas estão sujeitas ao longo da vida, e a fragilidade da estrutura do bastonete (concha vestigial) que impossibilitava o uso das técnicas abrasivas de corte e polimento e a reprodutibilidade das observações têm sido obstáculos difíceis de ultrapassar. Só recentemente, com a inclusão dos cortes de bastonetes em resina acrílica, que preserva a estrutura interna dos bastonetes, foi possível melhorar a técnica da estimação de idades usando esta estrutura. Com este trabalho analisámos a estrutura dos bastonetes em paralarvas e juvenis, de forma a medir e validar marcas de crescimento determinantes como a marca de desova. Para validar a marca de desova, observaram-se e mediram-se bastonetes de paralarvas com 1 e 3 dias de idade e paralarvas capturadas no meio selvagem com menos de 10 dias. Foi possível observar que na desova, e logo após a mesma, é possível distinguir uma zona nuclear de 5 μm de diâmetro e cuja dimensão é conservativa em relação à dimensão da paralarva e da concha vestigial. A definição desta região foi essencial para o passo seguinte de estimação de idades em sub adultos pós assentamento. O uso de técnicas de corte dos bastonetes idêntico aos métodos de corte de otólitos para a observação da microestrutura foi essencial para identificar pela primeira vez a área nuclear e pela primeira vez fazer uma estimativa directa da idade de juvenis e estimar o tempo que estes demoram a atingir o tamanho mínimo de captura, aproximadamente oito meses. Assim como para outras espécies do género *Octopus*, o exercício de observação da microestrutura de crescimento e estimação de taxas de crescimento permitiu mais uma vez confirmar que a variação individual das taxas de crescimento é elevada, o que torna difícil definir um modelo de crescimento que represente o crescimento da espécie ou de determinada população. É crítico neste caso, como se demostrou ao longo de toda a tese, que o ciclo de vida e a ecologia do *O. vulgaris*

seja muito dependente das condições ambientais físicas e biológicas locais, sendo importante que qualquer modelo de exploração que se venha a aplicar a esta espécie, integre e parâmetros ambientais, para que possamos prever a abundância, a disponibilidade do recurso e mesmo a quantidade de biomassa que se pode retirar em cada ano das populações residentes para que o recurso possa ser explorado de forma sustentável.

O capítulo cinco apresenta e engloba os resultados e conclusões obtidas no contexto do conhecimento mais recente que se tem da biologia e ecologia da espécie *O. vulgaris*.

LIST OF PAPERS

This thesis comprises the papers listed below. The papers are organized by theme in the chapter two, dedicated to the reproductive cycle, and in the chapter three dedicated to the habitat requirements of paralarvae and sub adults. The author of this thesis is the first author of two of the papers published and co-author of the other one. As first author of papers included in chapter two, the author of this thesis was responsible for the conception and design of the work, sample collection and processing, laboratory analytical procedures, data analyses and manuscript writing of those. As co-author of the paper included in chapter 3, the author of this thesis had participated actively in the sample collection, data analysis and results discussion. The co-authors of the three papers collaborated in some or several of these procedures.

Chapter 2:

- Lourenço, S. Moreno, A., Narciso, L., González, A.F., Pereira, J. 2012. Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. Fisheries Research 127-128:116-124.
- Lourenço, S. Narciso, L., Gonzalez, A. F., Pereira, J., Auborg, S., Xavier, J.C., 2014. Does the trophic habitat influence the biochemical quality of the gonad of *Octopus vulgaris*? Stable isotopes and lipid class contents as bio-indicators of different life cycle strategies. Hydrobiologia 725: 33-46.

Chapter 3:

- Moreno, A., Lourenço, S., Pereira, J., Gaspar, M., Cabral, H.N., Pierce, G.J., Santos A.M.P. 2014. Essential habitats for pre-recruit *Octopus vulgaris* along the Portuguese coast. Fisheries Research 152:74-85.

CHAPTER 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

1.1. OCTOPUS FISHERIES

Cephalopods world catches have been increasing constantly during the last 40 years (Norman *et al.*, 2014). This growing interest on cephalopods is related with the decline of the finfish catches and the perception of cephalopods as alternative protein sources for human consumption (Jereb *et al*. 2014). In this group, the octopuses (most belonging to the family *Octopodidae*) play an important role as a resource exploited by the small-scale fishing communities spread along the coastal areas, but and also by some industrial fleets (Fonseca *et al.*, 2008). Since the 1950's, the official statistics have been showing a global increase in octopus annual catches (FAO, 2011-2014). The Food and Agriculture Organization (FAO) estimated that between 2002 and 2012 an average 334 033 ton/year of octopuses were captured worldwide with an increasing tendency (FAO, 2011-2014). Despite that, since 2009 octopus landings have been decreasing, with 2012 registering annual global landings of 316 582 ton (Figure 1.1). A major problem is that only a few countries discriminate octopus catches by species, and the relative importance of the common octopus *Octopus vulgaris* is therefore unknown. Nevertheless due to its commercial value, it should be expected that the octopus landings from the eastern Atlantic and Mediterranean waters are dominated by *O. vulgaris* (Pierce *et al.*, 2010).

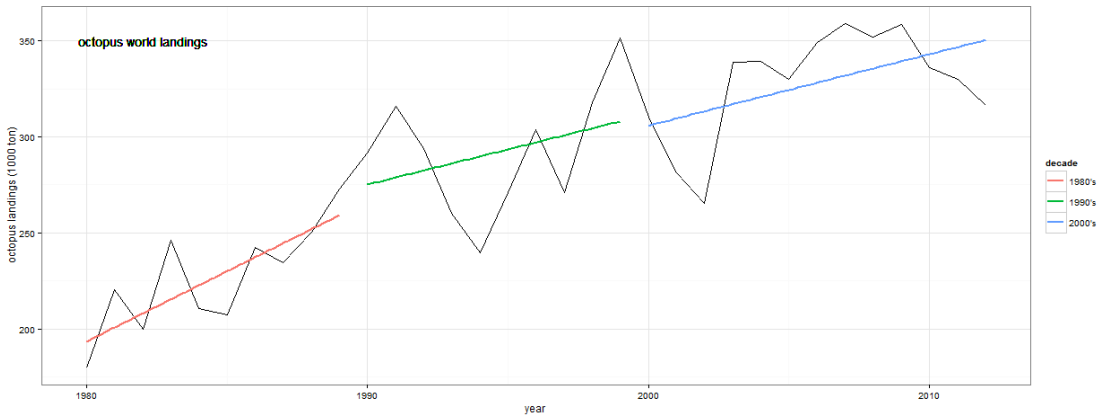


Figure 1.1– Evolution of the worldwide annual landings of the octopus species group between 1980 and 2012. The lines indicate the landings tendency by decade. The 2000's decade comprises the years between 2000 and 2012 (data source: FAO, 2014).

In Europe, *O. vulgaris* is the most important exploited octopus species (ICES, 2012a; Tsangridis *et al.*, 2002), the Italian, Spanish and Portuguese fleets being the most important contributors to the total catch (Pierce *et al.*, 2010). In the Iberian Peninsula, *O. vulgaris* reached an average 18 639 ton/year in the last 30 years, 6 % of the worldwide octopus landings in the same period. Here, the general tendency of the last 32 years (1982-2012) show that in Portugal *O. vulgaris* landings have been increasing, and Spanish Mediterranean and Northeast Atlantic catches have been decreasing both at approximately the same rate (Figure 1.2).

It is worth of note that the *O. vulgaris* catches can vary by more than 50% between consecutive years. Between 2008 and 2009 for instances, octopus landings of the Portuguese fleet decreased by 48% in landed weight, increasing by 36 % in 2010 (Figure 1.2; INE, 2014). The *O. vulgaris* life cycle characteristics, such as the short lifespan, the semelparity, and the lack of stock-recruitment relationship makes to be virtual impossible to predict fisheries outcomes from a year to another (Pita *et al.*, *in prep*).

In Portugal, *O. vulgaris* is captured mainly with pots and traps (around 90%) (ICES, 2012a) by the "local fleet" (small-size boats, the majority of which do not exceed 9 m in total length) and the "coastal fleet" (comprised of vessels generally ranging from 9-15 m in total length). The multi-specific trawling fleet is responsible for the other 10% of catches with an increasing tendency (Fonseca *et al.*, 2008; Pilar-Fonseca *et al.*, 2014). The traditional small-scale fishery targeting *O. vulgaris* in the northeast Spanish waters is identical to the Portuguese fleet (Freire & García-Allut, 2000). As in Portugal, the Northeast Spanish fleet is markedly local, multi-gear and multi-specific with an important economic and social role in the coastal fisher communities (Freire & García-Allut, 2000; Otero *et al.*, 2005). Nevertheless, the two neighboring areas seem to present distinct exploitation patterns, with the northeast Spanish catches being stable or slightly decreasing in the last 32 years, probably an influence of the fishing moratorium due to the Prestige oil spill, and the Portuguese catches still increasing (Figure 1.2). Another important aspect, is the fact that the northeast Spanish *O. vulgaris* catches present a marked seasonality (Otero *et al.*, 2005), which is only comparable in Portugal to the octopus landings pattern found in cephalopod targeting trawls (Fonseca *et al.*, 2008).

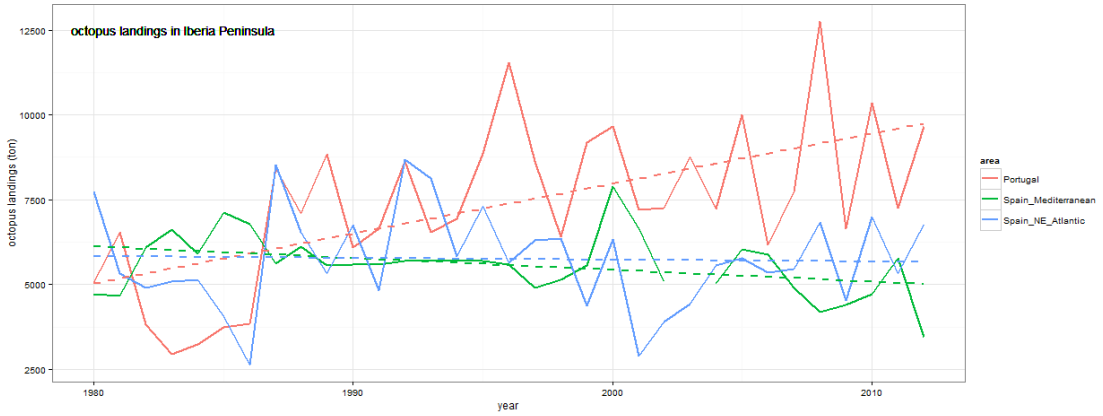


Figure 1.2 – Evolution of the octopus annual catches in the Iberian Peninsula. Annual landings data are discriminated by geographical area (Mediterranean and Northeast Atlantic) and by landing country (Sources: INE 2013; FAO, 2014).

In Portugal and despite the unpredictable variability in the stock availability, *O. vulgaris* is the most important target species in the entire Portuguese fisheries in terms of economic value, when all official and non-declared landings are considered (Pilar-Fonseca *et al.*, 2014) with evidences that the species' catches play an important role as guaranty revenue species to the non-targeting octopus fisheries. In fact, *O. vulgaris* has long been an important target species for artisanal fishers and, nowadays, it is an increasingly important fishery resource in terms of quantities landed and particularly in terms of commercial value. *O. vulgaris* is frequently the species with higher value in first sale auction (mean price of €4/kg) and, for instance in 2012, its official catches represented 14% (€38.7 million) of the official first sale revenue of all Portuguese fisheries, second only to sardines (INE, 2014).

The increasing dependence of the Portuguese small-scale and trawling fisheries of the *O. vulgaris* stock (Fonseca *et al.*, 2008; Pilar-Fonseca *et al.*, 2014) associated to the unpredictable variation in availability of the resource have, from time to time, lead to important pressure actions of fishers on governmental bodies, demanding protective measures. These fisher actions have, almost without exemption, lead to changes in the management rules that regulate fishing activity. This was the case in 1996, when fishers' worries about the risk of overexploitation of octopus brought them together to request protective measures for octopus, which resulted in the implementation of new legislation (Portaria nº 27/2001), and again in 2010, when a "bad" fishing year drove fishers' action to change the legislation (Portaria nº 230/2012) (Pita *et al.*, *in prep*).

Targeting small-scale fishery conducted with "shelter traps" (pots) (Pereira, 1999) targets the mature individuals of higher outcome (individual weight c.a. 1100 g, Pereira *pers. comm*), with virtually no by-catches or discards and minimum environmental damage. These characteristics elect the Portuguese octopus small-scale fishery as a good candidate for environmentally sustainable fisheries eco-labelling schemes (Matias, 2013). Although problems associated with the unpredictability of stock availability to fisheries, illegal and unreported catches, high auction first sale value and the lack of the formal producers organizations can be identified as constrains to the adoption of the eco-labelling.

1.2. EVOLUTION AND GENETICS

All modern cephalopods including the squids, cuttlefishes and octopuses, belong to the subclass Coleoidea (Boyle & Rodhouse, 2006; Kröger *et al.*, 2011). Fossil records and molecular studies show that the Coleoidea evolved along with their sister group, the Nautiloidea, in the late Paleozoic, 276 Ma ago (Kröger *et al.*, 2011) when in competition with modern fish, they apparently occupied the deep waters. The adaptation to this high pressure environment leaded to the progressive internalization of shell, trading the buoyancy for speed and the possibility of occupying the bentho-pelagic habitat (Kröger *et al.*, 2011; Young *et al.*, 1998).

With the loss of shell and its shield function, the modern cephalopods gain the ability to swim. Consequently, the exploration of larger areas demands for more energy and to fulfill the higher metabolic rates, the respiratory and circulation system evolved for capillary circulation with two branchial hearts located at the base of each gill to enhance circulation within a single pair of gills and a systemic heart. The more efficient ventilation of the gills allowed producing a more elaborate musculature in the mantle cavity leading to the evolution of the primitive expulsion chamber for jet propulsion (Boyle & Rodhouse, 2006). Swimming faster and becoming more maneuverable, the cephalopods also developed more sophisticated sensory systems, especially eyes and large brains (Nixon & Young, 2003).

Along with the internalization and reduction of shell, the adaptation to carnivory is also a unique event to all coleoids (Boyle & Rodhouse, 2006). The development of the beak during embryologic development in both the Teuthidae and Octopodidae supports this early adaptation in the feeding behavior (Franco-Santos & Vidal, 2014; Franco-Santos *et*

al., 2014). In fact, at hatch the beaks are fully developed only growing and hardening as individuals grow (Franco-Santos & Vidal, 2014; Franco-Santos *et al.*, 2014).

With the adaption to the pelagic realm, the coleoids evolved in two distinct groups, the ten-armed, Decabrachia and the eight-armed Vampyropoda. The latter group comprises the Octopodidae as well as the vampire squids (Vampyroteuthidae), that in fact have ten arms, but two arms are reduced sensory filaments (Kröger *et al.*, 2011).

The first modern octopuses appeared in fossil deposits of the late Cretaceous. The evolution of Vampyromorpha and Octopoda as sister groups shows a trend towards the reduction of the gladius. The modern fin supports of the Octopoda Cirrata, and the stylet-like shell vestiges of the Octopoda Incirrata, are considered to represent the remains of a teudopseid gladius (one of the three gladius morphotypes, Fuchs & Weis, 2010) (see Bizikov, 2004, for a review on stylet morphology, functional role and evolution of the Vampyropoda). The reduction of gladius was most probably accompanied by a shift in the locomotion mode. Some octopus of the modern Octopoda Incirrata had completely lost the ability to secrete organic shells resulting in a loss of attachment sites for fins. This radical shell reduction results in the increasing of extraordinary body plasticity (Kröger *et al.*, 2011).

During its evolutionary pathway, the modern coleoids adopted a fast growing and relatively short lifespan and reproduced once pattern called semelparity (Rocha *et al.*, 2001; Boyle & Rodhouse, 2006). The modern benthic octopuses (Octopoda, Incirrata) show two reproductive strategies. The holobenthic strategy, where the benthic adults produce few and large eggs resulting in well-developed benthic hatchlings (Villanueva & Norman, 2008) and a merobenthic strategy, where benthic adults produce batches of numerous small eggs (c.a. 350 000 eggs for *Octopus vulgaris* Mangold, 1983) hatching into free-swimming planktonic paralarvae (Villanueva & Norman, 2008) showing different strategies to the adaptation to the benthopelagic environment (Ibáñez *et al.*, 2014). Molecular studies show that in their adaption to the benthopelagic environment, the first octopuses would all have a planktonic paralarval phase and the holobenthic strategy evolved afterwards as a specialization (Ibáñez *et al.*, 2014).

The existence of a paralarval stage during the modern cephalopods life cycle is also a distinctive characteristic of this taxon. The term *paralarva* comes of the fact that those cannot be considered as a true larval stage as no truly metamorphic changes occur during

the transition between paralarva and juvenile (or sub-adult as defined in Robin *et al.*, 2014) and in some cases, as the in the sepiids, the hatchlings already resemble their parents. Nevertheless, the transition phase from hatchling or paralarvae to sub adult implies considerable physiologic, morphometric, allometric and habitat changes (with different degrees depending of the cephalopods families) supports the definition of a paralarva life stage between hatch and the adoption of the adult life characteristics (Young & Harman, 1988; Robin *et al.*, 2014).

The study conducted by Ibánez *et al.* (2004) supports the theory that successful colonization by the octopuses depend of the dispersal of the paralarva. The holobenthic octopuses are common in deep and cold waters while merobenthic octopuses are common in temperate and warm waters. And while in temperate and warm waters, shifts between strategies seem occurred through time, the deep and cold waters species all evolved into the holobenthic strategy the octopus. This differentiation of strategies seems to be related with environmental stability. In low temperature and low environment stochasticity as the deep-sea and polar environments, to survive the octopuses produce few large eggs and well-developed benthic hatchlings that improved the chance of survival and reproduction in the same benthic area. In high temperature and high environmental stochasticity, as the tropical and temperate habitats, to stay alive the octopuses produce numerous small eggs with free-swimming planktonic paralarvae that improved the chance of survival and reproduction using the transient opportunities of the environment between benthic and pelagic (Ibáñez *et al.*, 2014).

The *Octopus vulgaris* benthopelagic life cycle strategy most certainly explains its wide distribution and the existence of several cryptic species associated to the *O. vulgaris* species complex. This species group is composed by *O. vulgaris* sensu strictu (or sensu Cuvier, 1797) and several crypt species. In their study, Guzik *et al.* (2005) suggested to include *O. oculifer* from Galapagos, *O. cf. tetricus* from Western Australia, and *O. tetricus* from New South Wales in the species complex. Accordingly to Leite *et al.* (2008), this group also may contain the *O. insularis* in tropical western Atlantic. The wide range and number of cryptic species highlights' the evidence that the genus Octopus is polyphyletic (Guerra *et al.*, 2010). These recent findings on the species diversity and dispersion within the *O. vulgaris* species complex would suppose a reduction of the geographic range of *O. vulgaris s. str.* Nevertheless, the studies conducted by Warnke *et al.*(2004) and Guerra *et al.* (2010) showed evidences that *O. vulgaris s. str.* is monophyletic but also show evidences

of its wide distribution. The samples collected show that *O. vulgaris s. str.* can be found in coastal waters as distant as the North-eastern Atlantic waters can be from the Southern Indian Ocean or from North-western Pacific. Thus, the known distribution area of *O. vulgaris s. str.* is the Mediterranean Sea, the eastern Atlantic (from southern England to south-western Africa), the Azores, the Canary Islands, Cape Verde, St Helena, the Tristan da Cunha Islands, the southeast coast of South Africa in the Indian Ocean, and the north-western Pacific, namely the waters of Taiwan and Japan (Warnke *et al.*, 2004).

1.3. BIOGEOGRAPHIC CONTEXT OF THE STUDY AREA

To meroplanktonic organisms like *O. vulgaris*, that in their life cycle combine a planktonic paralarval stage relatively long with a benthic juvenile and adult phase with non-significant migratory patterns (Robin *et al.*, 2014), the oceanographic patterns and coastal topography play an important role in the survival and recruitment success of each generation (González *et al.,* 2005; Otero *et al.,* 2008; 2009; Roura et al., 2013). Moreover, in cases as this one where the recruitment to fisheries is independent of previous generation stock size but dependent of the local environmental conditions, it is worthwhile to look in detail to the regional oceanographic features that influenced the life cycle of *O. vulgaris* in the study area.

The Iberian Atlantic basin includes the western coast of Portugal and Spain. Considering the Estremadura Promontory (EP, 39ºN), the São Vicente Cape (CSV, 37º1'N; 8°59'W) and Sta Maria Cape (CSM, 36°57'N; 7°53'W) as references, the continental shelf is relatively wide (50 – 60 km) with several canyons (e.g the Nazaré and the Aveiro canyon) and a steep slope northern to the EP. Here the freshwater contribution is significant due to the river runoff from the Tagus, Douro and Minho but also from other smaller rivers, lagoons and inlets in its northern limits (the Rías) (Peliz *et al.*, 2005). South to the EP, the continental shelf is narrower (~25 km) with a moderate slope with no significant freshwater input (Peliz *et al.*, 2005). The CSV marks the transition of the coastline orientation from west to south, here the Portuguese and Spanish south coast's limit the northern boundary of the Gulf of Cadiz (GoC), the large bight that encloses the transition between the Atlantic and the Mediterranean. Between the CSV and the CSM, the shelf is narrow (~25 km wide), and east to the CSM it starts to widening till 40 km wide in the Spanish coast of the Gulf of Cádiz. East to the CSM, the continental shelf receives

important freshwater inputs from the Guadiana, Tinto, Odiel and Guadalquivir rivers (Pires *et al.*, 2013).

The entire Iberian Atlantic coast is under the influence of the Canary Current upwelling system, one of the four major eastern boundary upwelling systems (Arístegui et al., 2009) and comprises two sub-regional systems: The Western Iberia Upwelling System (WIUS) and the Gulf of Cadiz System (GCS) (Peliz *et al.*, 2005; Arístegui *et al.,* 2009) where the mesoscale oceanographic processes are major factors controlling the ecosystem functioning in those regions (Relvas et al., 2007).

The WIUS is a highly dynamic and productive system with marked seasonality that supports a complex food web (Bode *et al.*, 2004). The predominantly equatorward winds observed in summer off West Iberia, drive an offshore Ekman transport and force the upwelling of colder, nutrient-rich, sub-surface waters along the coast (Relvas *et al.*, 2007). Here, during the late spring and summer, a narrow band of cold water of relatively uniform width is observed along the coast and small scale (20-30 km) perturbations are usually seen along the thermal front. This cold front is responsible for the formation of major filament structures than can extend more than 200 km offshore (Relvas *et al.*, 2007). Those filaments can spread through the CSV to the east and influence the continental shelf circulation in the area between the CSV cape and the CSM. The filaments are responsible for exporting the new production offshore, although evidences show that the new production can be recirculated back in time scales of one month with interesting implications for the retention of fish larvae near shore (Relvas *et al.*, 2007). During winter, the Iberian Poleward Current (IPC) associated with the wind stress of south-westerly orientation dominates the WIUS system. The IPC increases its flow in September-October and persist in intensity till April. Nevertheless its influence do not reach the inner shelf, here the most important mechanism of forcing the shelf circulation is the wind stress. The prevailing winds in the west coast of Iberian Peninsula during winter are mainly south-westerlies, and the atmospheric circulation is dominated by the eastward displacement of cyclonic perturbations and their associated frontal systems (Relvas *et al.*, 2007). Several rivers drain in the west coast of the Iberian Basin, the contributions of the Tagus, Mondego, Douro and Minho, and the Galician rías feed the Western Iberian Buoyant Plume (WIBP). The WIBP is characterized by low salinities and is identified in winter sea surface temperature satellite imagery from its low temperature compared to the shelf waters and during summer the plume waters are warmer than surrounding waters. It

reaches its higher intensity during winter conditions when prevailing south-westerly winds generate an onshore Ekman component of transport, convergent to the coast and hence a saline front and the plume develops into a narrow (5–10 km) coastal current with strong poleward velocities (Relvas *et al.*, 2007). Sardine and other fish larvae are distributed vertically in the upper 20 m, associated with the WIBP water (Santos *et al.*, 2006). The surface stratification in the WIBP favours the optimal conditions for phytoplankton development (Ribeiro *et al.*, 2005) and the development of high zooplankton biomass.

Figure 1.3 shows the seasonality of both the Sea Surface Temperatures (SST) and the winds patterns in the northwest Portuguese coast based in data collected between January 2000 and December 2009.

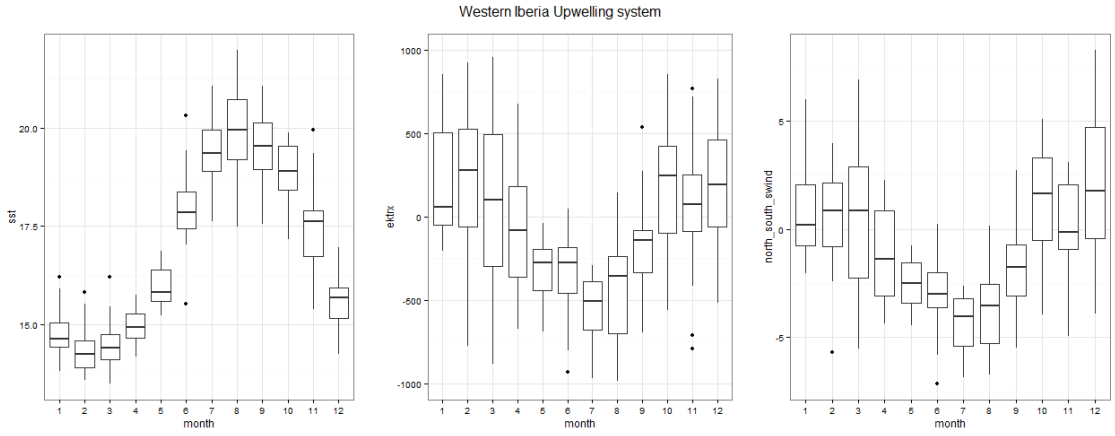


Figure 1.3 - Monthly mean Sea Surface Temperature (sst), East-West component of Ekman transport (ekmanx) and north-south wind stress component (north_south_swind) obtained from the SST and wind time series registered along the Portuguese coast under the influence of the WIUS. The mean monthly SST and ekmanx were obtained from the data registered in two points along the Portuguese coast between January 2000 and December 2009 (Source: NOAA ERDDAP database).

The south Portuguese coast is under the influenced by the Gulf of Cádiz System (GCS). The GCS can be defined as a sub- system embedded in the large-scale Canary Current upwelling system (Arístegui *et al.*, 2009) and its dynamic is highly influenced by this larger system. There are, however, two distinctive features that separates the GCS of the neighbors sub-systems: the coast orientation shift from north-south to west-east and the presence of the Strait of Gibraltar as the door to the Mediterranean. Here, the water masses exchange between the Atlantic and Mediterranean influences the large-scale circulation in the region. The meso-scale and inner-shelf circulation patterns are most influenced by the upwelling off CSV and the cold-water upwelling filaments from the eastern boundary, the runoff from the Guadiana and Guadalquivir rivers mouth, the coastal counter-current, and

the east and west wind dynamic. The interaction between those oceanic processes and the coast topography results in distinct circulation patterns east and west of the CSM (García-Lafuente & Ruiz, 2007) that influences the biological processes in the area. As in the western coast, the circulation is influenced by the seasonality of winds pattern and upwelling/downwelling events (Sánchez *et al.*, 2006) (Figure 1.4). During the spring-summer upwelling, the dominant westerlies winds resulted of a prevailing positive z-component of the wind curl in the zone of the CSV create conditions to bring cold and nutrient rich water to east of CSM and producing a cell over the western shelf associated with the upwelling off CSV. Nevertheless, this cell is not strong enough to influence the circulation and bring biological material eastward. During downwelling relaxation events, the easterlies are stronger and the coastal counter-current flows beyond CSM and invades the western shelf, providing transport of biological material from the east to the west and the biological connection between both shelves. During winter, the east-west counter-current and the east circulation cell are responsible for the introduction in the system of nutrient rich water from the Guadalquivir basin (García-Lafuente & Ruiz, 2006).

Despite the strong seasonality present in SST and wind pattern profiles (Ekman's transport and west-east wind stress component) (Figure 1.4), the upwelling/downwelling strength is not as intense as in the northwest coast. The water column stratification is also responsible by the higher temperatures attained in the end of summer in the coast oriented to south.

Both in the WIUS and the GCS, the retention and advection processes play the most important role in the control of the biological processes (Catalán *et al.*, 2006). And this advection and retention processes are related with the seasonality of the wind patterns and upwelling/downwelling processes. In the WIUS, the seasonality of the upwelling/downwelling processes sets the pace of the biological cycles. Here, the duality between the offshore transport of the upwelling filaments and the retention processes associated to the IPC and the westerly winter winds mark the seasonality of the biological cycles (Arístegui *et al.*, 2009) and sets the west coast of the Iberian Atlantic basin as the main spawning and recruitment areas for sardine (*Sardine pilchardus*, Bernal *et al.*, 2007; Silva *et al.*, 2009), horse mackerel (*Trachurus trachurus*, Murta *et al.*, 2008) and for the marine invertebrates (Marta-Almeida *et al.*, 2006; dos Santos *et al.*, 2008).

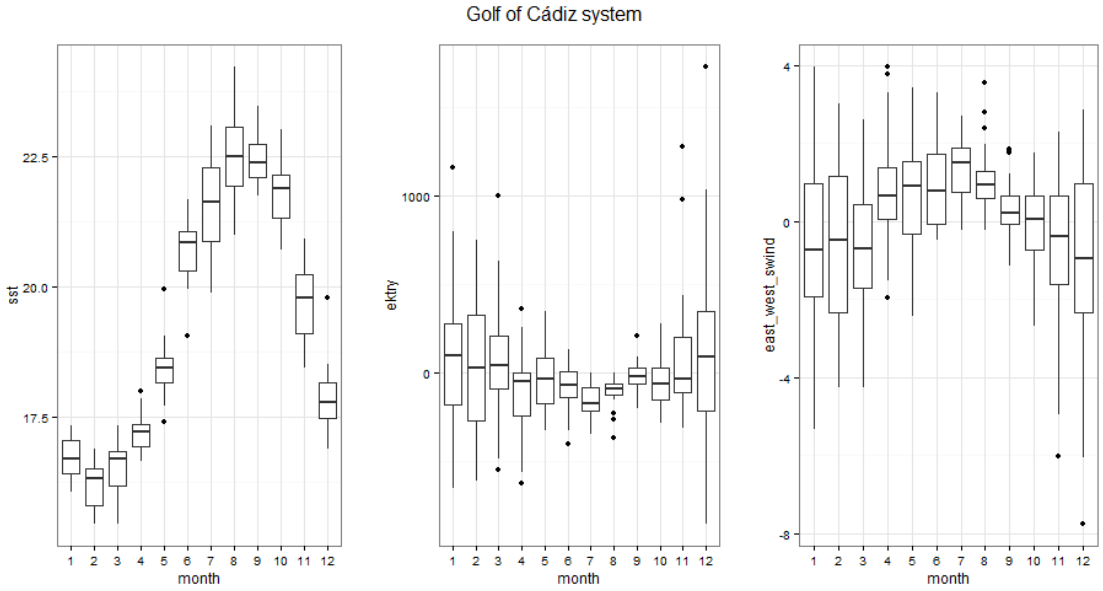


Figure 1.4 - Monthly mean Sea Surface Temperature (SST), East-West component of Ekman transport (ekmanx) and East-west wind stress component (east_west_swind) obtained from the SST and wind time series registered along the Portuguese coast under the influence of the GCS. The mean monthly SST and ekmanx were obtained from the data registered in two points along the Portuguese coast between January 2000 and December 2009 (Source: NOAA ERDDAP database).

In the GCS, the coastal orientation has a shadow effect in the upwelling conditions created by the prevalent northerly winds, and is the east-west wind pattern that controls the strength and influence of the two circulation cells east and west of the CSM. Here the biological cycles adapt to this east-west alternation. For instances, the anchovy *Engraulis encrasicolus* population in the northeastern area of the Gulf of Cádiz synchronizes its spawning cycle with summer to benefit of the warmer and rich waters and trophic regimes more suitable for the survival of the early stages in winter along the eastern basin (Ruiz *et al.*, 2006). As in the northwest coast, here the advective and retention processes are essential to explain not only the larval distribution and abundance as well as the survival of early life stages (Catalán *et al.*, 2006; Pires *et al.*, 2013). For instances, decapod crustacean larvae distribution and survival is closely related with the circulation processes and alternation along the south Portuguese coast under the GCS (Pires *et al.*, 2013).

Nevertheless, while the oceanographic processes dominate the biological processes in the pelagic habitat, the benthic and demersal habitat suffer the influence of the continental shelf width or the bottom type. In their work Sousa et al. (2005) defined five species assemblages: shallow northern, shallow southern, intermediate northern, intermediate southern and deep. Those assemblages are associated primarily with depth

gradient and latitude, but coastal topography, bathymetry, coastal morphology, river runoff and currents also play an important role. In those assemblages, the seasonality is particularly important for the shallow assemblages (Sousa *et al.*, 2005). The species richness in the demersal and benthic habitats is affected by the same factors (Sousa *et al.*, 2006) with the south coast presented higher species richness that the northwest coast, with species richness decreasing with the latitude increase. Being the latitude the main factor driving the decreasing gradient in species richness, this is a proxy of other factors with higher biological meaning as the shelf morphology, substrate characteristics, hydrology and oceanography, primary productivity and food availability. The narrower continental shelf associated to a higher productive area and shorter food web are the main factors that influence the low species richness of the northwest coast in relation to the south coast (Cunha, 2001; Sousa *et al.*, 2006).

1.4. ANALITICAL METHODS AND THEIR IMPORTANCE FOR ECOLOGICAL STUDIES

The *O. vulgaris* as other cephalopods, aside from being an important fisheries resource worldwide, has also an important ecological role in the marine benthic ecosystem as a predator of several species of fish, crustacean, molluscs and other cephalopods (Quetglas *et al.*, 1998; Velasco *et al.*, 2001; Smith, 2003). Their life cycle characteristics of fast growing, short life cycle and semelparity associated with the absence of known migratory patterns after settlement and their opportunistic feeding behaviour make of this a good model to assess environmental stability or shifts.

While the classical biological studies of growth and spawning associated to relatively long time series allows to follow changes in the life cycle that are closely related with local environmental conditions and/or environmental shifts, the studies based in the use of biochemical tracers helps to define species habitat and can be used add trophic information to the ecosystem based models (Jackson *et al.*, 2007). Defined as a predator of upper trophic level, *O. vulgaris* has the potential to be used to study habitats and food webs in relation to prey populations, oceanographic domains and environmental variability.

The biochemical tracers more frequently used to characterize cephalopods habitat are the Fatty Acids (FA), mainly Polyunsaturated Fatty Acids (PUFA) but also the Stable Isotopes signatures (SIS), mainly the $^{13}C/^{12}C$ ($\delta^{13}C$) and the $^{15}N/^{14}N$ ($\delta^{15}N$).

The FA have been used as trophic markers since 1960's (Dalsgaard *et al.*, 2003). The FA trophic markers (FATM), particularly PUFA are incorporated into consumers in a conservative manner, thereby providing information about predator-prey relationship not only in short-term but also over longer periods of time as FA are going to be sequestered in the predator reserves of lipid with time (Dalsgaard *et al.*, 2003). Considering that most FA and particularly the PUFA are primarily produced by microalgae and zooplankton (Bergé & Barnathan, 2013; Dalsgaard *et al.*, 2003; Piché *et al.*, 2010) and the variations in the biomass, distribution and species composition of microalgae are driven by hydrodynamic processes, these environmental processes are ultimately influencing the basic pattern of FA in the marine environment (Dalsgaard *et al.*, 2003). The second most important producers of FA are the herbivorous calanoid copepods that are in a central position within the food web linking the autotrophic organisms (phytoplankton and algae) and the second order producers (e.g. other zooplankton species as euphausiids and fish). These calanoid copepods feed on phytoplankton and also other copepods depending on food availability but also are able to biosynthesize monounsaturated FA (MUFA). The balance between different PUFA and between those and the MUFA in a specific moment or area is conservative through the upper levels of the food web and can be used as FA trophic marker (FATM) to assess the feeding conditions or productivity of a specific area or season (El-Sabaawi *et al.*, 2008; Gonçalves *et al.*, 2012), or being used to examine differences or changes in foraging patterns or diets, both within and between populations of predator species, without specifying what species are eaten (Budge *et al.*, 2006; Iverson *et al.*, 2007).

The FA profile in muscle and digestive gland of cephalopod species has been used to assess these species ecological role. In her work, Stowasser (2004) combined stomach analysis with analytical methods to determine the FA profile of muscle and digestive gland of several neritic and pelagic species of squids to validate the use of FA profile but also stable isotopes signatures as indicators of squids feeding history and habitat shifts. Using feeding experiments and samples of different locations, Stowasser proved that, although with some limitations, is possible to follow the feeding history of species with those analytical methods. In their work, Phillips *et al.* (2003a, 2003b, 2002, 2002, 2001a, 2001b) combined analytical methods with stomach content methods studying the feeding ecology in *Moroteuthis ingens* but also in other polar squids. Their studies showed that the cephalopods digestive gland is a rich source of fatty acid dietary tracers (Phillips *et al.*,

2002) and can provide the history of dietary intake and indirectly give an image of the history of environmental conditions through the individual life history. In the same line, Rosa *et al.* (2004a) investigated the lipid content and FA profile in the digestive gland and gonad of immature and mature *O. vulgaris* and *O. deffilipi*. In their study, they did not found a close relationship between immature and mature gonads in both species, showing evidences that the gonad FA profile is more closely related with the food availability at that time that with prior feeding history.

The stable isotopes signatures, the $^{13}C/^{12}C$ ($\delta^{13}C$) and the $^{15}N/^{14}N$ ($\delta^{15}N$) ratios are used here as complementary method to the lipid content and fatty acids profile giving information about trophic position and ontogenic changes of feeding habitat of *O. vulgaris*. The measurement of $\delta^{13}C$ and $\delta^{15}N$ of a consumer tissues in marine food webs provides important indications about the ecology of individuals and species and the trophic interactions within complex species communities (Hobson & Cherel, 2006a) as they reflect the consumers diet (Cherel and Hobson, 2005). Consumers are enriched in ^{15}N relatively to their food in 2.5 ‰ to 3 ‰ (Vanderklift & Ponsard, 2003) and consequently the $\delta^{15}N$ measurements indicate the consumer trophic position (Hobson & Welch, 1992). On the other hand, the $\delta^{13}C$ vary little along the food chain but can be used to determine primary sources in a trophic network. The $\delta^{13}C$ values indicate the lower versus higher latitudes plankton, and inshore versus offshore, or pelagic versus benthic contributions to food intake (Cherel *et al.*, 2000).

During several decades, the studies on food and feeding ecology of cephalopods were restricted to studies conducted in stomach contents (Sanchez & Obarti, 1993; Quetglas *et al.*, 1998; Smith, 2003; Rosa *et al.*, 2004b) or based on den ecology (Katsanevakis & Verripoulos, 2004). However, stomach content analysis is time consuming and prey determination is often difficult because cephalopods tear prey into small pieces by their chitinous beaks and items are often too digested. Another major limitation is that stomach contents represent the last feeding events with no indication of long-term dietary habits (Jackson *et al.*, 2007).

Recently, several studies have been applying the stable isotopes signatures in cephalopods mainly squids (Takai *et al.*, 2000; Cherel & Hobson, 2005; Hobson & Cherel, 2006; Ruiz-Cooley *et al.*, 2006; Stowasser *et al.*, 2006; Cherel *et al.*, 2009a) aiming to understand the role of cephalopods both as prey as well as predator in the marine food webs. Some of those studies investigate the contribution of squids to the diet of top

predators by investigating the stable isotope signatures in beaks found in the stomachs of sperm whales (Ruiz-Cooley *et al.*, 2006; Cherel *et al.*, 2009b) but many investigate the role of cephalopods as predators following the stable isotope signatures in both beaks and muscle (Takai *et al.*, 2000; Cherel & Hobson, 2005; Stowasser *et al.*, 2006). The study conducted by Hobson & Cherel, (2006) is particularly relevant. Here, the relationship between the isotopic signatures of prey and the beak and muscle of *Sepia officinalis* was assessed in a controlled environment experiment providing for the first time an estimate of isotopic discrimination between diet and hard and soft tissues of cephalopods (Hobson & Cherel, 2006). This study also proved that little or none discrimination between diet and tissue and that the differences found between beaks and muscle or between different beak regions are closely related with diet in a specific moment in the species life cycle and for that reason the stable isotope signature in the beaks can be used as life cycle recorder of the feeding and habitat, while the isotopic signature in muscle or flesh can be used as an indicator of present feeding behavior and habitat.

1.5. OBJECTIVES

O. vulgaris is one of the most studied marine species of the world. Important fisheries resource for Iberian and Mediterranean countries, potential candidate to aquaculture production, biological model for physiologic and behavior studies and emblematic species known by its intelligence and survival ability, the name *O. vulgaris* returns 1420 peer-reviewed papers in the web of knowledge database (724 in the last 10 years).

However, the *O. vulgaris* life cycle combining a pelagic paralarval stage with a benthic juvenile and adult stage, the high capacity to adapt to environmental conditions (plastic life cycle), the absence the of generations overlap and recruitment success highly dependent of the environmental conditions results in the necessity of study the adaptations of the population to the local conditions. Additionally, the paralarval dispersion processes and the grounds that offer the best settlement and growth conditions are still poorly known and appear to be quite variable. Lastly, the plastic life cycle characteristics result in highly variable growth rates and direct relationship between size and age has been difficult to address. This work addresses these three gaps in the knowledge about the *O. vulgaris* life cycle, particularly for the populations living along the Atlantic coast of the Iberian Peninsula. Under the influence of the coastal features of one of the four most productive

upwelling systems, the Canary Current upwelling system, the *O. vulgaris* population presents particular characteristics that makes of it one of the most resilient fisheries stocks for the coastal fisheries communities of Portugal and Galicia.

In the chapter two, the *O. vulgaris* reproductive season and nutritional requirements of mature females are address under the light of the different environmental conditions experienced in the Portuguese northwest (under WIUS) and south (under GCS) coasts. In this chapter, we aimed to identify different reproductive strategies adopted by those two populations defined as different spawning seasons but also addressing different strategies between males and females. After defining these different spawning strategies depending of environmental conditions, was time to understand how different environmental conditions affect the nutritional quality of food and if this affects the nutritional quality of eggs using the mature gonads as proxies of eggs quality. Following the hypothesis that the environmental conditions and trophic habitat influence the biochemical composition of storage organs and the nutritional quality of the eggs, we followed the EFA profile and the content of total lipids, cholesterol, triacylglycerol and phospholipids as bio-indicators of variations in the digestive gland and in immature and fully developed gonads of females of both populations. Here, the stable isotope signatures in the flesh and beaks were determined to set the trophic level of these octopus populations and proof the existence or not of differences in the trophic level of both populations.

Chapter three addresses the habitat and diet requirements of the *O. vulgaris* paralarvae and juveniles. The Ría de Vigo, an inlet ecosystem in the western coast of Galicia is an important nursery for the *O. vulgaris* population of the entire northeastern Atlantic coast of Iberian Peninsula. Here, we characterize the local factors that confirm the Ría de Vigo as an essential fish habitat for the *O. vulgaris* northeastern Atlantic population. The settlement process is still poorly unknown due to sampling limitations but the survival of juvenile during and post-settlement is important to the recruitment to fisheries success. For that reason, the identification of juvenile settlement and aggregation grounds is essential to an effective fishery management and to ensure sustainability of this heavily exploited species. Here, we aim firstly identify along the Portuguese coast areas where pre-recruits of *O. vulgaris* aggregate, and secondly, to analyse the relationships between pre-recruit abundance and several abiotic environmental variables to delineate their optimum habitat. The preferential preys of the *O. vulgaris* paralarvae have been recently identified in this area (Roura *et al.*, 2012), nevertheless the nutritional requirements of this phase of the

life cycle, essential to successfully rear *O. vulgaris* paralarvae and complete the life cycle under culture conditions, is still under debate. Here, we determine the lipid content and FA profile of wild captured paralarvae and their potential prey. After hatch and feed in the shallow waters of the Galician and Portuguese coast, the paralarvae leave this habitat and travel along shore until attained the reasonable size to settle.

The chapter four is dedicated to the ageing methods. It is generally accepted that the octopus, as generally all the cephalopods have a short life span of one to two years, nevertheless doubts arise when comparing senescent *O. vulgaris* with one kilogram and other with six kilograms of theoretically the same age. Well studied as a captivity animal, the pure observation of the animal in their natural status leads to conclude that the growth rate in captivity of controlled temperature and feeding *at libitum* hardly imitate the natural conditions. The latest achievements in determine age counting growth increments in the vestigial shell shows to be promising in the idea of determining age directly for this important resource. Nevertheless, an accurate and precise age determination needs to achieve the validation of different landmarks in the lifecycle of the individual as the hatch, the settlement of the onset of maturation. Considering this, firstly we aimed to develop a technique to locate the stylets in the muscle of paralarvae and to determining the stylet size at hatching as a reference to the nuclear area in the post-settled stylets. And secondly, to estimate the age of *O. vulgaris* juveniles (pre-recruits) of the Portuguese northwest coast (western Iberian upwelling system, WIUS) using SIA discussing the best methodology allowing to repeat and compare age estimates between populations and between Incirrata octopus species at the light of achieve higher precision in counts and closer age accuracy.

CHAPTER 2

REPRODUCTIVE CYCLE AND NUTRITIONAL HABITAT UNDER DIFFERENT OCEANOGRAPHIC CONDITIONS

2. REPRODUCTIVE CYCLE AND NUTRITIONAL HABITAT UNDER DIFFERENT OCEANOGRAPHIC CONDITIONS

2.1. SEASONAL TRENDS OF REPRODUCTIVE CYCLE OF *OCTOPUS VULGARIS* IN TWO ENVIRONMENTALLY DISTINCT COASTAL AREAS[1]

Sílvia Lourenço[a,b], Ana Moreno[b], Luís Narciso[a], Ángel F. Gonzalez[c], João Pereira[b]

[a] Centro de Oceanografia/Laboratório Maritimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Avenida Nossa Senhora do Cabo, nº939, 2750-374 Cascais – Portugal.

[b] Unidade de Recursos Marinhos e Sustentabilidade, INRB I. P. /L-IPIMAR, Av. de Brasília 1449-006 Lisboa, Portugal.

[c] Ecobiomar, Instituto de Investigaciones Marinas de Vigo, CSIC, C/Eduardo Cabello, 6. Vigo. E-36208, Spain.

ABSTRACT

Octopus vulgaris is an important fisheries resource, particularly in Iberian waters. Species life cycle is short with capacity to adapt to different environmental conditions and, considered a simultaneous terminal spawner. Data on maturation and other biological parameters collected from January 2007 to November 2010 are used to define spawning seasons for octopus landed by the small-scale trap fisheries in two oceanographically distinct Portuguese coastal areas: the northwest coast (western Iberia upwelling system) and the south coast (Gulf of Cadiz system). On a monthly basis, we followed the proportion of mature individuals, and the Gonad-Somatic and Hayashi Indices. Length-weight relationship, weight-at-maturity, body condition and energy allocation were other biological parameters studied. Spawning season was markedly different in both areas. The northwest population spawns from March to July, in synchrony with the northwest coast upwelling season, and the south coast population spawns mainly in summer, between August and September. A less intense spawning peak in early spring is present occasionally in the south coast. Weight-at-maturity is geographically indistinct, but in both areas males mature at smaller sizes than females. Body condition increases significantly during maturation and mass allocation for reproduction results indicate that males and females channel energy to reproduction from several sources.

Keywords: *Octopus vulgaris*, ecology, environment, reproduction, condition.

[1] Fisheries Research (2012) 127 – 128:116 - 124

1.5.1. Introduction

The common octopus *Octopus vulgaris* (Cuvier, 1797) is exploited as a target species by demersal trawl fleets and numerous small-scale fisheries, using hand jigs, pots, traps and trammel nets operating in southern Europe and northwestern Africa (Hastie *et al.*, 2009). In 2010, the Portuguese landings of common octopus in national ports were 12 602 tons (ten years average landings of 9 050 tons). The small-scale fleet, using traps and pots, is responsible for 95% of the common octopus landings (ICES, 2011). Nevertheless, a growing interest in this species as well as in other commercial cephalopod species is presently noticed for the bottom trawl fleet, with 10% of the landings, in 2007 (Fonseca *et al.,* 2008).

Cephalopods are characterized by a plastic life cycle with a recognized capacity to adapt to different ecosystems, due to a short life span, high growth rate and diverse diets. *O. vulgaris* is a merobenthic incirrate octopod, common in temperate shallow habitats between the coastline and 200 m depth (Roper *et al.*, 1984; Boletzky, 1992). Due to its wide distribution, *O. vulgaris* is one of the best models to investigate the adaptation of reproductive strategies to specific environmental features.

The species is characterized by a life span of one year in Saharan bank waters to two years in Galician waters attaining relatively large sizes, of up to 6000 g in weight and 25 cm mantle length (Domain *et al.*, 2000; Otero *et al.*, 2007). It is a simultaneous terminal spawner, laying on average 350 000 eggs in sheltered places between 20 and 120 m depth (Rocha *et al.,* 2001; Silva *et al.*, 2002). The spawning season is generally extended throughout the year with one or two peaks geographically variable in timing (Hastie *et al.*, 2009).

The Portuguese coast is a highly dynamic oceanographic region in the temperate northeast Atlantic, influenced by two important oceanographic systems the Western Iberia Upwelling System (WIUS) and the Gulf of Cadiz System (GCS). The WIUS is characterized by a strong influence of the spring-summer coastal upwelling (Fíuza, 1983; Mason *et al.*, 2005)**.** The predominantly equatorward winds observed in summer, drive an offshore Ekman transport and force the upwelling of colder, nutrient-rich, subsurface waters along the coast (Relvas *et al.*, 2007). The presence of the poleward current, responsible for the formation of convergence zones over the shelf-break, has an important ecological role for the retention and/or poleward transport of the phytoplankton (Ribeiro *et al.*, 2005) and zooplankton (Santos *et al.*, 2004), with zooplankton peaking in biomass during late spring and early summer near the outer-shelf (Cunha, 2001). All year round the coastal sea water temperature ranges between 14.4 ºC and 19.6

ºC (Moreno *et al.*, 2009) with a shallow thermocline developing during spring (Cunha, 2001). The south Portuguese coast on the other hand, is influenced by the GCS. The south coast orientation does not favour upwelling under northerly winds. Upwelling and downwelling events tend to be weak and the circulation is mainly wind forced and influenced by the local orography (García-Lafuente & Ruiz, 2007). The Cape Stª Maria (36º 57'N, 7º 53'W) divides the continental shelf in two, supporting different oceanographic processes (García-Lafuente & Ruiz, 2007). East to the Cape Stª Maria, the presence of the Huelva front as a warm coastal current flowing along the shore, the significant fresh water runoff forming buoyant river plumes in spring, the occasional occurrence of the Stafford Shear, a cold-warm frontal region associated to local upwelling systems (Mason *et al.*, 2005) and an extended Sea Surface Temperature (SST) range, between the 15.4 ºC and the 21.9 ºC (Moreno *et al.*, 2009) are the main characteristics of the GCS.

Studies on the reproductive features of commercially exploited marine species such as the common octopus are an important tool for the assessment of the stock status. Detailed information about the timing and the sites of spawning, size at maturity, and sex ratio at breeding, require a consistent assessment of the state of sexual maturity. These assessments usually involve several aspects of the reproductive development, such as the state of the gonad and the accessory reproductive structures, and are based on descriptive maturity scales and/or maturity condition indices (Boyle & Rodhouse, 2006). Due to the extensive distribution area of *O. vulgaris* and the commercial interest of the species in the Atlantic waters of the Iberian Peninsula, several studies have been conducted on the reproductive features of geographically distinct sub-populations. Based in her experiments in captivity Mangold (1983) reviewed all aspects of the *O. vulgaris* life cycle, and more recently other two reviews addressing *O. vulgaris* were published (Hastie *et al.*, 2009 and Pierce *et al.*, 2010), compiling recent advances on fisheries and life cycle aspects.

Regarding specifically *O. vulgaris* reproductive aspects, several papers targeting Mediterranean, Iberian and other regional sub-populations have been published since the late 70's, with diversified sampling designs and methodology used to identify spawning season mainly based on proportions of mature individuals and several condition indices, such as Gonad-somatic index or Hayashi index. Such studies rely mostly on landings from trawl fleets (e.g. Quetglas *et al.*, 1998) and small-scale fleets (e.g. Sánchez & Obarti, 1993; Hernández-García *et al.*, 2002; Fernandez-Rueda & Garcia-Flórez, 2007). A few studies were conducted with sampling designed specifically to *O. vulgaris* (e.g. Smith & Griffiths, 2002; Oosthuizen & Smale, 2003; Rodríguez-Rua *et al.*, 2005; Katsanevakis & Verripoulos, 2006).

Considering its regional proximity and the methodology used, the studies of Silva *et al.* (2002) and Otero *et al.* (2007) are particularly relevant for the present study. In the Northwest Galician waters, Otero *et al.* (2007) define a single spawning season during spring (May to June) related with the seasonal coastal upwelling in Galician waters, despite the presence of mature females all year round. In those waters, males mature at smaller sizes than females and the mass transfer during the maturation process occurs directly from feeding sources. According to Silva *et al.* (2002), in waters of the Gulf of Cadiz, the reproductive season covers most of the year, with two distinct peaks in April/May and August/September. As in the previous study, males mature at smaller sizes than females.

The present study aims to identify different reproductive strategies adopted by different populations, considering the distinct oceanographic features of the northwest coast within the WIUS and the South coast within the GCS. The reproductive strategies identified are described in terms of sex ratio, proportion of mature individuals and monthly mean gonad-somatic and Hayashi indices. At the individual level, length-weight relationships are determined by sex and area, as well as maturity ogives and the size effect at maturity. Additionally the effect of size and body condition is analyzed for each sex in both regions in order to identify sexual and regional differences regarding the use of storage mass for reproduction.

1.5.2. Material and Methods

1.5.2.1. Sampling

Samples were collected monthly from January 2007 to November 2010 from the small-scale trap fishery (Table 2.1) and frozen. Sampling sites were chosen considering the two different oceanographic regions, the Northwest coast, with Peniche as the landing port, and the South coast with Olhão as the landing port (Figure 2.1).

For all individuals, the dorsal mantle length (ML in mm), individual weight (W in g) and digestive gland weight (DGW in g) were measured to the nearest 5 mm and 0.1 g, respectively. To assess maturation, the following data were collected: testis and ovary weight (TW and OW), Needham's complex weight (NCW), oviduct weight (ODW) and oviducal complex weight (OCW), all weighed to the nearest 0.01g. A maturity scale of four stages for males (I: immature; II: maturing; III: mature; IV: post-spawning) and five stages for females (I: immature; II: maturing; III: pre-spawning; IV: mature; V: post-spawning) was used (adapted from Guerra, 1975).

Table 2.1 - Summary of sample collection between January 2007 and November 2010.

Month	Northwest region		South region		N total
	Females	Males	Females	Males	
Jan-07	7	12	42	41	102
Feb-07	13	10	16	12	51
Mar-07	10	20	-	-	30
Apr-07	17	10	-	-	27
May-07	12	19	33	27	91
Jun-07	44	38	-	-	82
Jul-07	12	15	15	18	60
Aug-07	8	9	-	-	17
Sep-07	19	12	13	19	63
Oct-07	16	16	11	25	68
Nov-07	13	9	-	-	22
Dec-07	-	-	-	-	-
Jan-08	12	22	42	32	108
Feb-08	12	10	39	26	87
Mar-08	15	14	15	11	55
Apr-08	14	12	12	16	54
May-08	13	9	11	12	45
Jun-08	9	26	16	4	55
Jul-08	15	15	24	15	69
Aug-08	6	11	-	-	17
Sep-08	12	23	25	7	67
Oct-08	18	7	18	35	78
Nov-08	16	14	18	36	84
Dec-08	-	-	8	20	28
Feb-09	7	20	16	14	57
Mar-09	15	16	28	20	79
Apr-09	16	10	32	21	79
May-09	4	21	18	24	67
Jun-09	5	4	22	20	52
Jul-09	11	12	17	11	51
Aug-09	10	21	23	28	82
Sep-09	12	17	60	38	127
Oct-09	21	13	39	55	128
Nov-09	19	18	40	42	119
Dec-09	-	-	20	35	55
Jan-10	10	12	44	52	118
Feb-10	17	13	-	-	30
Mar-10	12	22	34	25	47
Apr-10	10	22	-	-	32
May-10	15	23	-	-	38
Jun-10	18	22	13	25	78
Jul-10	13	10	-	-	23
Aug-10	16	19	-	-	35
Sep-10	23	24	26	29	102
Oct-10	24	18	20	29	91
Nov-10	17	19	16	21	73
N total	607	689	835	836	2967

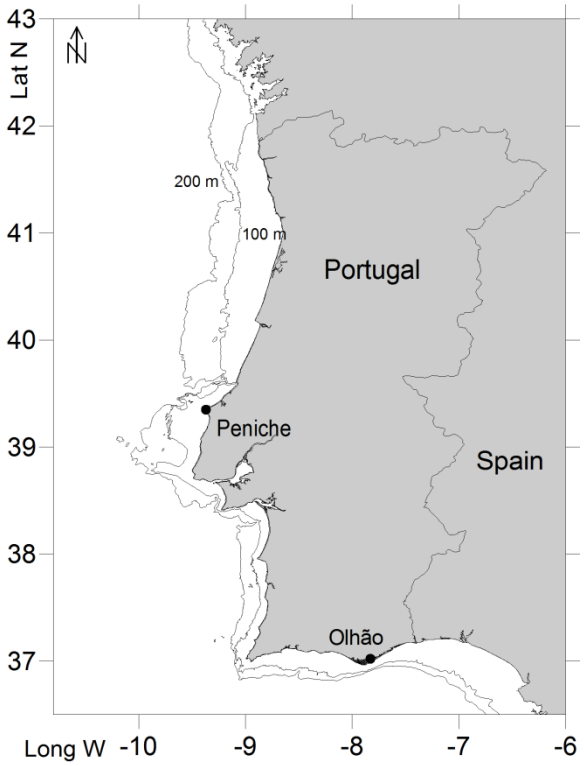


Figure 2.1 - Sampling ports and coastal characteristics

1.5.2.2. Data Analysis

a) Sex ratio and spawning season

Individual weight data aggregated in 200 g weight classes by study area were analyzed using RMIX, an algorithm designed to identify and separate normal distributions in size frequency distributions (MacDonald and Green, 1988; MacDonald, 2008). RMIX identifies a set of overlapping component distributions that give the best fit to the histogram. The modal weight classes, identified by visual inspection of the weight distribution, were used as the initial weight parameters. Constraints were applied in the analysis, such as a fixed variance coefficient for each component. Only components with n > 50 were considered and all components were assumed to be lognormal.

The sex ratio by month was estimated as the ratio between males and females. Significant deviations from 1:1 were tested by a χ^2 test with $\alpha = 0.05$. In order to define the spawning season by study area, the proportion of mature individuals (MI) was determined monthly as: $MI_{fi} = (\Sigma M_i/N_i)_f$, with *i* as month, and M as the frequency of mature females, *f* (maturity stage III and IV) in each month; $MI_{mi} = (\Sigma M_i/N_i)_m$, with *i* as month, and M as the frequency of mature males, *m* (maturity stage III) in each month. The spawning season was defined empirically as the sampling months in which $MI_{fi} > 0.1$ and $MI_{mi} > 0.1$.

Both the Gonad-somatic index (GSI) and the Hayashi index (HI) are important tools to assess the reproductive and maturation condition both at the individual and population level. The GSI, most indicated to assess the maturation condition in females (Guerra, 1975), was determined individually for all females in the form GSI = OW*100/ (BW-OW) (Otero *et al.*, 2007). The monthly GSI was determined as the monthly mean of the individual GSI and analyzed separately for each study area. The HI, most indicated to assess the maturation condition in males (Guerra, 1975) was determined individually for all males in the form HI = NCW/(NCW+TW) (Otero *et al.*, 2007). The monthly HI was determined as the monthly mean of the individual HI_m and analyzed separately for each study area. The standard error was adopted as the measure of variability for the determination of the monthly mean of GSI and HI, and determined as $SE = \sqrt{(Var(GSI_i)/n_i)}$ or $SE = \sqrt{(Var(HI_i)/n_i)}$ with *i* as month (Zar, 1999).

b) Length-weight relationship and weight-at-maturity

The effect of both study area and sex in the length-weight relationship and maturity proportion was investigated with Scheffé F-tests at a significance level $\alpha = 0.05$. In order to normalize the data, ML, W and MI by 200 g weight class were log transformed and compared with a two-way ANOVA test at a significance level $\alpha = 0.05$.

Length-weight relationships for combined sexes as well as for all females and all males were determined by means of non-linear estimates by maximum likelihood of the form log (W) = $a+b$*log(ML). Goodness of fit was expressed by r^2 and the significance level of the parameters estimated by a *t-Student* test.

A maturity curve, for each sex in each study area, was fitted to the weight frequency distribution of the proportion (P_i) of mature individuals by 200 g weight class, with a logistic model: $Pi = 1/(1+\exp(\alpha+\beta W_i)$ by weight class, *i*. Differences in the maturity curves between areas and between sexes were tested by means of the non-parametric Kruskal-Wallis test for successive comparisons. The Weight-at-Maturity was defined as the weight class where 50% of individuals are mature and determined from $W_{50\%} = -\alpha/\beta$.

c) Body condition and energy allocation

The body condition was determined for each study area for females and males separately, as the value of the residuals obtained from the geometric mean regression of log(BW) by log(BW)-log(OW) for the females, replacing log(OW) by log(TW) for the males. The residuals

resulting from this analysis allow a comparison of individuals independently of body weight: positive residuals indicate animals that are heavier than predicted by the model, and negative residuals indicate individuals that weight less than predicted. Variations in body condition were compared by maturity stage using a one-way ANOVA and Tukey's honesty significant difference HSD post hoc test (McGrath Steer & Jackson, 2004; Zar, 1999).

To study the relative energy allocation, the variation in weight between organs in mature animals was assessed. Two geometric regressions were performed on mature individuals of each study area, for each sex separately: (A) the body weight *vs.* gonad weight and (B) the body weight *vs.* digestive gland weight, after log transformation. The residuals obtained from these two regressions, provide a size-independent estimate of the relative weight of the gonads and digestive gland. To determine if there is a mass transfer between organs during the reproductive process, the residuals derived from both regressions were compared to the body weight (W), the reproductive complex weight (OW+OCW for females and TW+NCW for males) and the digestive gland weight using the Pearson correlation test procedure (Zar, 1999).

1.5.3. Results

The weight of females ranged between 625 g (135 mm ML, maturity stage II, northwest coast) and 6189 g (247 mm ML, maturity stage IV, south coast). The weight of males ranged between 635 g (100 mm ML, maturity stage II, south coast) and 5612 g (248 mm ML, maturity stage III, south coast). The weight distributions were significantly different between areas ($\chi^2_{\text{Kruskal-Wallis}} = 19.62$, df = 6, $p < 0.05$) and within each area between sexes (Northwest coast, $\chi^2_{\text{Kruskal-Wallis}} = 28.55$, df = 6, $p < 0.05$; South coast, $\chi^2_{\text{Kruskal-Wallis}} = 42.07$, df = 6, $p < 0.05$) (Figure 2.2). Weight frequency analysis indicates that the weight distribution was bimodal in both areas. On the Northwest coast the weight distribution presents two modal peaks in the 1100 g weight class (modal weight, 1147 g) and in the 2200 g weight class (modal weight, 2216 g). On the South coast the weight distribution presents a mode in the 900 g weight class (modal weight of 979 g) and another in the 1700 g weight class (modal weight of 1724 g) (Table 2.1).

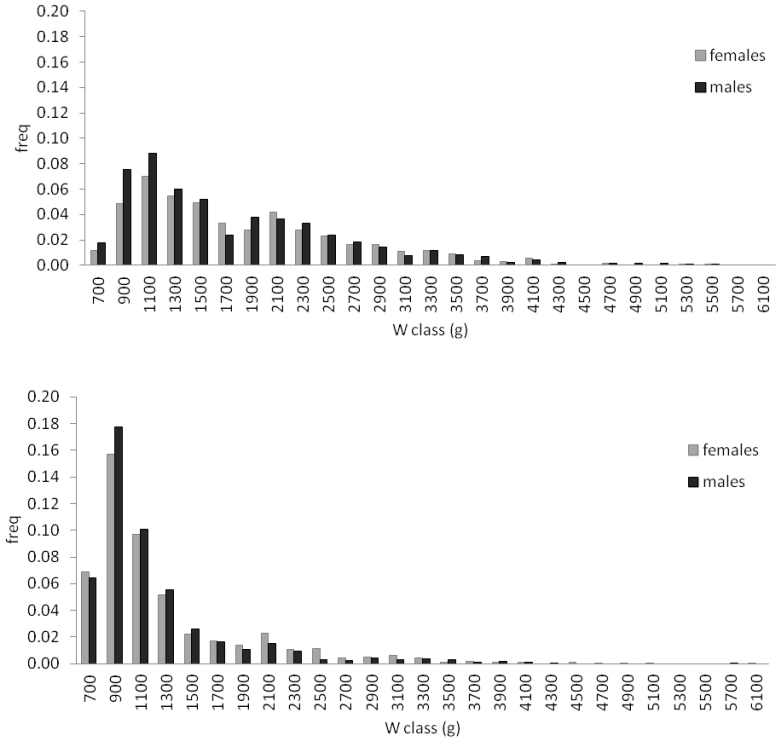


Figure 2.2- Weight distribution of the females and the males sampled in the northwest coast and the south coast.

Table 2.2- Weight frequency analysis results for the Northwest coast and for the South coast: A, B: distribution components, SE: standard error, χ^2: chi-square test value, *df*: degrees of freedom.

	Northwest Coast			**South Coast**		
	Mean weight (±SE)	Probability (±SE)	Standard Deviation (±SE)	Mean weight (±SE)	Probability (±SE)	Standard Deviation (±SE)
A	1147 (±15)	0.40 (±0.03)	221.1 (±14.0)	978.8 (±6)	0.66 (±0.03)	179.6 (±9.1)
B	2216 (±47)	0.60 (±0.03)	866.1 (±25.6)	1724.1 (±80)	0.33 (±0.03)	981.0 (±41.4)
Significance test	$\chi^2 = 114.29$, $df = 19$, p-value < 0.05			$\chi^2 = 115.83$, $df = 20$, p-value < 0.05		

a) Sex ratio and spawning season

The overall sex ratio (F:M) showed that in the Northwest coast sample, males are significantly more abundant than females, 0.88:1. In these samples, more males occurred in all months with the exception of April, October and November when the sex ratio was balanced towards females. On the other hand, despite the fact that the overall sex ratio on the South coast

was 1:1, females were more abundant than males except for the months of August, October, November and December, where the sex ratio was balanced towards males (Table 2.3).

Table 2.3- Sex ratio and χ^2 test for deviance from 1:1 sex ratio for Northwest and South coast by month sampled.

	Northwest Coast			South Coast		
Month	F:M	χ^2	p-value	F:M	χ^2	p-value
Jan	0.63:1	71.49	<0.01	1.16:1	248.87	<0.01
Feb	0.92:1	98.12	<0.01	1.37:1	118.37	<0.01
Mar	0.72:1	120.35	<0.01	1.38:1	128.72	<0.01
Apr	1.05:1	106.98	<0.05	1.19:1	76.88	<0.01
May	0.61:1	112.51	<0.01	0.98:1	121.05	<0.01
Jun	0.84:1	162.19	<0.01	1.06:1	96.98	<0.01
Jul	0.96:1	98.08	<0.01	1.27:1	95.80	<0.01
Aug	0.67:1	96.43	<0.01	0.82:1	47.27	<0.01
Sep	0.86:1	138.17	<0.01	1.35:1	211.73	<0.01
Oct	1.46:1	248.87	<0.01	0.61:1	228.50	<0.01
Nov	1:1	158.02	<0.01	0.74:1	132.32	<0.01
Dec				0.51:1	79.68	<0.01
Overall	0.88:1	1329.12	<0.01	1:1	1630.00	<0.01

Table 2.4 presents the proportion of mature individuals found by month in both sampling sites. In the Northwest coast, mature males were present every month with the exception of November 2007. For females, it is possible to identify a consistent pattern of a higher proportion of mature individuals (proportion mature > 0.10) between early spring and late summer. In the south coast, no mature males were found in the samples in September 2008 and October 2008, between March 2009 and May 2009, and in September 2010 and November 2010. In this region, there was a non-negligible presence of mature females in late winter and earlier spring months of 2007, 2008 and 2010, although the major proportion of mature females occurs in a single month in summer or late summer in each year.

Considering the overall results, females in maturity stage I present a mean GSI of 0.198 (± 0.004, SE), in stage II of 0.507 (± 0.022), in stage III of 3.947 (± 0.215), and for fully mature females (stage IV) of 9.134 (± 0.566), revealing a clear separation between maturing females and pre-spawning females. In males, the HI maturity index also presents increasing values from immature to mature males, with HI values of 0.357 (± 0.004) for stage II males, and 0.429 (± 0.005) for stage III males, although in this case with overlapping extremes. For both maturity stages I and IV, not enough data were available for the determination of HI by maturity stage (n = 2 for maturity stage I, and n = 1 for maturity stage IV).

In the northwest coast (Figure 2.3) females present higher GSI between early spring and summer, with two GSI peaks, during the studied period. After the second GSI peak, values decrease to near 0.1, comparable to the mean GSI for stage I. Regarding males in the northwest

coast, HI present higher values roughly between late winter and late summer, comparable to those of stage III males.

Table 2.4- Monthly proportion of mature females and males between January 2007 and November 2010 in the Northwest region and the South region.

Month	Northwest region		South region		Month	Northwest region		South region	
	Females	Males	Females	Males		Females	Males	Females	Males
Jan-07	0.000	0.250	0.048	0.512	Jan-09	-	-	-	-
Feb-07	0.077	0.500	0.125	0.667	Feb-09	0.000	0.400	0.063	0.143
Mar-07	0.500	0.350	-	-	Mar-09	0.333	0.313	0.000	0.000
Apr-07	0.294	0.800	-	-	Apr-09	0.688	0.600	0.000	0.000
May-07	0.250	0.368	0.030	0.333	May-09	0.250	0.524	0.000	0.000
Jun-07	0.341	0.500	-	-	Jun-09	1.000	1.000	0.348	0.200
Jul-07	0.667	0.533	0.400	0.333	Jul-09	1.000	0.667	0.176	0.273
Aug-07	0.625	0.555	-	-	Aug-09	0.800	0.238	0.130	0.286
Sep-07	0.053	0.167	0.000	0.210	Sep-09	0.000	0.176	0.450	0.108
Oct-07	0.000	0.125	0.000	0.160	Oct-09	0.000	0.385	0.026	0.164
Nov-07	0.000	0.000	-	-	Nov-09	0.105	0.556	0.000	0.190
Dec-07	-	-	-	-	Dec-09	-	-	0.000	0.314
Jan-08	0.083	1.000	0.024	0.312	Jan-10	0.200	0.500	0.077	0.227
Feb-08	0.083	0.700	0.077	0.154	Feb-10	0.650	0.789	-	-
Mar-08	0.333	0.857	0.200	0.545	Mar-10	0.583	0.579	0.206	0.160
Apr-08	0.429	0.750	0.000	0.375	Apr-10	0.500	0.636	-	-
May-08	0.231	0.333	0.000	0.417	May-10	0.400	0.870	-	-
Jun-08	0.444	0.731	0.000	0.500	Jun-10	0.333	0.737	0.077	0.240
Jul-08	0.333	0.333	0.000	0.200	Jul-10	0.308	0.700	-	-
Aug-08	0.833	0.545	-	-	Aug-10	0.688	0.368	-	-
Sep-08	0.000	0.130	0.440	0.000	Sep-10	0.130	0.292	0.154	0.000
Oct-08	0.000	0.286	0.000	0.000	Oct-10	0.000	0.222	0.150	0.034
Nov-08	0.000	0.286	0.000	0.055	Nov-10	0.059	0.526	0.000	0.000
Dec-08	-	-	0.000	0.050					

In the south coast (Figure 2.4), the plot of mean monthly GSI for females shows that there is a spawning peak in the summer or late summer months, although in adjacent months values are always high and near the average for mature animals, indicating the occurrence of mature females throughout the year. A secondary peak occurs in some spring months as is evident in March 2008 and June to July 2009. As for the HI of males, no specific season stands out for a higher proportion of mature specimens, although peaks are found in January 2007, from March 2008 to July 2008, and from April 2009 to March 2010, indicating that mature males are present independently of the season.

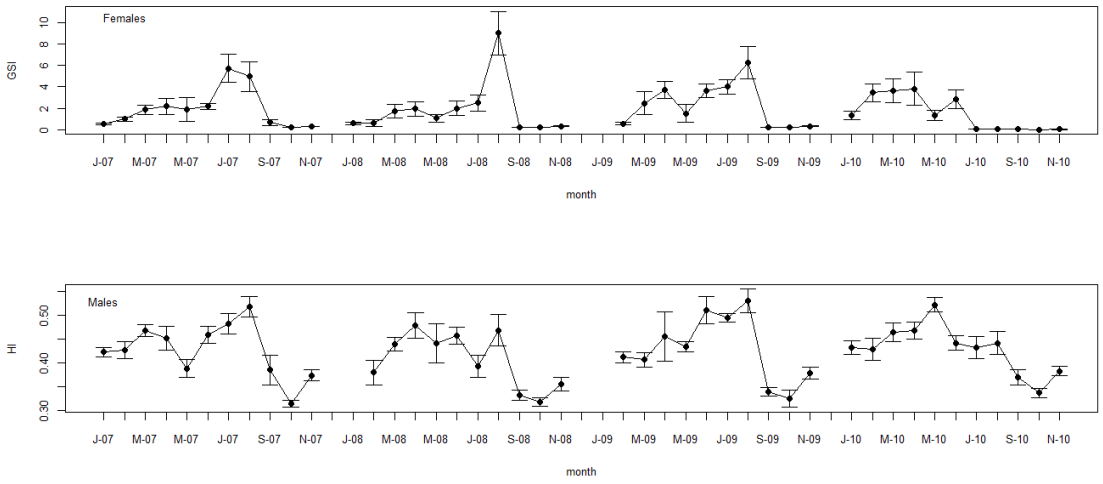


Figure 2.3- Monthly evolution of the mean Gonad-Somatic index (±SE) for females (upper plot) and Hayashi index (±SE) for males (lower plot) in the northwest coast.

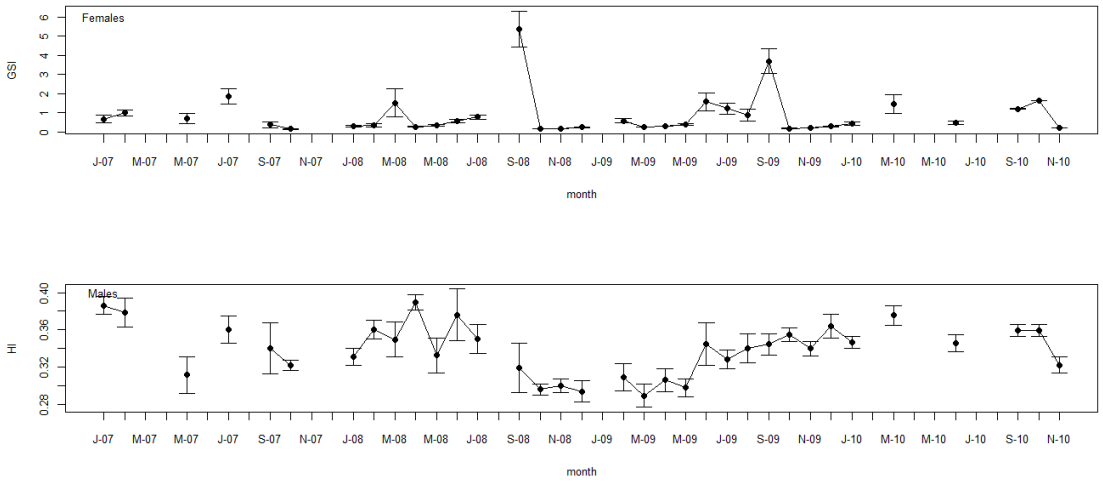


Figure 2.4- Monthly evolution of the mean Gonad-Somatic index (±SE) for females (upper plot) and Hayashi index (±SE) for males (lower plot) in the south coast.

b) Length-weight relationship and weight-at-maturity

Length-weight relationship parameters by area and by sex as well as the statistical tests to the parameters estimated are summarized in Table 2.5. The ANCOVA analysis of the influence of geographic location and sex in length-weight relationships shows that the combined effect of both factors is significant (area : sex, $F = 11.83$, p-value < 0.05), with the effect of the single factor area also being significant ($F = 46.93$, p-value < 0.05) but not the effect of the single factor sex ($F = 2.47$, p-value > 0.05), resulting in different length-weight relationships in each area, and for males and females in the northwest coast. In every case the increase in weight with length may be characterized as positively allometric ($b > 1$).

Regarding weight-at-maturity, the Kruskal-Wallis one-factor analysis of variance shows no effect of geographic area of origin ($\chi^2_{Kruskal\text{-}Wallis} = 0.20$, $p > 0.05$) but a significant difference between sexes ($\chi^2_{Kruskal\text{-}Wallis} = 8.34$, $p < 0.05$). Considering each sex separately, the effect of geographic area was tested and a no significant effect of area was shown for both females ($\chi^2_{Kruskal\text{-}Wallis} = 0.11$, $p > 0.05$) and males ($\chi^2_{Kruskal\text{-}Wallis} = 0.003$, $p > 0.05$). Therefore there are only distinct maturity ogives for males and females with different values of weight at maturity, 2548.01 g for females and 1577.54g for males (Figure 2.5).

Table 2.5 - Length-weight relationships determined for northwest coast and south coast considering both sexes (M+F), males and females separately: *a* and *b* as parameters of the length-weight relationships; *df* – degrees of freedom; r^2 – linear regression coefficient; t_a and t_b – *t*-test value for the regression parameters; and p-value.

		a	*b*	*df*	r^2	Parameter significance level
Northwest coast	Males (M)	2.47	2.77	687	0.83	$t_a = 29.04$, p-value < 0.05 $t_b = 60.33$, p-value < 0.05
	Females (F)	2.28	2.57	605	0.84	$t_a = 24.86$, p-value < 0.05 $t_b = 56.88$, p-value < 0.05
	M+F	2.37	2.66	1294	0.83	$t_a = 37.55$, p-value < 0.05 $t_b = 81.66$, p-valeu < 0.05
South Coast	Males (M)	1.96	2.34	834	0.78	$t_a = 21.37$, p-value < 0.05 $t_b = 53.97$, p-valeu < 0.05
	Females (F)	1.83	2.24	833	0.83	$t_a = 23.65$, p-value < 0.05 $t_b = 64.49$, p-valeu < 0.05
	M+F	1.89	2.34	1669	0.83	$t_a = 31.86$, p-value < 0.05 $t_b = 83.89$, p-valeu < 0.05

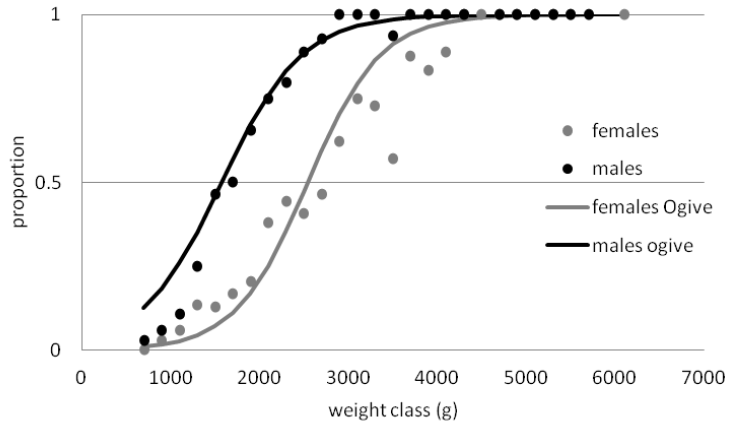


Figure 2.5 - Mature proportion by weight class and maturity ogives for females and males. The weight-at-maturity is the weight at which 50% of the individuals in the population are mature.

c) Body condition and energy allocation

In females, body condition increases significantly between the early maturation (maturity stage II) and late maturation (maturity stage III) ($\mu_{stageII} \neq \mu_{stageIII,}$ $p < 0.05$). Males also present a considerable increase in body condition between maturity stage II and stage III ($\mu_{stageII} \neq \mu_{stageIII,}$ $p < 0.05$) (Figure 2.6).

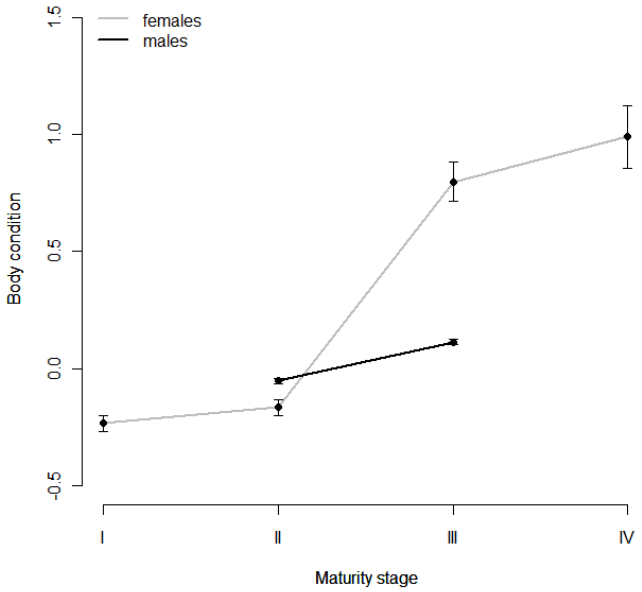


Figure 2.6 - Mean body condition (±SE) with maturity stages.

The Pearson Correlation index for female energy allocation shown by the size-independent digestive gland weight, evidences a negative correlation to individual total weight (-0.109, df =160, $p < 0.05$) and to the weight of reproductive structures (-0.226, df =160, $p < 0.05$). In the case of males, no significant correlation was found between the size-independent digestive gland weight and total weight or reproductive complex weight. Energy allocation shown by the size-independent gonad weight evidences a negative correlation with total weight for both females (-0.428, df = 261, $p < 0.05$) and males (-0.256, df = 492, $p < 0.05$), and similarly with the digestive gland weight (females: -0.256, df = 261, $p < 0.05$; males: -0.412, df = 492, $p < 0.05$).

1.5.4. Discussion

The definition of different spawning seasons in octopus populations within relatively short distances off the Portuguese coast confirms the plasticity of environmental responses of the *O. vulgaris* life cycle, as observed in other cephalopods such as *Loligo forbesi* and *Loligo vulgaris* (Boyle *et al.*, 1995; Moreno *et al.*, 2005). In the northwest coast, females mature preferentially

between early spring and summer in a long spawning season that encompasses two spawning peaks, one in February-April and another in June-July. In this region, mature males are found all year round and are generally more abundant in the fishery than females, due to the spawning behaviour of the latter, which decreases their availability at spawning. In autumn, the proportion of immature females in the population increases with a decrease of mature individuals, both males and females.

In the south coast, the reproductive season occurs during summer peaking in September, despite the non-negligible occurrence of mature females during late winter or early spring, which would indicate that more favourable conditions could generate a secondary peak. Similar situations have been described for other populations in oligotrophic conditions as in the eastern Mediterranean waters and south-eastern coast of South Africa (Sanchez & Obarti, 1993; Oosthuizen & Smale, 2003; Katsanevakis & Verripoulos, 2006). Here, the overall sex ratio is balanced towards males, which seems particularly common in commercially exploited *O. vulgaris* populations (Hernández-García *et al.*, 2002; Fernández-Rueda & García-Flórez, 2007; Otero *et al.*, 2007). This variation in sex ratio, other than purely derived from the behavioral unavailability of females to commercial fisheries, has also been explained by a combination of different factors, such as different growth rates, migrations, feeding or post-spawning behavior (Mangold, 1983) although we have not found any study that indisputably proves the existence of ontogenic or reproductive migrations in populations of the common octopus.

Our results seem to indicate that it is mainly the females that display environmentally adaptive reproductive strategies. In the Northwest coast where the ocean is more dynamic, the spawning season is extended over more than 6 months usually with two reproductive peaks similarly to the results of Hernández-García *et al.* (2002) and Fernández-Rueda & García-Flórez (2007) and Otero *et al.* (2007). In this area, the reproductive cycle of *O. vulgaris*, therefore appears to be highly connected with the long upwelling season as in other regions, such as the adjacent Galician waters and the Sahara Bank (Hatanaka, 1979; Demarq & Faure, 2000; Otero *et al.*, 2007), synchronizing hatching with ideal conditions for food supply (Otero *et al.*, 2008; Moreno *et al.*, 2009) combined with reduced exchanges with offshore areas (Demarq and Faure, 2000). Ecologically, the WIUS is characterized by a persistent nutrient and zooplankton shoreward transport (Almeida & Queiroga, 2003; Santos *et al.*, 2004; Queiroga *et al.* 2007), favored by the westerly winds in late winter and spring, that create a continuous supply of food and potentiate the survival of two cohorts of paralarvae, hatching approximately four months apart, in July and November (Mangold & Boletzky, 1987; Otero *et al.*, 2008; Moreno *et al.*, 2009).

In the South coast, where conditions are more stable but less productive, females concentrate spawning over a shorter period of time. The factors that trigger the onset of the reproduction seem to be related with the seasonal occurrence of favourable oceanographic conditions, such as higher temperatures and salinities, favoured by the easterly winds which are common during summer in the coastal areas close to Cape Stª Maria (Sánchez & Relvas, 2003; García-Lafuente *et al.* 2006). Ecologically, the GCS is a less dynamic and oligotrophic system, in which species tend to direct all of their energy onto a single spawning peak, such as in the case of the sardine *Sardina pilchardus*, the anchovy *Engraulis encrasicolus* and the wedge sole *Dicologlossa cuneata* (Baldó *et al.*, 2006). In the case of the common octopus, individuals spawn in late summer, with the next generation hatching in winter, when the predominant westerly winds favor the transport of nutrients and zooplankton near to the Cape Stª Maria (García-Lafuente & Ruiz, 2007).

Actually, these differences of timing and spawning strategies adopted by neighboring *O. vulgaris* populations is also observed on the South African coast, where the southwest coast is bathed by the cold-water northward flowing Benguela Current producing long and strong upwelling events, and the southeastern coast is influenced by the Agulhas Current, a western boundary current that transports warm equatorial water pole wards (Gibbons *et al.*, 2010). On the west coast, *O. vulgaris* females spawn throughout the year but mainly between spring and summer (Smith & Griffiths, 2002), and in the southeast coast, females spawn in summer, in a shorter and more intense spawning season (Oosthuizen & Smale, 2003).

The length-weight relationship in the present study varies not only with sex but also with region, apparently reflecting environmental conditions. For the northwest region we determined different length-weight relationships for females and males, both positively allometric, but showing that males increase in weight faster than females ($b_{males} > b_{females}$). In the south coast, no differences were found in the length-weight relationship between sexes and both present lower increases in weight with length than either sex in the northwest coast.

It is particularly interesting to note that in this study, geographic area is not reflected in maturity ogives, which may suggest that biological constraints could be imposing limits to minimum maturation body size, as occurs in other cephalopod species (see e.g. Boavida-Portugal *et al.*, 2010 and Moreno *et al.*, 2005). The weight-at-maturity for females is 2550 g and 1577 g for males. The differences found between sexes are probably linked with the simultaneous terminal spawning strategy, with females having to store reserves that are then mobilized to produce yolk, and for behavioral traits related to egg laying and protection. Additionally males probably reach maturity earlier in the life cycle, followed by an earlier decrease in growth rate

(Mangold, 1983; Smith *et al.*, 2005). However, weight-at-maturity registered for both sexes in our samples is higher than previously published for adjacent waters. In the northerly neighboring waters of Galicia, females mature at 1788.3 g and males at 903.4 g (Otero *et al.*, 2007), and in the southeastern neighboring waters of the Gulf of Cadiz females mature at 2023 g and males at 671 g (Silva *et al.*, 2002). The differences found in the weight-at-maturity in different regions may be related with the sampling strategy followed by each author, since weight-at-maturity decreases at peak population maturity (Moreno, 2008) and thus the proportion of samples taken from peak reproduction seasons in relation to those obtained throughout the year affects the size-composition of the sample of mature females. It can also indirectly result from the gears with which the samples were collected, since some gears are less likely to collect fully mature females (Fernández-Rueda & García-Flórez, 2007). It is therefore likely that there is high variability between individuals in any population and that sample characteristics, among which size and timing, may strongly influence the results. It seems reasonable to assume, and has been demonstrated, that maturity is first of all driven by a minimum weight limit and subsequently modulated by biological and environmental conditions. In our samples, there is a significant increase in body condition for both females and males between stage II (immature) and stage III (pre-spawning), indicating that the relative success of somatic growth in this pre-spawning stage may be crucial to determine subsequent events (McGrath Steer & Jackson, 2004; Otero *et al.*, 2007).

Mass allocation data show that reserves are heading for reproduction in both sexes, although some differences can be found between males and females, in accordance with the studies conducted by Otero *et al.* (2007) and Rosa *et al.* (2004), and similarly to *Loligo forbesi* (Collins *et al.*, 1995; Smith *et al.*, 2005). Considering that males mature at smaller sizes than females, and can maintain this stage for several months until a female is available to breed, there seems to be a differentiated breeding strategy between males and females. Other studies, such as Gonçalves *et al.* (2002) and Rodríguez-Rua *et al.* (2005), additionally suggest that mature males are able to breed with immature females which receive and store the mature spermatophores inside the oviducal glands until the oocytes mature. However, only the mass transfer between organs was considered here, and this should be complemented by a study of the types of energetic nutrients that are transferred between organs.

The present study has shown the potential influence and relative importance of environmental factors on the reproduction cycle of *O. vulgaris*, modulating timing, intensity and synchronism. Other studies have been constantly disclosing interactions that may improve our understanding of the main mechanisms. Nevertheless, in terms of population segregation, it

seems evident that even under conditions of apparent genetic homogeneity (Cabranes *et al.*, 2008), populations subjected to fisheries in different geographic areas must be managed differently. And if we agree on the extent of its distribution pattern, *O. vulgaris* is one of the most diversely impacted cephalopods in terms of environmental exposure.

ACKNOWLEDGEMENTS

The authors wish to express their acknowledgment to Pedro Mendonça for assistance with the biological sampling. The authors also thank four anonymous referees for their comments, which greatly improved the final version of this paper. Data collection was conducted under the CEPHVAR (FAIR-CT96-1520), the Data Collection Framework (DCF)/DG FISH (EC Regulation No. 665/2008 of the 14 July 2008) and PRESPO projects (INTERREG IVB - Atlantic Area Program, co-financed by the European Union ERDF funds). The first author is supported by a PhD fellowship granted by the Portuguese Foundation for Science and Technology (FCT).

BIBLIOGRAPHIC REFERENCES

ALMEIDA, M.J. & QUEIROGA, H., 2003. Physical forcing of onshore transport of crab megalopae in the northern Portuguese upwelling system. *Estuarine, Coastal and Shelf Science* **57**, 1091-1102.

BALDÓ, F., GARCÍA-ISARCH, E., JIMÉNEZ, M.P., ROMERO, Z., SÁNCHEZ-LAMADRID A. & CATALÁN, I.A. 2006. Spatial and temporal distribution of the early life stages of three commercial fish species in the northeastern shelf of the Gulf of Cádiz. *Deep-Sea Research* II **53**, 1391–1401.

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.

BOLETZKY, S.v.-. 1992. Evolutionary aspects of development, life style, and reproductive mode in incirrate octopods (Mollusca, Cephalopoda). *Revue Suisse Zoologie* **99** (4), 755-770.

BOYLE, P.R., PIERCE, G.J. & HASTIE, L.C. 1995. Flexible reproductive strategies in the squid *Loligo forbesi*. *Marine Biologie* **121**, 501-508.

BOYLE, P. & RODHOUSE, P. 2006. Cephalopods: Ecology and Fisheries. Second Ed. Blackwell Publishing, 452 p.

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**(1), 12-16.

COLLINS, M.A., BURNELL, G.M. & RODHOUSE, P.G. 1995. Reproductive strategies of male and females *Loligo forbesi* (Cephalopoda: Loliginidae). *Journal of the Marine Biological Association UK* **75**, 621-634.

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. A thesis submitted to the Faculdade de Ciências da Universidade de Lisboa forthe degree of Doutor em Biologia (ramo Ciências do Mar). 307 p.

DEMARCQ, H.& FAURE, V. 2000. Coastal upwelling associated retention indices derived from satellite SST. Application to *Octopus vulgaris* recruitment. *Oceanologica Acta* **23** (4), 391-408.

DOMAIN, F., JOUFFRE, D. & CAVERIVIÈRE, A. 2000. Growth of *Octopus vulgaris* from tagging in Senegalese waters. *Journal of the Marine Biological Association UK* **80**, 699–705.

FERNÁNDEZ-RUEDA, P. & GARCÍA-FLÓREZ, L. 2007. *Octopus vulgaris* (Mollusca: Cephalopoda) fishery management assessment in Asturias (North-west Spain). *Fisheries Research* **83**, 351-354.

FIÚZA, A.F.G. 1983. Upwelling patterns off Portugal. In: Suess, E., Thiede, J. (Eds). Coastal Upwelling: Its Sediment Records, Part A. Plenum, New York, pp. 85-98.

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.

GARCÍA-LAFUENTE, J., DELGADO, J., CRIADO-ALDEANUEVA, F., BRUNO, M., 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. & RUIZ, J. 2007. The Gulf of Cádiz pelagic ecosystem: A review. *Progress in Oceanography* **74**, 228-251.

GIBBONS, M.J., BUECHER, E., THIBAULT-BOTHA, D. & HELM, R. 2010. Patterns in marine hydrozoan richness and biogeography around southern Africa: implications of life cycle strategy. *Journal of Biogeography* **37**, 606-616.

GUERRA, A.1975. Determinación de las diferentes fases del desarollo sexual de *Octopus vulgaris* Lamarck, mediante uníndice de madurez. *Investigacion Pesquera* **39** (2), 397-416.

GONÇALVES, J., 1993. *Octopus vulgaris* Cuvier, 1797 (polvo-comum): sinopse da biologia e exploração. Trabalho de síntese para as provas de aptidão Pedagógica e Capacidade Científica, para acesso à categoria de assistente de carreira docente universitária. Departamento de Oceanografia e Pescas da Universidade dos Açores.

GONÇALVES, I., SENDÃO, J. & BORGES, T.C. 2002. *Octopus vulgaris* (Cephalopoda: Octopodidae) Gametogenesis: A histological approach to the verification of the macroscopic maturity scales. *Abh. Geol. B. –A.* **57**, 79-88.

HASTIE, L.C., PIERCE, G.J., WANG, J., BRUNO, I., MORENO, A., PIATKOWSKI & ROBIN, J-P. 2009. Cephalopods in the North-eastern Atlantic: species, biogeography, ecology, exploitation and conservation. Oceanogr. *Marine Biology* **47**, 11-190.

HERNÁNDEZ-GARCÍA, V., HERNÁNDEZ-LÓPEZ, J.L. & CASTRO-HERNÁNDEZ, J.L. 2002. On the reproduction of *Octopus vulgaris* off the coast of the Canary Islands. *Fisheries Research* **57**, 197-203.

ICES. 2011. Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH), 28 February–03 March 2011, Lisbon, Portugal. ICES CM 2011/SSGEF: 03. 118 pp.

KATSANEVAKIS, S. & VERRIPOULOS, G. 2006. Seasonal population dynamics of *Octopus vulgaris* in the eastern Mediterranean. *ICES Journal of Marine Science* **63**, 151-160.

MACDONALD, P. D. M. & GREEN, P. E. J. 1988. User's Guide to Program MIX: An Interactive Program for Fitting Mixtures of Distributions. Ichthus Data Systems, 58 pp.

MACDONALD, P. 2008. Finite Mixture Distribution. 37 pp. (http://www.r-project.org, http://www.math.mcmaster.ca/peter/mix/mix.html, 20-11-2011)

MANGOLD, K. 1983. *Octopus vulgaris*. In P.: Boyle, P. (ed) Cephalopod Life cycle: Species accounts. Vol 1. Academic press, London, pp. 335-363.

MANGOLD, K. & BOLETZKY, S.v-. 1987. Cephalopods. In: Fischer, W. *et al.* (Eds) Fiches FAO d'Identification des Espèces pour les Besoins de la Pèche (Rev. 1) Méditerranée et mer Noire. Zone de Pêche 37. FAO. Rome, 633–714.

MASON, E., COOMBS, S. & OLIVEIRA, P.B. 2005. An overview of the literature concerning the oceanography of the eastern north Atlantic region. Relatórios científicos e técnicos do IPIMAR - Série Digital, 33, p. 58. (http://www.inrb.pt/fotos/editor2/Reln33final.pdf, 20-11-2011).

MCGRATH STEER, B.L. & 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.

MORENO, A. 2008. Geographic variation in cephalopod life history traits. Dissertação original apresentada para acesso à categoria de Investigador auxiliary. Unidade de Recursos Marinhos e Sustentabilidade. INRB/L-IPIMAR. 181 pp.

MORENO, A., PEREIRA, J. & CUNHA, M. 2005. Environmental Influences on age and size at maturity of *Loligo vulgaris*. *Aquatic Living Resources* **18**, 377-384.

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.

OOSTHUIZEN, A. & SMALE, M.J. 2003. Population biology of *Octopus vulgaris* on the temperate south-eastern coast of South Africa. *Journal of the Marine Biological Association UK* **83**, 535-541.

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., WEATLEY, B. & GUERRA, A. 2008. Bottom-up control of common *Octopus vulgaris* in the Galician upweeling system, Northwest Atlantic Ocean. *Marine Ecology Progress Series* **362**, 181-192.

PIERCE, G.J., ALLCOCK, L., BRUNO, I., BUSTAMANTE, P., GONZÁLEZ, A., GUERRA, A., JEREB, P., *et al.* 2010. Cephalopod biology and fisheries in Europe. ICES Cooperative Research Report No. 303. 175 pp.

QUEIROGA, H., CRUZ, T., DOS SANTOS, A., DUBERT, J., GONZÁLEZ-GORDILLO, J.I., PAULA, J., PELIZ, A. & SANTOS, A.M.P. 2007. Oceanographic and behavioral processes affecting invertebrate larval dispersal and supply in the western Iberia upwelling ecosystem. *Progress in Oceanography* **74**, 174-191.

QUETGLAS, A., ALEMANY, F., CARBONELL, A., MERELLA, P. & SÁNCHEZ, P. 1998. Biology and Fishery of *Octopus vulgaris* Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, Western Mediterranean). *Fisheries Research* **36**, 237-249.

RELVAS, P., BARTON, E.D., DUBERT, J., OLIVEIRA, P.B., PELIZ, A., DA SILVA, J.C.B. & SANTOS, A.M.P. 2007. Physical Oceanography of the western Iberia ecosystem: Latest views and challenges. *Progress in Oceanography* **74** (2-3), 149-173.

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.

ROCHA, F., GUERRA, A. & GONZÁLEZ, A.F. 2001. A review of the reproductive strategies in cephalopods. *Biological Reviews* **76**, 291-304.

RODRÍGUEZ-RUA, POZUELO, I., PRADO, M.A., GÓMEZ, M.J., BRUZÓN, M.A. *et al.*, 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.

ROPER, C.F.E., SWEENEY, M.J. & NAUEN, C.E., 1984. Cephalopods of the world. An annotated and illustrated catalogue of species of interest to Fisheries. FAO Fisheries Synopsis. Vol. 3. 227 pp.

ROSA, R., COSTA, P.R. & NUNES, M.L. 2004. Effect of the sexual maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca: Cephalopoda). *Marine Biology* **145**, 563-574.

SÁNCHEZ, P. & OBARTI, R. 1993. The biology and fishery of *Octopus vulgaris* caught with clay pots on the Spanish Mediterranean coast. In: Okutami, T., O'Dor, R., Kubodera, T. (Eds). Recent Advances in Cephalopod Fisheries Biology. Tokai University Press, Tokyo. 477–487.

SÁNCHEZ, R.F. & RELVAS, P. 2003. Spring-summer climatological circulation in the upper layer in the region of Cape St Vincent, Southwest Portugal. *ICES Journal of Marine Science* **60**, 1232-1250.

SANTOS, A.M.P, PELIZ, A, DUBERT,J., OLIVEIRA, P.B., ANGELICO, M.M. & RÉ, P. 2004. Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. *Continental Shelf Research* **24**, 149–165.

SILVA, L., SOBRINO, I. & RAMOS, F. 2002. Reproductive biology of the common octopus, *Octopus vulgaris* Cuvier, 1797 (Cephalopoda: Octopodidae) in the gulf of Cádiz (SW Spain). *Bulletin of Marine Science* **71**, 837-850.

SMITH, C.D. & GRIFFITHS, C.L. 2002. Aspects of the population biology of *Octopus vulgaris* in False bay, South Africa. *South African Journal of Marine Science* **24**, 185-192.

SMITH, J.M., PIERCE, G., 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.

ZAR, J. 1999. Biostatistical Analysis. 4th Edition. Prentice Hall. 663pp.

2.2. DOES THE TROPHIC HABITAT INFLUENCE THE BIOCHEMICAL QUALITY OF THE GONAD OF OCTOPUS VULGARIS? STABLE ISOTOPES AND LIPID CLASS CONTENTS AS BIO-INDICATORS OF DIFFERENT LIFE CYCLE STRATEGIES[2]

Sílvia Lourenço[1,2,3], Luís Narciso[1], Ángel F. Gonzalez[2], João Pereira[3], Santiago Auborg[2], José C. Xavier[4]

[1]Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa,Avenida Nossa Senhora do Cabo 939, 2750-374 Cascais, Portugal

[2] Instituto de Investigaciones Marinas de Vigo, CSIC, C/Eduardo Cabello, 6. Vigo. E-36208, Spain.

[3] Departamento do Mar e Recursos Marinhos, Instituto Português do Mar e da Atmosfera, I.P.Avenida de Brasília, 1449-006, Lisboa, Portugal.

[4]Institute of Marine Research, University of Coimbra, Department of Life Sciences, 3001-401 Coimbra, Portugal.

ABSTRACT

This study aims to test whether environmental conditions including the trophic habitat and diet, impact the biochemical composition of storage organs and affect the nutritional quality of eggs of *Octopus vulgaris*. Trophic habitat and gonad quality of neighbouring populations off the Portuguese coast, subject to different oceanographic regimes, were compared using the digestive gland and beaks as recorders of trophic and habitat preferences, and gonads as indicators of egg quality. Cholesterol, phospholipids' and triacylglycerol content, essential fatty acid (EFA) profile of the digestive gland and stable isotopes, $\delta^{15}N$ and $\delta^{13}C$, in the buccal mass flesh and beaks were indicators of the differences in the trophic habitat between populations. For gonad quality, the same bio-indicators were used to identify differences with maturation. The study shows that, although diet influences the EFA profile of the gonads to a certain degree, the main lipid content, phospholipids and cholesterol content in the gonads are not influenced by habitat conditions. This therefore suggests that *O. vulgaris* is able to influence the quality of egg content independently of diet. The species is believed to be an income breeder which attains maturity upon reaching a sufficient condition level, then channelling energy directly from food to gonad development.

Keywords: *Octopus vulgaris*, trophic habitat, gonad quality, bio-indicators.

[2] Hydrobiologia 725 (2013): 33-46.

2.2.1. Introduction

The common octopus, *Octopus vulgaris* Cuvier (1797) is an important fisheries resource and a key component of coastal food webs as a prey and generalist predator (Smith, 2003; Katsanevakis & Verripoulos, 2004). The species is believed to channel energy to growth and reproduction directly from food (Quetglas *et al.* 2011; Smith *et al.* 2011). It is a species with high feeding and turnover rates (Semmens *et al.* 2004), short life cycle and high plasticity to environmental conditions. Studies on the molecular and nutritional composition of reserve and reproductive organs reflect the benthic characteristics of the species. The habitat biodiversity and energy flow to which the populations are exposed are mirrored in the molecular and energetic composition of the tissues (e. g. Rosa *et al.* 2004a; Bandarra *et al.* 2006; Cherel *et al.* 2009; García-Garrido *et al.* 2010).

The diet of *O. vulgaris* depends on the life-cycle stage, size, depth of occurrence, habitat and seasonal availability of their prey (Nixon, 1985; Smith, 2003). The cephalopod hepato-pancreas or digestive gland has different functions in the physiology *Octopus* spp., including the synthesis and secretion of digestive enzymes, the reabsorption and metabolism of nutrients; synthesis and storage of lipids like cholesterol, lipoproteins, glycogen, pigments, vitamins and protein-bound Fe, Cu, Ca and non-physiological heavy metals; and excretion and rejection of waste products of the digestion and cell metabolism (Blanchier & Boucaud-Camou, 1984; Budelmann *et al.* 1997; Moltschaniwskyj & Johnston, 2006). Its function as a storage organ indicates its utility as the ideal source of dietary tracers such as essential fatty acids (EFA) (Phillips *et al.* 2001) and dietary lipids (e.g. triacylglycerol and cholesterol) that are deposited in this organ with little or no modification of the lipid content (Boucaud-Camou & Boucher-Rodoni, 1983; Phillips *et al.* 2003).

Lipids are important dietary constituents, providing energy, vitamins and EFA. Cholesterol is the predominant sterol in the cephalopods lipids reserves (Sieiro *et al.* 2006) and proxy of the production of hormones in marine invertebrates (Kanazawa, 2002). The endogenous synthesis of cholesterol seems to be absent in cephalopods, suggesting that it is an essential dietary nutrient (Villanueva & Norman, 2008). Triacylglycerol is a neutral lipid involved in fatty acid (FA) storage and metabolism (Lee *et al.* 2006).The FAs incorporated within the phospholipids act as building blocks for the membrane lipid bilayer (Athenstaedt & Daum, 2006; Bergé & Barnathan, 2005; Dalsgaard *et al.* 2003). The triacylglycerol and phospholipids seasonal variability is related with cellular mechanisms under different environmental conditions and because of that, they are good indicators of condition (Shulman & Love, 2006). The most important EFA are arachidonic acid C20:4n6 (ARA), eicosapentaenoic acid C20:5n3 (EPA) and docosahexaenoic acid C22:6n3

(DHA). The requirements on ARA, EPA and DHA and the balance between them are important for growth (Navarro & Villanueva, 2000; Shulman & Love, 2006). These EFA are related with the energy channelling, the cellular membrane structure and function, and are integral elements of phospholipids as components of lipid bilayers (Tocher, 2010).

Reproduction timing in *O. vulgaris* depends on the local oceanographic regime: a long season in productive systems, frequently with two reproductive peaks; and a shorter season (one to two months) in oligotrophic systems (Lourenço *et al.* 2012). The formation of the yolk is extremely important during maturation and egg development, because the newly hatched paralarvae are not truly lecithotrophic depending to some extent on these reserves to survive (Boletzky, 1975, Villanueva & Norman, 2008). The nutritional content of the yolk is mainly protein, but lipids are also important for membrane formation and energetic supply (11-14% of dry weight) particularly the of polyunsaturated fatty acids (PUFA), phospholipids and cholesterol (Navarro & Villanueva, 2000).

The $\delta^{13}C$ and $\delta^{15}N$ signatures in different tissues of a predator like *O. vulgaris* reflect its habitat and trophic position respectively (Cherel & Hobson, 2005). Consumers or predators are enriched in ^{15}N relative to their food and consequently the $\delta^{15}N$ measurement is an indicator of the consumer trophic position (Hobson & Cherel, 2006; Vander Zanden & Rasmussen, 2001). With little variation along the food chain, the $\delta^{13}C$ is used to determine primary sources in the trophic web indicating the habitat of the organism, and the inshore *vs* offshore, or pelagic *vs* benthic contribution of food intake (Cherel & Hobson, 2007; Jackson *et al.* 2007). The determination of $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures both in muscle and in cephalopod beaks are complementary approaches to stomach content and fatty acid analysis in studies of trophic dynamics and feeding ecology, permitting the identification of ontogenic migration and feeding shift events (Stowasser, 2004; Jackson *et al.* 2007). In species like *O. vulgaris*, diet studies are difficult to carry out due to the diversity of preys (Smith, 2003); the fast digestion rate (Boucaud-Camou and Boucher-Rodoni, 1983); and the large number of empty stomachs. For these cases, an analytic approach combining fatty acids and stable isotopes analyses provides overall information on the average diet regarding both trophic level and habitat of a particular population.

Here we hypotheses that environmental conditions, including the trophic habitat, influence the biochemical composition of storage organs and the nutritional quality of the eggs (indirectly potentially affecting the next generation). The Portuguese coast presents an advantageous geographical setting where it is possible to follow *O. vulgaris* populations which are subjected to distinct environmental conditions: in the northwest coast, one of the populations is in a

productive system integrated in the Western Iberia Upwelling System (Relvas *et al.* 2007); in the south, the other is integrated in the Gulf of Cadiz System influenced by the oligotrophic and warmer waters of the Huelva front where downwelling and upwelling events are weaker and not seasonal (García-Lafuente *et al.* 2006). To test our hypothesis, we followed the EFA profile and the content of total lipids, cholesterol, triacylglycerol and phospholipids as bio-indicators of variations in the digestive gland and in immature and fully developed gonads of females of both populations. We also followed the $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures in the upper and lower beaks and buccal mass tissue of immature and mature individuals to identify the possible differences in the overall diet between population and maturity stages.

2.2.2. Material and Methods

2.2.2.1. Sampling

All tissue samples were obtained from females captured in the small-scale *O. vulgaris* fisheries from March to April 2011 within the two study areas represented by landings in ports of Peniche (northwest coast area; lat: 39º 21.40 N, long: 9º 20.38 W) and Olhão (south coast area; lat: 36º 59.34 N, long: 7º 50.44 W). Within each area, five immature females and five mature females were selected and digestive gland tissue and gonads were collected, freeze dried and stored at -20ºC.

Cleaned upper and lower beaks and buccal mass muscular tissue (referred as flesh hereafter) samples were collected from frozen animals and kept in 70% ethanol for isotopic analysis. Sampling was conducted considering the same explanatory factors, area and maturity in an unbalanced sampling design, collecting 27 flesh samples (4 mature and 6 immature samples in the northwest coast and 5 mature and 12 immature samples in the south coast) and 33 beak samples (8 mature and 9 immature in the northwest coast and 5 mature and 12 immature in the south coast). The dorsal mantle length and individual weight were measured to the nearest 5 mm and 0.1 g, respectively. Immature and maturing females were classified as immature whereas mature and spawning females were classified as mature (according to Guerra 1975).

2.2.2.1. Stable isotope analyses

Beaks and flesh samples were freeze-dried and homogenized prior to analysis. To avoid the depletion of $\delta^{13}C$ values due to lipids presence, flesh samples were rinsed successively in a 2:1 chloroform-methanol solution (Cherel *et al.* 2005). Nitrogen and carbon isotope ratios were determined via Finningan conflo II interface to a Thermo Delta V S mass spectrometer coupled to a Flash EA1112 Series elemental analyser. Approximately 0.3 mg of each sample was combusted in a tin cup for the simultaneous determination of nitrogen and carbon isotope ratios. Isotope ratios are presented in the usual δ notation relative to the PeeDee Belemnite (PDB) for carbon and atmospheric N_2 (AIR) for nitrogen, and expressed as ‰. Replicate measurements of internal laboratory standards (acetanilide) indicate a precision of < 0.2‰ both for $\delta^{13}C$ and $\delta^{15}N$. The C/N mass ratio was used to check the effectiveness of the lipid extraction in the flesh and in the beaks (Post *et al.* 2007; Cherel *et al.* 2009).

Prior to the statistical analysis, the assumptions of normality and sample variance homogeneity were assessed by Shapiro-Wilk's test, and Bartlett´s test, respectively. A paired t-test was performed to assess possible differences in stable isotope signatures between upper and lower beaks. A two-way analysis of variance (ANOVA) was performed to assess the effect of area, maturity and the interaction between the two factors in the ratios $\delta^{13}C$ and $\delta^{15}N$ in the upper and lower beaks and flesh. The hypothesis for the two-way ANOVA was formulated under the assumption of: H_{0A}: there is no main effect of the factor area on the stable isotope ($\delta^{13}C$ or $\delta^{15}N$) mean value in the beaks upper and lower beaks and flesh; H_{0B}: there is no main effect of the factor maturity on the stable isotope mean value in the upper and lower beaks and flesh; H_{0C}: there is no additive effect of the interaction between area and maturity on the stable isotope mean value in the upper and lower beaks and flesh. If H_{0C} was rejected, a *post-hoc* Tukey test for multiple comparisons was performed to determine which combination of factors were significantly different (p-value < 0.05).

2.2.2.2. Lipid class analyses

The total lipid (TL) fraction was extracted by the Bligh & Dyer (1959) method. Samples of ≈ 1 g and ≈ 2 g of dry tissue of digestive glands and gonads were used respectively. The results were expressed as g lipid/100 g dry weight. Lipid classes were determined by different spectrophotometric methods. The phospholipids fraction was purified from the total lipid extract

according to Auborg *et al.* (1996). Total phospholipids were quantified by measuring the organic phosphorus in total lipid extracts according to the Raheja *et al.* (1973) method based on a complex formation with ammonium molybdate. Results are expressed as g PL/100 g dry weight. Total cholesterol was determined in the total lipids extracts by the method of Huang *et al.* (1961) based on the Liebermann-Buchardt reaction. Results are expressed in g cholesterol/100 g dry weight. FA composition of lipids present in the Bligh & Dyer extract was determined converting total lipids into fatty acid methyl esters (FAME), according to the method described by Lepage & Roy (1984). FAME were analysed by gas chromatography. Peaks corresponding to FAs were identified by comparison of their retention times with standard mixtures. Peak areas were automatically integrated with C19:0 being used as an internal standard for quantitative analysis. The concentration of each fatty acid or fatty acid group was expressed as g/100g total FAME.

Prior to the statistical analysis, the assumptions of normality and sample variance homogeneity were assessed with Shapiro-Wilk's and Bartlett´s tests, respectively. Whenever these assumptions failed the data were log-transformed to guarantee normality and variance homogeneity. Two-way ANOVA was performed to assess the effect of area, maturity and the interaction between the two factors in the lipid classes in the digestive gland and in the gonad. The hypothesis for the two-way ANOVA was formulated under the assumption of: H_{0A}: there is no main effect of the factor area on the lipid class content in the tissue; H_{0B}: there is no main effect of the factor maturity on the lipid class content in the tissue; H_{0C}: there is no additive effect of the interaction between area and maturity on the mean lipid class content in the tissue. If H_{0C} was rejected, a *post-hoc* Tukey test for multiple comparisons was performed to determine which factor combinations were significantly different.

Major FAs were defined as those presenting concentration by area and by maturity stage higher than 1g/100g of FAME. For each FA, the assumption of sample normality and homogeneity was tested by the Shapiro-Wilk's and Bartlett´s respectively by tissue and factor (area and maturity), when these assumptions failed, the variable was log-transformed. Differences in mean concentration of each FA between area and maturity stage were tested with *t*-test (Zar, 1999). The major fatty acids profile was compared by explanatory factor, area and maturity and the interaction between area and maturity by means of Multivariate ANOVA (MANOVA), followed by Discriminant Function Analysis (DFA). The MANOVA and DFA are complementary approaches based in the separation of observation groups represented by their centroids obtained under the effect of two or more factors levels (Quinn and Keough, 2002). DFA is particularly useful to detect the variables that better discriminates between different observation groups (Zuur *et al.* 2007).

2.2.3. Results

2.2.3.1. Diet and Habitat

According to the mean C:N mass ratio, lipids were effectively removed from flesh (C:N = 3.13 ± 0.06 , mean ± standard deviation, SD) and from beaks (upper beak: 3.38 ± 0.07; lower beak: 3.33 ± 0.08). The flesh of *O. vulgaris* females presents a mean $\delta^{15}N$ of 11.86 ± 0.66 (SD) ‰, and the mean $\delta^{13}C$ value of -16.77 ± 0.74 (SD) ‰ (Figure 2.7) independently of area, maturity stage or the interaction of both factors (Table 2.6). Upper and lower beaks present different $\delta^{15}N$ and $\delta^{13}C$ signatures between then (for $\delta^{15}N$: paired t = -2.12; df = 32, p-value < 0.01; for $\delta^{13}C$: paired t = -4.14, df = 32, p-value < 0.01). The factorial ANOVA shows that area has a significant effect in $\delta^{15}N$ signature (Table 2.6), with both beaks presenting higher values in south coast (Figure 2.7). The $\delta^{13}C$ signature is not affected by area in both beaks (Table 2.6).

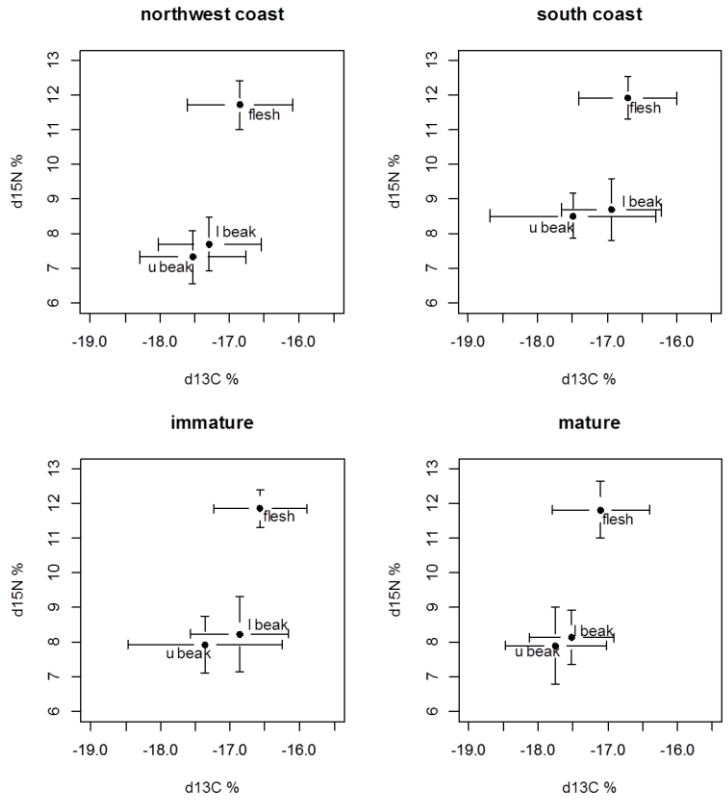


Figure 2.7 - *Octopus vulgaris* flesh and upper and lower beaks stable isotope $\delta^{15}N$ and $\delta^{13}C$ signatures by explanatory factors area and maturity. The upper right and left graphics represent the stable isotope signatures for the northwest and south coast, and the lower left and right graphics represent the stable isotope signatures for immature and mature individuals. The dot indicates the mean value for the $\delta^{15}N$ and $\delta^{13}C$ for each tissue studied; the error bars indicate the magnitude of standard deviation on either axis.

The mean individual weight of the females collected was 2340.30 ± 979.45 (SD) g in the northwest coast and 2637.40 ± 479.00 (SD) g in the south coast. Digestive gland and gonad presented different mean content in total lipids and lipid classes, with the digestive gland presenting higher mean content of total lipids, cholesterol and triacylglycerol (Figure 2.8). Despite that, the gonads were richer in phospholipids than the digestive gland (Figure 2.8).

In the digestive gland the mean total lipid content and mean cholesterol content was depended on area. The digestive glands collected in the northwest coast presented a significantly lower content of total lipids ($t = -2.42$, $df = 18$, p-value < 0.01), and higher mean content in cholesterol ($t = 7.91$, $df = 18$, p-value < 0.01, see Figure 2.8) when compared with south coast. The two-way ANOVA for the interaction between the factors area and maturity showed significant differences in the mean content of cholesterol and triacylglycerol due to the additive effect of the two factors (Table 2.6).

In the digestive gland, the fatty acids (FA) represented 31μg/mg and 46 μg/mg of the total lipid content in the northwest coast and the south coast, respectively. The most abundant FA were the saturated fatty acids (SFA), followed by the polyunsaturated fatty acids (PUFA) and the monounsaturated fatty acids (MUFA) (Table 2.7). The mean content of myristic acid, C14:0, palmitoleic acid, C16:1n7, gondoic acid C20:1n9, ARA, C20:4n6 and DHA were significantly different between areas, as well as the ratio DHA/EPA mainly due to the increase in DHA content in digestive glands of the south coast (Table 2.7). Results of the MANOVA analysis showed that content levels depend both on area and maturity, with no effect on the interaction between both factors (Table 2.8). In the DFA, the first discriminant function maximizes the differences between groups. The DFA for region groups show that the first function explained 84% of the variability, with a good separation between the northwest coast group (class score = 0.92) and the south coast group (class score = -0.92). The FA that contributed the most to the group separation was the DHA which was positively correlated with the south coast and negatively correlated with northwest coast. The DFA for maturity groups showed a good separation between immature (class score = - 0.85) and mature digestive glands (class score = 1.02) with 86 % of explained variation. The FAs that most contributed to the group separation were EPA and DHA and C18:0. The DFA for area x maturity groups (Figure 2.9, Digestive gland) showed a poor separation between groups with an explained variance of 51% in accordance with MANOVA results. Nevertheless, the FAs that contributed the most to the group separation were: DHA, positively related with immature females of the south coast area and negatively related with the immature and mature females from northwest coast: EPA, positively

related with the immature females, and negatively related with the northwest coast mature females.

Table 2.6 - Two-way analysis of variance and hypothesis testing considering the applied bio-indicators, δ15N and δ13C, total lipids content (TL), Triacylglycerol content (tag), Cholesterol content (cho), Phospholipids content (phosp) determined for the upper and the lower beaks and the flesh, digestive gland and gonad by area and maturity stage (mat). Values in bold indicate statistical significance.

Tissue	Indicator	Between groups DF	Residual DF	ANOVA					
				area		mat		area x mat	
				F	P-value	F	P-value	F	P-value
Upper beak	$\delta^{13}C$	1	29	1.16	0.29	**5.68**	**0.02**	0.02	0.88
	$\delta^{15}N$	1	29	**25.99**	**<0.01**	0.03	0.87	0.06	0.80
Lower Beak	$\delta^{13}C$	1	29	2.03	0.16	**7.68**	**<0.01**	0.02	0.89
	$\delta^{15}N$	1	29	**11.98**	**<0.01**	0.08	0.78	0.06	0.80
Flesh	$\delta^{13}C$	1	23	0.28	0.60	4.00	0.06	0.44	0.51
	$\delta^{15}N$	1	23	0.62	0.44	0.02	0.88	1.83	0.19
Digestive Gland	TL	1	16	**5.88**	**0.03**	**5.88**	**0.03**	2.48	0.13
	tag	1	16	1.85	0.29	3.62	0.07	**4.68**	**0.05**
	cho	1	16	**62.54**	**<0.01**	0.13	0.72	**6.44**	**0.02**
	phosp	1	16	3.49	0.08	4.15	0.06	2.26	0.15
Gonad	TL	1	16	3.48	0.08	2.35	0.14	0.49	0.49
	tag	1	16	1.87	0.19	1.72	0.21	0.04	0.84
	cho	1	16	1.22	0.29	0.01	0.92	0.13	0.72
	phosp	1	16	0.94	0.34	**14.68**	**< 0.01**	1.44	0.25

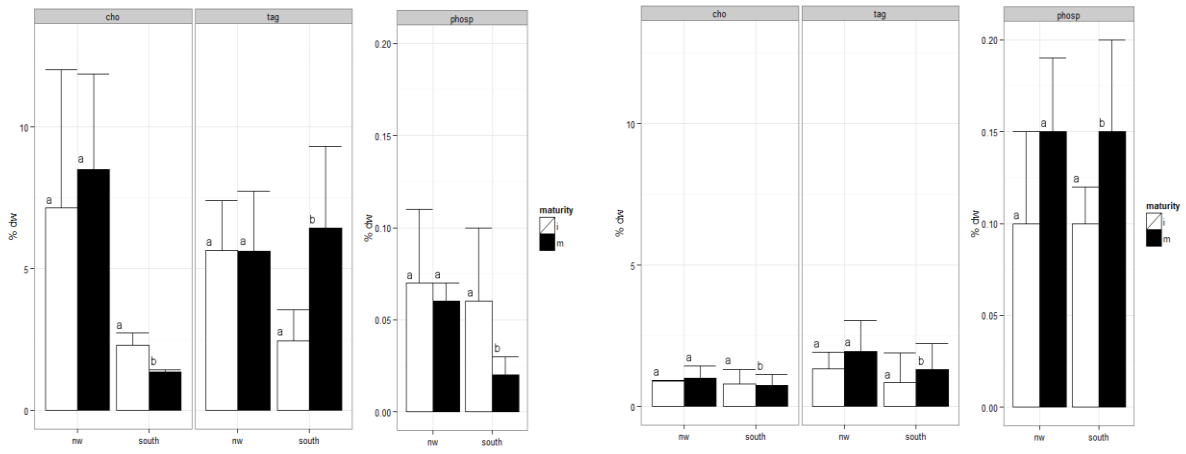


Figure 2.8 - Mean lipid classes' content in % dw (± SD) aggregated by tissue (digestive gland; gonad), by area (nw – Northwest coast; south – South coast) and by maturity stage (maturity; i - immature, m - mature). Different superscript letters indicate significant statistical differences ($p < 0.05$). The acronym cho stands for cholesterol, tag for triacylglycerol and phosp for phospholipids.

Table 2.7 - Total lipid content (mean ± SD, % dw) and major fatty acid (mean ± SD, g/100 g FAME) profiles in the digestive gland and gonad of immature and mature females collected in the northwest and south Portuguese coast. * indicates significant statistical difference (p-value < 0.05) between immature and mature individuals; ** indicates significant statistical difference (p-value < 0.05) in the fatty acid content between sampling areas.

	Digestive Gland						Gonad					
	northwest coast			south coast			northwest coast			South coast		
	immature	mature	mean	immature	Mature	mean	immature	mature	mean	immature	mature	mean
Total Lipid (TL)	14.60 ± 4.44	15.39 ± 4.22	15.00 ± 4.11**	16.97 ± 4.30*	24.42 ± 5.76*	20.69 ± 6.20**	15.39 ± 2.36	12.53 ± 3.27	13.69 ± 3.08	12.16 ±3.62	11.03±0.99	11.59 ± 2.57
Saturated fatty acids (SFA)												
C 14:0	4.99±1.58	4.89±2.05	4.94±1.75**	1.88±0.96	3.25±1.62	2.51±1.42**	4.19±0.48	4.09±0.36	4.14±0.40	4.06±0.72	3.94±0.52	3.99±0.58
C 16:0	22.62±4.35	18.34±5.71	20.48±5.33	22.08±14.61	18.48±9.58	20.44±12.14	19.25±1.98*	23.85±1.18*	21.73±2.83	18.78±2.44	22.31±3.50	20.83±3.48
C 18:0	13.73±4.27	11.63±0.32	12.68±3.09	12.88±1.58	11.21±1.78	12.12±1.80	8.68±1.33*	6.36±0.93*	7.43±1.62	7.47±1.26	5.90±1.00	6.56±1.33
Other SFA	6.21±2.98	6.60±2.66	6.40±2.70**	3.85±1.08	3.58±0.62	3.72±0.87**	2.19±0.22	2.25±0.45	2.22±0.35**	2.04±0.14	1.93±0.38	1.98±0.30**
Σ SFA	47.55±8.68	41.46±8.25	44.51±8.68	40.68±13.32	36.52±13.50	38.79±12.90	34.32±1.33*	36.55±0.85*	35.52±1.56**	32.36±1.58	34.08±3.41	33.37±2.83**
Monounsaturated fatty acids (MUFA)												
C 16:1n7	6.61±1.51	6.78±2.84	6.69±2.17**	3.05±0.52	4.11±1.34	3.53±1.08**	2.68±0.34*	2.05±0.42*	2.34±0.49	2.79±0.51	2.63±0.73	2.70±0.63
C 17:1	1.77±0.62	1.15±0.10	1.46±0.53	1.56±0.22*	1.25±0.26*	1.42±0.28	2.53±0.40	2.62±0.55	2.58±0.47**	2.26±0.42	1.90±0.42	2.05±0.44**
C 18:1n9	11.49±2.85	10.49±6.77	10.99±4.98	8.54±3.46	14.51±3.22	11.26±4.45	6.23±1.33	5.37±0.56	5.76±1.05	5.34±1.32	4.41±1.74	4.80±1.59
C 20:1n9	2.25±0.60	2.72±0.94	2.49±0.79**	3.33±1.08	3.98±0.96	3.34±1.08**	5.50±0.63*	4.48±0.21*	4.95±0.69**	6.11±1.18	7.90±6.51	7.15±4.95**
other MUFA[2]	2.19±0.98	1.78±0.86	1.98±0.90	2.23±0.78	3.04±1.08	2.60±0.97	3.50±0.40*	2.46±0.36*	2.94±0.65**	2.88±0.38	2.13±0.65	2.44±0.66**
ΣMUFA	24.30±3.76	22.92±9.26	23.61±6.78	18.19±3.22*	26.88±4.86*	22.14±5.94	20.45±1.21*	16.99±1.17*	18.59±2.13	19.39±1.45	18.97±7.69	19.15±5.31
Polyunsaturated fatty acids (PUFA)												
C: 20:4n6[3]	4.34±1.55	5.18±2.62	4.76±2.09**	7.64±3.22	6.06±2.06	6.92±2.75**	9.86±1.71*	7.51±0.96*	8.59±1.78	9.80±1.00	9.18±0.68	9.48±0.87
C 20:5n3	8.54±4.06	17.45±11.53	13.00±9.46	10.82±9.46	11.00±6.08	10.90±5.02	1.64±0.32	1.47±0.28	1.55±0.30	2.58±2.20*	1.14±0.20*	1.74±1.53
C 22:6n3	11.43±7.92	8.42±6.23	9.92±6.97**	19.54±8.70	16.28±8.97	18.06±8.54**	1.09±0.30*	0.27±0.22*	0.65±0.49	1.27±0.44*	0.67±0.31*	0.92±0.47
Other PUFA[4]	2.32±0.77	3.15±1.75	2.73±1.36**	1.92±0.30	1.88±0.41	1.91±0.34**	n.d	n.d	n.d	n.d	n.d	n.d
ΣPUFA	26.63±11.34	34.19±16.02	30.41±13.81	39.92±15.68	35.23±16.73	37.79±15.52	12.60±1.64	9.25±1.04	10.79±2.16	13.75±3.26	10.99±0.82	12.14±2.50
Σ FAME (ug/mg PS)	31.35±9.55	33.14±8.30	31.25±8.53**	48.79±11.56	43.40±9.59	46.34±10.56**	63.74±15.67	67.99±9.53	66.03±12.35	58.27±16.11	67.56±15.02	63.69±15.50
Σn-3/Σn-6	2.03±1.46	1.11±0.55	1.57±1.15	2.11±0.83	2.07±0.98	2.09±0.85	3.52±0.67*	5.07±0.76*	4.35±1.06	3.66±0.53	4.01±0.36	3.86±0.52
DHA/EPA	1.31±0.53	0.63±0.37*	0.97±0.56**	1.76±0.34	1.57±0.31	1.68±0.36**	0.66±0.10*	0.18±0.15*	0.40±0.28	0.65±0.03	0.57±0.05	0.60±0.23

[1] – other minor SFA C15:0, C17:0 and C24:0;
[2] – other minor MUFA C18:1n7, C22:1n9 and C24:1n9;
[3] – as the FA C20:3n3 and the FA C20:4n6 have the same retention time, and the concentration of the FA C20:4n6 is dominant in marine products, the concentration presented here is representative of C20:4n6.
[4] – other minor FA C18:2n6 and C20:2n6.

Table 2.8 - Multivariate analysis of variance (MANOVA) and hypothesis testing to the major fatty acids profile in the digestive gland and in the gonads grouped by the factors area and maturity (mat). P-value < 0.05 indicates significant statistical difference.

Tissue	Num Df	Denom DF	MANOVA								
			Area			maturity			area x mat		
			λ_{Wilks}	F_{Pillai}	p-value	λ_{Wilks}	F_{Pillai}	p-value	λ_{Wilks}	F_{Pillai}	p-value
Digestive Gland	10	12	0.13	7.96	0.001	0.17	5.84	0.003	0.36	1.79	0.186
Gonad	10	14	0.20	5.62	0.002	0.26	4.09	0.008	0.24	3.74	0.017

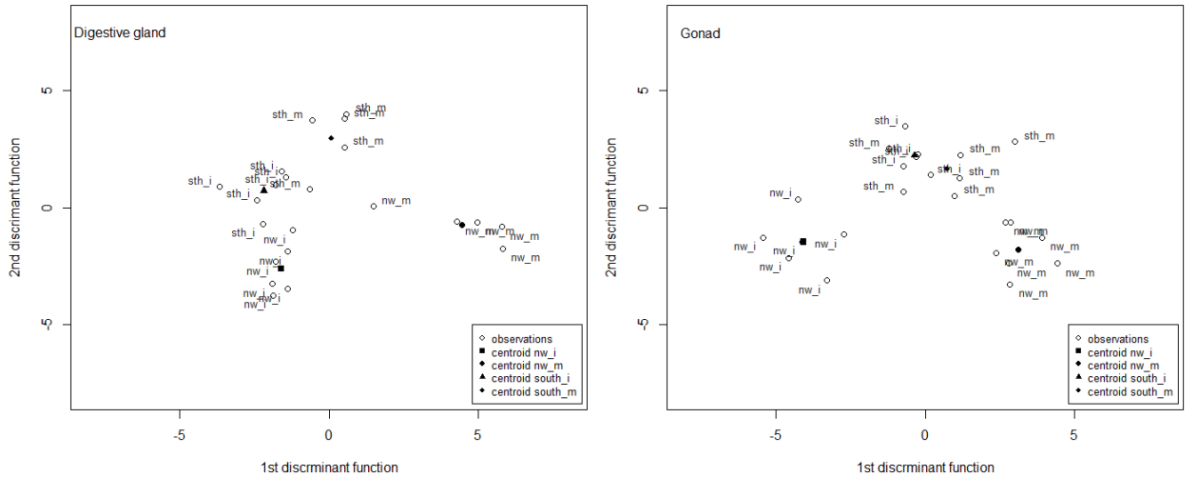


Figure 2.9 - Discriminant function analysis biplots of the fatty acid profiles of the digestive gland (on the left) and gonad (on the right). The acronym nw_i stands for the group immature females of northwest coast, nw_m stands for the group mature females of northwest coast, sth_i stands for the immature individuals of the south coast and sth_m stands for the mature individuals of south coast.

2.2.3.2. Maturity

Stable isotope signatures showed that the $\delta^{13}C$ signature is statistically significant different with the maturity stage (Table 2.6), with the beaks of immature females presenting significant higher $\delta^{13}C$ signature (Figure 2.7).

The effect of different nutritional levels on maturity was studied by assessing differences in the digestive gland and gonads with maturity. For the digestive gland, there was no effect of this factor in the lipid classes content studied (Table 2.6). Total lipid, cholesterol and triacylglycerol content tended to increase from immature to mature females although this increase is not statistically significant. In relation to the phospholipid content, the digestive glands of mature females of south coast were significantly poor in

phospholipids in relation to the immature ones ($t = 3.50$, $df = 18$, p-value < 0.01) (Figure 2.8). Nevertheless, when comparing the digestive glands lipid content between mature females of northern coast with mature females of south coast, some differences arose. Particularly, the total lipid content ($t = -2.82$, $df = 18$, p-value $= 0.01$), the cholesterol content ($t = 10.52$, $df = 18$, p-value < 0.01) and the phospholipids content ($t = 6.04$, $df = 18$, p-value < 0.01) were significantly higher in the mature digestive glands of south coast (Figure 2.8).

Mature gonads of the northwest coast present higher contents in triacylglycerol, cholesterol and phospholipids, although no significant differences were found for the mean content of those bio-indicators between immature and mature individuals (Figure 2.8, Table 2.6). In the south coast, the gonads of mature individuals are significant higher than immature individuals in triacylglycerol ($t = -2.86$, $df = 18$, p-value < 0.01) and phospholipids ($t = 3.50$, $df = 18$, p-value < 0.01).

The FA profile in the gonads was affected by area, maturity, and by the interaction of both factors (Table 2.8). Mature gonads were rich in SFA, showing the capacity to produce saturated fatty acids namely palmitic acid C16:0, increasing significantly from immature to mature females in the northwest coast. In opposition, the stearic acid C18:0 in the northwest coast decreased from immature to mature gonads (Table 2.7). MUFA were the second most abundant fatty acids in the gonads, with the presence of vaccenic acid C18:1n7 as a minor FA, presenting significant differences between the northwest coast and the south coast namely in the FA C17:1 and C20:1n9. The FA C16:1n7 was significantly more abundant in immature than in mature gonads from the northwest coast. The PUFA represented between 9% and 13% of the FAME present in the total lipid content of the gonads, with the content in ARA, DHA and EPA decreasing significantly from immature to mature gonads in both study areas. In particular, the significant decrease in DHA in the northwest coast leaded to significant differences between the DHA/EPA ratio of immature and mature females in that area (Table 2.7).

The DFA on the FA profile of the females' gonads between areas showed that the first function explained 79% of the variability , with a good separation between the northwest coast group (class score = 0.82) and the south coast group (class score = -0.97). The FA that contributed the most to the group separation is the C18:0 which is positively correlated with the northwest coast. The DFA for maturity groups showed a good separation between immature (class score = - 0.94) and mature individuals (class score =

0.79) with 74 % of the variability explained. The FA that contributed the most to the group separation was the C18:0, positively correlated to the immature individuals. The DFA for area x maturity groups (Figure 2.9, Gonad) showed a good separation between groups with an explained variability of 95% in concordance with MANOVA results. The FAs that contributed the most to the group separation were C16:0, 18:0 and ARA that were positively correlated with immature gonads from both areas.

2.2.4. Discussion

The $\delta^{15}N$ and $\delta^{13}C$ signatures in the flesh seem to be adequate short term indicators of diet and habitat. Cherel & Hobson (2005), working on *Psychroteuthis glacialis*, suggested that the difference between the $\delta^{15}N$ of the flesh relative to the beaks relates to the chitin synthesis and consequent N accretion in the beaks. In the more recently formed regions of the beaks, such as the wing, the $\delta^{13}C$ signature of the predator closely matches that of the prey (Hobson and Cherel, 2006), therefore also reflecting to an extent the recent dietary composition. The $\delta^{15}N$ and $\delta^{13}C$ of beaks are therefore used as indicators of habitat and diet preference (Post, 2002; Jackson *et al.* 2007). In our southern population of *O. vulgaris*, beak $\delta^{13}C$ signature is different between maturity stages, probably a reflection of the recent trophic history of the different maturation stages. The higher $\delta^{15}N$ ratio in the beaks from the south coast in relation to the northwest coast is probably related to the higher frequency of occurrence of crustacean prey (e.g. decapod crabs) found in the diet of *O. vulgaris* sampled (Lourenço, *unpublished data*). Rosa *et al.* (2004b) show that the decapod crabs are important prey of the *O. vulgaris* populations along the Portuguese coast and the presence of crustacean prey in the diet of *Loligo forbesi* is known to increases the $\delta^{15}N$ content of the beaks and flesh of that species (Stowasser 2004). Darnaude *et al.* (2004) relate increased $\delta^{15}N$ to a significant input from river plumes, through the added input of $\delta^{15}N$ rich particulate organic matter. This however does not appear to be the case of our study, since river input is more important on the west than on the south coast.

Considering that the digestive gland is mainly a lipid reservoir and therefore has a high lipid content, it is the ideal organ to trace fatty acids as diet bio-indicators (Phillips *et al.* 2001; Moltschaniwskyj & Johnston, 2006; García-Garrido *et al.* 2010). In our study the total lipid content ranged between 14.60 % in northwest coast immature females, in line with values obtained by Rosa *et al.* (2004a), and 24.42 % in south coast mature females.

Contrary to what might be expected for a nutrient rich area, the digestive glands of the northwest coast population present relatively low lipid content in comparison to the south coast population, in particular of the storage lipid triacylglycerol. This is probably related to nutrient seasonality. In March and April the pre-upwelling conditions in the northeast coast determine a low fat content in sardine *Sardina pilchardus* (Bandarra *et al.* 2006) and horse-mackerel *Trachurus trachurus* (Bandarra *et al.* 2001), while on the other hand phytoplankton peaks in the south coast are common in early spring associated with the warmer and nitrogen rich river plumes. Conditions in the south are then relatively favourable to the development of earlier phytoplankton blooms dominated by dinoflagellates (Navarro and Ruiz, 2006; Crespo *et al.* 2012), which is corroborated by the higher relative content in ARA, EPA and DHA produced by the community of primary producers (Dalsgaard *et al.* 2003).

The high digestive gland content of C18:1n9 (≈ 11 % in both study areas), which is indicative of deep waters prey species, and C16:0 (≈ 20% in both study areas) which is indicative of the presence of herbivorous preys, actually suggest a diverse diet (Piché *et al.* 2010). Comparing both areas, the DHA/EPA ratio is exceptionally low in the north-western population. According to Dalsgaard *et al.* (2003), the DHA content should be higher relative to the EPA for a carnivorous species. In their study Rosa *et al.* (2004b) observed the possibility of a carnivorous preys on the northwest coast, while this study highlights a dominance of a carnivorous preys towards the south coast. A higher triacylglycerol content in the South can in addition be related with the dominance of crustacean preys (Phillips *et al.* 2003) in the diet of the population of this area, which is also suggested by the $\delta^{15}N$ and $\delta^{13}C$ of the beaks.

It is therefore likely that during the period in analysis the diet of the south coast population had a relatively higher dominance of crustacean prey, those in turn recently fed on lipid-rich plankton.

As in the squids *Sepioteuthis lessoniana* and *Photololigo* sp. (Semmens, 1998), or the cuttlefish *Sepia officinalis* (Blanchier & Boucaud-Camou, 1984), the lipid class contents in the digestive gland do not correlate directly with the lipid contents of the oocytes in the gonad, especially for total lipid content, triacylglycerol and phospholipids (see Figure3.8).The lipid class contents of the digestive gland of mature females shows significant differences between regions but this regional difference is not found in the gonads of the same females, which seems to indicate that the factors triggering oocytes

maturity are independent of the nutritional value of reserves, even though females generally reach maturity beyond the optimum body mass and independently of gonad size (Lourenço *et al.* 2012). As income breeders that channel the energy directly from food to the gonad during maturation (Houston *et al.* 2006; Quetglas *et al.* 2011), the different levels in triacylglycerol and cholesterol noted in the *O. vulgaris* digestive glands between geographical areas should be expected to show in the gonads as a reflection of diet. Despite that, only the gonad fatty acid profile presents significant differences related with both area and maturity stage evidencing that the composition changes as maturation progresses but also that there are differences between areas at every stage of the process. The gonads of the south coast are richer in PUFA and in MUFA and the gonads of the northwest coast are richer in SFA especially C16:0 and C18:0. Nevertheless, the differences found in the ratio DHA/EPA between areas are marginally insignificant ($t = -1.86$, $df = 18$, p-value = 0.08), indicating a low impact of trophic habitat in the quality of oocytes. These results partially agree with the results obtained by Farías *et al.* (20115). In that study, the authors found a direct effect of diet in the DHA/EPA ratio for the Patagonian red octopus *Enteroctopus megalocyathus,* here, although FA profile in the gonads revealed different between areas, the contents in DHA, EPA or DHA/EPA ratio are identical between the studied areas.

In conclusion, this study shows that, despite the variability observed in the stable isotopic signatures studied, $\delta^{15}N$ signature in beaks is a valuable bio-indicator to follow the diet history of different *O. vulgaris* populations, and the $\delta^{13}C$ signature has potential to assess feeding behaviour changes related with maturity in *O. vulgaris* females. Furthermore, specific lipid classes as the triacylglycerol and the cholesterol and its content in the digestive gland can be used as indicators of their nutritional availability in the local habitat. The fatty acid profile is a good indicator of both nutritional availability in the environment and also of gonads quality as shown by the main EFA content and the EPA/DHA ratio. The environmental availability of these EFA depends on seasonal cycles. Upwelling/downwelling events for example determine changes in the lipidic contents of the plankton and in turn of the prey of octopus, as evidenced by the higher total lipids and cholesterol content in the digestive glands of the animals from the south coast. In spite of their characteristics as income breeders, it is difficult to observe a direct relationship between the lipid class contents in the digestive gland and in the gonad, suggesting that a measure of regulation is exerted between food intake and oocyte formation.

ACKNOWLEDGEMENTS

The authors wish to express their acknowledgment to the anonymous reviewers for their comments, which greatly improved the final version of this paper. The authors also want to thank to António Pedro Mendonça for assistance with the biological sampling and to Cristina Vidal for teaching and supervising the biochemical analyses. Data collection and analytical analysis were conducted under the Data Collection Framework (DCF/DG FISH (EC Regulation No. 665/2008 of the 14 July 2008)) and LARECO project (CTM2011-25929), supported partially by FEDER funds. The first author is supported by a PhD fellowship granted by the Portuguese Foundation for Science and Technology (FCT).

BIBLIOGRAPHIC REFERENCES

ATHENSTAEDT, K. & DAUM, G. 2006. The life cycle of neutral lipids: synthesis, storage and degradation. *Cellular and Molecular Life Sciences* **63**, 1355–1369.

AUBORG, S., MEDINA, I. & PÉREZ-MARTIN, R. 1996. Polyunsaturated fatty acids in tuna phospholipids: Distribution in Sn – 2 location and changes during cooking. *Journal of Agricultural and Food Chemistry* **44**, 585-589.

BANDARRA, N., BATISTA, I., NUNES, M. & EMPIS, J. 2001. Seasonal variation in the chemical composition of horse-mackerel (*Trachurus trachurus*). *European Food Research and Technology* **212**, 535–539.

BANDARRA, N. M., BATISTA, I., NUNES, M. L., EMPIS, J. M. & CHRISTIE, W.W. 2006. Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). *Journal of Food Science* **62**, 40–42.

BERGÉ, J.-P. & BARNATHAN, G. 2005. Fatty Acids from Lipids of Marine Organisms: Molecular Biodiversity, Roles as Biomarkers, Biologically Active Compounds, and Economical Aspects, in: Ulber, R., Gal, Y. (Eds.), *Marine Biotechnology* I. pp. 49–125.

BLANCHIER, B. & BOUCAUD-CAMOU, E. 1984. Lipids in the digestive gland and the gonad of immature and mature *Sepia officinalis* (Mollusca: Cephalopoda). *Marine Biology* **80**, 39–43.

BOLETZKY, S. V, 1975. A contribution to the study of yolk absorption in the Cephalopoda. *Zeitschrift für Morphologie der Tiere* **80**, 229-246.

BOUCAUD-CAMOU, E. & BOUCHER-RODONI, R. 1983. Feeding and Digestion in Cephalopods, in: Wilburn, K.M (Ed). The Mollusca. Academic Press, pp. 149–187.

BUDELMANN, B.U., SCHIPP, R. & BOLETZKY, S. v. 1997. Cephalopoda, in: Harrison F. W.(Ed). Microscopic Anatomy of Invertebrates. Wiley-Liss, pp. 119–414.

CHEREL, Y. & HOBSON, K. A. 2005. Stable isotopes, beaks and predators: a new tool to study the trophic ecology of cephalopods, including giant and colossal squids. *Proceedings of the Royal Society B: Biological Sciences* **272**, 1601–1607.

CHEREL, Y., HOBSON, K. A. & WEIMERSKIRCH, H. 2005. Using stable isotopes to study resource acquisition and allocation in Procellariiform seabirds. *Oecologia* **145**, 533-540.

CHEREL, Y. & HOBSON, K. A. 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Marine Ecology Progress Series* **329**, 281–287.

CHEREL, Y., RIDOUX, V., SPITZ, J. & RICHARD, P. 2009. Stable isotopes document the trophic structure of a deep-sea cephalopod assemblage including giant octopod and giant squid. *Biology Letters* **5**, 364–367.

CRESPO, B.G., ESPINOZA-GONZÁLEZ, O., TEIXEIRA, I.G., CASTRO, C.G. & FIGUEIRAS, F. G. 2012. Structure of the microbial plankton community in the NW Iberian margin at the end of the upwelling season. *Journal of Marine Systems* **95**, 50–60.

DALSGAARD, J., ST JOHN, M., KATTNER, G., MÜLLER-NAVARRA, D. & HAGEN, W. 2003. Fatty acid trophic markers in the pelagic marine environment. *Advances in marine biology* **46**, 225–340.

DARNAUDE, A.M., SALEN-PICARD, C., POLUNIN, N.V.C. & HARMELIN-VIVIEN, M. L. 2004. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). *Oecologia* **138**, 325–332.

FARÍAS, A., NAVARRO, J.C., CERNA, V., PINO, S. & URIARTE, I. 2011. Effect of broodstock diet on the fecundity and biochemical composition of eggs of the Patagonian red octopus (*Enteroctopus megalocyathus* Gould 1852). *Ciencias Marinas* **37**, 11–21.

GARCÍA-GARRIDO, S., HACHERO-CRUZADO, I., GARRIDO, D., ROSAS, C. & DOMINGUES, P. 2010. Lipid composition of the mantle and digestive gland of *Octopus vulgaris* juveniles (Cuvier, 1797) exposed to prolonged starvation. *Aquaculture International* **18**, 1223–1241.

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 Part II* **53**, 1182–1197.

GUERRA, A. 1975. Determinación de las diferentes fases del desarollo sexual de *Octopus vulgaris* Lamarck, mediante unín dice de madurez. *Investigacion. Pesquera* **39**, 397-416.

HOBSON, K.A. & CHEREL, Y. 2006. Isotopic reconstruction of marine food webs using cephalopod beaks: new insight from captively raised *Sepia officinalis*. *Canadian Journal of Zoology* **84**, 766–770.

HOUSTON, A.I., STEPHENS, P.A., BOYD, I.L., HARDING, K.C. & MCNAMARA, J. M. 2006. Capital or income breeding? A theoretical model of female reproductive strategies. *Behavioral Ecology* **18**, 241–250.

JACKSON, G.D., BUSTAMANTE, P., CHEREL, Y., FULTON, E.A., GRIST, E.P.M., JACKSON, C.H., NICHOLS, P.D., PETHYBRIDGE, H., PHILLIPS, K., WARD, R.D. & XAVIER, J. C. 2007. Applying new tools to cephalopod trophic dynamics and ecology: perspectives from the Southern Ocean Cephalopod Workshop, February 2–3, 2006. *Reviews in Fish Biology and Fisheries* **17**, 79–99.

KANAZAWA, A. 2002. Sterols in marine invertebrates. *Fisheries Science* **67**, 997–1007.

KATSANEVAKIS, S., & VERRIPOULOS, G. 2004. Den ecology of *Octopus vulgaris* Cuvier, 1797, on soft sediment: availability and types of shelter. *Scientia Marina* **68**, 147–157.

LEE, R.F., HAGEN, W. & KATTNER, G. 2006. Lipid storage in marine zooplankton. *Marine Ecology Progress Series* **307**, 273–306.

LOURENÇO, S., MORENO, A., NARCISO, L., GONZALEZ, Á. & PEREIRA, J. 2012. Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. *Fisheries Research* **127-128**, 116–124.

MOLTSCHANIWSKYJ, N. & JOHNSTON, D. 2006. Evidence that lipid can be digested by the dumping squid *Euprymna tasmanica*, but is not stored in the digestive gland. *Marine Biology* **149**, 565–572.

NAVARRO, G. & RUIZ, J. 2006. Spatial and temporal variability of phytoplankton in the Gulf of Cádiz through remote sensing images. *Deep Sea Research Part II* **53**, 1241–1260.

NAVARRO, J.C. & VILLANUEVA, R. 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. *Aquaculture* **183**, 161–177.

NIXON, M., 1985. Capture of prey, diet and feeding of *Sepia officinalis* and *Octopus vulgaris* (Mollusca: Cephalopoda) from hatchling to adult. *Vie et Milieu* **35**, 255–261.

PHILLIPS, K.L., JACKSON, G.D. & NICHOLS, P. D. 2001. Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Islands: stomach contents and fatty acid analyses. *Marine Ecology Progress Series* **215**,179–189.

PHILLIPS, K.L., NICHOLS, P.D. & JACKSON, G.D. 2003. Dietary variation of the squid *Moroteuthis ingens* at four sites in the Southern Ocean: stomach contents, lipid and fatty acid profiles. *Journal of the Marine Biological Association of the UK* **83**, 523–534.

PICHÉ, J., IVERSON, S.J., PARRISH, F.A. & DOLLAR, R. 2010. Characterization of forage fish and invertebrates in the Northwestern Hawaiian Islands using fatty acid signatures: species and ecological groups. *Marine Ecology Progress Series* **418**, 1–15.

POST, D. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* **83**, 703–718.

POST, D. M., LAYMAN, C. A., ALBREY, ARRINGTON, D. A., TAKIMOTO, G., QUATTROCHI, J. & MONTAÑA, J. C. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotopes analyses. *Oecologia* **152**, 179-189.

QUETGLAS, A., ORDINES, F. & VALLS, M. 2011. What drives seasonal fluctuations of body condition in a semelparous income breeder octopus*? Acta Oecologica* **37**, 476–483.

QUINN, G. & KEOUGH, M. 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press.

RELVAS, P., BARTON, E.D., DUBERT, J., OLIVEIRA, P.B., PELIZ, Á., DA 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.

ROSA, R., COSTA, P.R. & NUNES, M. L. 2004a. Effect of sexual maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca: Cephalopoda). *Marine Biology* **14**, 563-574.

ROSA, R., MARQUES, A.M., NUNES, M.L., BANDARRA, N. & SOUSA REIS, C. 2004b. Spatial-temporal changes in dimethyl acetal (octadecanal) levels of *Octopus vulgaris* (Mollusca, Cephalopoda): relation to feeding ecology. *Scientia Marina* **68**, 227–236.

SEMMENS, J. 1998. An examination of the role of the digestive glands of two loliginid squids, with respect to lipid: storage or excretion? *Proceedings of the Royal Society B* **265**, 1685–1690.

SEMMENS, J., PECL, G., VILLANUEVA, R., JOUFFRE, D., SOBRINO, I., SOBRINO, I., WOOD, J.B., & RIGBY, P. R. 2004. Understanding octopus growth: patterns, variability and physiology. *Marine and Freshwater Research* **55**, 367–377.

SHULMAN, G.E. & LOVE, R. M. 2006. The Biochemical ecology of Marine fishes Advances in Marine Biology. Advances in Marine Biology 36: 351 pp.Sieiro, M.P., Aubourg, S.P. & F. Rocha, 2006. Seasonal study of the lipid composition in different tissues of the common octopus (*Octopus vulgaris*). *European Journal of Lipid Science and Technology* **108**, 479–487.

SMITH, C.D. 2003. Diet of *Octopus vulgaris* in False Bay, South Africa. *Marine Biology* **143**, 1127–1133.

STOWASSER, G. 2004. Squid and their prey: insights from fatty acid and stable isotope analysis. Thesis presented for the degree of doctor of Philosophy at the University of Aberdeen. University of Aberdeen. Aberdeen.

TOCHER, D.R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research* **41**, 717–732.

VANDER ZANDEN, M.J. & RASMUSSEN, J. B. 2001. Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. *Limnology and oceanography* **46**, 2061–2066.

VILLANUEVA, R. & NORMAN, M. D. 2008. Biology of the planktonic stages of benthic octopuses. *Oceanography and Marine Biology: An Annual Review* **46**, 105–202.

ZAR, J. 1999. Biostatistical Analysis. 4th Edition. Prentice Hall.

ZUUR, A.F., IENO, E.N. & SMITH, G. M. 2007. Analysing Ecological data. Springer.

CHAPTER 3

HABITAT REQUIREMENTS OF PARALARVAE AND JUVENILES OF *OCTOPUS VULGARIS*

3. HABITAT REQUIREMENTS FOR *OCTOPUS VULGARIS* PARALARVAE AND JUVENILES

3.1. THE RÍA DE VIGO OCTOPUS VULGARIS SPAWNING GROUND: LOCAL FACTORS INFLUENCING THE DISTRIBUTION AND ABUNDANCE OF PARALARVAE

ABSTRACT

The Ría de Vigo (Southwestern Galicia) is an important spawning ground for the *Octopus vulgaris* population of the north-western Atlantic coast. The large-scale oceanographic conditions that are linked to the abundance and distribution peaks of the paralarvae have been extensively discussed. Nevertheless, the local micro-scale factors that favour the paralarvae concentration in Ría de Vigo have been less well studied. With this study, the correlation between local variables on *O. vulgaris* paralarvae abundance were analysed. The correlation of water column depth, distance from coast, and zone were analysed during the season of higher paralarvae abundance (the autumn). Our results have shown that the depth of the water column and the distance to the coast have a significant correlation with the paralarvae abundance. The importance of those results is discussed under the insight of the role of the Ría de Vigo as an essential habitat for the *O. vulgaris* population of the northwestern Atlantic coast.

Keywords: spawning ground, paralarvae, local abundance.

3.1.1. Introduction

Octopus vulgaris is a neritic benthic species, common in the coastal waters between shore and approximately 100 m depth (Mangold, 1983). Its distribution extends to the edge of the continental shelf but most animals aggregate in the coastal waters at a relatively short distance from the coastline (Quetglas *et al.*, 2000). The paralarvae are pelagic from hatching to settlement (Villanueva & Norman, 2008), and this transition to the juvenile benthic life style depends biologically on feeding opportunities (energy input) and environmentally on the local temperature (Villanueva, 1995; Faure *et al.*, 2000; Iglesias *et al.*, 2004). The choice of the ground to settle depends of the dispersal of the paralarvae during the pelagic phase, but bottoms of large grain sediments, namely gravel and coarse sand, and the proximity to rocky reefs appear to be beneficial to the juvenile and sub-adult recruitment success (Katsanevakis & Verriopoulos, 2004; Villanueva & Norman, 2008; Moreno *et al.*, 2014).

Different environmental factors (e.g. sea surface (SST) and bottom (SBT) temperatures, depth, water-column stratification) have different impacts in *O. vulgaris* survival and growth. The level of impact depends of the life cycle stage following important shifts in each life cycle transition (see Robin *et al.,* 2014 for a review in life cycle transitions). Nevertheless, in the case of *O. vulgaris,* the degree of influence of environmental (mainly oceanographic) conditions is considered to be higher during the paralarval stage affecting the present generation recruitment to fisheries (Otero *et al.*, 2008; Roura *et al.*, 2013). In the last fourteen years several studies have been conducted to understand the effect of the oceanographic parameters on the distribution and abundance of *O. vulgaris* paralarvae in the vicinities of important fishing areas. Those studies have been targeting the effect of large and meso-scale indicators such as sea surface temperature (Demarcq & Faure, 2000; Moreno *et al.*, 2009) or coastal upwelling (Faure *et al.*, 2000; Gonzalez *et al.*, 2005; Otero *et al.*, 2008, 2009), but few have been conducted on the environmental conditions at the local scale as *in situ* temperature, water column stratification, bottom characteristics or distance to the coast line.

The coastal areas, particularly those in the vicinity of important estuaries, or those featuring large inlets (e.g. Northwest Spanish coast) are considered important spawning grounds and nurseries for most of the exploited marine fish and invertebrate species (Able, 2005; Pallas *et al.*, 2006; Vasconcelos *et al.*, 2008; Morais *et al.*, 2011). Larvae and

juvenile stages of several species grow in these areas to the adult stage, then often undergoing ontogenic migrations to different habitats (Able, 2005).

For marine invertebrate species with complex life cycles, with pelagic phases that carry out medium- or large-scale dispersion movements and benthic phases of limited mobility, the definition of nursery ground is more complex (Stoner, 2003; Pallas *et al.*, 2006). These species depend on larval dispersion to enhance survival and the definition of a nursery ground should add temporal (e.g. season) and physical dimensions (e.g. temperature, dispersion currents, water column stratification) to the spatial factors (e.g. sediments, depth).

Shallow inlets such as the "Rías" are structurally complex environments where many biotic (e.g. predation, food availability) and abiotic factors (e.g. physical disturbance) determine habitat quality and generate different stress gradients that determine community structure and population dynamics (Pallas *et al.*, 2006). The most important stress gradients impact at the micro (meters) or meso-scales (hundreds of meters or kilometers) and as a response to this variability, benthic organisms might select their habitat to reduce the trade-offs among different stress factors (Pallas *et al.*, 2006).

The identification of essential habitat (essential fish habitats, EFH) for *O.vulgaris* requires knowledge about how environmental and biological factors affect the survival of pre- and post-settlement juveniles both at the micro-scale and meso-scales (Moreno *et al.*, 2014). Considering this, the aim of this study is to assess the local effect of some factors (water column depth, zone and distance to coast) in the abundance of *O. vulgaris* paralarvae in the spawning grounds around the Cies Islands, in Ría de Vigo.

3.1.2. Material and Methods

To determine *Octopus vulgaris* paralarvae abundance near the Cies islands, a survey was conducted using a 750 mm diameter bongo net of 375 μm, equipped with a mechanical flowmeter (Figure 3.1). Sampling was conducted at night in four transects and two tows per transect at 2 knots. The tows were conducted during 20 min near surface (between 10 and 15 m depth) according to the depth strata of higher abundance of paralarvae (see Otero *et al.*, 2009 and Roura, 2013 for methods).

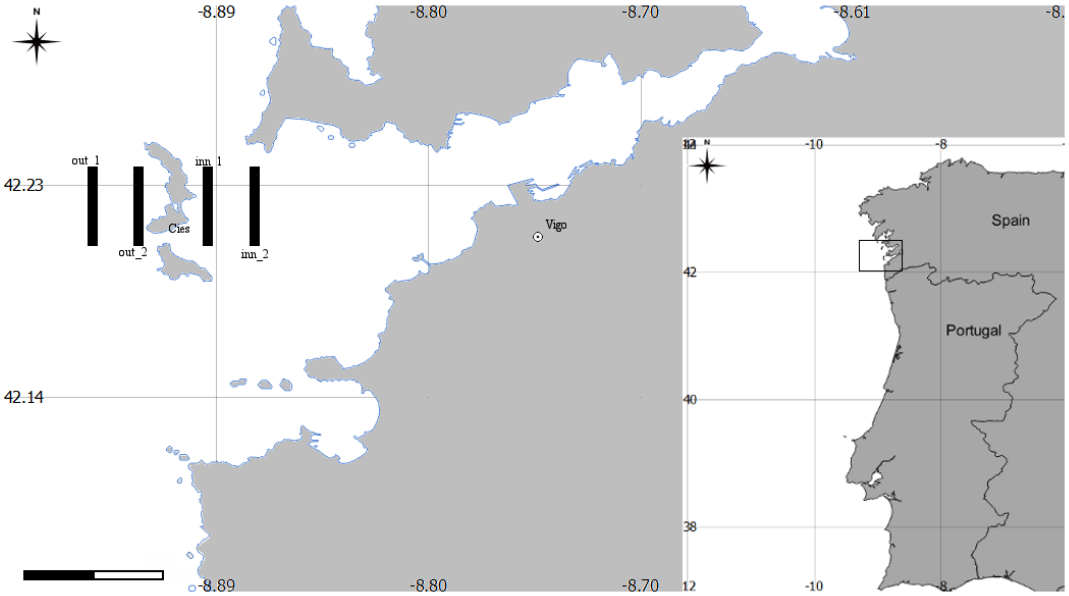


Figure 3.1 - Geographical position of the sampling area in Ría de Vigo, Spanish northwest Atlantic coast. Legend: lines indicate the sampling transects (out and inn transects). The scale bar corresponds to 3.5 km.

Mean abundance (n/1000 m^3) of *O. vulgaris* paralarvae were determine by transect and compared considering the factors transect water column depth (depth), distance to the Cies Islands (dland) and sampling zone (zone). Sample abundance results were tested for the assumptions of normality and homogeneity with Shapiro-Wilk's and Bartlett´s tests, respectively. The main effects and interaction between the factors depth, dland and zone on the mean abundance were determined by two-way ANOVA with permutation test, re-sampling the original data 10 times to generate a sampling distribution F against which to test H0 and obtain a probability measure. The hypothesis for the two-way ANOVA was that there is no main effect of depth, dland and zone and no interaction effect of depth × dland, depth × zone and dland × zone on paralarval mean abundance. The existence of collinearity between factors was assessed by determining the spearman correlation index ($r_{spearman}$), assuming that two factors were collinear when $r_{spearman}$ was significantly higher than 0.90.

3.1.3. Results

The average abundance (n/1000 m^3 ± SD) of *O. vulgaris* paralarvae was determined by transect (Table 3.1). Higher paralarval abundance was observed in station inn_1 in the inner zone (19.73 ± 4.32 n/1000 m^3) followed by station out_2 close to the outside part of

the islands in the Ría de Vigo. The lower average abundance occurs in the more distant transect of outer zone, out_1 with 0.92 ± 1.30 n/1000^3.

Despite the differences found, there is no statistical evidence that the outer zone and the inner zone present significantly different levels of paralarval abundance (Table 3.2). The transects both sides of the Cies islands presented higher abundances than the more distant transects (out_2 and inn_1 transects) (Table 3.1), this relationship observed between the average abundance of paralarvae and the vicinity of the Cies Islands is proved with the ANOVA results for the factor dland in Table 3.2.

The shallower transects (out_2 and inn_1) were the ones where paralarval average abundance was higher. Those differences were confirmed by the ANOVA results, presenting statistical differences between transects with different water column depths. It is important to note here that the factors dland and depth are not collinear ($r_{spearman}$= 0.44, p-value = 0.27).

Table 3.1 - Stations geographic position, zone, depth and distance to the Cies Island and *Octopus vulgaris* paralarvae mean abundance determined by station. Legend: Dland – distance to the shoreline.

Transect	Zone	Latitude (dg N)	Longitude (dg W)	Depth (m)	Dland (m)	Mean abundance (a ± SD, n/1000^{3}m)
out_1	out	42.22	8.94	80	421.2	0.92 ± 1.30
out_2	out	42.22	8.92	60	143.7	4.55 ± 6.44
inn_1	inn	42.22	8.89	15	143.7	19.73 ± 4.32
inn_2	inn	42.22	8.87	34	421.2	2.31 ± 1.31

Table 3.2- Results of the two-way analysis of variance for the factors main effects and interaction effects between sampling zone (zone), distance to coast (dland) and transect water column depth (depth) in terms of abundance of *Octopus vulgaris* paralarvae. Legend: DF_{WG} – Within groups degrees of freedom; DF_{BG} – Between groups degrees of freedom; $DF_{residual}$ – Residual degrees of freedom; * identifies statistical significance (p-value < 0.05).

	Factor	DF_{WG}	DF_{BG}	$DF_{residual}$	p-value
Main effects	Zone	1		6	0.086
	Dland	1		6	< 0.001*
	$depth_{wc}$	1		6	< 0.001*
Factors interaction	dland x zone		1	4	0.047*
	depth x zone		1	4	0.047*
	depth x dland		1	4	0.047*

The two-way ANOVA results on the interactions between the three factors show that those interactions have a significant effect on the abundance of the paralarvae (Table 3.2 and Figure 3.2). Despite the fact that the factor zone had not a significant effect on the paralarvae abundance, the inner zone of the Ría de Vigo is associated with shallower waters that were associated with the higher abundances of *O. vulgaris* paralarvae (Figure 3.2), clearly benefiting of the barrier and protection effect of the Cies Islands.

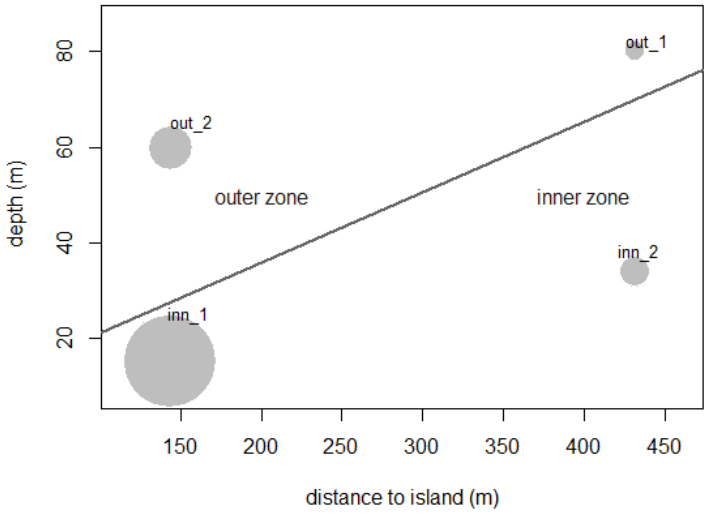


Figure 3.2 - Interaction effects of the factors distance to island, water column depth and zone in the *O. vulgaris* paralarvae abundance. Different circle sizes mean different abundances levels in n/10003m. Legend: out_1_{Abund} = 0.13 n/1000^{3}m; out_2_{Abund} = 4.55 n/1000^{3}m; inn_1_{Abund} = 19.73 n/1000^{3}m; inn_2_{Abund} = 2.23 n/1000^{3}m.

3.1.4. Discussion

The analysis conducted here showed that additionally to SST, physical disturbance or productivity, there are local factors as the water column depth and the distance to the coast that have an important effect in the distribution and abundance of paralarvae, at least at the local scale.

All of the paralarvae captured here and the majority of the paralarvae captured in previous studies conducted in the same study area (Gonzalez *et al.*, 2005; Roura, 2013) show 3 suckers on the arms and weigh on average 2.5 mg indicating that they are newly hatched (Villanueva 1995; Villanueva & Norman, 2008; Roura, 2013) and confirming this area as a spawning ground. During the autumn hatch peak (our sampling season), Roura (2013) described the dispersion mechanism of paralarvae from this area. According to his

work, during September/October upwelling conditions, the paralarvae concentrate in the surface waters and are washed out from the inlet due to the offshore currents. This is, probably, the mechanism responsible for "feeding" up some of the winter juvenile (pre-recruits) grounds identified by Moreno *et al*. (2014).

According to Villanueva & Norman (2008) most benthic octopus species with pelagic paralarvae spawn in shallow rocky or coral substratum areas, and consequently their hatchlings are more abundant near the coastline.

This link between important spawning grounds, feeding grounds (or nurseries) and favorable transport conditions was also identified by Garofalo *et al.* (2010) for the *O. vulgaris* population of the Strait of Sicily. There, the spatial distribution of the *O. vulgaris* spawning grounds and nurseries is controlled by the meso-scale eddies that favor the migration of the paralarvae to south-western feeding grounds. The circulation is further modified by the formation of meso-scale eddies, which appear to control the spatial distribution of the spawning and nursery grounds of *O. vulgaris* in the strait of Sicily. Spawning octopuses concentrate in the northern and central sectors of the Strait of Sicily at the margin of fronts or eddies, the presence of which favors larval retention and benthic settlement, promoting the formation of nursery areas close to the spawning grounds (Garofalo *et al.*, 2010).

In the present study, we show that the Cies Islands influence the abundance of the paralarvae inside the Ría de Vigo system. The Cies Islands possibly act as a barrier and protection for the *O. vulgaris* breeding females and consequentially for the newly hatched paralarvae wich have limited swimming capacity. Here, the paralarvae start to feed mainly of zoeae (Roura *et al.*, 2012) of decapod species of the families Paguridae, Palaemonidae and Procellanidae which live in these shallow inlets (Lindley, 1982; Sampedro *et al.*, 1997; Valdés *et al.*, 2007).

The functional role of the Ría de Vigo as a nursery is not fully understood because few or no paralarvae with more than 3 suckers per arm (sucker number at hatching, Villanueva, 1995) have been found in the several studies conducted until now. Nevertheless, there is evidence (Guerra *pers comm*) of mature females looking for shallow grounds in the surroundings of the Cies Islands to spawn. Furthermore, Otero *et al.* (2008) and Roura *et al.* (2013) found out that the local environmental conditions as wind stress and the strength of downwelling/upwelling events prior and during hatch in this region highly explains the abundance and biomass paralarvae abundance and particularly the

year-to-year variability of the adult catches for the entire Galician coast. Additionally, comparing the studies conducted by Moreno *et al*. (2009) and by Roura (2013), the abundances obtained by the latter for the Ría de Vigo (a maximum abundance of in Out_2 transect of 395 n/1000^3 m in 2008) seem to be higher than the abundances estimates obtained by the former for the entire portuguese coast (maximum abundance in autum in the north coast of 6 n/100^3) for a 18 years survey data.

Although oceanic features appear to be the main driving forces that explain why Rías Baixas is an important spawning ground, this preliminary study showed that at the local level other factors determine the abundance of newly hatched *O. vulgaris* and these may also contribute to explain why this area can be considered an EFH to the Northwestern Atlantic coastal population of *O. vulgaris.*

ACKNOWLEDGMENTS

The author wants to acknowledge the help ofthe crew of RV "Mytilus", Alvaro Roura and Marcos Regueira while conducting zooplankton surveys. Data collection was supported by the Portuguese Foundation for Science and Technology (FCT). The first author is supported by a PhD fellowship granted by FCT.

BIBLIOGRAPHIC REFERENCES

ABLE, K.W. 2005. A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. *Estuarine, Coastal and Shelf Science* **64**: 5–17.

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.

FAURE, V., INEJIH, C.A., 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.

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.

GONZALEZ, A.F., OTERO, J., GUERRA, Á., PREGO, R., ROCHA, F. & 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.

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.

KATSANEVAKIS, S. & VERRIOPOULOS, G. 2004. Abundance of *Octopus vulgaris* on soft sediment. Scientia Marina **68**: 553–560.

LINDLEY, J.A. 1982. Population dynamics and production of euphausiids. *Marine Biology* **66**: 37–46.

MANGOLD, K. 1983. *Octopus vulgaris*. In: Boyle, P. (ed) Cephalopod Life Cycles. Academic Press, London, pp. 335–386.

MORAIS, P., DIAS, E., BABALUK, J. & ANTUNES, C. 2011. The migration patterns of the European flounder Platichthys flesus (Linnaeus, 1758) (Pleuronectidae, Pisces) at the southern limit of its distribution range: Ecological implications and fishery management. Journal of Sea Research **65**: 235–246.

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.

MORENO, A., LOURENÇO, S., PEREIRA, J., GASPAR, M.B., CABRAL, H.N., PIERCE, G.J. & SANTOS, A.M.P. 2014. Essential habitats for pre-recruit *Octopus vulgaris* along the Portuguese coast. *Fisheries Research* **152**: 74–85.

OTERO, J., ÁLVAREZ-SALGADO, X., GONZÁLEZ, Á.F., GILCOTO, M., GUERRA, Á. 2009. High-frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. *Marine Ecology Progress Series* **386**: 123–132.

OTERO, J., ÁLVAREZ-SALGADO, X., GONZÁLEZ, Á.F., MIRANDA, A., GROOM, S., CABANAS, J., CASAS, G., WHEATLEY, B. & GUERRA, Á. 2008. Bottom-up control of common octopus *Octopus vulgaris* in the Galician upwelling system, northeast Atlantic Ocean. *Marine Ecology Progress Series* **362**: 181–192.

PALLAS, A., GARCIA-CALVO, B., CORGOS, A., BERNARDEZ, C. & FREIRE, J. 2006. Distribution and habitat use patterns of benthic decapod crustaceans in shallow waters: a comparative approach. *Marine Ecology Progress Series* **324**: 173–184.

QUETGLAS, A., CARBONELL, A. & SÁNCHEZ, 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.

ROBIN, J-P., ROBERTS, M., ZEIDBERG, L., BLOOR, I., RODRIGUEZ, A., BRICEÑO, F., DOWNEY, N., MASCARÓ, M., NAVARRO, M., GUERRA, A., HOFMEISTER, J., BARCELLOS, D.D., LOURENÇO, S.A.P., ROPER, C.F.E., MOLTSCHANIWSKYJ, N.A., GREEN, C.P., MATHER, J. 2014. Chapter Four - Transitions During Cephalopod Life History: The Role of Habitat, Environment, Functional Morphology and Behaviour, In: Erica A.G. Vidal (Ed.), *Advances in Marine Biology* **67**,361–437.

ROURA, Á., 2013. Ecology of planktonic cephalopod paralarvae in coastal upwelling systems. Universidade de Vigo, Vigo, Spain.

ROURA, Á., ÁLVAREZ-SALGADO, X.A., GONZÁLEZ, Á.F., GREGORI, M., ROSÓN, G. & GUERRA, Á. 2013. Short-term meso-scale variability of mesozooplankton communities in a coastal upwelling system (NW Spain). *Progress in Oceanography* **109**: 18–32.

ROURA, Á., GONZÁLEZ, Á.F., REDD, K. & GUERRA, Á. 2012. Molecular prey identification in wild *Octopus vulgaris* paralarvae. *Marine Biology* **159**: 1335–1345.

SAMPEDRO, M. P., FERNANDEZ, L., FREIRE, J. & GONZALEZ-GURRIARAN, E. 1997. Fecundity and reproductive output of Pisidia longicornis (Decapoda, Anomura) in the Ría de Arousa (Galicia, Nw Spain). *Crustaceana* **70**: 95 -110.

STONER, A.W. 2003. What constitutes essential nursery habitat for a marine species? A case study of habitat form and function for queen conch. *Marine Ecology Progress Series* **257**: 275–289.

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.

VASCONCELOS, R.P., REIS-SANTOS, P., TANNER, S., MAIA, A., LATKOCZY, C., GÜNTHER, D., COSTA, M.J. & CABRAL, H. 2008. Evidence of estuarine nursery origin of five coastal fish species along the Portuguese coast through otolith elemental fingerprints. *Estuarine, Coastal and Shelf Science* **79**: 317–327.

VILLANUEVA, R., 1995. Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to settlement. *Canadian Journal of Fisheries Aquatic Science* **52**: 2639–2650.

VILLANUEVA, R. & NORMAN, M.D. 2008. Biology of the planktonic stages of benthic octopuses. *Oceanography and Marine Biology: An Annual Review* **46**: 105–202.

3.2. NUTRITIONAL PROFILE OF WILD OCTOPUS VULGARIS PARALARVAE AND THEIR POTENTIAL PREY OFF WESTERN IBERIAN UPWELLING SYSTEM [3]

Sílvia lourenço*[1,2,3], Álvaro Roura[2], Maria José Fernández-Reiriz[2], Luís Narciso[1], Ángel F. González[2]

[1] Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Avenida Nossa Senhora do Cabo 939, 2750-374 Cascais, Portugal

[2] Instituto de Investigaciones Marinas de Vigo, CSIC, C/Eduardo Cabello, 6. Vigo. E-36208, Spain

[3] Departamento do Mar e Recursos Marinhos, Instituto Português do Mar e da Atmosfera, I.P. Avenida de Brasília, 1449-006, Lisboa, Portugal

ABSTRACT

The Iberian Atlantic coast and particularly the Rías Baixas (Southwestern Galicia, Spain) constitute an important spawning ground for *Octopus vulgaris*. Unfed hatchlings of *O. vulgaris* were directly collected by scuba diving from wild ripe eggs in the Ría de Vigo in Rías Baixas. At the same time, plankton samples were collected in the same area to capture wild paralarvae and their potential prey. The calorimetric analytical methods were used to assess the nutritional quality (carbohydrates, proteins and lipid classes content) of unfed hatchlings, planktonic paralarvae and their potential prey aiming to interpret the nutritional requirements of *Octopus vulgaris* paralarvae. Multivariate analyses show that *O. vulgaris* hatchlings are richer in carbohydrates (and glycogen), proteins, total lipids, cholesterol and phospholipids although maintaining the protein:lipid ratio of their potential prey. The fatty acids (FA) content was also determined and compared between unfed hatchlings, planktonic paralarvae and potential prey to understand if there specific FA were incorporated by the planktonic *O. vulgaris* through their diet. There are differences in the n-3 Highly Unsaturated Fatty Acids (HUFA) content, with mesozooplankton samples being richer in C22:6n3 (EPA), poorer in C20:5n3 (DHA) and also in C20:4n6 (ARA) than hatchlings and paralarvae. The planktonic paralarvae have an FA profile markedly different from that of the hatchlings and mesozooplankton, being particularly rich in C16:1n7, C18:1n9 and ARA, evidencing their capability to biosynthesizing Polyunsaturated FA (PUFA). Considering that few days of life separate the *O. vulgaris* hatchlings from the planktonic paralarvae, the observed changes in *O. vulgaris* FA profile occur in a relative small step of time, thus showing that their natural prey greatly influence specific FA enrichment. This kind of selective enrichment could be carried out under culture conditions in order to enhance survival during the first critical days.

Keywords: *Octopus vulgaris,* paralarvae, prey, fatty acids, diet profile.

[3] Submitted to the Journal of Experimental Marine Biology and Ecology.

3.2.1. Introduction

The common octopus, *Octopus vulgaris*, is an important resource for the artisanal and industrial fisheries in the Atlantic margin of the Iberian peninsula, with official average annual landings of 9185 tons in Portugal (INE, 2013) and 4000 tons in Galicia (Gonzalez *et al*., 2005). In 2011, the Portuguese artisanal fleet was responsible for 95 % of the common octopus landings in this country (ICES, 2011). Landings statistics also show the increasing importance of common octopus in catches of the trawling fleet in recent years (ICES, 2012; Fonseca *et al*., 2008; Pilar-Fonseca *et al*., 2014). *O. vulgaris* is also considered one of the species with most aquaculture production potential due to its high growth and high food convertion rates (Vaz-Pinto *et al.*, 2004). Nevertheless, there is a bottleneck in the transfer of knowledge to an industrial scale because of the high mortality rates prior to settlement in captivity, even after the cycle has been closed. This is believed to be due to feeding and nutritional constrains at the paralarval stage (Iglesias *et al.*, 2007).

O. vulgaris is defined as an "r" life-history strategy organism with the production of numerous small eggs that hatch into pelagic paralarvae (although not strictly, due to the maternal egg care). The factors responsible for the recruitment success in cephalopods are most dependent on the interaction between egg masses, hatchling and pre-recruit juveniles and the physical and biological environment prevailing during each phase (Gonzalez *et al*., 2005); sea temperature playing an important role (Forsythe, 2004). In the north-east Atlantic, the *O. vulgaris* population is characterized by a bimodal life cycle with an extended reproduction phase with two spawning peaks, one in early spring and another in late summer (Otero *et al*., 2007; Lourenço *et al*., 2012) resulting in two hatching peaks in July and September/October (Gonzalez *et al*., 2005; Moreno *et al*., 2009) and two recruitment peaks in coastal areas (Demarcq & Faure, 2000) linked with the seasonality and strength of the seasonal coastal upwelling of the Western Iberia (Demarcq & Faure, 2000; Otero *et al*., 2008, 2009).

The Iberian Atlantic coast is known as a hatchery and nursery area for *Octopus vulgaris* (Moreno *et al*., 2014), and the Rías Baixas in the Galician coast are believed to be particularly important (Otero *et al*, 2008). Here as in the coastal waters, the dynamics of the coastal upwelling/downwelling events favours the cross-shelf dispersion of *O. vulgaris* paralarvae (Moreno *et al.,* 2009; Roura, 2013). After hatching, *O. vulgaris* paralarvae remain pelagic for 2 to 4 months, depending on the temperature, contributing to the mesozooplankton community (Villanueva, 1995; Demarcq & Faure, 2000; Katsanevakis &

Verriopoulos, 2006). In the first days of life, *O. vulgaris* paralarvae combine endogenous (yolk) with exogenous (prey) feeding (Villanueva & Norman, 2008). Using molecular methods, Roura *et al.* (2012) concluded that the main preys of *O. vulgaris* paralarvae, at least in this region, are larval stages of decapods of the families Crangonidae, Alpheidae, Brachyura, Paguridae, Thalassinidae and Porcellanidae, followed by euphausiids and fish larvae (Roura *et al.,* 2012). With the exception to the euphausiids, all the species detected contributed less than 4,3 % to the abundance of the mesozooplanktonic community in this region, suggesting that *O. vulgaris* paralarvae are specialist predators in the wild (Roura *et al.,* 2012).

The mesozooplankton community is a key component of the coastal ecosystems, linking the microbial food web to the macrozooplankton and neritic food webs. In the Rías Baixas (Galicia), the mesozooplankton community is closely linked to the upwelling/downwelling events associated to the Western Iberian Upwelling System, where primary production is increased by wind-driven currents that bring nutrient-rich sub-surface water up into the photic layer during the spring/summer coastal upwelling events (Relvas *et al*., 2007; Álvarez-Salgado *et al*., 2009). In this region Roura *et al*. (2013) identified 3 mesozooplankton communities following the bathymetric gradient, named as coastal, frontal and oceanic. These communities differed in both species composition and abundance, and are influenced by the regional meteorology and hydrography.

Despite the extended knowledge about the environmental physical factors that drive the distribution, abundance and recruitment success of *O. vulgaris* paralarvae (González *et al.,* 2005; Otero *et al.,* 2008, 2009; Moreno *et al.,* 2009; Roura 2013), there is a gap in studies regarding the feeding requirements of wild *O. vulgaris* paralarvae and the nutritional profile of their natural preys. The studies conducted to date were undertaken on reared eggs and individuals in captivity, showing that an *O. vulgaris* paralarva weighs about 1.4 mg at hatching with approximatelly 80% of water (Navarro & Villanueva, 2003; Villanueva, 1995) and protein (between 46 % and 69 % of the dry weight). Lipid classes are minor nutrients accounting for 12 % of the paralarva dry weight, mainly cholesterol and polar lipids (phospholipids) (Navarro & Villanueva, 2003; Villanueva *et al*., 2004).

Accordingly, our study aims to identify the composition profiles of *O. vulgaris* paralarvae collected from wild ripe eggs (unfed hatchlings) and the nutritional profiles of their potential preys present in the mesozooplankton (species bigger than 1 mm) of the Ría de Vigo. The profiles were assessed in terms of lipid classes (phospholipids, cholesterol, triacylglycerol, wax esters and fatty acids profile), proteins, carbohydrates and glycogen

content. Condition indices and trophic markers based in the protein, lipids and fatty acids (FA) classes (Saturated, Polyunsaturated, PUFA and Highly Unsaturated) were determined and compared between potential preys and *O. vulgaris* hatchlings to understand the nutritional requirements of the paralarvae. Additionally, we follow the variation in the FA profile between unfed hatchlings and older paralarvae aiming to understand which FA were incorporated into planktonic *O. vulgaris* hatchlings through their diet, in order to understand their nutritional requirements for aquaculture.

3.2.2. Material and Methods

3.2.2.1. Zooplankton sampling

Mesozooplankton samples were collected at the surface (5 m depth) with a multitrawl (MultiNet®) sampler (0.71 × 0.71 m opening frame, 200 μm mesh) in three surveys conducted under the LARECO project (CTM2011-25929) in autumn 2012 (September 17th, October 1st and 5th) in the outer part of Ría de Vigo (Figure 3.3). Samples were collected east (inner sample) and west of Cies Islands (outer sample). Zooplankton samples were visually examined on board, looking for *Octopus vulgaris* paralarvae. Up to 40 *O. vulgaris* paralarvae were found and stored at -80ºC in a solution of Methanol: Dicloromethane (2:1) solution for analytical purposes. Samples with paralarvae were split into two subsamples. One was washed with sea water and filtered through a 1000 μm sieve to retain individuals larger than 1000 μm (considered hereafter as the potential prey of *O. vulgaris* paralarvae), frozen at -80ºC, freeze dried for 48 h and stored again at -80ºC for further analysis. The second subsample was stored in 70% ethanol and used to identify the mesozooplankton community accompanying the paralarvae. Organisms were identified under a binocular (Nikon SMZ800) or inverted microscope (Nikon Eclipse TS100) to the lowest possible taxonomic level. To determine the base biochemical profile of the *O. vulgaris* paralarvae, newly hatched paralarvae were obtained from ripe eggs collected by scuba diving in October 9th 2012 off the Ría de Vigo (site coordinates: 42º14'N, 8º 54'W) (Figure 3.3). The hatchlings were counted and pooled in three samples for biochemical analysis.

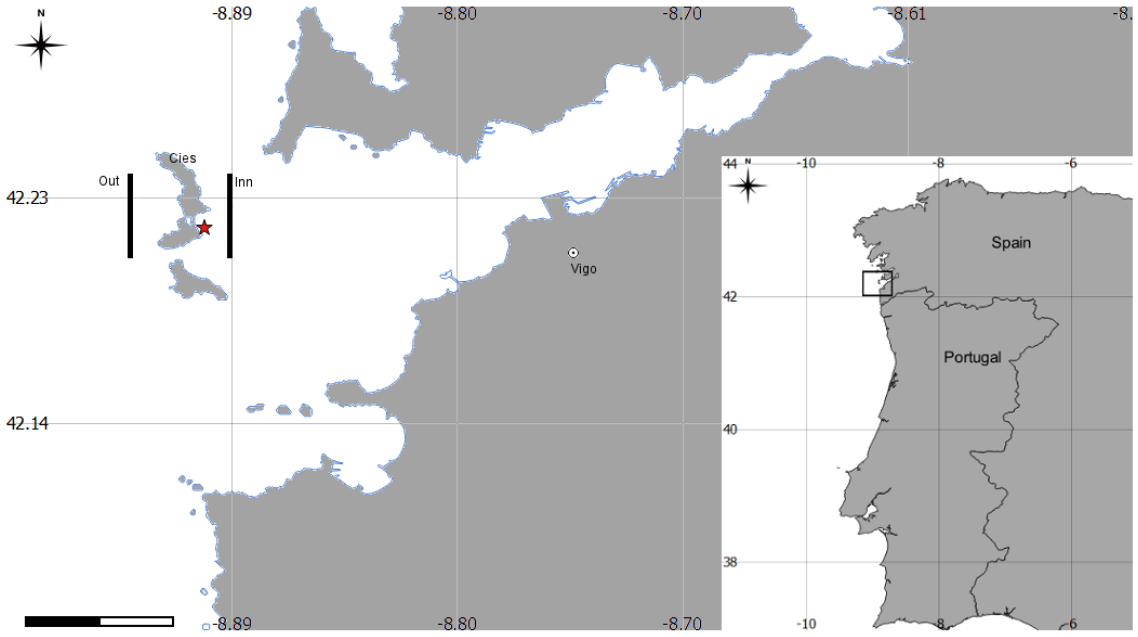


Figure 3.3 – Geographical position of the sampling área in the Ría de Vigo, Spain northwest Atlantic coast. Legend: lines indicate sampling transects (out and inn transects). The symbol * indicates the sampling site for the *O. vulgaris* paralarvae collected during diving. The scale bar corresponds to 3.5 km.

3.2.2.2. Biochemical methods

Proteins, carbohydrates, total lipids and the lipid classes phospholipids (phos), wax esters (wax), cholesterol (chol), triacylglycerol (tag), free fatty acids (ffa) and fatty acids (FA) were determined from the samples.

The protein content was determineded through the method of Lowry *et al.* (1951) after alkaline hydrolysis with 0.5 N NaOH at 30°C for 24 h. Bovine albumin (Sigma), which underwent the same treatment as samples, was used as a standard.

Carbohydrates were quantified according to the phenolsulphuric acid method (Strickland and Parsons, 1968) using glucose as the standard. Glycogen was quantifieded through the same assay as carbohydrates, after sample precipitation with 100 % ethanol.

Lipids were first extracted with chloroform:methanol (1:2) and after centrifugation, the precipitate was re-extracted with chloroform:methanol (2:1). Both supernatants were subsequently washed with chloroform:methanol:water (8:4:3) as described by Fernández-Reiriz *et al*. (1989). Total lipids were quantified following the method described by Marsh & Weinstein (1966) with a tripalmitine standard (Sigma Aldrich Inc., Buchs, Switzerland). Lipid classes were studied by thin-layer chromatography (TLC)/densitometry. Silica gel 60 W plates (Merck 16486) of 20 × 20 cm by 0.25 mm in thickness, were used. Samples were

applied by an automatic TLC sampler (Camag 27220). The chromatographic stain was made according to Freeman & West (1966). The plates were stained with a 10% $CuSO_4$ solution in 0.85% H_3PO_4 by heating to 180ºC (Bitman & Wood, 1982). Standards employed for the quantitative analysis of the sterol and waxes esters, sterols, free fatty acids and triacylglicerol were cholesterol, palmitate, cholesterol, palmitic acid and cod liver (CLO, Sigma), respectively. A standard obtained from a natural sample was used for phos. The plates were scanned with a Shimadzu CS9000 densitometer, using a monochromatic 370 nm beam of 0.4 × 0.4 mm working in the zigzag mode, reading the whole spot, and with automatic autozero for baseline correction. All solvents, reagents and fatty acid standards used in this work were of analytic grade (Merck, Darmstadt, and Sigma).

The results of the biochemical analyses, expressed in units of weight per individual, were transformed into their energy equivalents using the following factors: 18, 17.2 and 35 KJ/g for protein, carbohydrates and lipids, respectively, based on Beukema & De Bruin (1979).

The FA composition of the total lipid fraction was determined converting total lipids into FA methyl esters, according to the method described by Lepage & Roy (1984). Fatty acid methyl esters (FAME) were analysed by gas chromatography. Peaks corresponding to FAME were identified by comparison of their retention times with standard mixtures. Peak areas were automatically integrated with C19:0 being used as an internal standard for quantitative analysis. The concentration of each fatty acid or fatty acid group was expressed as g/100g total FAME.

Nutrient and FA ratios are used as biomarkers of condition (Shulman & Love, 1999) and represent the trophic position or dietary quality of a species population (e.g. El-Sabaawi *et al.*, 2008). The content in saturated FA (SFA), Monounsaturated FA (MUFA), polyunsaturated FA (PUFA) including n-3 Highly Unsaturated (n-3 HUFA), n-6 HUFA, Docosahexaenoic acid C22:6n3 (DHA), Eicosapenteonoic acid C22:6n3 (EPA) and Arachidonic acid C20:4n6 (ARA) are particularly important to determine biomarkers of condition. Here were determined the protein:lipid, tag:lipid, n-3 HUFA:n-6 HUFA, SFA:PUFA; DHA:EPA and the DHA:ARA ratios for mesozooplankton and *O. vulgaris* hatchlings and paralarvae. Although the number of wild *O. vulgaris* paralarvae collected form the zooplankton samples was apparently high (n=40), their low weight prevented us from carrying the whole biochemical analyses and only FA profiles could be obtained.

3.2.2.3. Statistical analysis

The species composition and abundance of zooplankton samples, macronutrients, lipid classes and FA profiles were analysed using metric multidimensional techniques. Prior to analysis, the zooplankton database was log (x+1) transformed and screened to select those taxa that appeared in at least 10% of the samples. A zooplankton dissimilarity matrix was calculated using the Bray-Curtis dissimilarity index (Zuur *et al.*, 2007) and analysed with principal coordinate analysis (PCO). The dimensions (axes) eigenvalues and species scores in each dimension of a full model were used to reduce the matrix size to the species that most correlate with the PCO axes in a reduced model (Table 3.3). The macronutrients and lipid classes content dataset was normalized according to Zuur *et al*. (2007). The similarity matrix was calculated based in the Euclidean distance matrix (Zuur *et al*., 2007) and analyzed with principal component analysis (PCA). The groups separated by PCA were tested for differences related with sampling area and species composition by non-parametric permutational ANOVA (PERMANOVA).

The FA profile database was screened to select the major FA (of which the mean content is > 1g/100g FAME) and data were normalized according to Zuur *et al*. (2007). The FA similarity matrix was calculated based in the Euclidean distance matrix (Zuur *et al*., 2007) and further analyzed with PCA. The dimension (axes) eigenvalues and FA scores in each dimension of the full model were used to reduce the FA matrix size to the FA that explain most of the variance in a reduced model (Table 3.3). Both zooplankton species and FA profile reduced models were tested for differences related with sampling area and species composition by non-parametric permutational ANOVA (PERMANOVA) considering type I errors.

A constrained canonical analysis (CCA) was applied to the zooplankton reduced model using the macronutrients, lipid classes and FA as explanatory variables to identify significant correlations between the content of those nutrients and the zooplankton species composition and abundance in each sample. The CCA was conducted on the site scores of the species composition reduced model, applying the macro-nutrients and lipid classes content as explanatory variables in the nutrients model; and the FA's content as explanatory variables for the FA model. The analysis was conducted applying the “envfit” function of the VEGAN package in R (Oksanen *et al.*, 2013).

The macronutrients, lipid classes and FA profile of zooplankton and *O. vulgaris* paralarvae (unfed hatchlings and feeding paralarvae) were compared using PCA to

determine which FA could differentiate between the groups and then, the arising groups were tested with PERMANOVA. The CCA was used to assess the correlation between different groups, the condition indices (protein:lipid and tag) and trophic markers (SFA:PUFA; n-3 HUFA:n-6 HUFA; DHA:ARA; DHA:EPA) studied.

Table 3.3 - Metric multidimensional scaling results for the zooplankton species model and zooplankton fatty acids full models and their respective reduced models. * Identify species/FA selected for the the reduced models.

Zooplankton PCO (Bray- Curtis dissimilarity matrix)						Fatty acid PCA (Euclidean distance matrix)		
Full Model						**Full Model**		
	Axis 1			Axis 2			Axis 1	Axis 2
Proportion explained	0.33			0.24		Proportion explained	0.51	0.30
Cumulative proportion	0.33			0.57		Cumulative proportion	0.51	0.81
Species scores						*Species scores*		
species	Axis 1	Axis 2	species	Axis 1	Axis 2	Fatty Acid	Axis 1	Axis 2
cop_ac	0.08	0.04	cop_peh*	-0.18	-0.04	C14:0*	0.14	-0.14
cop_cca*	-0.14	-0.04	cop_pe	0.003	0.08	C16:0	0.08	0.02
cop_ch*	-0.14	-0.01	cop_pce	-0.03	0.02	C16:1n7*	0.15	-0.15
cop_cch	-0.03	-0.02	cheat	0.02	0.07	C17:0*	-0.10	0.13
cop_cla*	-0.19	-0.005	siph*	-0.22	0.29	C17:1*	0.21	0.05
cnid*	0.19	0.23	cop_sec	0.05	0.02	C18:0	-0.05	0.002
cop_cory*	0.21	-0.17	cop_tl*	-0.11	0.17	C18:1n9	-0.07	0.10
cop_cv*	-0.14	-0.06	gamm	0.08	0.05	C18:1n7*	0.16	0.13
nyc_couchii	-0.004	0.001	cirrip	0.02	0.05	C18:2n6	-0.02	-0.005
mis	-0.07	0.05	polich	0.05	-0.05	C18:4n3	0.005	-0.05
cop_pp	-0.02	0.002	brachy_zoea	0.01	0.02	C18:3n3	0.09	0.02
crang_zoea	0.04	-0.06	p_platy_zoea	0.04	-0.05	ARA*	0.10	0.10
pag_zoea*	0.10	0.07	process_zoea	0.02	0.003	EPA	0.04	0.07
palaem_zoea*	0.10	0.01	salp	0.06	0.11	DHA*	-0.19	0.08
p_long_zoea*	0.10	-0.02						
Sites scores						*Sites Scores*		
	Axis 1			Axis 2			Axis 1	Axis 2
Out_d1	-0.55			0.15		Out_d1	-0.21	-0.06
Out_d2	-0.20			-0.78		Out_d2	-0.11	-0.31
Out_d3	-0.14			0.34		Out_d3	-0.01	0.38
Inn_d1	-0.14			0.43		Inn_d1	-0.05	-0.25
Inn_d2	0.23			-0.29		Inn_d2	-0.16	0.25
Inn_d3	0.78			0.14		Inn_d3	0.53	-0.02
Reduced Model						**Reduced Model**		
	Axis 1			Axis 2			Axis 1	Axis 2
Proportion explained	0.61			0.30		Proportion explained	0.64	0.31
Cumulative proportion	0.61			0.91		Cumulative proportion	0.64	0.95

3.2.3. Results

Specific composition of the zooplankton with size > 1 mm (Table 3.4) showed a dominance of holoplankton species both in the inner zone (67.65 %) and the outer zone (83.61%) with the copepods *Paracalanus parvus* and *Acartia clausii,* and the euphausid *Nyctiphanes couchii* being the most frequent species. The meroplankton species contributed with 32.35 % in the inner zone and 16.39 % in the outer zone, with the most frequent larvae being cirripids and brachyuran zoeae, mainly in the inner zone stations. The mean holo/meroplankton ratio of the > 1 mm fraction of the zooplanktonic community ranged between 2.12 in the inner zone and 6.33 in the outer zone indicating that the two sampling groups belong to the same coastal community. The similarity observed between inner and outer zone groups was confirmed by the PERMANOVA for differences between sampling zone (F = 1.57, p-value 0.25, 199 perm). Although differences between areas were not significant, the PCO on the zooplankton reduced model allowed the samples to be separated considering the presence and abundance of species in two different groups: the copepod group that aggregates the samples dominated by the copepods species (samples out_d1, out_d2 and inn_d2); and the zoeae group which aggregates the samples for which the larval stages of crustacean, Cnidaria and Siphonophora and the copepod *Temora longicornis* are the most important species (samples inn_d1, inn_d3 and out_d3) (Figure 3.4, zooplankton reduced model).

The zooplankton samples are rich in protein and total lipids, with a Protein: Lipid ratio of 2.99. Lipids are the second most abundant nutrient and an important energy source (7.50 KJ/g) (Table 3.5 - Macronutrient composition and energy content of the zooplankton samples and *Octopus vulgaris* hatchlings. Different superscripts indicate significant statistical differences ($p < 0.05$) between studied groups. * indicates a significance level of $p < 0.05$ between groups; ** indicates a significance level of $p < 0.01$.), with tag being the most abundant lipid class, followed by the ffa and waxes (Figure 3.5). Individual FA average concentration (g/100g FAME ± SD) obtained by GC are presented in Table 3.6. The zooplankton fatty acids profile was analysed with PCA and results show that the first and second dimensions explained 81% of variation due to the Myristic acid C14:0, Palmitic acid C16:0, Palmitoleic acid C16:1n7, Margaric acid C17:0, C17:1 and Vaccenic acid C18:1n7 variation between groups (Table 3.3).

Table 3.4 - Mesozooplankton community and abundance (n/1000 m^3 and % to sample total abundance) identified by sample (out_d1, out_d2, out_d3, inn_d1, inn_d2, inn_d3). Species which individuals are bigger than 1 mm were selected as potential preys of the *Octopus vulgaris* paralarvae and analysed for their nutritional profile (identified with a species code).

	species code	out_d1	out_d2	out_d3	inn_d1	inn_d2	inn_d3
Holoplankton							
Acartia clausi	cop_ac	186 (6.93)	90 (6.52)	288 (10.87)	162 (5.25)	549 (24.05)	468 (18.27)
Calanoides carinatus	cop_cca	72 (2.68)	24 (1.74)	6 (0.23)	24 (0.78)	18 (0.79)	3 (0.12)
Calanus helgolandicus	cop_ch	6 (0.22)	12 (0.87)	12 (0.45)	12 (0.39)	3 (0.13)	
Centropages chierchiae	cop_cch	3 (0.11)				3 (0.13)	
Clausocalanus spp.	cop_cla	48 (1.80)	97 (0.65)	3 (0.11)	21 (0.68)	9 (0.39)	
Corycaeus spp.	cop_cor		18 (1.30)	3 (0.11)		24 (1.05)	36 (1.41)
Paracalanus parvus	cop_pp	624 (23.3)	327 (23.70)	390 (14.72)	183 (5.93)	225 (9.86)	372 (14.52)
Paraeuchaeta hebes	cop_peh	54 (2.01)	3 (0.22)				
Paraeuchaeta sp.	cop_pe	3 (0.11)		6 (0.23)			3 (0.12)
Ctenocalanus vanus	cop_cv	30 (1.12)	6 (0.43)	24 (0.91)		24 (1.05)	
Pseudocalanus elongatus	cop_pce	6 (0.22)	6 (0.43)	12 (0.45)	24 (0.78)	30 (1.31)	3 (0.12)
Subeucalanus crassus	cop_sec	3 (0.11)		3 (0.11)		9 (0.39)	3 (0.12)
Temora longicornis	cop_tl	21 (0.78)	3 (0.22)	93 (3.51)	57 (1.85)	9 (0.39)	3 (0.12)
Candacia armata							3 (0.12)
Oithona plumífera		3 (0.11)	3 (0.22)	12 (0.45)	12 (0.39)	12 (0.52)	6 (0.23)
Oncaea media		21 (0.78)		291(10.99)	99 (3.21)	6 (0.267)	
Harpacticoida					3 (0.10)	6 (0.26)	3 (0.12)
Copepodid stages	cop	72 (2.68)	42 (3.04)		78 (2.53)	45 (1.97)	6 (0.23)
Evadne nordmanni		306(11.41)	63 (4.57)	9 (0.34)	831(26.92)	156 (6.83)	54 (2.11)
Podon intermedius			15 (1.09)		21 (0.68)	42 (1.84)	33 (1.29)
Nyctiphanes couchii calyptopa		51 (1.90)	102 (7.39)	600 (22.65)	144 (4.66)	48 (2.10)	24 (0.94)
Nyctiphanes couchii furcilia	nyc_cou	300(11.18)	129 (9.35)	99 (3.74)	69 (2.24)	69 (3.02)	222 (8.67)
Mysidacea	mys	3 (0.11)		3 (0.11)			
Cnidaria	cnid			27 (1.02)	15 (0.49)		81 (3.16)
Chaetognatha	chaet	108 (4.03)	21 (1.52)	72 (2.72)	66 (2.14)	84 (3.68)	90 (3.51)
Syphonophora	syph	33 (1.23)		30 (1.13)	96 (3.11)		
Tunicata	salp	81 (2.93)	12 (0.86)	30 (1.14)	117 (3.65)	63 (2.68)	156 (5.74)
Platyhelminthes							12(0.47)

Table 3.4 (continue) - Mesozooplankton community and abundance (n/1000 m^3 and % to sample total abundance) identified by sample (out_d1, out_d2, out_d3, inn_d1, inn_d2, inn_d3). Species which individuals are bigger than 1 mm were selected as potential preys of the *Octopus vulgaris* paralarvae and analysed for their nutritional profile (identified with a species code).

	species code	out_d1	out_d2	out_d3	inn_d1	inn_d2	inn_d3
Meroplankton							
Amphioxus		3 (0.11)					
Gammaridea					6 (0.19)	3 (0.13)	3 (0.12)
Cirripida cipris	cirripid	57 (2.12)	3 (0.22)		27 (0.87)	15 (0.66)	24 (0.94)
Polichaeta larvae	polich		9 (0.65)	12 (0.45)	6 (0.19)	12 (0.53)	3 (0.12)
Bivalvia larvae		429 (16.00)	381 (27.61)	312 (11.78)	699 (22.64)	582 (25.49)	642 (25.06)
Gastropoda		57 (2.13)	69 (5.00)	117 (4.41)	84 (2.72)	138 (6.04)	243 (9.48)
Ophiuridea larvae		45 (1.68)	3 (0.22)	3 (0.11)	159 (5.15)	6 (0.26)	
Equinoidea larvae					9 (0.29)		
Cirripida nauplius		18 (0.67)	9 (0.65)	171 (6.46)	51 (1.65)	27 (1.18)	21 (0.82)
Brachyura zoeae	brach_zoea	30 (1.13)	6 (0.43)	15 (0.57)	6 (0.19)	24 (1.05)	18 (0.70)
Crangonidae zoeae	crang_zoea					12 (0.53)	
Paguridae zoeae	pag_zoea				3 (0.10)		6 (0.23)
Palaemonidae zoeae	palam_zoea			3 (0.11)		6 (0.26)	3 (0.12)
Bryozan larvae		6 (0.22)	12 (0.87)			15 (0.66)	9 (0.35)
Pisidia longicornis zoeae	p_long_zoea					3 (0.13)	3 (0.12)
Porcellana platycheles zoeae	p_platy_zoea	3 (0.11)	3 (0.22)				6 (0.23)
Processidae zoeae	process_zoea				3 (0.10)	6 (0.26)	
fish eggs				3 (0.11)			
fish larvae						3 (0.13)	
Holoplankton/ Meroplankton		**2.22**	**1.78**	**2.50**	**1.55**	**1.36**	**1.87**

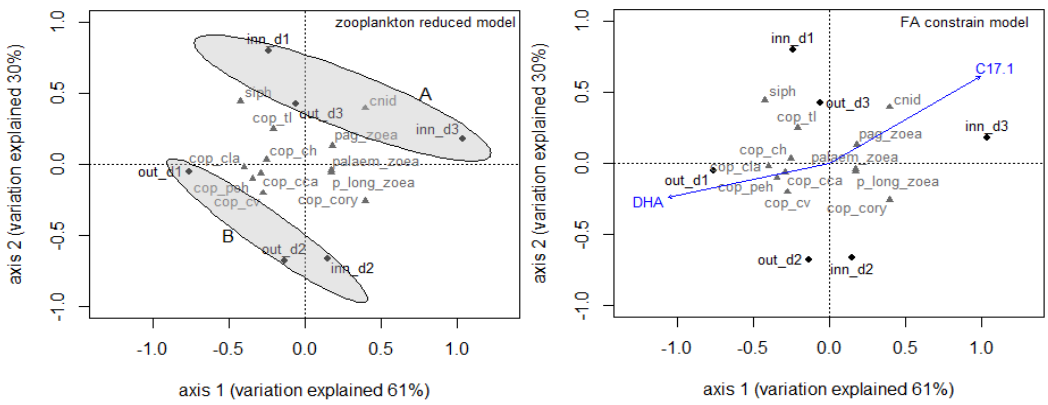


Figure 3.4 – Zooplankton principal coordinate reduced model biplot showing sites and species correlation with PCO axes and the canonical constrain analysis output comparing the zooplankton reduced model sites scores with the most significant FA, C17:1 and DFA. Legend: out_d1, out_d2, out_d3, inn_d1, inn_d2 and inn_d3 represent the sites sampled by sampling day; A and B represent the two samples groups identified: A - zoeae group and B - copepods group.

Table 3.5 - Macronutrient composition and energy content of the zooplankton samples and *Octopus vulgaris* hatchlings. Different superscripts indicate significant statistical differences ($p < 0.05$) between studied groups. * indicates a significance level of $p < 0.05$ between groups; ** indicates a significance level of $p < 0.01$.

			Carbohydrates	Glycogen	Protein	Lipids
Zooplankton	% DW		4.07 ± 1.56[a]	1.86 ± 0.81[a]	28.26 ± 4.60[a]	9.45 ± 1.85[a]
	KJ/g		1.63 ± 0.54	0.75 ± 0.30	10.95 ± 1.65	7.50 ± 1.39
Hatchlings	sample	% DW	9.11 ±3.32[b]**	7.77 ± 1.83[b]**	43.23 ± 10.35[b]**	12.06 ± 0.97[b] *
		KJ/g	2.54 ± 0.58[b]**	2.37 ± 0.52[b] **	13.09 ± 2.52[b] **	4.45 ± 1.07[b] *
	Individual[1]	% DW	0.84 ± 0.34	0.30 ± 0.10	5.94 ± 0.86	1.98 ± 0.46
		KJ/g	0.76 ± 0.34	0.34 ± 0.09	5.11 ± 0.74	3.49 ± 0.81

[1]Considering as a reference the mean individual weight of hatchlings of *Octopus vulgaris*in the Ría de Vigo equals to 2.42 mg.

The CCA model output (Figure 3.4 – FA constrain model) shows that the FA selected correlate with sites scores although with different significance levels (C16:1n7 $r^2 = 0.34$ p-value = 0.58; C18:1n7 $r^2 = 0.80$, p-value = 0.14; C17:1 $r^2 = 0.85$, p-value = 0.03; DHA $r^2 = 0.74$ p-value = 0.08; ARA $r^2 = 0.64$, p-value = 0.24). The C17:1 and DHA allow the separation of the zooplankton groups and significantly correlate with biplot axes (blue arrows in the FA constrain model biplot, Figure 3.4). The correlation results show that C17:1 is highly correlated with zoeae, Cnidaria and Siphonophora species, and that the DHA is closely related with the copepods samples group (marginally significant).

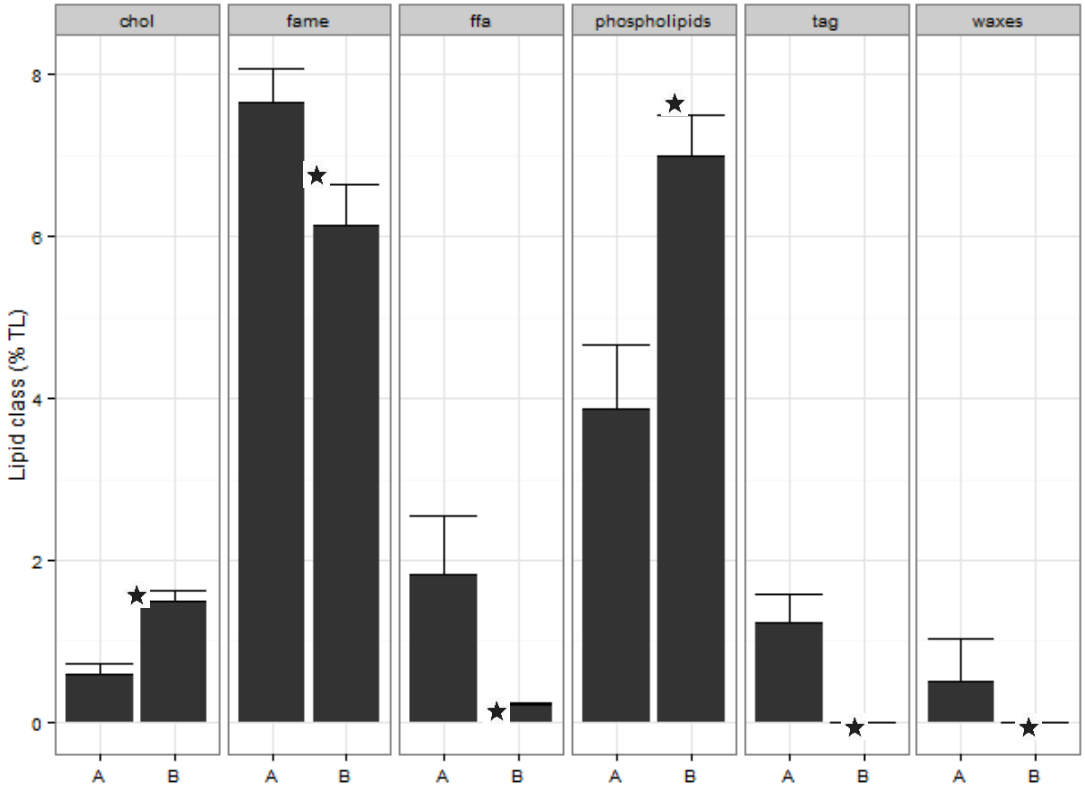


Figure 3.5 - Zooplankton and *Octopus vulgaris* hatchlings lipid classes in % Total Lipids (TL) (± SD). **A** represents de mesozooplankton samples; **B** represents the *O. vulgaris* hatchlings samples and * indicates a significance level of $p < 0.05$ between groups.

The macronutrient content of *O. vulgaris* hatchlings shows that the paralarvae are rich in protein (43.23 %DW) and lipids (12.06 % DW) with an average Protein: Lipid of 3.65 (Table 3.5). For the lipid classes' content, *O. vulgaris* hatchlings are rich in phospholipids and fatty acids, followed by cholesterol and a very low content in ffa. No tag and waxes esters were detected (Figure 3.5).

Both macronutrients and lipid classes content are statistically different between zooplankton samples and hatchlings with the latter being richer in carbohydrates (and glycogen), proteins, total lipids, chol and phos (Table 3.5, Figure 3.5). PCA shows that the different macronutrients and lipid classes content allow zooplankton samples to be separated from *O. vulgaris* hatchlings, explaining 92 % of the model variation (Figure 3.6: nutrients model) further supported by PERMANOVA ($F = 8.86$, p-value = 0.025, 199 perm). The canonical constrain analysis shows that the nutrients that better explain the separation between zooplankton and hatchlings are tag ($r^2 = 0.94$, p-value = 0.003) and waxes ($r^2 = 0.99$, p-value = 0.002) (arrows in Figure 3.4 nutrients model). The tag is absent

in the hatchlings and one of the zooplankton samples, the out_d2 sample, is particularly reach in waxes.

Table 3.6 - Fatty acid concentration (mean ± SD, g/100 g FA) of zooplankton community and *Octopus vulgaris* hatchlings in the Ría de Vigo. Different superscripts indicate significant statistical differences ($p < 0.05$) between studied groups.

	Mesozooplankton	*O. vulgaris*	
		hatchlings	paralarvae
Saturated fatty acids (SFA)			
C14:0	5.46 ± 0.84	2.47 ± 0.17[b]	2.33
C15:0	0.63 ± 0.10	0.33 ± 0.03[b]	0.65
C16:0	18.88 ± 1.21	19.35 ± 1.46[b]	21.97
C17:0	1.35 ± 1.18	1.42 ± 0.11[b]	1.49
C18:0	4.57 ± 0.41	9.96 ± 0.75[b]	10.58
C24:0	0.77 ± 0.11	0.65 ± 0.06[a]	
ΣSFA	31.69 ± 6.97	34.19 ± 2.59	37.02
Monounsaturated fatty acids (MUFA)			
C15:1		0.37 ± 0.04[b]	0.46
C16:1n7	7.18 ± 1.10	0.54 ± 0.05[b]	1.31
C17:1	1.21 ± 0.20	3.29 ± 0.26[b]	3.03
C18:1n7	3.15 ± 0.50	1.67 ± 0.19[b]	1.94
C18:1n9	4.61 ± 0.51	2.82 ± 0.25[b]	7.65
C20:1n9	0.73 ± 0.41	4.09 ± 0.29[b]	4.61
C22:1n9	0.62 ± 0.66	0.90 ± 0.14[a]	
C24:1n9	0.65 ± 0.09	0.52 ± 0.03[a]	0.63
Σ MUFA	19.40 ± 2.41	15.66 ± 1.38	21.10
Poli-unsaturated fatty acids (PUFA)			
C18:2n6	1.78 ± 0.04	0.37 ± 0.02[a]	0.67
C18:4n3	2.61 ± 0.28	0.39 ± 0.03[b]	
C18:3n3	1.41 ± 0.12		
C20:2n6		0.76 ± 0.07	
C20:4n6[1] (ARA)	1.79 ± 0.19	5.32 ± 2.40[b]	5.06
C20:4n3	0.81 ± 0.12		
C20:5n3 (EPA)	22.23 ± 1.51	18.43 ± 1.44[b]	15.40
C22:5n3	0.90 ± 0.05	1.63 ± 0.13[b]	1.78
C22:6n3 (DHA)	17.34 ± 2.69	23.25 ± 1.78[b]	18.97
Σ PUFA	50.17 ± 8.17	50.15 ± 5.86	41.88

[1]the FA C20:4n6 and FA C20:3n3 have the same retention time, and the concentration of FA C20:4n6 is dominant in marine products, the concentration presented here is representative of C20:4n6.

Comparing *O. vulgaris* hatchlings (with embryonic yolk) with the zooplankton, most FA show different concentrations with the exception of lignoceric acid C24:0 (minor concentration FA), erucic acid C22:1n9, nervonic acid C24:1n9 and linoleic acid C18:2n6. Some FA were only identified for *O. vulgaris* hatchlings as oleic acid C18:1n9 and eicosadienoic acid C20:2n6 (a minor concentration FA), and others were only identified for the zooplankton community such as α-linoleiric acid C18:3n3 and eicosatetraenoic acid C20:4n3. The overall FA profile is significantly different when comparing zooplankton samples and *O. vulgaris* hatchlings (PERMANOVA, F = 266.79, p-value = 0.03, 199 perm). The biplot in figure 3.6 showed that the first axis explained about 98 % of the variation observed and the zooplankton samples are correlated with higher content of

short-chain C14:0, C16:1n7 and the family of denatured stearic acid C18:0 with exception of C18:1n9 that was only detected in *O. vulgaris* hatchlings. The *O. vulgaris* hatchlings are particularly rich in the long-chain fatty acids erucic acid C20:1n9, docosapentanoic acid C22:5n3 and ARA.

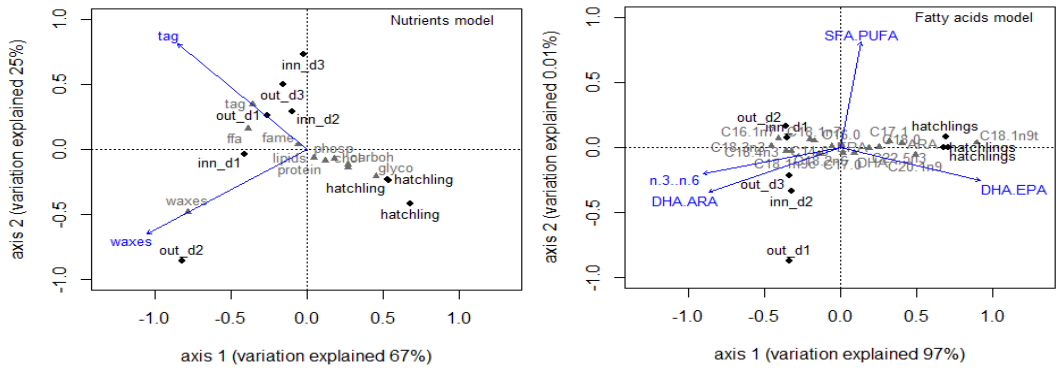


Figure 3.6 - Biplots of principal component model of macro-nutrients and lipid classes profiles (nutrients model) and the fatty acids profile (Fatty acids model) comparing zooplankton samples and *Octopus vulgaris* hatchlings. The vectors represent the most correlated variables obtained in the canonical constrained analysis. Legend: out_d1, out_d2, out_d3, inn_d1, inn_d2 and inn_d3 represent the zooplankton samples and hatchlings represents the *O. vulgaris* hatchlings samples. The codes in grey represent the nutrients scores (nutrients model) and the fatty acids names (fatty acids model).

The condition indices (Protein: lipid) and trophic markers (SFA: PUFA, n-3HUFA: n-6HUFA, DHA: EPA and DHA: ARA) showed that in terms of protein: lipid ratio, the protein content was three-fold the lipid content in the zooplankton compared with the hatchlings. The ratio n-3 HUFA: n-6 HUFA is twice as large in the zooplankton community than in *O. vulgaris* hatchlings, which influences in the same degree the DHA: ARA ratio. *O. vulgaris* hatchlings are richer in DHA in comparison with the EPA content (DHA: EPA ratio > 1) (Table 3.7). Those conditions indices and trophic markers were compared with the nutrients (macro-nutrients and lipid classes) and with the fatty acid profile of zooplankton samples and *O. vulgaris* hatchlings as explanatory variables of the observed fatty acid profile. Results show that those can be used to differentiate between samples (Figure 3.6), particularly the tag content, n-3HUFA: n-6HUFA, DHA: EPA and DHA: ARA (blue arrows in Figure 3.6 FA model) and tag content (blue arrow Figure 3.6 nutrients model).

Table 3.6 presents the fatty acids profile identified in the sample of 40 *O. vulgaris* paralarvae collected from the plankton. Some of the minority (< 1 g/100g FAME) FA identified in *O. vulgaris* hatchlings were not identified in the planktonic paralarvae. It is

noteworthy that C16:1n7 and C18:1n9 content is particularly high in the paralarvae in comparison both with the hatchlings and the zooplankton samples. The ARA content is identical in both hatchlings and paralarvae and higher than in the zooplankton samples. The DHA content of paralarvae is identical to that of the zooplankton and lower to that of the hatchlings. EPA content in wild paralarvae is particularly low in comparison with the other groups (Table 3.6). Overall, planktonic *O. vulgaris* have increased concentrations of MUFAs and lower concentrations of PUFAs when compared with the hatchlings. The PCA reflects those differences separating the planktonic paralarvae from hatchlings and zooplankton samples, mainly based in the differences in the content of Oleic acid (Figure 3.7). The PERMANOVA results showed that the FA profile is different between these three groups in terms of both FA identified and FA content (F = 119.5, p-value = 0.005, 199 perm). The trophic markers (blue arrows in the Figure 3.7 biplot) are highly correlated with axis 1 (95 % explained variation), indicating that differences found in these trophic markers ratios are more significant between *O. vulgaris* samples and mesozooplankton samples than between hatchlings and feeding paralarvae.

Table 3.7 - Mean (± SD) condition and trophic indicators determined for the mesozooplankton and *Octopus vulgaris* hatchlings in the present study in comparison with the results for other potential preys and benthic octopus paralarvae obtained in recent octopus paralarvae feeding studies (Navarro and Villanueva, 2000; Uriarte *et al.*, 2011; Couto, 2012; Iglesias *et al* 2013). Legend: n-3:n-6 stands for the n-3HUFA: n-6HUFA ratio; HUFA - High-Unsaturated Fatty Acids; SFA - Saturated Fatty Acids; PUFA - Poly-Unsaturated FattyAcids; DHA: C22:6n3; EPA: C20:5n3; ARA: C20:4n6.

		Protein: Lipid	TAG	n-3: n-6	SFA: PUFA	DHA: EPA	DHA: ARA
Present study	zooplankton	3.02	1.22	12.74	0.65	0.78	9.88
	O. vulgaris hatchlings	3.65		7.27	0.66	1.26	3.74
	O. vulgaris paralarvae			6.31	0.88	1.23	3.75
Navarro and Villanueva, 2000	Mysids zoeae[1]		~17	10.00	0.54	1.10	18.46
	Pagurid zoeae[2]		~11	3.60	0.56	1.20	3.48
	Hatchlings[3]		~ 6	2.90	0.55	1.70	2.90
Iglesias *et al.* 2013	Crab zoeae[4]	2.72	10.23	4.74	0.66	0.66	1.95
Couto, 2012	zooplankton[5]	2.68	20.24	14.36	0.55	0.81	9.93
Uriarte *et al.* 2011	Paralarvae[6]	4.50		7.66	1.23	0.52	5.64

1 *Acanthomysis longicornis* zoeae

2 *Pagurus prideaux* zoeae

3 Newly hatched *Octopus vulgaris* paralarvae

4 *Maja brachydactyla* zoeae

5 Multispecies pool with higher abundance of Siphonophora, copepods and crustacean larvae.

6 *Robsonella fontaniana* newly hatched paralarvae

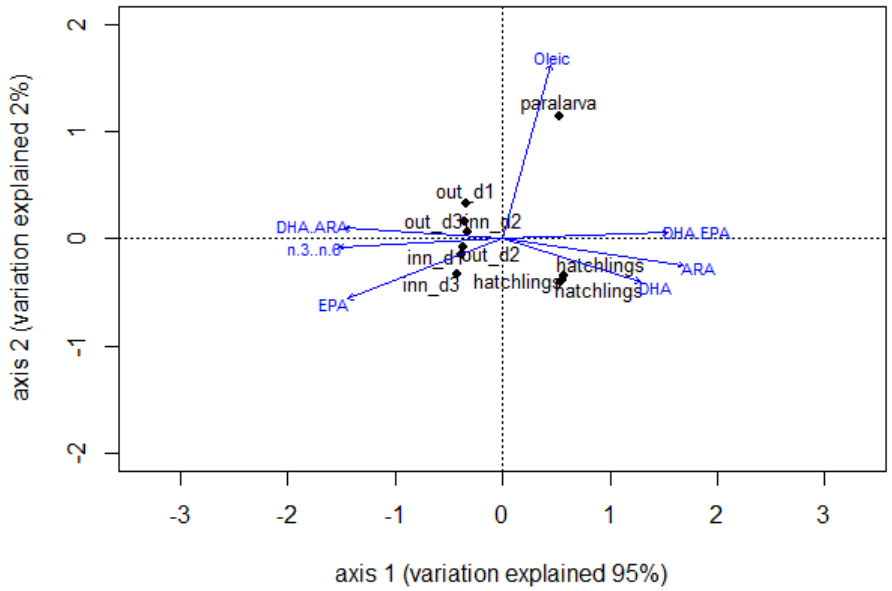


Figure 3.7 - Biplot of principal component model of fatty acids profile comparing zooplankton samples, *Octopus vulgaris* hatchlings and paralarvae. The vectors represent the most correlated variables obtained in the canonical constrained analysis. Legend: out_d1, out_d2, out_d3, inn_d1, inn_d2 and inn_d3 represent the zooplankton samples scores by sampling day, hatchlings represents the *O. vulgaris* hatchlings samples scores and paralarvae represent the *O. vulgaris* paralarvae sample scores.

3.2.4. Discussion

The zooplankton species identified represent a small time and size window of the mesozooplankton community of the Ría de Vigo during the season of higher *O. vulgaris* abundance. In the western Iberian Atlantic coast, *O. vulgaris* paralarvae hatch preferentially in September/October in synchrony with the higher levels of food and energy availability as a result of the previous spring/ summer upwelling (Gonzalez *et al*., 2005). *O. vulgaris* paralarvae start to feed from some hours to a few days after hatching (Villanueva *et al.*, 1996; Roura *et al.*, 2012), hunting preferentially on crustacean species larger than 1mm in length, which coincides with observations documented in captivity (e.g. Villanueva *et al*., 1996; Hernández-García *et al.*, 2000, Iglesias *et al.*, 2006; Roura *et al.*, 2010). Accordingly, we analysed as potential prey for *O. vulgaris* only the zooplankton species that were larger than 1 mm. It included a heterogeneous assemblage of organisms such as cnidarians, appendicularians, siphonophores, crustaceans, chaetognaths, polychaetes, echinoderms and fish larvae. Although *O. vulgaris* paralarvae appear to be specialist predators feeding preferentially on decapod crab larvae (Roura *et al.*, 2012), we

included the entire zooplankton assemblage to understand what was the nutritional profile of the community.

Our samples were dominated by two copepods *Paracalanus parvus* and *Acartia clausi,* the euphausiid *Nyctiphanes couchii,* chaetognaths and small Tunicata (see Table 3.4). In spite of their abundance, copepods were not detected in the gut contents of the early stages of octopus (Roura *et al.*, 2012), but these organisms may constitute an important part of the diet of the paralarvae later in their planktonic stage (Roura *et al.*, 2013), as studies in captivity show that *O. vulgaris* feds on copepod species in absence of other preys (Iglesias *et al.*, 2007). Additionally, the identification of the species composition in our samples linked with the FA profile, permitted the identification of the source of some of the most limiting FA for the optimal growth of *O. vulgaris* paralarvae such as DHA (Monroig *et al.*, 2012a) which is biosynthesized by the more abundant copepod species (Dalsgaard *et al.*, 2003; Bergé & Barnathan, 2005).

The lipid classes content in the zooplankton community denotes a rich source of phospholipids and free FA that can be easily incorporated by the *O. vulgaris* paralarvae and fuel their high growth rates. Furthermore, this earlier autumn zooplankton community is richer in tag than wax esters, which is characteristic of a community that experienced a peak of food availability (phytoplankton) (Lee *et al.*, 2006). The higher content in tag relatively to wax esters is also related with the increasing presence of the meroplankton species in some samples, particularly cirripedes and brachyuran larvae that are known to store tag in large lipid globules (Lee *et al.*, 2006), in opposition to the copepod dominated samples richer in wax esters from PUFA (Lee *et al.*, 1970, 1971).

The zooplankton community FA profile showed a dominance of the saturated FA (SFA) and the polyunsaturated FA (PUFA). This is related with the dominance of calanoid copepod species but also due to the sampling season. In this season there is a higher availability of bacteria, detritus and green algae in the water which are richer in short-chain SFA (Falk-Petersen *et al.*, 2002) ando n which the zooplankton feeds, influencing the high content in SFA (~ 30%) and PUFA (~ 49 %) (Falk-Petersen *et al.*, 2002; Gonçalves *et al.,* 2012).

The biochemical profile and nutritional ratios determined for *O. vulgaris* hatchlings allowed predicting the nutritional requirements during the paralarval stage (Sargent *et al.*, 1999). *O. vulgaris* hatchlings collected here are particularly rich in carbohydrates (and glycogen), proteins and phos, with proteins comprising the major energy source for

hatchlings individually (see Table 3.5 and Figure 3.5). Navarro and Villanueva (2000, 2003) already showed that newly hatched and older *O. vulgaris* paralarvae in captivity, are relatively low in lipid contents with relatively large phos and chol fractions and small lipid reserves (e.g. tag). Other early stages of cephalopod species such as *Galiteuthis glacialis* show the same pattern (Piatkowski & Hagen, 1994).

On the other hand, these *O. vulgaris* hatchlings depend on the inner yolk rich in lipoproteins to survive through the first hours of life (Boletzky, 2003; Lee, 1991; Villanueva & Norman, 2008). The digestive gland is not fully developed (Moguel *et al.*, 2010) and paralarvae get energy from the lipoproteic yolk rich in phosp and chol (Lee, 1991) which explains the relatively high content of those nutrients. Tag and waxes are only vestigial in content and were not quantified by the method employed. We believe that tag and waxes had been greatly consumed due to stress during collection as at least tag should be expected as a natural low percentage reserve of mature *O. vulgaris* females (Lourenço *et al.*, 2014).

Accordingly to Iglesias *et al.* (2013) wild zooplankton populations in general, present the suitable biochemical composition for the diet of *O. vulgaris* paralarvae, presenting lower levels of total lipids, phos and tag, and higher percentage of PUFAs (DHA and EPA). Here, it is important to note that potential prey and hatchlings present identical Protein/ Lipid ratios. On the other hand, the differences found in the trophic ratios such as n-3HUFA/n-6HUFA, DHA/ARA and DHA/EPA found between potential preys and *O. vulgaris* hatchlings are small but significant as they allow differentiating between the two groups.

Notwithstanding being a single pooled sample, this is the first time that the FA profile of wild planktonic *O. vulgaris* paralarvae is determined. Keep in mind the preliminary characteristics of these results, the FA profile of these paralarvae shows some particular aspects when compared with the hatchlings and the zooplankton groups. Compared with the *O. vulgaris* hatchlings and paralarvae present higher content in C16:0, C18:0, C16:1n7 and C18:1n9. This relative enrichment is related with their diet which includes herbivorous species such as decapod zoeae and other omnivorous and carnivorous holo and meroplankton species (responsible for the denaturation of PUFA and MUFA into SFA) (Dalsgaard *et al.*, 2003; Bergé & Barnathan, 2005). On the other hand, the ARA content of the paralarvae is identical to the observed in the hatchlings and both significantly higher than in the potential preys. This fact clarifies the ARA role as an

essential fatty acid. The high ARA content was already observed in mature females gonads (Rosa *et al.*, 2004; Lourenço *et al.*, 2014) and in hatchlings collected off the Gran Canaria Island (J. Estefanell, *pers comm*).

There are two possible sources for this input of ARA in paralarvae. One of the sources can be the PUFA rich tag intake through the meroplankton larvae. Alternatively, it may be a product of the desaturation and chain elongation of 18:2n6 and 18:3n3 present in the zooplankton (Dalsgaard *et al.*, 2003; Almansa *et al.,* 2006). As found in captive feeding experiments (e.g. Navarro & Villanueva, 2000, 2003; Almansa *et al.*, 2006) *O. vulgaris* hatchlings and paralarvae are particularly rich in n-6HUFA in relation to their prey including ARA, EPA and DHA. The work conducted by Monroig and colleagues (Monroig *et al.* 2012a, 2012b) showed that *O. vulgaris* are capable of the biosynthesis of several essential PUFA particularly C20:3n3, ARA and EPA from HUFA of the families C18 and C20. This fact can explain the high content in ARA found in the hatchlings and paralarvae in relation to their potential preys. According to Monroig *et al.,* (2012a) the most common pathway to the biosynthesis of ARA is through the elongation of n-6HUFA, particularly C20:4n6 that we failed to identify both in hatchlings and paralarvae samples. Probably other pathway can be active in the paralarvae, biosynthesizing ARA through the n-3HUFA, although this option is less clear (Dalsgaard *et al*., 2006; Monroig *et al.,* 2012a). On the other hand, the higher peak of ARA identified in the hatchlings and paralarvae sample can be associated with the of C20:3n3, normally a minor FA in marine product, that is also synthesized by *O. vulgaris* (Monroig *et al.*, 2012a) and was not separate from ARA in the GC column.

The comparison of the conditions indices and trophic markers with other studies conducted on *O. vulgaris* paralarvae and their potential preys (see Table 3.7) ashowed a consistency between studies in the case of the protein: lipid ratio (Iglesias *et al.*, 2013) and for the FA trophic markers SFA: PUFA, DHA: EPA and DHA: ARA (Navarro & Villanueva, 2000; Uriarte *et al.*, 2011; Couto, 2012; Iglesias *et al.*, 2013). It is worth noting the variability observed in the tag content obtained here as compared to results obtained in the previously cited studies. Both for the potential preys and for the *O. vulgaris* hatchlings, our results show a significantly lower content in tag by comparison with the feeding experiments conducted by Navarro & Villanueva (2000), Couto (2012) and Iglesias *et al.* (2013).

The high natural availability of HUFA n-3 (HUFA n-3: HUFA n-6 ~ 12) supports the results obtained by Seixas *et al.* (2010) as they failed to observe any enhanced survival or growth when paralarvae were fed with *Artemia* enriched with DHA. DHA and other HUFA n-3 are naturally abundant in the prey of *O. vulgaris* paralarvae as well as in the summer phytoplankton community, so it is unlikely that these FA are limiting nutrient for the development of the species. In this case, the protein: lipid ratio of the diet was found to be more important to promote a good growth and survival of the paralarvae. A significant positive linear correlation was found between an increasing Protein: Lipid ratio in the diet and the dry weight of paralarvae, at 15 and 25 days of rearing, whereas no correlation could be established between dietary DHA content and growth or survival of paralarvae (Seixas, 2009).

We believe that the biochemical profile and trophic ratios determined here both for the potential preys and for the *O. vulgaris* hatchlings allow an understanding of the nutritional requirements of the paralarval stage. Several studies showed that the lipid content of preys has a clear influence on the lipid composition of octopus paralarvae both at the level of lipid class and FA composition (Navarro & Villanueva, 2000, 2003; Iglesias *et al.*, 2013). On the other hand, the nutritional profile of newly hatched paralarvae from the wild permits the identification of the specific nutritional requirements under culture conditions, in order to attain a good nutritional condition independently of external factors (Uriarte *et al.*, 2011).

Studies such as this one are difficult to perform due to constrains related with the low number of wild *O. vulgaris* paralarvae that can normally be captured and to the high water content of their tissues. Nevertheless, the colorimetric methods applied herein facilitate the study of these small-sized *O. vulgaris* paralarvae. Further biochemical and physiological studies on wild paralarvae after first feeding are however still needed to untangle the nutritional deficiencies verified under culture conditions for *O. vulgaris* paralarvae.

ACKNOWLEDGEMENTS

The authors want to acknowledge the crew of RV "Mytilus" and Marcos Regueira during zooplankton surveys, Manuel Garcia and Jorge Hernández Urcera the divers that collect the hatchlings and to Lourdes Nieto for her availability to help with the biochemical analyses. We also want to thank the comments of Juan Carlos Navarro (Instituto de Acuicultura “Torre de la Sal”, IATS). Data collection and analytical analysis were conducted under the LARECO project (CTM2011-25929) and supported

partially by FEDER funds and by the Portuguese Foundation for Science and Technology (FCT). The first author is supported by a PhD fellowship granted by FCT (SFRH/BD/44182/2008).

BIBLIOGRAPHIC REFERENCES

ALMANSA, E., DOMINGUES, P., SYKES, A., TEJERA, N., LORENZO, A. & ANDRADE, J.P. 2006. The effects of feeding with shrimp or fish fry on growth and mantle lipid composition of juvenile and adult cuttlefish (*Sepia officinalis*). *Aquaculture*, **256**, 403–413.

ÁLVAREZ-SALGADO, X., BORGES, A., FIGUEIRAS, F. & CHOU, L. 2009. Iberian margin: The rías, in: Carbon and Nutrient Fluxes in Continental Margins. A Global Synthesis.

BERGÉ, J.-P., & BARNATHAN,G., 2005. Fatty Acids from Lipids of Marine Organisms: Molecular Biodiversity, Roles as Biomarkers, Biologically Active Compounds, and Economical Aspects, in: Ulber, R., Gal, Y. (Eds.) Marine Biotechnology I. pp. 49–125.

BEUKEMA, J.J. & DE BRUIN, W. 1979. Calorific values of the soft parts of the tellinid bivalva *Macoma balthica* (L.) as determined by two methods. *Journal of Experimental Marine Biology and Ecology*, **37**, 19-30.

BITMAN, J., & WOOD, D.L.1982. An improved copper reagent for quantitative densitometric thin-layer Chromatography of lipids. *Journal of chromatography* **5**, 1155–1562.

BOLETZKY, S., 2003. Biology of early life stages in cephalopod molluscs. *Advances in Marine Biology* **44**, 144–203.

BOUCAUD-CAMOU, E. & BOUCHER-RODONI, R., 1983. Feeding and Digestion in Cephalopods, in: Wilburn, K.M (Ed). The Mollusca. Academic Press, pp. 149–187.

COUTO, A.T., 2012. Study on the wild diet of *Octopus vulgaris* paralarvae in Ría de Vigo (NW spain). Masters in Science thesis. Instituto de Ciências Biomédicas Abel Salazar. Universidade Do Porto, Porto, Portugal.

DALSGAARD, J., ST JOHN, M., KATTNER, G., MÜLLER-NAVARRA, D. & HAGEN, W., 2003. Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* **46**, 225–340.

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.

EL-SABAAWI, R., DOWER, J.F., KAINZ, M. & MAZUMDER, A., 2008. Characterizing dietary variability and trophic positions of coastal calanoid copepods: insight from stable isotopes and fatty acids. *Marine Biology* **156**, 225–237.

FALK-PETERSEN, S., DAHL, T.., SCOTT, C., SARGENT, J.R., GULLIKSEN, B., KWASNIEWSKI, S., HOP, H. & MILLAR, R., 2002. Lipid biomarkers and trophic linkages between ctenophores and copepods in Svalbard waters. *Marine Ecology Progress Series* **227**, 187–194.

FERNÁNDEZ-REIRIZ, M.J., PEREZ-CAMACHO, A., FERREIRO, M.J., BLANCO, J., PLANAS, M., CAMPOS, M.J. & LABARTA, U.,1989. Biomass Production and variation in the biochemical

profile (Total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. *Aquaculture*, **83**, 17-27

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., 2004. Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice. *Marine & Freshwater Research* **55**, 331–339.

FREEMAN, C.P. & WEST, D., 1966. Complete separation of lipid classes on a single thin-layer plate. *Journal of Lipid Research* **7**, 324-327.

GONÇALVES, A.M.M., AZEITEIRO, U.M., PARDAL, M.A. & DE TROCH, M., 2012. Fatty acid profiling reveals seasonal and spatial shifts in zooplankton diet in a temperate estuary. *Estuarine Coastal and Shelf Science* **109**, 70–80.

GONZALEZ, A.F., OTERO, J., GUERRA, Á., PREGO, R., ROCHA, F. & 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.

HERNANDEZ-GARCIA, V., MARTIN, A.Y. & CASTRO, J.J. 2000. Evidence of external digestion of crustaceans in *Octopus vulgaris* paralarvae. *Journal of the Marine Biological Association of the United Kingdom* **80**, 559–560.

ICES, 2011. Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH), 28 February–03 March 2011, Lisbon, Portugal. ICES CM 2011/SSGEF:03. 118 pp.

ICES, 2012. Report of the working group on Cephalopod Fisheries and Life History (WGCEPH), 27-30 March 2012 (No. ICES CM 2012/SSGEF:04). ICES, Cadiz, Spain.

IGLESIAS, J., FUENTES, L., SÁNCHEZ, J., OTERO, J. J., MOXICA, C. & LAGO, M.J. 2006. First feeding of *Octopus vulgaris* Cuvier 1797 paralarvae using *Artemia*: Effect of prey size, prey density and feeding frequency. *Aquaculture* **261**: 817-822.

IGLESIAS, J., SÁNCHEZ, F.J., BERSANO, J.G.F., CARRASCO, J.F., DHONT, J., FUENTES, L., LINARES, F., MUÑOZ, J.L., OKUMURA, S., ROO, J., VAN DER MEEREN, T., VIDAL, E.A.G. & VILLANUEVA, R., 2007. Rearing of *Octopus vulgaris* paralarvae: present status, bottlenecks and trends. *Aquaculture* **266**, 1-15.

IGLESIAS, J., PAZOS, G., FERNÁNDEZ, J., SÁNCHEZ, F.J., OTERO, J.J., DOMINGUES, P., LAGO, M.J. & LINARES, F., 2013. The effects of using crab zoeae (*Maja brachydactyla*) on growth and biochemical composition of *Octopus vulgaris* (Cuvier 1797) paralarvae. *Aquaculture International*

KATSANEVAKIS, S. & VERRIOPOULOS, G., 2006. Seasonal population dynamics of *Octopus vulgaris* in the eastern Mediterranean. *ICES Journal of Marine Science* **63**, 151–160.

LEE, R.F., NEVENZEL, J.C., PAFFENHÖFER, G.-A & BENSON, A.A. 1970. The metabolism of wax esters and other lipids by the marine copepod *Calanus helgolandicus*. *Journal of Lipid research* **11**: 237-240.

LEE, R.F., NEVENZEL, J.C. & PAFFENHÖFER, G.-A. 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. *Marine biology* **9**: 99-108.

LEE, P.G., 1991. Lipoproteins from the hemolymph and ovaries of marine invertebrates, in: Advances in Comparative and Environmental Physiology. Springer, London U.K, pp. 187–208.

LEE, P.G., 1995. Nutrition of cephalopods: Fueling the System. Marine and Freshwater Behaviour and Physiology **25**, 35- 51.

LEE, R.E., HAGEN, W. & KATTNER, G., 2006. Lipid storage in marine zooplankton. Marine Ecology Progress Series **307:** 273-306.

LEPAGE, G. & ROY, C.C., 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *Journal of Lipid Research* **25:** 1391-1396.

LINDLEY, J.A., 1982. Population dynamics and production of euphausiids. *Marine Biology*, **66:** 37–46.

LOURENÇO, S., MORENO, A., NARCISO, L., GONZÁLEZ, Á.F. & PEREIRA, J., 2012. Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. *Fisheries Research* **127-128:** 116–124.

LOURENÇO, S., NARCISO, L., GONZALEZ, Á.F., PEREIRA, J., AUBORG, S. & XAVIER, J.C., 2014. Does the trophic habitat influence the biochemical quality of the gonad of *Octopus vulgaris*? Stable isotopes and lipid class contents as bio-indicators of different life-cycle strategies. *Hydrobiologia*, **725:** 33-46.

MOGUEL, C., MASCARÓ, M., AVILA-POVEDA, O., CAAMAL-MONSREAL, C., SANCHEZ, A., PASCUAL, C. & ROSAS, C., 2010. Morphological, physiological and behavioral changes during post-hatching development of *Octopus maya* (Mollusca: Cephalopoda) with special focus on the digestive system. *Aquatic Biology* **9:** 35–48.

MONROIG, Ó., NAVARRO, J. C., DICK, J. C., ALEMANY, F. & TOCHER, D. R. 2012a. Identification of a Δ5-like fatty acyl desaturase from the Cephalopod *Octopus vulgaris* (Cuvier 1797) involved in the biosynthesis of essential fatty acids. *Marine Biotechnology* **14**, 411-422.

MONROIG, Ó., GUINOT, D., HONTORIA, F., TOCHER, D. R., NAVARRO, J.C., 2012b. Biosynthesis of essential fatty acids in *Octopusvulgaris*(Cuvier, 1797): Molecular cloning, functional characterisation and tissue distribution of a fatty acyl elongase. *Aquaculture* **360-361**, 45-53.

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.

MORENO, A., LOURENÇO, S., PEREIRA, J., GASPAR, M.B., CABRAL, H.N., PIERCE, G.J. & SANTOS, A.M.P., 2013. Essential habitats for pre-recruit *Octopus vulgaris* along the Portuguese coast. *Fisheries Research* **152:** 74-85.

NAVARRO, J.C. & VILLANUEVA, R., 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. *Aquaculture* **183:** 161–177.

NAVARRO, J.C. & VILLANUEVA, R., 2003. The fatty acid composition of *Octopus vulgaris* paralarvae reared with live and inert food: deviation from their natural fatty acid profile. *Aquaculture* **219:** 613–631.

OKSANEN, J., BLANCHET, F. G., KINDT, R., LEGENDRE, P., MINCHIN, P. R., O'HARA, R. B., SIMPSON, G. L., SOLYMOS, P., PETER R., STEVENS, P. R., & WAGNER, H., 2013. Vegan: Community Ecology Package. R package version 2.0-10. http://CRAN.R-project.org/package=vegan

OTERO, J., ÁLVAREZ-SALGADO, X., GONZÁLEZ, Á.F., GILCOTO, M. & GUERRA, Á., 2009. High-frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. *Marine Ecology Progress Series* **386:** 123–132.

OTERO, J., GONZÁLEZ, Á.F., SIEIRO, M.P. & GUERRA, Á., 2007. Reproductive cycle and energy allocation of *Octopus vulgaris* in Galician waters, NE Atlantic. *Fisheries Research* **85:** 122–129.

PHILLIPS, K.L., NICHOLS, P.D., & JACKSON, G.D., 2003. Dietary variation of the squid *Moroteuthis ingens* at four sites in the Southern Ocean: stomach contents, lipid and fatty acid profiles. *Journal of the Marine Biological Association of the United Kingdom* **83:** 523–534.

PIATKOWSKI, U. & HAGEN, W., 1994. Distribution and lipid composition of early life stages of the cranchiid squid *Galiteuthis glacialis* (Chun) in the Weddell Sea, Antarctica. *Antarctic Science* **6:** 235–239.

PILAR-FONSECA, T., CAMPOS, A., PEREIRA, J., MORENO, A., LOURENÇO, S. & AFONSO-DIAS, M. 2014., Integration of fishery-dependent data sources in support of octopus spatial management. *Marine Policy* **45:** 69–75.

RELVAS, P., BARTON, E.D., DUBERT, J., OLIVEIRA, P.B., PELIZ, Á., DA 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.

ROURA, A. 2013. Ecology of planktonic cephalopod paralarvae in coastal upwelling systems. Dissertation to obtain the PhD degree with International Mantion in the University of Vigo. Vigo. 224p

ROURA, Á., ÁLVAREZ-SALGADO, X.A., GONZÁLEZ, Á.F., GREGORI, M., ROSÓN, G. AND GUERRA, Á. 2013. Short-term meso-scale variability of mesozooplankton communities in a coastal upwelling system (NW Spain). *Progress in Oceanography* **109:** 18–32.

ROURA, Á., GONZÁLEZ, Á.F., REDD, K. & GUERRA, Á. 2012. Molecular prey identification in wild *Octopus vulgaris* paralarvae. *Marine Biology* **159:** 1335–1345.

ROURA, Á. GONZÁLEZ, Á.F., GUERRA, Á. 2010. A molecular approach to identifying the prey of cephalopod paralarvae. *ICES Journal of Marine science* **67**: 1408–1412.

SAMPEDRO, M. P., FERNANDEZ, L., FREIRE, J. & GONZALEZ-GURRIARAN, E. 1997. Fecundity and reproductive output of Pisidia longicornis (Decapoda, Anomura) in the Ría de Arousa (Galicia, Nw Spain). *Crustaceana* **70**: 95110.

SEIXAS, P. 2009. Composición bioquímica y crecimento de paralarvas de pulpo (*Octopus vulgaris* Cuvier, 1797), alimentadas con juveniles de Artemia enriquecidos con microalgas y otros suplementos

nutricionales. Memoria que para optar al grado de doctor en Biología. Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

SEIXAS, P., REY-MÉNDEZ, M., VALENTE, L.M.P. & OTERO, A. 2010. High DHA content in *Artemia* is ineffective to improve *Octopus vulgaris* paralarvae rearing. *Aquaculture* **300:** 156–162.

SHULMAN, G.E., & LOVE, R.M. 2006. The Biochemical ecology of Marine fishes Advances in Marine Biology. *Advances in Marine Biology* **36**: 351 pp.

URIARTE, I., FARÍAS, A., PASCHKE, K., NAVARRO, J.C. & ROSAS, C. 2011. Observations on feeding and biochemical characteristics to improve larviculture of *Robsonella fontaniana* (Cephalopoda: Octopodidae). *Aquaculture* **315:** 121–124.

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 the zooplankton time series telling us? *Progress in Oceanography* **74**: 98-114.

VAZ-PINTO, SEIXAS, P. & BARBOSA, A. 2004. Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. *Aquaculture* **238**: 221-238.

VILLANUEVA, R. 1995. Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to settlement. *Canadian Journal of Fisheries and Aquatic Science* **52**: 2639-2650.

VILLANUEVA, R., NOZAIS, C. & BOLETZKY, S.v. 1996. Swimming behaviour and food searching in planktonic *Octopus vulgaris* Cuvier from hatching to settlement. *Journal of Experimental Marine Biology and Ecology* **208**: 169-184.

VILLANUEVA, R. & NORMAN, M. 2008. Biology of the planktonic stages of benthic octopuses. *Oceanography and Marine Biology - An Annual Review* **46**: 105–202.

VILLANUEVA, R., RIBA, J., RUÍZ-CAPILLAS, C., GONZÁLEZ, A.V. & BAETA, M. 2004. Amino acid composition of early Stages of cephalopods and effect of amino acid dietary treatments on *Octopus vulgaris* paralarvae. *Aquaculture*, **242**: 455–478.

ZUUR, A.F., IENO, E.N. & SMITH, G.M. 2007. Analysing Ecological data. Springer. 672 p.

3.3. ESSENTIAL HABITATS FOR PRE-RECRUIT OCTOPUS VULGARIS ALONG THE PORTUGUESE COAST[4]

Moreno A[1], Lourenço S[1,2], Pereira J[1], Gaspar MB[1], Cabral HN[3], Pierce GJ[4] and, Santos AMP[1]

[1]Instituto Português do Mar e da Atmosfera (IPMA), Av. Brasília, 1449-006 Lisboa, Portugal

[1,2]Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa,Avenida Nossa Senhora do Cabo 939, 2750-374 Cascais, Portugal.

[3]Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

[4]Oceanlab, University of Aberdeen, Newburgh, Aberdeenshire, AB41 6AA, UK

ABSTRACT

The exploitation of the common octopus *Octopus vulgaris* in Portugal increased 50% in the last 20 years, largely motivated by the depletion of many fish stocks. Recently, the biomass of this fishery resource sharply decreased in some areas causing a major concern among local fishermen and an effort is underway to advice on novel and sustainable management measures. In this context, the octopus pre-recruit aggregations along the Portuguese coast are identified using georeferenced fishery-independent data, from autumn and winter sampling between 1996 and 2008. The relationships between pre-recruit aggregations and several environmental variables are analysed to characterize their essential habitats (EFH). Pre-recruits are distributed throughout the Portuguese coast aggregated in 8 distinct recruitment grounds located on the middle-shelf at 11-19 km from the coastline, which are characterised by average bottom depths of 65-110 m and to be associated to major rivers and lagoon systems. Within each season pre-recruit abundance is much higher in the south region, while pre-recruit aggregations on the northwest coast showed high inter-annual and seasonal variation driven by environmental variability. The western zone adjacent to Ria Formosa lagoon (southern coast) was identified as the main recruitment ground for *O. vulgaris* along the Portuguese coast. This is supported either by the higher abundance of pre-recruits and by the recurrence of their presence in this area over the years analysed, both in autumn and winter. The effects of physical variables on pre-recruit abundance modelled with generalized additive models (GAM) showed important regional differences. Bottom salinity and river runoff are the environmental variables that have more impact on pre-recruit distribution and abundance on the west coast, regardless of any seasonal effects. On the other hand, temperature imposes distinct seasonal and regional limitations to pre-recruit distribution, both on the NW and S regions. Pre-recruit preferential habitat is characterised by bottom temperatures of 14 ºC, salinity values around 36.0, low precipitation (average <200 mm), and coarse sediments (in which they find shelters to escape predation). Some of the octopus recruitment grounds identified are located in areas under intense fishing pressure, both by artisanal fisheries using traps and by bottom trawling. Thus, their value for the sustainability of the octopus fishery should be taken into consideration in future marine management strategies.

Keywords: *Octopus vulgaris*, pre-recruits, recruitment grounds, habitat, EFH, Portugal.

[4] Fisheries Research 152 (2014) 74-85

3.3.1. 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 - 2011). 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 (DGRM, 2012). The exploitation of octopus in Portugal increased 50% in the last 20 years, largely motivated by the depletion of many finfish stocks. Recently, the landed biomass of this fishery resource has sharply decreased in some areas causing a major concern among local fishermen and an effort is underway by scientists to advice on novel and sustainable management measures.

O. vulgaris has a short life cycle of 12-18 months (Iglesias *et al.*, 2004; Katsanevakis & Verriopoulos, 2006). The spawning season extends, in general, throughout the year (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*., 2012). Paralarvae are planktonic and settle in the benthic habitat of adults at ca 173 mg of total weight (Mangold, 1983; Villanueva, 1995). After settlement animals grow fast at ca. 1.2-1.6% d^{-1} reaching the fisheries minimum landing weight (750 g) at 9-10 months old (Domain *et al*., 2000; Katsanevakis & Verriopoulos, 2006). *O. vulgaris* is mainly a coastal species (0-100 m depth) with a wide geographic distribution. Density is low between 100 and 200 m and only few specimens have been found beyond the continental shelf break (Belcari & Sartor, 1999; Quetglas *et al*., 2000; Silva *et al*., 2002; Garofalo *et al*., 2010). Juveniles and spawners have generally a distinct depth range distribution (Sanchez & Obarti, 1993; Hernandez-Garcia *et al*., 1997; Faraj & Bez, 2007). Of the environmental variables that may affect the spatial distribution and abundance of juveniles, seawater temperature is thought to be the most important one (Garofalo *et al*., 2010).

Habitat modelling for marine animals is increasingly used as a management tool, to support conservation and sustainable exploitation. “Essential Fish Habitat” (EFH) is the habitat identified as essential to the ecological and biological requirements for critical life

history stages of exploited species, and which may require special protection to improve stock status and long term sustainability (e.g. Ardizzone, 2006; Valavanis & Smith, 2007). EFH may be modelled based on the abundance of animals at specific life cycle stages (e.g. juveniles) and the habitat identified as the aggregation of abiotic and biotic parameters where individuals of that specific life cycle stage are concentrated (e.g. recruitment grounds). Successful modelling depends on knowledge of species biology and ecology, thus permitting selection of appropriate variables, measured at an appropriate scale (see Valavanis *et al.*, 2008).

The *O. vulgaris* life cycle characteristics increase this species resilience to high fishing pressure. At the same time *O. vulgaris* populations are vulnerable to overfishing because of non-overlapping generations (Boyle & Rodhouse, 2005), and the juvenile phase (the pre-recruits) is therefore a key life stage on which we may concentrate fishery management to ensure sustainability of this heavily exploited species. The present study aims to, firstly identify along the Portuguese coast areas where pre-recruits of *O. vulgaris* aggregate, using georeferenced fishery-independent data, that may be protected in future management options; and secondly, to analyse the relationships between pre-recruit abundance and several abiotic environmental variables to delineate their optimum habitat.

3.3.2. Material and Methods

3.3.2.1. Survey sampling

Two sets of bottom trawl surveys carried out on the Portuguese continental coast onboard R/V Noruega and R/V Capricórnio were selected for the analysis of *Octopus vulgaris* pre-recruit aggregations, the autumn and winter surveys. The main objective of these research surveys is to estimate indices of abundance and biomass of the most commercially important fish and crustacean species. For our study we selected only the surveys which used a bottom trawling net suitable to sample benthic species, the "FGAV019", 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 sampling area covered latitudes 36.7º to 41.8º N and longitudes 7.47º to 10.0º W in the NE Atlantic. 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 3.8). The tow duration varied between 20 and 60 min, but no significant differences were previously found in the

mean abundance and length distribution for several species due to different tow duration (Cardador, *pers comm*). The number of hauls by research cruise and other characteristics of the sampling procedure are detailed in Table 3.8. In each sampling station, all of the *O. vulgaris* captured were weighted (individual body weight, to the nearest g) and measured (mantle length, to the nearest 0.5 mm).

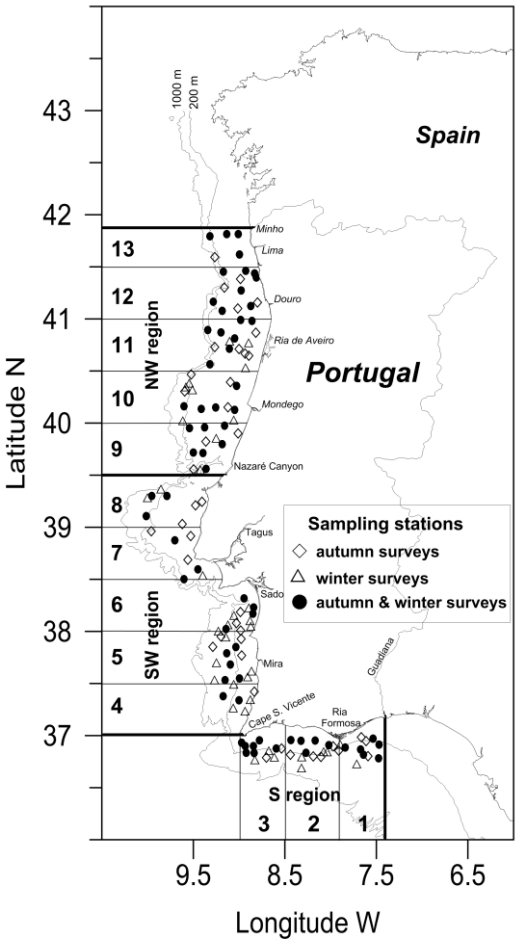


Figure 3.8 - Sampling stations in autumn and winter surveys. Location of survey sampling areas (1 to 13) and regions (NW, SW, S) and landing ports for fishery sampling.

3.3.2.2. Environmental data

The following environmental variables were selected and assigned to each sampling station. Satellite-derived sea surface temperature data (SST) were obtained for each sampling station from the AVHRR Pathfinder V.5 provided by NASA-JPL-Physical Oceanography Distributed Active Archive Center (PO.DAAC). Sea bottom temperature (SBT), sea surface salinity (SSS), and sea bottom salinity (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. Bottom sediment type (BS) in each sampling station was classified based on the fishery charts from the Instituto Hidrográfico as gravel (gv), coarse sand (cs), sand (s), mud (md) and rocky mud (rm). River runoff (RR) from the main rivers and rainfall (RA) were extracted from the Sistema Nacional de Informação de Recursos Hídricos (http://snirh.pt).

Table 3.8 - *Octopus vulgaris* sampling details for each research survey: number of fishing hauls, number of fishing hauls with *O. vulgaris* catches (Fishing hauls +), *in situ* temperature and salinity data (CTD), number of individuals captured and sampled (N), percentage of pre-recruits and mean body weight of the pre-recruits (BWJ).

Cruise	Start date	End date	Fishing hauls	Fishing hauls +	CTD	N	% pre-recruits	BWJ (g)
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
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

3.3.2.3. Data analysis

Octopus with body weight below the fisheries minimum landing weight (750 g) captured on the survey cruises listed in Table 3.8, were classified as pre-recruits and their distribution and abundance used to identify *O. vulgaris* recruitment grounds. Catches were converted into an abundance index as number of pre-recruits per hour trawling.

The spatial distribution of pre-recruits was analysed and mapped with the geostatistics interpolation method, Krigging (Cressie, 1991), implemented in the software SURFER 9.0. Pre-recruit abundance (PR) data were interpolated separately by Krigging, using a linear variogram with no nugget effect, for each cruise and also averaged for autumn and winter cruises. Distinct recruitment grounds were spatially identified as those areas centered at pre-recruit distribution centroids with a mean PR $\geq$ 5 ind.h^{-1} (in at least

one season). Additionally, to assess the relevance of each recruitment ground for the common octopus pre-recruits, an index of exclusiveness, EI was calculated as the ratio between PR and the total species abundance. To verify whether the recruitment grounds were located in the same area consistently through time, an index of persistence, PI (adapted from Garofalo *et al.*, 2010) was also estimated as the proportion of cruises within each season a given recruitment ground was actually used.

To analyze the spatial distribution, the sampled area was divided into 3 regions, which are environmentally different and 13 areas. To analyze distribution with depth (DepthZ), the sampling stations were also assigned to the inner-shelf (is, <40 m), middle-shelf (ms, 40-90 m), outer-shelf (os, 90-200 m), and slope (s, > 200 m). The distance of each sampling station to the coast line (Dcoast) was also estimated. Total pressure (PRS) in each sampling station was estimated from bottom depth. A measure of RR by cruise and area was calculated as the total runoff in each of the 13 areas. A measure of RA by cruise and area was calculated as the total rainfall for the period of each survey cruise recorded in the main coastal city in each of the 13 areas. These values were replicated and assigned to each sampling station within each area. Mean pre-recruit abundance was calculated in relation to categorized temporal, spatial and physical variables. The effects of these explanatory variables in pre-recruit abundances 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 Region and the other variables and the interactions between Season and the other variables, were also tested by two-way ANOVA. The relationships between log transformed pre-recruit abundance (logPR) and the continuous physical explanatory variables: Dcoast, PRES, SST, SBT, SSS, SBS, RA, and log transformed RR (logRR) were further investigated using Generalised Additive Models (GAMs), thereby allowing non-linearity in the relationships to be taken into account. Since logPR was normally distributed we used a Gaussian GAM with identity link. The nominal variables Year and BS were also included in models. For all continuous explanatory variables, degrees of freedom were constrained to be less than 5 to avoid overfitting. Models were fitted using a backwards selection starting with full models and removing explanatory variables with non-significant partial effects. The Akaike Information Criterion (AIC) was used to choose the best fitting model (lowest AIC). More information about these techniques can be found in Zuur *et al.* (2007). Preferred habitat (highest mean

PR) and habitat limits (PR<1 ind.h^{-1}) for *O. vulgaris* pre-recruits within the sampled area were estimated as proxies of EFH for this key life stage.

3.3.3. Results

3.3.3.1. Environmental variation along the Portuguese continental shelf

Over the Portuguese continental shelf SST is much higher in autumn than in winter and was ca. 2-4 ºC lower in the NW region than in the S region. Within the period analysed autumn SST ranged between 14.0 ºC and 21.7 ºC and winter SST ranged between 11.6 ºC and 16.6 ºC (Figure 3.9 a). There was little seasonal variation in the mean SBT (13.9±0.9 ºC), but SBT was on average ~1.5ºC lower in the NW region than in the S region. Autumn SBT ranged between 11.4 ºC and 17.3 ºC, and winter SBT ranged between 11.7 ºC and 15.8 ºC (Figure 3.9 b). In autumn the water column was still stratified and important differences existed between SST and SBT, namely in the SW and S regions (~5 ºC). On the contrary SST was only slightly higher than SBT during the winter period of water column mixing, namely on the NW region (~0.5 ºC).

The expected positive correlation between temperature and salinity may be observed by the spatial variation of salinity similar to that of temperature, decreasing with increasing latitude and also away from the influence of the Mediterranean salty waters. Despite this fact, both mean SSS and mean SBS along the Portuguese shelf were lower in autumn (when temperature was higher) than in winter (Figure 3.9 c, d). Rainfall levels during autumn and winter were similar between areas 1 and 10, but towards the north rainfall increased substantially, especially during autumn (Figure 3.10 a). The higher river runoff levels occurred in autumn located at areas 7, 10, 12 and 13 (Figure 3.10 b), associated mainly to the rivers Tagus, Mondego, Douro and Lima, respectively. The low saline waters from these river discharges generally spread along the west coast with a poleward direction, therefore with no influence in the areas south of area 7. The seasonal differences in salinity derive most likely from the higher levels of autumn rainfall and consequent river discharges, which contributed to a decrease in salinity of coastal waters more significant in autumn than in winter.

3.3.3.2. Recruitment grounds

O. vulgaris pre-recruits were quite frequent (55-83% of total octopus catches) both during the autumn and winter research surveys and were represented by specimens with a mean body weight of 300 - 450 g (Table 3.8). They were widely distributed along the Portuguese coast, but an important seasonality in their distribution and abundance was apparent, in winter, octopus pre-recruits were more abundant, spread and shifted towards the south (Figure 3.11). In winter abundance was twice as high (mean PR ≈ 6ind.h^{-1}) as in autumn (mean PR ≈ 3ind.h^{-1}), in particular during the winter2005 and winter2008 surveys. However, on the NW region, pre-recruit abundance was higher in autumn, namely in the autumn1996 survey. Within each season abundance was much higher on the S region, especially at area 2, where the concentration of pre-recruits was recurrent each year.

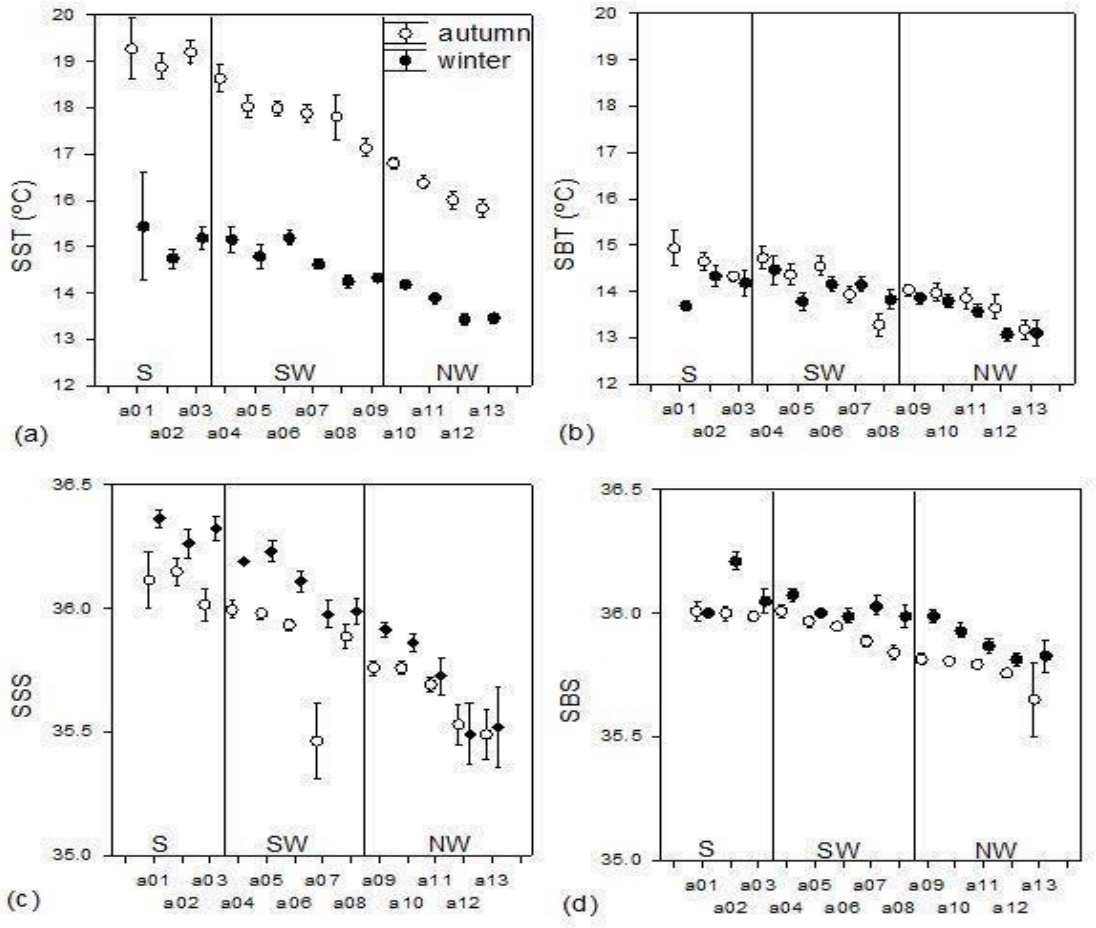


Figure 3.9 - Spatial variation of (a) mean sea surface temperature (SST), (b) mean sea bottom temperature (SBT), (c) mean sea surface salinity (SSS), and (d) mean sea bottom salinity (SBS) during the autumn and winter surveys in areas a01 to a13 within the northwest (NW), southwest (SW) and south (S) regions. Vertical bars denote standard error.

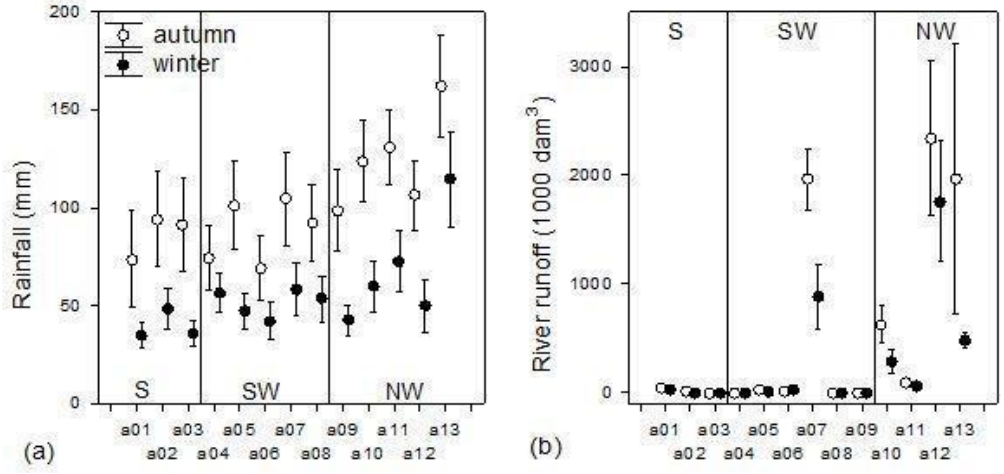


Figure 3.10 - Spatial variation of (a) mean rainfall and (b) mean river runoff in autumn and winter surveys in areas a01 to a13 within the northwest (NW), southwest (SW) and south (S) regions. Vertical bars denote standard error.

Pre-recruits were found aggregated in eight distinct grounds, A to H. On the NW region 3 recruitment grounds were identified during autumn (Figure 3.12 a), located close to the mouth of river Lima (A, only present in autumn1996 survey); close to river Douro and the lagoon system of Ria de Aveiro (B); and south of river Mondego (C). During winter (Figure 3.12 b), the recruitment ground B shifted to the southern part of Ria de Aveiro, partially merging with C. On the SW region, 3 recruitment grounds were also identified: D located at ≈ 140 m in autumn in the vicinity of the Nazaré canyon and shifted southwards closer to the mouth of river Tagus in winter; E at area 6, close to the estuary of the river Sado with higher PR in winter; and the recruitment ground F at areas 4 and 5 extending from the coastline to offshore of the 130 m isobath in winter. On the S region the highest concentration of pre-recruits was located west of the lagoon system of Ria Formosa (H) in autumn (mean above 30 ind.h^{-1}). The whole S region could be considered a single recruitment ground in winter. Despite this, based on spatial differences in abundance within this region, two distinct areas of concentration of pre-recruits may be identified: the recruitment ground G centred at area 3; and H with the highest concentration of pre-recruits (winter mean above 80 ind.h^{-1}), centred at area 2 west of the lagoon system of Ria Formosa, as was in autumn, but spreading eastwards into area 1.

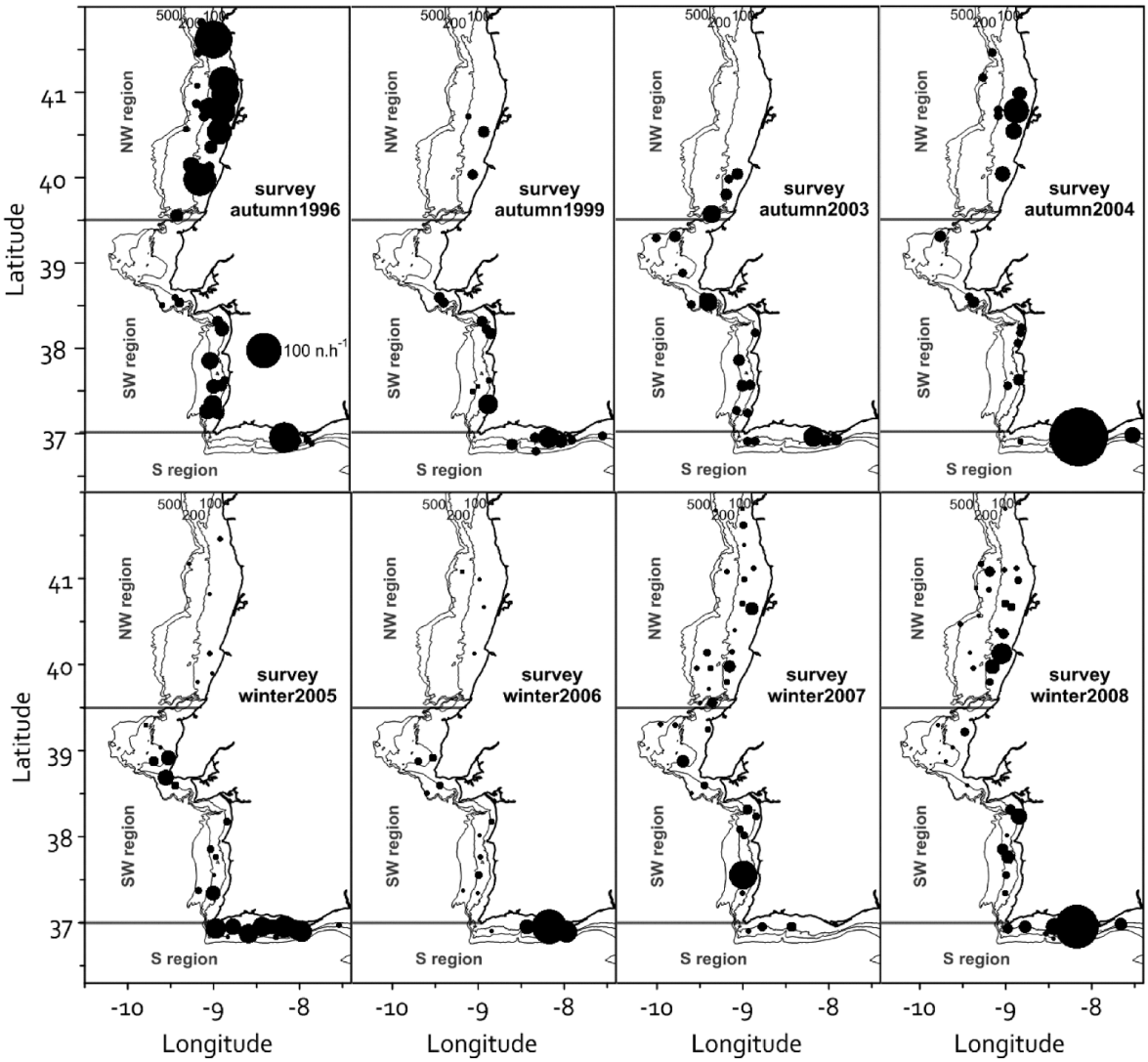


Figure 3.11 - Distribution and abundance of *O. vulgaris* pre-recruits in (a) the autumn and (b) the winter survey cruises.

The location of each recruitment ground is summarized in Table 3.9. Overall, the most persistent recruitment grounds, with PI=1, were the recruitment grounds F and G in winter and H both in autumn and winter. Additionally, these grounds were important habitats in particular for pre-recruits showing high levels of exclusiveness (EI). The recruitment grounds on the NW region showed high EI but low PI giving evidence of higher inter-annual variation in pre-recruit distribution within this region.

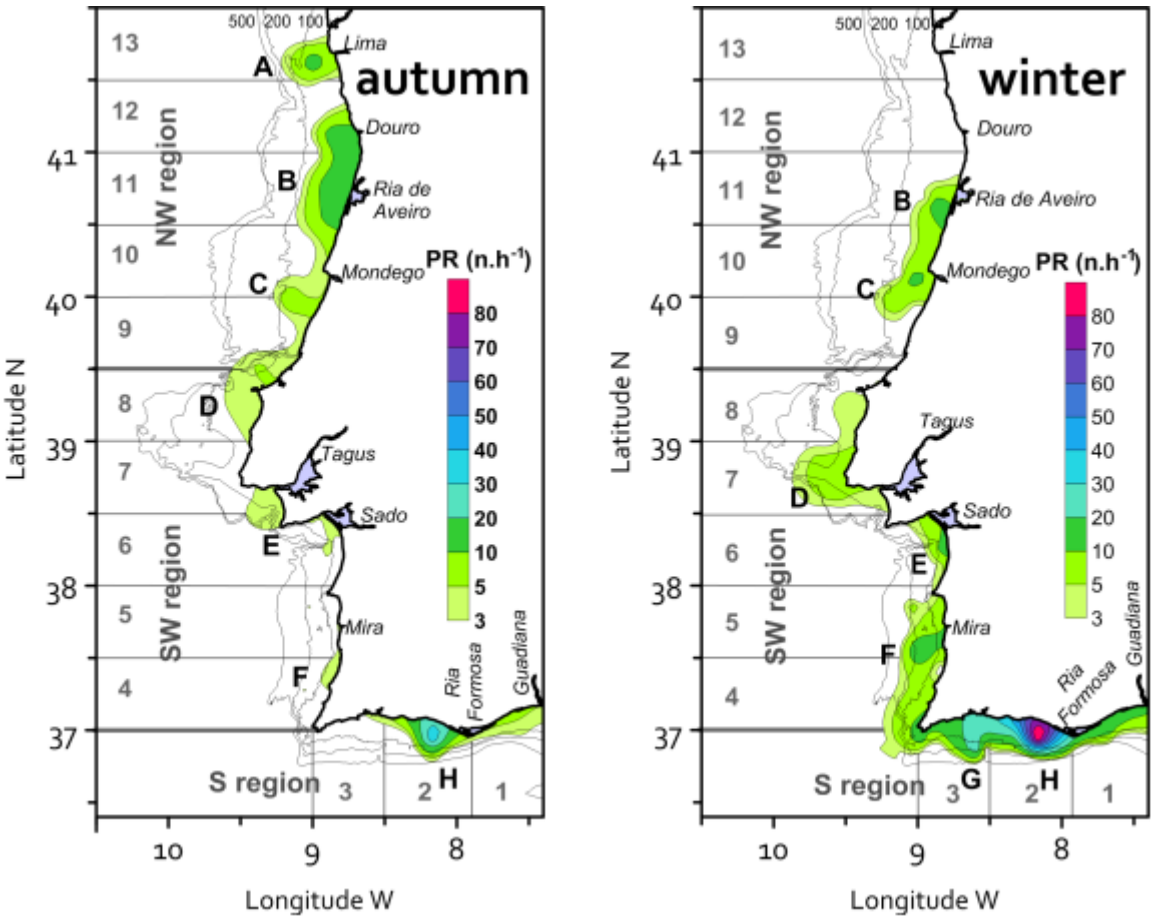


Figure 3.12 - Recruitment grounds of *O. vulgaris* identified by mapping of pre-recruit mean abundances in (a) autumn and (b) winter survey cruises.

3.3.3.3. Environmental effects and essential habitat

ANOVA results indicated that the individual effects of all temporal, spatial and physical variables analysed on pre-recruit abundance were significant. The effects of the explanatory variables Year, DepthZ, Dcoast, BS, SST and SBT were significantly dependent of Region, but independent from any seasonal effects (except for SST) (Table 3.10). The effects of the physical variables related to water salinity: SSS, SBS, RA, and RR, were independent both of Region and Season.

To further investigate the relationships between physical variables and pre-recruit abundance, GAMs were fitted separately for the NW, SW and S regions given the above mentioned "explanatory variable": Region interactions. The optimum models are listed in Table 3.11. The best-fit GAM for pre-recruit abundance on the NW region explains about 47% of deviance and has the form: logPR ~ 1 + factor(Year) + factor(Season) + factor(BS)

+ s(Dcoast) + s(SST) + s(SBS) + s(logRR). The best-fit GAM for the SW region explains ca. 44% of deviance and has the form: logPR ~ 1 + factor(Year) + s(Dcoast) + s(SBS) + s(logRR). Finally, the best-fit GAM for the S region explains ca. 69% of deviance and has the form: logPR ~ 1 + factor(BS) + s(PRS) + s(SST).

Table 3.9 - Recruitment grounds identified by krigging of pre-recruit abundance (PR). PI = Persistence Index and EI = Exclusiveness Index.

Region	Recruitment ground	Season	Centroid PR ($n.h^{-1}$)	Centroid lat°N/long°W	Centroid depth (m)	PI	EI
NW	A	autumn	12.5	41.624/8.997	51	0.33	0.93
		winter	-	-	-	0	-
	B	autumn	14.4	40.798/8.736	68	0.5	0.64
		winter	12.5	40.617/8.871	30	0.5	0.83
	C	autumn	7.8	39.983/9.164	84	0.5	0.95
		winter	11.2	40.101/9.028	51	0.5	0.93
SW	D	autumn	5.1	39.520/9.373	82	0.25	0.78
		winter	9.3	38.716/9.566	55	0.75	0.72
	E	autumn	-	-	-	0	-
		winter	12.3	38.258/8.808	5	0.75	0.63
	F	autumn	-	-	-	0	-
		winter	16.3	37.566/8.998	181	1	0.86
S	G	autumn	-	-	-	0	-
		winter	26.5	36.878/8.591	185	1	0.90
	H	autumn	33.9	36.991/8.152	36	1	0.64
		winter	87.0	36.990/8.152	39	1	0.83

Preferred habitat and habitat limits of *O. vulgaris* pre-recruits are summarized in Table 3.12. The variation in pre-recruit abundance with the categorical variables Year, Season, DepthZ, Dcoast, and BS in each region is depicted in Figure 3.13. Pre-recruit abundance presented an important inter-annual variation within each region (Figure 3.13 a), particularly high in autumn 1996 on the NW region and in autumn 2004 on the S region. During winter, the abundance was very high in 2005, 2006 and 2008 on the S region and in 2007 on the SW region. On the west regions the Year effect explained some variability not accounted by the other explanatory variables considered (Table 3.11). On the other hand, the Year effect was not retained in the optimum GAM for the S region.

Table 3.10 - Effects of temporal, spatial and environmental variables on pre-recruit abundance. Summary of ANOVA results for significant effects ('***'<0.001, '**'<0.01, '*'<0.05).

Pre-recruits ~	Mean Square	df	Residuals Mean Square	Residuals df	F value	p
Year	1.078	7	0.204	378	5.293	***
Season	1.884	1	0.215	384	8.753	**
Region	4.747	2	0.196	383	24.230	***
Area	1.192	12	0.188	373	6.328	***
DepthZ	2.109	2	0.210	383	10.058	***
Dcoast	1.242	10	0.192	375	6.459	***
BS	1.649	4	0.205	381	8.064	***
SST	0.582	7	0.213	378	2.734	**
SBT	0.967	4	0.212	380	4.563	**
SSS	1.136	3	0.190	324	5.980	***
SBS	3.543	2	0.177	323	19.994	***
RA	1.125	4	0.210	381	5.357	***
RR	0.965	3	0.215	377	4.498	**
Region*Year	0.860	14	0.151	362	5.709	***
Region *Season	3.148	2	0.174	380	18.134	***
Region*DepthZ	1.615	5	0.122	564	13.247	***
Region*Dcoast	0.281	17	0.144	545	1.955	*
Region*BS	0.727	7	0.173	372	4.198	***
Region*SST	0.572	13	0.166	363	3.170	***
Season*SST	1.241	2	0.202	375	6.134	**
Region*SBT	1.041	7	0.179	371	5.822	***

Pre-recruit' habitat includes a variety of bottom sediments, but they seemed to concentrate preferably over large grain sediments in relation to fine sand or mud (Figure 3.13 b). Their abundance was the highest over gravel on the NW region and coarse sand on the S region (gravel not sampled). The effect of BS on the SW region was not significant (Table 3.11), but the overlay of the recruitment grounds on a bottom sediments type map (online figure) showed that those on the SW region were located over several BS, all neighbouring rock outcrops.

Pre-recruits were concentrated mainly in the middle-shelf at a mean depth of 79 m (±44 SD) on the NW region, 108 m (±45 SD) on the SW region, and 66 m (±31 SD) on the S region, with insignificant seasonal variation in the preferred depth zone (Figure 3.13 c). The highest abundances were located at 19 km (±9 SD) from the coastline on the NW region, 11 km (±6 SD) on the SW region, and 13 km (±3 SD) on the S region (Figure 3.13 d). The distribution limit was slightly deeper in winter (360 m) than in autumn (332 m), but abundances on the slope deeper than 350 m (PRS > 35.7 atm) were consistently rather low (Table 3.9, PR < 1 ind.h^{-1}). On the SW region, because the continental shelf is narrow and steep, pre-recruits aggregated on the middle-shelf deeper than in the other regions (see also Table 3.9). Nevertheless, distribution on the west coast was mainly affected by the distance

to the coast line (Dcoast) rather than by total pressure (PRS). Abundance decreased linearly with increasing distance to the coast line; gradually on the wider continental shelf of the NW region (Figure 3.14 a), and more obviously on the narrow and steeper continental shelf of the SW region (Figure 3.14 e). On the other hand, pre-recruit distribution on the S region was mainly affected by PRS, with abundance abruptly decreasing until depths of 25 atm and with no further effect in deeper waters (Figure 3.14 h).

Table 3.11 - Results for the optimum GAMs fit to logPR (log transformed pre-recruit abundance) on the northwest (NW), southwest (SW) and south (S) regions. Estimates, SE, t-ratio and associated parameters are given for the nominal variables. The reference year is 1996, the reference season is winter, and the reference bottom sediment types are gravel (NW and SW) or coarse sand (S). Edf, F-statistic and associated probabilities are given for smoothers and the parametric terms ('***'<0.001, '**'<0.01, '*'<0.05, '*ns*'>0.05).

Region	GAM variables	Estimate	SE	t-ratio	p-value	Edf	F-statistic	p-value	Deviance explained	AIC
NW	Year					7	7.61	***	46.5%	104.5
	1999	-0.368	0.082	-4.48	***					
	2003	-0.312	0.077	-4.07	***					
	2004	-0.220	0.066	-3.32	**					
	2005	0.072	0.075	0.96	*ns*					
	2006	-0.132	0.048	-2.74	**					
	2007	0.276	0.062	4.46	***					
	2008	0.043	0.058	0.75	*ns*					
	Season					1	4.76	*		
	autumn	0.201	0.092	2.18	*					
	BS					4	4.37	***		
	coarse sand	-0.193	0.088	-2.18	*					
	sand	-0.325	0.089	-3.65	***					
	mud	-0.301	0.088	-3.42	***					
	rocky mud	-0.042	0.216	-0.19	*ns*					
	Dcoast					1.00	21.3	***		
	SST					3.32	3.81	**		
	SBS					2.24	5.43	**		
	logRR					2.09	8.95	***		
SW	Year					7	3.73	***	44.3%	91.3
	1999	-0.173	0.107	-1.62	ns					
	2003	-0.056	0.114	-0.49	ns					
	2004	-0.287	0.107	-2.68	**					
	2005	0.128	0.115	1.11	ns					
	2006	-0.151	0.116	-1.30	ns					
	2007	0.010	0.114	0.08	ns					
	2008	0.082	0.118	0.70	ns					
	Dcoast					1	24.80	***		
	SBS					3.61	4.28	**		
	logRR					1.50	3.85	*		
S	BS					3	4.39	***	68.7%	83.4
	sand	-0.559	0.175	-3.20	**					
	mud	-0.357	0.115	-3.12	**					
	rocky mud	-0.228	0.141	-1.62	ns					
	PRS					3.77	22.50	***		
	SST					3.08	9.83	***		

SE, standard error; Edf, estimated degrees of freedom; AIC, Akaike Information Criterion.

Table 3.12 – Preferred habitat and habitat limits of *O. vulgaris* pre-recruits. Regional and/or seasonal differences reported only when significant.

Variables	Sampled habitat	Preferred habitat (mean±SD)	Limits (PR<1 ind.h^{-1})
Region	NW,SW,S	S	-
Area	1-13	2	-
DepthZ	NW&SW:is,ms,os,s; S:ms,os,s	ms	-
Depth	NW:20-650m; SW:35-650m; S:45-675m	NW:79±44m; SW:108±45m; S:66±31 m	> 350m
Dcoast	NW:2-60km; SW:2-45km; S:4-43km	NW:19±9km; SW:11±6km; S:13±3km	None
BS	gv,cs,s,md,rm	NW:gv; S:cs	None
SST	NW:11.6-19.6ºC; SW:13.1-20.6ºC; S:13.7-22.0ºC autumn:14.0-22.0ºC; winter:11.6-16.9ºC	NW:15.3±1.3ºC; SW:16.0±1.6ºC; S:16.0±1.8ºC autumn:17.3±1.5ºC; winter:14.8±0.8ºC	< 13ºC < 13ºC
SBT	NW:11.4-17.3ºC; SW:10.3-16.7ºC; S:11.7-17.0ºC	NW:14.0±0.7ºC; SW:14.1±0.7ºC; S:14.6±0.9ºC	< 12ºC
SSS	33.26-36.90	35.99±0.31	<34.70 & >36.47
SBS	33.90-36.60	36.03±0.16	<35.60 & >36.30
RA	36-730mm	166±82mm	> 438mm
RR	0-3357835dam^3	155904±394096dam^3	None

Over the continental shelf pre-recruits were found associated preferably with SST of 15.3 ºC (±1.3 SD) on the NW region and with SST of 16.0 ºC (±1.6 SD) on the SW (Table 3.12). On the NW region, the effect of SST was non-linear showing some seasonal dependence: abundance increases with increasing local winter SST and with increasing local autumn SST, i.e. main recruitment grounds are located under the warmest temperatures available and the general SST effect seems a more complex nonlinear relationship (Figure 3.14 b). On the other hand, abundance decreases with increasing SST on the S region (Figure 3.14 i). In this region, over the continental shelf, pre-recruits were found associated preferably with SST of 16.0 ºC (±1.8 SD). The effect of SBT showed some regional dependence, but regional SBT differences were of small magnitude and the effect of this explanatory variable on GAMs for each region was negligible (Table 3.11). Pre-recruits were found associated preferentially with SBT of 14.0 ºC (±0.7 SD) on the NW region, 14.1 ºC (±0.7 SD) on the SW region and 14.6 º C (±0.9 SD) on the S region. In general, pre-recruit habitat seems to be limited to temperatures above 12.0 ºC, SST>13ºC and SBT>12ºC (Table 3.12).

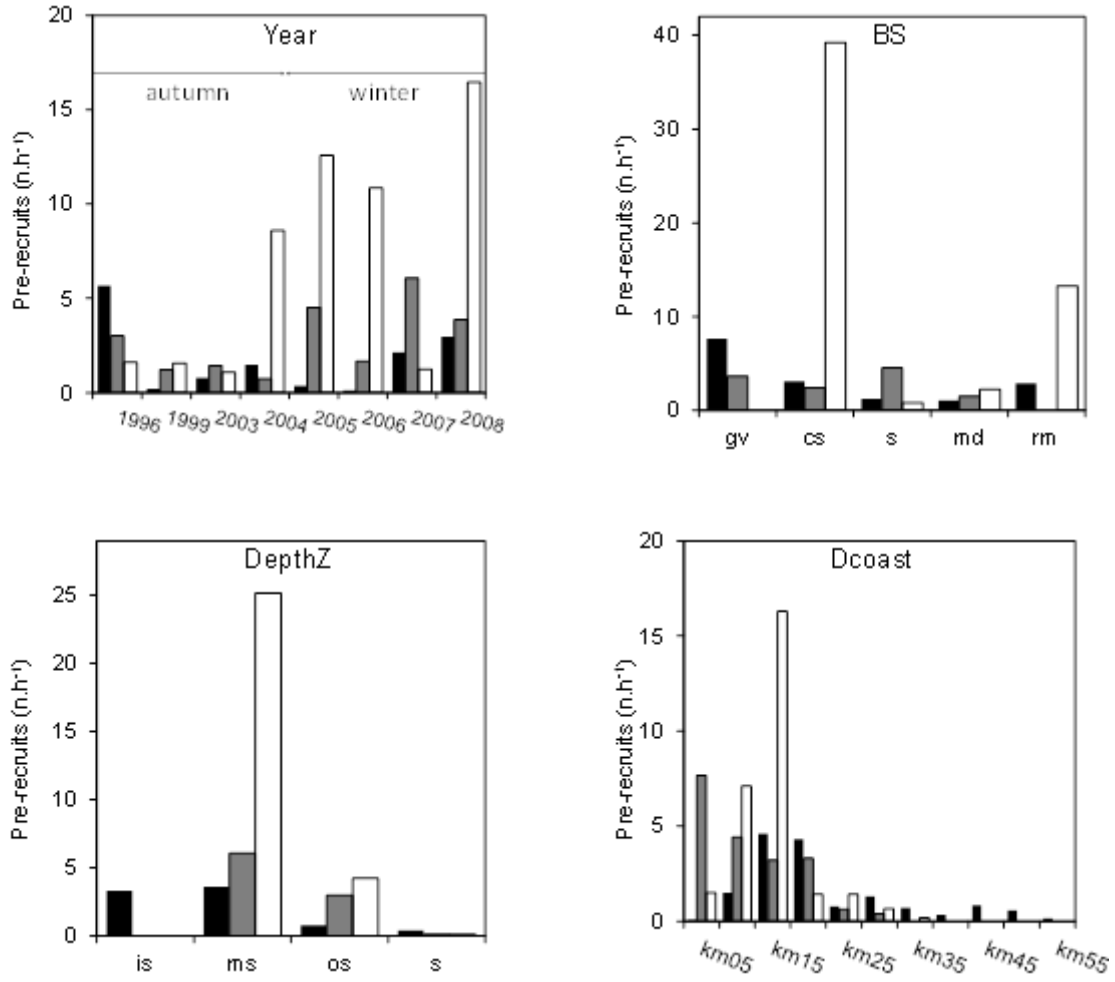


Figure 3.13 – Variation of mean abundance of *O. vulgaris* pre-recruits on the NW (black bar), SW (grey bar) and S (white bar) regions by (a) in each research cruise (Year), (b) bottom sediment (BS), (c) depth zone (DepthZ), and (d) distance to coast line (Dcoast).

Pre-recruit abundance was consistently higher in the more saline waters, with salinity ≈ 36.0, independently of any regional or seasonal effects. The effect of SSS was not relevant to explain pre-recruit distribution, but on the NW region abundance increased with SBS between 35.5 and 36.2 where most data were obtained. Moreover, the exceptional occurrence of recruitment ground A was probably related to the unusual high salinities in area 13 verified in autumn1996 survey. Episodic lower salinities also introduced some complexity to the SBS effect on this region (Figure 3.14c). SBS explained also the location of recruitment grounds on the SW region: pre-recruit abundance increased with increasing SBS until a maximum of ca. 36.1, decreasing towards the saltiest waters (Figure 3.14 f). In general, pre-recruit habitat was limited by SBS below 35.60 and above 36.30 (Table 3.12).

Higher concentrations of pre-recruits were also related to areas with relatively low rainfall (preferred RA = 166±82 mm), such as on the S region (Table 3.12). However, rainfall was not relevant to explain pre-recruit distribution within each region (Table 3.11). Similarly, the highest concentrations of pre-recruits were found on the S region, where there is considerably less river runoff than on the west coast (Figure 3.10 b). Both on the NW and SW regions, distribution and abundance were significantly affected by RR. Despite the main recruitment grounds were located close to the main rivers and lagoon systems, i.e. associated to areas with important river runoff, abundance decreased under the highest runoff levels of the NW region (Figure 3.14 d). This negative effect was not observed on the SW region, (Figure 3.14 g), despite the important RR levels which occurred in area 7 (Figure 3.10 b).

3.3.4. Discussion

O. vulgaris pre-recruits were widely distributed along the Portuguese coast. The abundance was substantially higher in the south coastal waters than on the west coast as expected, considering that the general distribution of this species in the Eastern Atlantic have the highest densities towards the south, namely on the north-western coasts of Africa (Balguerias *et al.*, 2000). Pre-recruit distribution in Portuguese waters was patchy and animals aggregated in several recruitment grounds. The area surrounding the lagoon system "Ria de Aveiro" seems to be the most important recruitment ground for *O. vulgaris* in the NW region, and on the SW region pre-recruits aggregated in recruitment grounds close to Tagus and Sado estuaries. Moreover, on the S region, despite the whole continental shelf showed a high abundance of small octopus, they were aggregated more abundantly close to the lagoon system "Ria Formosa". Consequently, some of the most important recruitment grounds of *O. vulgaris* are located in the vicinity of estuarine or lagoon systems. These are highly productive ecosystems with high abundance and diversity of marine fish and invertebrate species, providing excellent feeding conditions to the young 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 may take advantage of these productive ecosystems by living in their vicinity.

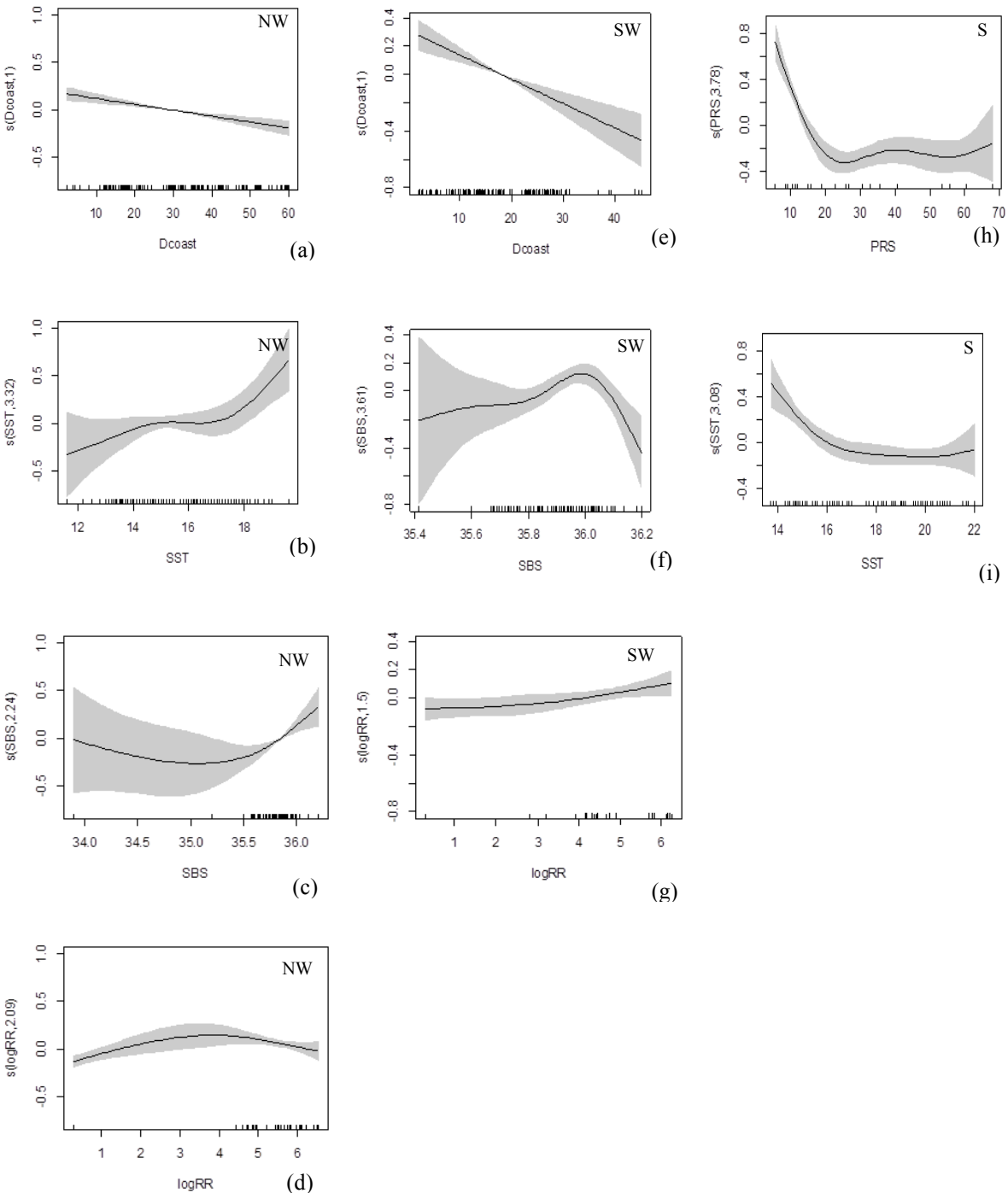


Figure 3.14 – GAMs results: smoothing curve for partial effect of (a,e) distance to the coastline (Dcoast), (b,i) sea surface temperature (SST), (c,f) sea bottom salinity (SBS), (d,g) log transformed river runoff (logRR), and (h) total pressure (PRS) on pre-recruit abundance (PR) on the northwest (NW), southwest (SW) and south (S) coasts. Shaded area indicates 95% confidence intervals around the main effects. The density of tick marks on the x-axis indicates the data points available for different values of x.

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).

Likewise in Portuguese waters, in other areas of *O. vulgaris* distribution, juveniles and sub-adults generally aggregate in coastal waters at a relatively short distance from the coastline. It is the case of several Mediterranean areas, where the highest abundances of *O. vulgaris* captured by bottom trawl surveys (mostly pre-recruits) were found within the depth range of 10-50 m by Belcari *et al.* (2002). Similarly, sub-adult densities are higher in shallow waters (20-40 m) than further offshore in the NW African coasts (Faraj & Bez, 2007). Even so, these authors located the preferred distribution of small octopus inshore of the main recruitment grounds identified along the Portuguese coast. In contrast, a different demographic distribution was described by Belcari *et al.* (2002), Quetglas *et al.* (1998) and Sanchez & Obarti (1993) for the Western Mediterranean and by Hernandez-Garcia *et al.* (1997) for the Canary Islands, as they all observed a wider bathymetric distribution of the small animals. In Portuguese waters we also found a wide distribution of *O. vulgaris* pre-recruits, with aggregations centred at mean depths of ~80 m, and approximately at 10-15 km from the coast. However, the surveyed area of the cruises analysed poorly covers the inner-shelf, namely on the S region (see Figure 3.8), the region where the abundance was the highest, which may have contributed to an overestimation of the pre-recruit preferred depth or distance to the coast line. Overcoming this sampling constrain, both the spatial distribution predicted by geostatistics interpolation of abundance and the GAM modelling of the Depth or Dcoast effects on PR for each region indicated that PR increases towards shallow waters, and for that reason the main recruitment grounds should be in general located in even more coastal areas.

O. vulgaris adults are equally abundant over a variety of sediment types (Mangold, 1983). Nevertheless, we observed that sub-adults have a preference for large grain sediments, namely gravel and coarse sand. Despite the lack of sampling over the rocky ocean floor, we found also the pre-recruit distribution to be closely related to the proximity of rocky bottoms and abundance over mud with rock outcrops significantly higher than over soft mud. This positive relationship between small size octopus density and sediment grain size was also documented by Katsanevakis & 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 shelters ("dens") in the substratum (Mather, 1988).

The relationships between pre-recruit abundance and several abiotic environmental variables were analyzed to delineate pre-recruit optimum habitat and eventual

environmental limitations to their distribution. Using presence-absence data, Hermosilla *et al.* (2011) modelled the distribution and large scale 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.

In Portuguese waters salinity proved to be an important determinant for the aggregation of *O. vulgaris* pre-recruits. Their distribution was limited by SBS below 35.60 and above 36.30 and the preferred habitat was characterized by salinities of ≈ 36.0. The higher abundance was hence observed on the S region, where both rainfall and river runoff are generally low and mean seasonal SBS has a narrow range (36.01-36.21, Figure 3.9 d). Within its global distribution this species prefers habitats with bottom salinity between 30 and 45 (Hermosilla *et al.*, 2011), and is fairly abundant under salinities at least up to 39 (e.g. in the Greek Seas, Katsanevakis & Verriopoulos, 2004a). Thus, the reason why we observed a negative effect of salinities above 36.03 in Portuguese waters may be partially related to the low availability of more saline waters in the study area, or that negative effect relates to regional environmental tolerances. At the same time, low salinity is a limitation for octopus survival (Chapela *et al.*, 2006; Vaz-Pires *et al.*, 2004) and hence constrains its distribution. The negative effect of SBS was mainly observed on the west coast. Here, salinities generally fall below 36.0 in autumn and winter, namely on the northern area caused by heavy rainfall and subsequent important river discharges. Despite this, as mentioned before, several recruitment grounds were identified close to large rivers and lagoon 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 episodes 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 effect of temperature on pre-recruits distribution was also important, even if less significant than salinity. It was observed that SST affects 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 temperature near the bottom. In that sense, pre-recruits were found associated preferably with SBT between 15

and 16 ºC and the main recruitment grounds were limited to mean SBT > 13.6 ºC. Similarly, to the general distribution of the species in relation to the available environment described by Hermosilla and co-workers (2011), we found that the pre-recruits of *O. vulgaris* concentrate also under the warmest temperatures, i.e. prefer areas where SBT have an average greater than those of the environment. On the other hand, if compared with the habitat modelling in Hermosilla *et al*. (2011), cold water (SBT < 12 ºC) seems to impose a more pronounced limitation to pre-recruit distribution than to adult distribution. During the surveys analysed, SBT was quite stable within each region and no significant effect of this variable on PR was retained by GAMs. Even so, on the NW region we observed important seasonal and inter-annual variation in distribution and abundance of pre-recruits which may stand for seasonal and yearly differences in SBT (e.g. unusual low SBT in autumn 2004). Even if temperature at surface supposedly affects less the life stages living on the bottom, we found a significant effect of SST on pre-recruit distribution (positive on the west regions and negative on the S region). Pre-recruit preferred habitat was characterized by SSTs of 15.3-16.0 ºC and limited by SST<13 ºC. Furthermore, SSTs above 21 ºC seem to have constrained the eastwards spread of the recruitment ground H on the S region in 2003. In this region the Year effect was not kept in the optimum GAM for the S region and consequently the inter-annual variation in pre-recruit abundance in this region must be explained essentially by SST (the only variable in the model passive of yearly changes).

Other studies which modelled *O. vulgaris* abundance in relation to environmental variables found that oceanographic conditions play a very significant role in determining the distribution and abundance during the paralarval phase (e.g. Moreno *et al.*, 2009; Otero *et al.*, 2009). Inter-relationships between paralarval distribution, settlement and recruitment ground locations are still largely unknown. This is a field in which much work is still required and that we expect will be evolving in the near future.

Based on pre-recruit abundance, PI and EI together we may consider the winter recruitment grounds D and F on the SW region, and G and H on the S region, as the main pre-recruit hot spots on the Portuguese continental shelf, where these animals meet their optimum habitat. Ria Formosa recruitment ground (H) is probably the most important *O. vulgaris* pre-recruit hot spot along the Portuguese coast showing the highest abundances both in autumn and in winter. Important recruitment grounds were also observed on the NW region close to Ria the Aveiro (B) and south the mouth of the river Mondego (C), but

pre-recruit abundance showed larger inter-annual variation, probably associated to the environmental dynamics of the local upwelling ecosystem.

Overall, the distribution pattern of pre-recruits predicted by the spatial interpolation might have missed the identification of some important aggregation areas. Indeed, the model predicted low concentration of small octopuses in the eastern part of the south coast. Notwithstanding, according to fishermen surveys, in this part of the coast aggregations occur in very shallow waters (6–12m depth) between October and December, with higher concentrations in front of the inlets of the Ria Formosa lagoon and near the estuary of the river Guadiana. In these areas, there is high abundance of prey, mainly bivalves (Rufino *et al.*, 2010), and the substrate is coarse (Rosa *et al.*, in press) with a high fraction of bioclasts constituted by large molluscan shells fragments where small octopuses can hide. Altogether, food availability and habitat characteristics might explain the high concentration of pre-recruits in this part of the coast. Similarly, fishermen also reported that small-sized octopuses are numerous during winter in the westernmost part of the south coast (between Lagos and Sagres), especially near the rocky outcrops in inshore waters, where they find refuge from predators.

The recruitment grounds 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 recruitment grounds (Pilar-Fonseca *et al.*, 2014). In principle, 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 recruitment 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* pre-recruits essential habitat. 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 & Verriopolus, 2004b). Additionally, it would be desirable to include 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. More input derived from "Fishers ecological knowledge" (FEK) would be desirable in future studies.

ACKNOWLEGEMENTS

We are very grateful to the survey cruise teams for all the octopus sampling. We also thank Corina Chaves for her help with the survey database CRUZDEM. The work was undertaken within the scope of the Project "Desarrollo Sostenible de las Pesquerías Artesanales del Arco Atlántico - PRESPO" (Programme INTERREG IV B, co-financed by European Union, ERDF funds), was a contribution to the project LongUp (PTDC/AAC-CLI/105296/2008) funded by FCT, and was partially financed by the "Data Collection for Fisheries Sector Programme" (DCF-EC/DG Maritime Affairs & Fisheries/Joint Research Center).

BIBLIOGRAPHIC REFERENCES

ARDIZZONE, G.D., 2006 (Inédito). Sensitive and Essential Fish Habitats in the Mediterranean Sea. Working document to the STECF/SGMED-06-01 sub-group meeting on sensitive and essential fish habitats in the Mediterranean; 2006. Rome, 17.

BALGUERÍAS, E., QUINTERO, M.E., HERNÁNDEZ-GONZÁLEZ, C.L., 2000. The origin of the Saharan Bank cephalopod fishery. *ICES Journal Marine Science* **57**, 15-23.

BELCARI, P., SARTOR, P., 1999. *Octopus vulgaris*, in: Relini, G., Bertrand, J.A., Zamboni, A. (Eds.), Synthesis of the knowledge on bottom fishery resources in Central Mediterranean (Italy and Corsica), *Biologia Marina. Mediterranea*. **6**, 757-766.

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.

BOYLE, P.R., RODHOUSE, P., 2005. Cephalopods: Ecology and Fisheries, John Wiley & Sons, New York.

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.

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.

CRESSIE, N., 1991. Statistics for spatial data, J. Wiley, New York.

DGRM, 2012. Recursos da pesca, Ano 2011. Série Estatística 24A-B (online in http://www.dgrm.min-agricultura.pt/).

DOMAIN, F., JOUFFRE, D., CAVERIVIÈRE, A., 2000. Growth of *Octopus vulgaris* from tagging in Senegalese waters. *Journal of the Marine Biological Association U.K*. **80**, 699-705.

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

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 Marine Science* **67**, 1363-1371.

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.

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

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 Marine Biology - An Annual Review*. **47**, 119-190.

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.

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.

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., 2006. Modelling the effect of temperature on hatching and settlement patterns of meroplanktonic organisms: the case of octopus. *Scientia Marina* **70**, 699-708.

LOURENÇO, S., MORENO, A., NARCISO, L., GONZÁLEZ, A.F., PEREIRA, J., 2012. Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. Fisheries Research. **127-128**, 116-124.

MANGOLD, K., 1983. *Octopus vulgaris*, in: Boyle, P.R. (Ed.), Cephalopod Life Cycles Volume I: Species Accounts. Academic Press, New York.

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

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.

OTERO, J., ÁLVAREZ-SALGADO, X.A, GONZÁLEZ, Á.F., GILCOTO, M., GUERRA, Á., 2009. High-frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. *Marine Ecology Progress Series* **386**, 123-132.

PILAR-FONSECA, T., CAMPOS, A., PEREIRA, J., MORENO, A., LOURENÇO, S., AFONSO-DIAS, M. 2014. Exploitation patterns and size composition of *Octopus vulgaris* in Portuguese waters (Northeast Atlantic) derived from fine-scale geo-referenced bottom-trawl commercial catches. *Marine Policy* **45**, 69-75.

QUETGLAS, A., ALEMANY, F., CARBONELL, A., MERELLA, P., SÁNCHEZ, 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.

ROSA, F., RUFINO, M., FERREIRA, Ó., MATIAS, A., BRITO, A.C., GASPAR, M.B., in press. The influence of coastal processes on inner shelf sediment distribution: The Eastern Algarve Shelf (Southern Portugal). *Geologica Acta*, 10.

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).

RUFINO, M.M., GASPAR, M.B., PEREIRA A.M., MAYNOU, F., MONTEIRO, C.C., 2010. Ecology of megabenthic bivalve communities from sandy beaches on the south coast of Portugal. *Scientia Marina* **74**, 163-177.

SÁNCHEZ, P., OBARTI, R., 1993. The biology and fishery of *Octopus vulgaris* caught with clay pots on the Spanish Mediterranean coast, in: Okutani, T., O'Dor, R.K., Kubodera, T. (Eds.), Recent Advances in Fisheries Biology, Tokai University Press, Tokyo.

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.

VALAVANIS, V.D., SMITH, C., 2007. Essential Fish Habitats, in: Papaconstantinou, C., Zenetos, A., Tserpes, G., Vassilopoulou, V. (Eds) State of the Hellenic Fisheries, HCMR Publ, Athens.

VASILIS, D.V., PIERCE, G.J., ZUUR, A.F., PALIALEXIS, A., SAVELIEV, A., KATARA, I., WANG, J., 2008. Modelling of essential fish habitat based on remote sensing, spatial analysis and GIS. *Hydrobiologia* **612**, 5-20.

VAZ-PIRES, P., SEIXAS, P., BARBOSA, A., 2004. Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. *Aquaculture* **238**, 221-238.

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.

ZUUR, A.F., IENO, E.N., SMITH, G.M., 2007. Analysing Ecological Data, Springer, New York.

CHAPTER 4

ASSESSING AGE IN *OCTOPUS VULGARIS* JUVENILES IN THE PORTUGUESE NORTHWEST COAST

4. ASSESSING AGE IN *OCTOPUS VULGARIS* JUVENILES IN THE PORTUGUESE NORTHWEST COAST

4.1. STYLET (VESTIGIAL SHELL) SIZE IN OCTOPUS VULGARIS (CEPHALOPODA) HATCHINGS USED TO LOCATE STYLET NUCLEUS IN ADULTS[5]

Sílvia Lourenço[1,2,3], Ana moreno[2], Luís Narciso[1], João Pereira[2], Rui Rosa[1], Ángel González[3]

[1]Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Avenida Nossa Senhora do Cabo 939, 2750-374 Cascais, Portugal

[2]Departamento do Mar e Recursos Marinhos, Instituto Português do Mar e da Atmosfera, I.P.Avenida de Brasília, 1449-006, Lisboa, Portugal.

[3]Instituto de Investigaciones Marinas de Vigo, CSIC, C/Eduardo Cabello, 6. Vigo. E-36208, Spain.

ABSTRACT

The estimation of age and growth of cephalopod stocks is a key issue for their sustainable management. Recently, several studies have successfully validated the daily deposition of growth rings in the vestigial shell or stylets of several octopus species. *Octopus vulgaris* eggs were incubated to hatching at two different temperatures, 18°C and 22°C, to determine stylet size at hatching and assess the effect of temperature in the stylet dimensions. The 3 days-old hatchlings were sectioned transversally and 6 μm sections were stained to enhance the stylet position and visibility. The sections were observed under transmitted light microscopy at 1000x magnification, and the stylets identified as blue/green structures inside of the mantle – funnel retractor muscle. The transversal sections of the whole paralarva allowed the diameter of the embryonic stylet of an octopus species to be measured for the first time. The mean stylet diameter in three-day old paralarvae is 3.99 μm independently of the thermal conditions. Moreover, significant differences in stylet size between captive and wild paralarvae were observed; the latter showed significantly larger stylets, an indication that they are over three-days old. Our results also evidence that the stylet nucleus of hatchlings is much smaller than previously thought based on measurements in stylets of juveniles and adults, and its' dimensions are independent of paralarva and stylet size.

Keywords: *Octopus vulgaris*, hatchlings, vestigial shell, stylet, age.

[5] Submitted to the Journal of the Marine Biological Association UK.

4.1.1. Introduction

The assessment of growth and age provides important input data for many stock assessment models and thus is very important for the sustainable management of fisheries stocks. In cephalopods, considering that the success of recruitment depends almost entirely on environmental conditions, it is quite important to understand how reproduction, life span and in particular growth, are affected by those conditions.

Octopus vulgaris Cuvier 1797 is an important resource for the artisanal and industrial fisheries in all of the Atlantic margin of the Iberian peninsula, with annual average landings of 9185 tons in Portugal (INE, 2013) and 4000 in Galicia (Otero *et al.*, 2005). Females of this species brood thousands of small eggs usually in rocky crevices (Rocha *et al.*, 2001). After hatching, the jet-propelled hatchlings (paralarvae) stay in the pelagic realm, where they are highly vulnerable to predation by the macro zooplankton (mainly decapods) and mid water fish (Roura, 2013; Villanueva & Norman, 2008). During the first days, paralarvae still carry the yolk sac within the visceral mass and combine endogeneous (yolk) with exogenous (prey) feeding until the yolk is completely consumed (Villanueva & Norman, 2008). Thereafter, the paralarvae grow exponentially until settlement, followed by a logarithmic phase where the instantaneous growth rate decreases until the maturation phase is complete (Mangold, 1983; Villanueva, 1995). The life span of *O. vulgaris* was estimated in one to two years (Domain, Jouffre, & Caveriviére, 2000; Katsanevakis & Verriopoulos, 2006). Direct ageing methods based on statolith increment analysis were not found to be useful in incirrate octopods, while approaches using beaks in *Octopus vulgaris* still need accuracy validation, in particular due to erosion by feeding (Raya & Hernández-González, 1998; Perales-Raya *et al.*, 2010; Canali *et al.*, 2011). A further alternative to perform direct age assessments is the use of the vestigial shell or stylet (Sousa Reis & Fernandes, 2002). Stylets are slender, needle–shaped and bent rods, a non-calcified α-chitin structure lying obliquely on the dorso-lateral side of the mantle, that arose from the reduction of the shell in the Incirrata (Budelmann *et al.*, 1997; Naef, 1921, 1923 in Bizikov, 2004). Its function is unclear in finless octopus but its position in the insertion of the funnel retractors, visceral sac and gillnets in the mantle is an indication of a structural function (Budelmann *et al.*, 1997; Bizikov, 2004). Growth of stylets progresses from the centre (stylet primordium) located in the bend through the deposition of concentric layers of semi-transparent chitin. Growth increments and apical lines are clearly visible inside both shoulders under transmitted light (Bizikov, 2004). In the case of *Octopus pallidus* the

nucleus looks pale with no discernable increments (Doubleday *et al.*, 2006). The chemical composition of the matrix of the stylets is based on hydrated calcium phosphates (e.g. hydroxiapatite) and a fraction of the vestigial elements P, Cl and Ca, which can be used to differentiate increment bands (Doubleday *et al.*, 2008; Marquez & Re, 2009; Napoleão *et al.*, 2005).

Stylets have recently been used successfully to assess age in wild populations of some octopus species (e.g. *O. pallidus*, Leporati *et al.*, 2008; *O. cyanea*, Herwig *et al.*, 2012). The fast degradation of the structure upon contact with air and the abrasive techniques used to expose the growth structures are major concerns to the standardization of the techniques and their regular implementation. Nevertheless, new preparation methods are being developed, which appear to produce good quality stylet sections (Barratt & Allcock, 2010) and consequently the age determination by stylet increment analysis (SIA) is potentially an effective tool for the age determination in *O. vulgaris*, as was first advanced by Sousa-Reis & Fernandes (2002). It is also worth noting that the daily deposition of growth increments in the stylets of adults of this species was validated by Hermosilla *et al.* (2010) by staining the stylets with oxytetracycline and tetracycline, and comparing the number of rings produced after staining with the number of days elapsed. However, this marking of adult structures does not validate the age of an individual at first increment formation, essential for a rigorous age validation of the SIA in each species (Campana, 2001). The difficulties and potential inaccuracies associated with determining the age of merobenthic octopuses (such as *O. vulgaris*) using SIA and the importance of validating age at first increment formation are discussed in Doubleday *et al.* (2011).

The present study aimed firstly to develop a technique to rapidly locate the stylets in the muscle of paralarvae, and secondly to determine the stylet size at hatching in newly hatched *O. vulgaris* paralarvae as a tool to define the starting point for age determination in stylets of later stages. Additionally, the stylets of three-day old paralarvae were compared to unknown age paralarvae captured in the wild to determine if the stylet nuclear area is conservative between paralarvae of different sizes and ages and between animals incubated at different temperatures.

4.1.2. Material and Methods

Recently spawned eggs of *O. vulgaris* were raised in captivity at 18ºC and 22ºC. After hatching, three day-old paralarvae from both experimental temperatures were collected. Three-day old paralarvae were chosen to ensure some growth past the hatch check and permit the observation of increments if already formed. All paralarvae were preserved in 70% ethanol. Wild and captive paralarvae were measured under transmitted light binocular microscopy at 30 x magnification. Measurements were taken as follows: total length (TL in mm), mantle ventral length (ML in mm), eye diameter (D-eye in mm) and total weight (W in mg).

To establish the most adequate protocol that could simultaneously permit the location and examination of several sections of the paralarval stylet, six captive individuals were embedded in paraffin and sectioned (in 6 µm width sections) according to three morphological planes: (A) the sagittal, (B) transversal, and (C) frontal planes (Figure 4.1). Sections were stained with acetic alcian blue solution (n = 3) and Masson's trichrome (n = 3) in order to enhance the fibrous nature of the stylets, by staining fibrin tissue in a solution of acetic alcian blue (adapted from Vecchione, 1991) or light green/blue (Jones, 2002), respectively. It was expectable that the staining would improve the identification of the structures inside the mantle and funnel muscles. Stained sections were observed under a binocular microscope equipped with transmitted light, at 400 x and 1000 x magnification. All sections were sequentially photographed. Taking into account the results of the experiment above, two groups of 3 day-old paralarvae (18ºC group and 22ºC group, n=20) and wild paralarvae (n=9) were subsequently sectioned in the transversal plane in 6 µm sections and stained with Masson's Trichrome method. All sections were observed under transmitted light at a magnification of 400 x and 1000 x and photographed.

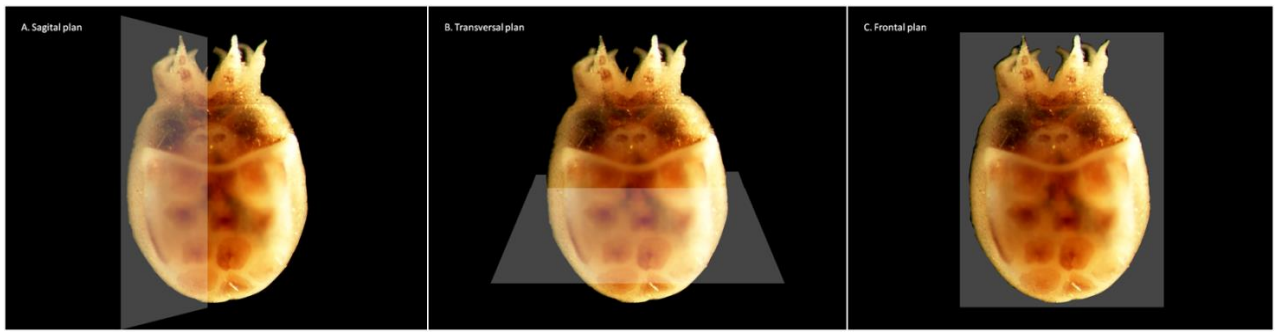


Figure 4.1 – *Octopus vulgaris* paralarva and section plans used to obtain transversal sections of the stylets. (A) sagital plan; (B) transversal plan; (C) frontal plan.

The elected transversal sections of the stylet (the best transversal section closer to the stylet bend), were used to identify the embryonic primordium or nucleus of the stylet and to estimate the diameter of the stylet at hatching. The nucleus is limited by a discontinuity which appears as a high-contrast micro-increment with a deeply darker zone under transmitted light, or an abrupt change in the micro-structural growth pattern (Panfili *et al.*, 2002).

Stylet measurements were taken under 1000x magnification from the selected section of the stylet, as follows: stylet diameter (SD in μm), stylet perimeter (SP in μm), stylet area (SA in μm^2), stylet major radius (SRmax in μm) and the radius of the nucleus (SRnucleus in μm).

To assess the effect of temperature on paralarva and stylet sizes, size data were grouped according to the incubation temperature and sampling source, as "18ºC" and "22ºC" groups for paralarvae raised in captivity and "wild" group for the paralarvae collected during the mesozooplankton surveys. Prior to the statistical analysis, the assumptions of sample normality and homogeneity were assessed by group with Shapiro-Wilk's and Bartlett´s tests, respectively. A non-parametric Kruskal-Wallis test was used to identify differences in mean measurements between groups, followed by a non-parametric post-hoc test whenever significant differences were found between mean values.

Additionally, wild paralarvae of unknown age were collected in July and September 2010 in Ría de Vigo (Southwest Galicia, Spain) during mesozooplankton surveys. These paralarvae were collected in depth and surface strata using a multitrawl (MultiNet®) sampler (0.71 × 0.71 m opening frame, see Roura (2013) for details). The wild paralarvae were stored in 70 % ethanol and measured similarly to the captive paralarvae. These were then transversally sectioned according to the protocol defined for captive paralarvae and stained with Hemotoxylin & Eosin. Selected sections were measured following the same procedure defined for the captive paralarvae. The wild paralarvae and respective stylet dimensions were compared with the captivity group with a non-parametric Kruskal-Wallis test of variance.

A Spearman correlation index was used to identify cases of collinearity between the measurements taken from the whole paralarva or the stylet, as well as to identify strong correlations between the size of the paralarva and measurements of the respective stylet.

4.1.3. Results

As in adults, the stylets of *O. vulgaris* paralarvae were located at the insertion of the funnel retractor muscles, in the posterior region of the mantle. In relation to those of adults, in paralarvae these structures were situated more dorsally and mid region of the mantle (Figure 4.2). In the paralarva, the stylet bend (where the primordium of the structure is located) was found to lie between 100 μm and 200 μm from the tip of the mantle.

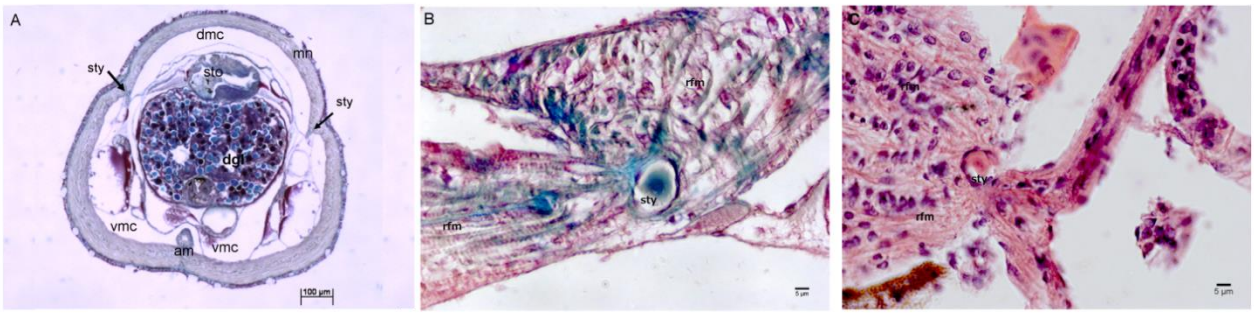


Figure 4.2 – Transverse section (A) of an *Octopus vulgaris* paralarva (magnification: 400x). The stylets are well inserted in the antero-dorsal region of the mantle. Detail of a section of an *Octopus vulgaris* stylet (B, magnification: 630x) obtained from the transverse section of the paralarva. Cross- section of an *Octopus vulgaris* stylet (C, magnification: 630x) belonging to a wild paralarva of unknown age. Acronyms: am – aductor muscle; dgl - digestive gland; dmc - dorsal mantle cavity; mn - mantle; rfm - funnel retractor muscle; sto - stomach; sty – stylet; vmc - ventral mantle cavity (after Bizikov, 2004).

The use of Masson's trichrome as a stain clearly improved the ability to locate the stylet inside the mantle – funnel retractor muscle insertion (compared to alcian blue). Due to its α-chitin nature similar to cartilage tissue, the stylet appeared stained in most paralarvae sections as green/blue. The stylet is anterior-posteriorly oriented in the mantle with the anterior branch (or rostrum) inserted deep inside the mantle muscle, the bend was located inside the mantle – funnel retractor muscle insertion, and the post-rostral branch positioned more superficially along the interior wall of the mantle (Figure 4.3). Several orientation experiments were made to obtain the best section of the stylet, frontal (Figure 4.3), sagittal (Figure 4.4) and transversal planes of the paralarva (Figure 5). Although both sagittal and transversal plane sections gave good results in terms of locating the primordium, the transversal section improved the chances of obtaining good cross sections of the stylet near the bend (Figure 4.5). This transversal plane allowed firstly to identify the stylet at the bend level in the mantle-funnel retractor muscle insertion and then to identify the best section where it was possible to detect the hatch check in the stylet and to measure the diameter, perimeter, area and major radius of the stylet.

In the three day-old paralarvae, the mean diameter of the stylet (measured between the most distant points) was 3.99 ± 0.46 μm and the mean area measured was 13.00 ± 6.13 μm^2. In those stylets, the nucleus was only identifiable in the sections near the bend. It was identified as a distinctively darker area circumscribed by one highly-contrasted micro-increment (with a deeply darker zone), and within which first order growth rings are not observed. The mean diameter of the nucleus was 2.71 ± 0.42 μm.

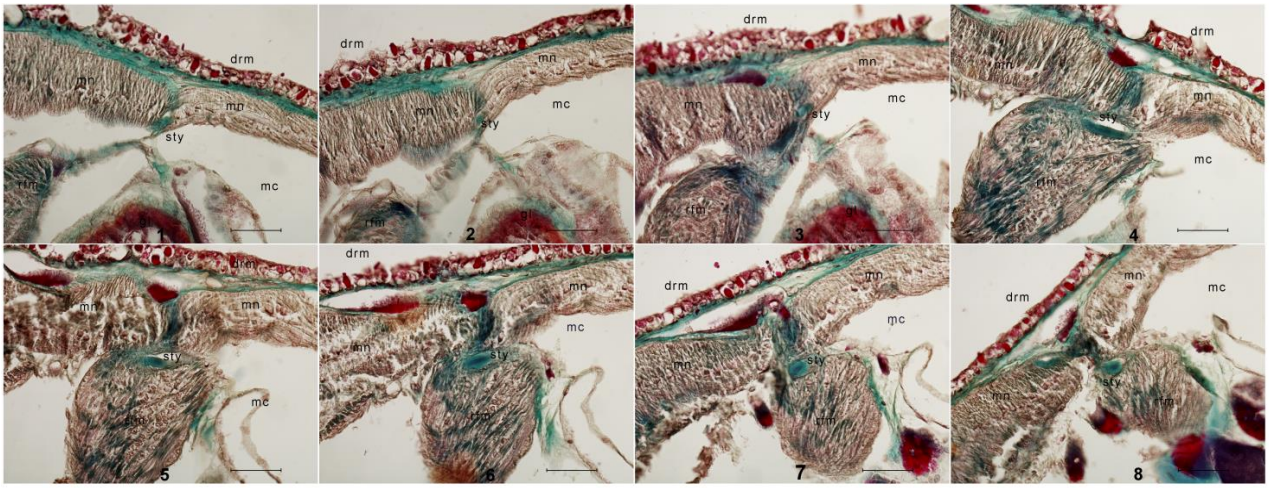


Figure 4.3 – Sequence of frontal sections (magnification: 400x) of a one day-old *Octopus vulgaris* paralarva indicating the sequential position of the stylet in the insertion between the mantle and retractor funnel muscle. Scale bar indicates 20 μm. Acronyms: drm: dermis; dgl_ digestive gland; gl: gills; mc: mantle cavity; rfm: retractor funnel muscle; sty: stylets (after Bizikov, 2004).

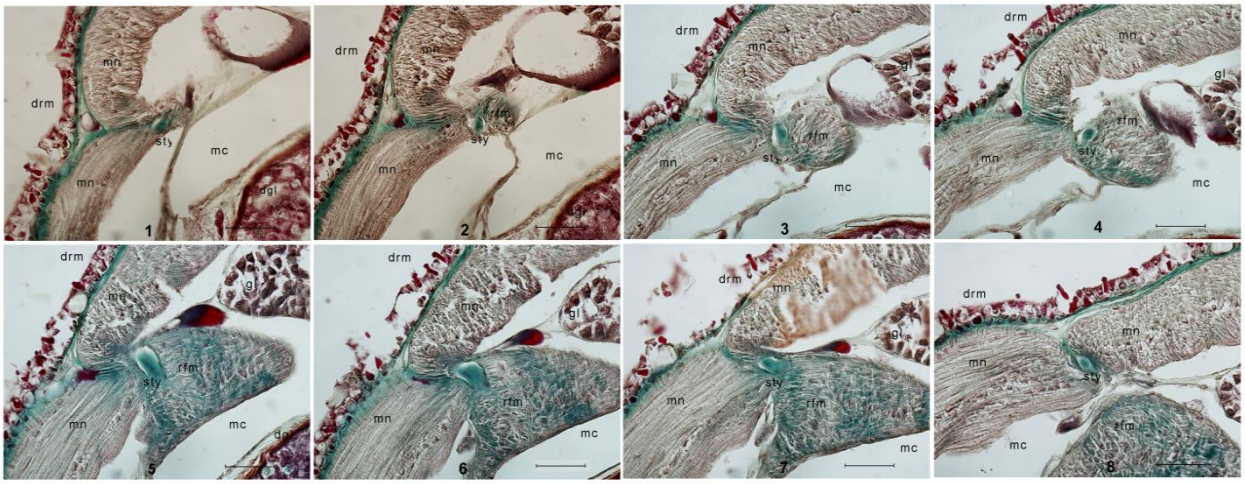


Figure 4.4 – Sequence of sagittal sections (magnification: 400x) of a one day-old *Octopus vulgaris* paralarva indicating the sequential position of the stylet in the insertion between the mantle and retractor funnel muscle. Scale bar indicates 20 μm. Acronyms: drm: dermis; gl: gills; mc: mantle cavity; rfm: retractor funnel muscle; sty: stylets (after Bizikov, 2004).

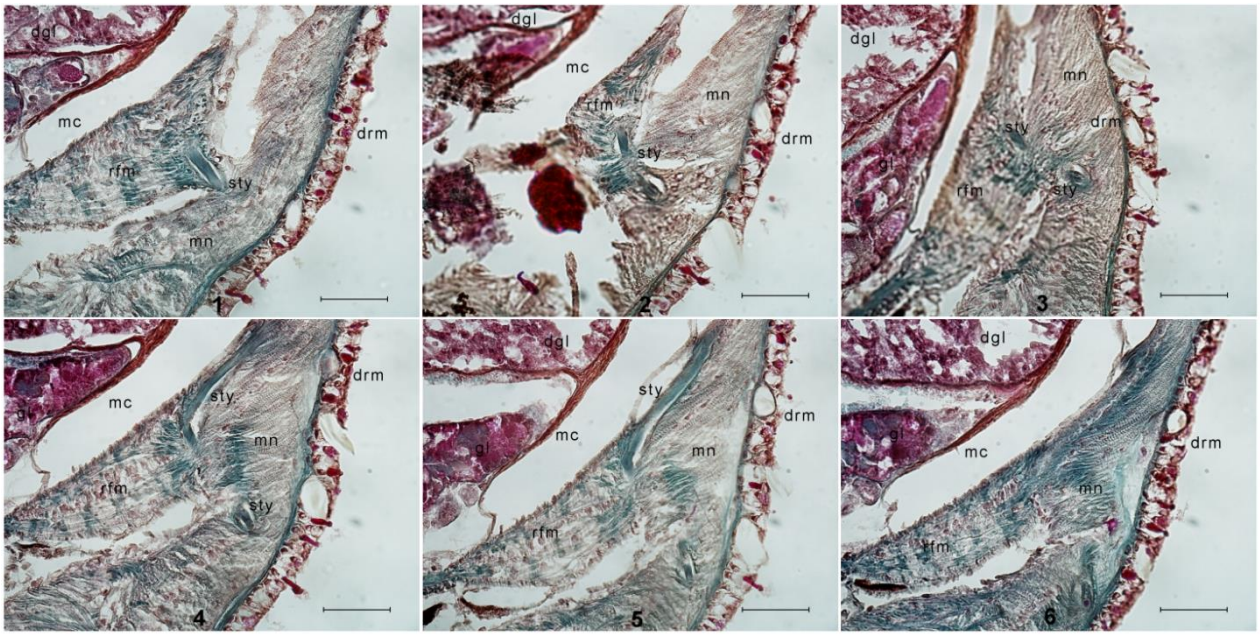


Figure 4.5 – Sequence of transversal sections (magnification: 400x) of a one day-old *Octopus vulgaris* paralarva indicating the sequential position of the stylet in the insertion between the mantle and retractor funnel muscle. Scale bar indicates 20 µm. Acronyms: drm: dermis; dgl_ digestive gland; gl: gills; mc: mantle cavity; rfm: funnel retractor muscle; sty: stylets (after Bizikov, 2004).

To determine the effect of temperature on stylet diameter as well as its variability, twenty cross sections of the stylet were measured and compared to the corresponding paralarval dimensions between "18ºC" and "22ºC" groups. Both paralarval dimensions and stylets measurements did not present any significant difference between the two groups (see Kruskal-Wallis comparison results for groups "18ºC" and "22ºC" in Table 4.1).

Table 4.1 – *Octopus vulgaris* paralarvae and stylets mean (± Standard deviation) dimensions by group. Different superscripts indicate statistically significant differences between groups tested by a Kruskal-wallis test with significance level p-value < 0.05.

			Paralarvae			
Group	Mantlelength	Total length	Eyediameter		Weight	
18ºC	0.96 ± 0.15[a]	1.9 ±0.07[a]	0.33 ± 0.03[a]		1.05 ± 0.05[a]	
22ºC	1.09 ± 0.10[a]	1.95 ± 0.07[a]	0.32 ± 0.03[a]		1.13 ± 0.10[a]	
Wild	1.61 ± 0.19[b]	2.41 ± 0.30[b]	0.44 ± 0.05[b]		2.45 ± 0.30[b]	
			Stylet			
Group	Stylet diameter	Stylet Perimeter	Stylet Area	Stylet major radius	Stylet nucleus radius	Stylet nucleusdiameter
18ºC	3.91 ±1.19[a]	14.71 ± 4.58[a]	12.88 ± 7.56[a]	2.28 ±0.84[a]	1.26 ± 0.24[a]	2.52 ± 0.48[a]
22ºC	4.06 ± 0.76[a]	16.12 ± 3.40[a]	13.11 ± 4.94[a]	2.43 ±0.64[a]	1.41 ± 0.46[a]	2.82 ± 0.92[a]
Wild	5.88 ± 0.95[b]	22.23 ± 4.86[a]	27.54 ± 8.62[b]	3.39 ±0.72[b]	1.51 ± 0.27[a]	3.02 ± 0.55[a]

The nuclear area previously defined in the captive paralarvae was easily identified in the nine stylets of wild paralarvae by its microstructure. In the wild paralarva group, the diameter of the stylet measured 5.98 ± 0.95 μm and the area measured 27.54 ± 8.62 μm^2. The diameter of the stylet nucleus measured 3.02 ± 0.55 μm, and, it was only possible to identify the deposition of one growth increment in the post-nuclear area (Figure 2C) of the stylets of two wild paralarvae.

When comparing paralarval and stylet measurements between the three-day old captive paralarvae and the unknown age wild paralarvae, statistically significant differences arise. Paralarvae captured in the Rias Baixas presented significantly higher mean ML, TL, D-eye and weight in relation to the paralarvae for which the embryologic development occurred in captivity. Nevertheless, the diameter of the nucleus did not show significant differences between a combined captive group ("18ºC" and "22ºC" groups pooled together) and wild paralarvae (Table 4.1).

The comparison between paralarval measurements did not show any significant collinearity (Table 4.2). On the other hand, for the stylet dimensions, significant collinearity was found between SD and SP (r_s= 0.90, N = 29, P< 0.001), between SD and SA (r_s= 1.00, N = 29, P< 0.001), between SD and SRmax (r_s= 0.94, N = 29, P< 0.001), and between SP and SRmax (r_s= 0.90, N = 29, p < 0.001) (Table 4.2). SRnucleus, was dependent on SDnucleus, but showed strong independence from other stylet dimensions. When comparing stylet dimensions with other paralarval dimensions, all combinations of measurements presented positive correlations to one another. The stronger correlations presented were between the diameter of the eye and the diameter and section area of the stylet and between the weight of the paralarva and stylet diameter and area (Table 4.2).

Table 4.2 – Spearman correlation (r_s) matrix. For comparisons between measurements of the same structure, r_s> 0.75 indicates collinearity. For comparisons between measurements of different structures, r_s> 0.50 indicates correlation. For all cases, N = 29. * indicates significant correlation between variables (P < 0.05).

		Paralarvae				Stylet				
		ML	TL	D_eye	W	SD	SP	SA	SRmax	SRnucleus
Paralarvae	ML		0.73*	0.61*	0.78*	0.41*	0.35	0.40*	0.32	0.23
	TL			0.61*	0.74*	0.53*	0.52*	0.53*	0.52*	0.09
	D_eye				0.62*	0.60*	0.55*	0.60*	0.52*	0.22
	W					0.60*	0.55*	0.58*	0.57*	0.31
Stylet	SD						0.90*	1.00*	0.94*	0.13
	SP							0.62*	0.90*	0.77
	SA								0.63*	0.13
	Srmax									0.22

4.1.4. Discussion

To our knowledge, this is the first time that the vestigial shell (or stylet) was identified in pelagic paralarvae of a merobenthic octopus, proving its formation in a prior embryonic stage. In the adults of *Octopus vulgaris*, the stylet is a recognizable structure in the dorso-anterior region of the mantle, easily extracted by dissection. However, in newly hatched individuals, the body size and the fragile structure of non-mineralized chitin of the stylet make it particularly difficult to collect the stylets by dissection. Several methods to isolate and collect the stylet from the body of the paralarva were tried, including staining with an acetic alcian blue solution, in an adaptation of the method used by Vecchione (1991) to identify stomach contents in squids. According to that author, alcian blue efficiently stains cartilaginous structures such as eye rings and funnel/mantle-locking cartilages in squid paralarvae. We observed that, although the alcian blue successfully stained the eye lenses of *O. vulgaris* paralarvae, the staining achieved for the stylets was not effective and resulted in unclear structures.

To overcome this difficulty and considering the fragile nature of newly-hatched paralarvae with the beaks and radula still under-developed, we chose to adopt a histological approach to obtain and observe sections of the stylets. Nevertheless, other challenges arise with this approach. As in adults, the stylets of paralarvae are spine-like rods with an irregular shape, presenting a middle bended region with concave and convex arms in the insertion area of the mantle-funnel retractor muscles. Both sagittal and transversal cutting planes result in good cross sections of the stylet, but only the transversal plane allowed a greater number of sections in the vicinity of the primordium. Additionally, this sectioning plane allowed the definition of a methodology to identify the bend and the closest section in which it is possible to identify the nucleus and to measure the structure in a replicable manner.

The nucleus (primordium) is visible in the nearest section to the stylet bend, with a mean diameter of 2.71 μm independently of the developmental temperature. The diameter of the stylet in newly-hatched (three-days old) paralarvae is close to 5 μm. Under a magnification of 1000x, the stylet does not have visible growth rings in the majority of the sections. In only two stylets of the wild paralarvae group were post-nuclear growth increments visible. Smooth core regions seem to be particularly common in stylets of holobenthic octopus such as *O. pallidus* (Doubleday *et al*., 2006) and merobenthic octopus such as *Macroctopus maorum* (Doubleday *et al.*, 2011). These observations are in

agreement with observations made in the stylets of adults, where it is possible to identify a core region with few irregularly marked increments (Figure 4.6). Nevertheless, one should hypothesise that the absence of visible growth increments near the nucleus may reflect an inadequate resolution power of light microscopy to resolve distances of less than 1 μm (Campana, 1992; Doubleday *et al.*, 2011) rather than an actual feature of the structure. The use of scanning electronic microscopy associated with cryo-sectioning of the paralarvae could be useful tools to improve the analysis of the stylet.

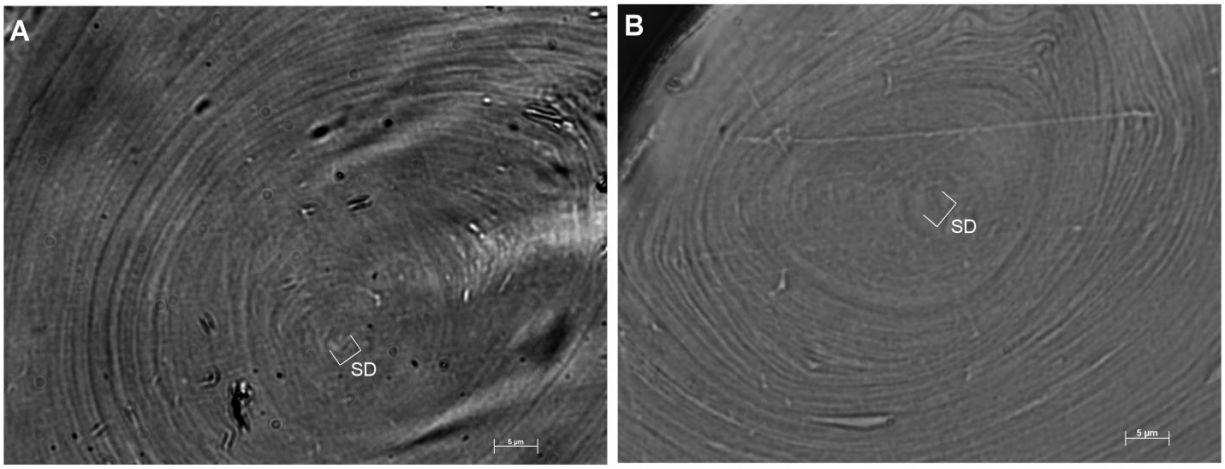


Figure 4.6 – *Octopus vulgaris* juvenile and adult stylet sections showing the central area which corresponds in size to the stylet diameter at hatching (magnification 630x). SD – diameter of the stylet at hatching; A – stylet section of a juvenile weighing 384 g (SD = 3.5 μm); B – stylet section of a juvenile weighting 700 gr (SD = 3.39 μm).

In *Octopus vulgaris* a merobenthic species, both stylet diameter and nuclear region of hatchlings are considerably smaller than in *O. pallidus*, a holobenthic species, but identical to stylet sizes and characteristics described by Doubleday *et al*. (2011) for *Macroctopus maorum*, a merobenthic octopus living in temperate and subantarctic waters off the Australian coast. In comparison with *O. pallidus*, *O. vulgaris* hatchlings are small and pelagic until settlement 30 to 60 days after hatching (Villanueva, 1995; Villanueva & Norman, 2008), while *O. pallidus* hatchlings are larger in relation to their adults and benthic from hatching. This results in two orders of magnitude difference in weight (2 mg weight for *O. vulgaris* hatchlings *vs.* 0.10 to 0.54 g for *O. pallidus* hatchlings, Semmens *et al.* 2011) at hatching and fully accounts for size differences between stylet diameter and nuclear area. Such differences illustrate the importance of investigating and validating growth structures and check marks in the stylets of each species.

Comparing our observations between *O. vulgaris* paralarva and juvenile stylet sections it is possible to observe correspondences of the nuclear area among the two life stages (Figure 6). In the juveniles, the identified nucleus ranges between 3 and 5 μm in diameter, followed by a smooth region where growth increments are hardly visible, necessarily larger than observed in these paralarvae. Nevertheless, the dimensions of the nuclear area are within the same range in both stages (paralarvae and juveniles).

The three day-old captivity-hatched paralarvae of which the embryologic development was conducted under controlled temperatures did not show significant differences in either body size or stylet dimensions. These findings are consistent with results obtained in laboratory essays under similar developmental temperatures: 18ºC and 21ºC. Although embryos at warmer temperatures grow faster and hatch earlier, hatchlings do not present significant differences in size or weight (Baptista *et al.*, *pers. comm.*). It is important to note that these paralarvae were not fed during the first three days of life, which could influence growth. Working with squid, Vidal *et al.* (2002) suggested that in the first hours to days of life, even if they start to feed, paralarvae depend almost entirely on their yolk reserves, going through a two-stage critical period with a "no net growth", comprised of a negative phase (decreasing in weight) and a posterior positive phase (weight recovery). In our study, the paralarvae were not fed and there was no recovery phase, so only a negative grow-phase was present. However the two groups might have been different at hatching and different net negative growth phase between the 18ºC and 22ºC could have resulted in similar weights and sizes at the end of the three days.

We were not able to determine the age of the nine paralarvae captured in the Cies Islands. Those paralarvae were in all cases larger in size and weight than the ones hatched in captivity, indicating that they may be over three-days old. The results of studies conducted by Villanueva (1995) with *O. vulgaris* hatchlings reared at 21ºC, suggest that these paralarvae could be approximately 10-days old (Figure 4.7). Nevertheless, these larvae were captured in July and October experiencing mean surface temperatures between 16.5 ºC (for the July paralarvae) and 19.2 ºC (for the October paralarvae) during embryologic development (SST data source: Seawatch buoy located off Cape Silleiro , 42º 7.80 N, 9º 23.40 W, www.puertos.es). This means that on average these paralarvae experienced lower temperatures that those in captivity, resulting in a longer embryologic development (Villanueva & Norman, 2008; Moreno *et al.*, 2012) and therefore larger sizes at hatching (Leporati *et al.*, 2007). Even though the nucleus is the same diameter between

wild-caught and captive-hatched paralarvae, body size, stylet area and diameter and major radius are all larger in the wild paralarvae, which indicates that deposition of new material in the stylet after hatching had occurred, although no increments were observed. One can hypothesize that at this stage the deposition of growth increments does not happen daily or that their contrast is not sufficient to show. During the paralarval stage, individuals go through a dramatic change from endogenous feeding to exogeneous feeding (Boletzky, 2003; Villanueva & Norman, 2008) associated to a no-net growth (Moguel *et al.*, 2010) and a learning to hunt process that is not cyclic. Either during this process growth increment deposition depends on other factors that are not regulated by circadian mechanisms, or the composition of the deposition layers is not very different. Either possibility may have resulted in a single increment deposition having been observed in two paralarvae.

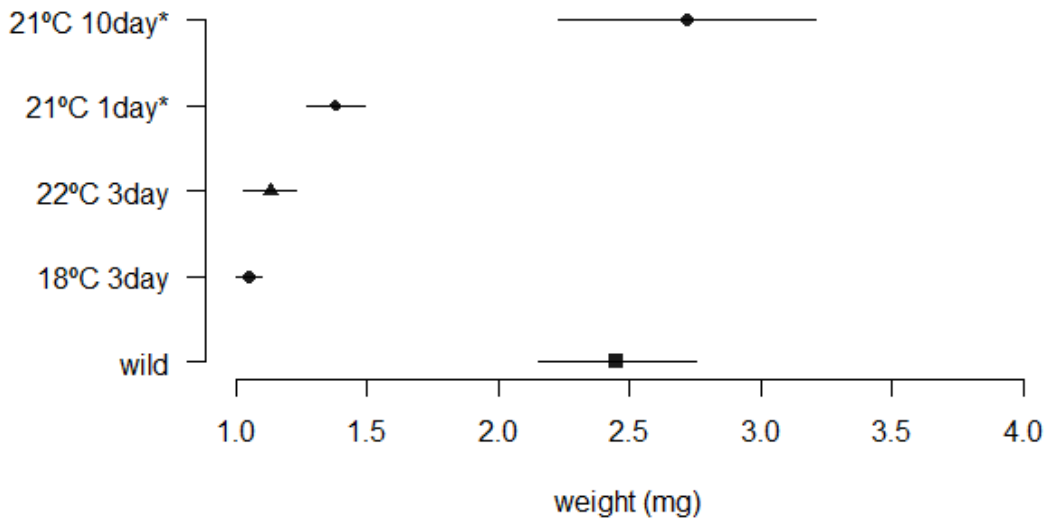


Figure 4.7 - Comparison of the mean weight of *Octopus vulgaris* paralarvae of known age reared under different temperatures (18ºC 3day, 22ºC 3day, 21ºC 1 day* and 21ºC 3 day*). Data for the 21ºC 1 and 21ºC 10 day paralarvae were published in Villanueva (1995).

Despite the significant differences in paralarvae and stylet measurements between groups, the dimension of the nuclear region in the stylet seem to be conservative between them and independent of both biological and environmental factors, suggesting that the nuclear region (corresponding to stylet size at hatching) can be used as a reference point to determine age and growth and related measurements. More studies on the stylet structure

are however needed to understand how the structure grows in both girth and length at this pre-settlement stage.

ACKNOWLEDGMENTS

The first author is grateful to José António Durán for teaching and support with histological methodologies. This study was supported by the projects CAIBEX (CTM2007-66108-C02-01) and LARECO (CTM2011-25929). The MultiNet® sampler used to collect wild paralarvae was funded by FEDER funds. The Portuguese Foundation for Science and Technology (FCT) supported this study through a project grant PTDC/BIA-BEC/103266/2008 to Rui Rosa and a PhD grant to the first author.

BIBLIOGRAPHIC REFERENCES

BARRATT, I. M & ALLCOCK, A.L. 2010.Ageing octopods from stylets: development of a technique for permanent preparations. ICES *Journal of Marine Science*, **67**:1452-1457.

BIZIKOV, V. A. 2004. The Shell in Vampyropoda (Cephalopoda): Morphology, functional role and evolution. *Ruthenica*, supplement **3**. 87p.

BOLETZKY, v. S. 2003. Biology of early life stages in cephalopod molluscs. *Advances in Marine Biology*, **44**: 144–203.

BUDELMANN, B. U., SCHIPP, R. & BOLETZKY, v S. 1997.Cephalopoda. In: Harrison, F. W., Kohn A. J. (Ed) Microscopic Anatomy of Invertebrates. Vol 6A: Molusca II. 119 - 414.

CAMPANA, S. E. 1992. Measurement and Interpretation of the Microstructure of Fish Otoliths. In: Stevenson, D., and Campana, S.E. (Eds). Otolith Microstructure Examination and Analysis. *Canadian Special Publication of Fisheries and Aquatic Sciences* **117:** 59 - 72.

CAMPANA, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59**: 197–242.

CANALI, E., PONTE, G., BELCARI, P., ROCHA, F. & FIORITO, G. 2011. Evaluating age in *Octopus vulgaris*: estimation, validation and seasonal differences. *Marine Ecology Progress Series* **441**: 141–149.

DOMAIN, F., JOUFFRE, D. & CAVERIVIÉRE, A. 2000. Growth of *Octopus vulgaris* from tagging in Senegalese waters. *Journal of Marine Biological Association UK* **80**: 699–705.

DOUBLEDAY, Z., SEMMENS, J.M., PECL, G. & JACKSON, G. 2006. Assessing the validity of stylets as ageing tools in *Octopus pallidus*. *Journal of Experimental Marine Biology and Ecology* **338**: 35–42.

DOUBLEDAY, Z., BELTON, D., PECL, G. & SEMMENS, J. 2008.Quantitative elemental imaging of

octopus stylets using PIXE and the nuclear microprobe. *Nuclear Instruments Methods in Physic Research Section B* **266**:67–72.

DOUBLEDAY, Z. A., WHITE, J., PECL, G. T. & SEMMENS, J. M. 2011. Age determination in merobenthic octopuses using stylet increment analysis: assessing future challenges using *Macroctopus maorum* as a model. *ICES Journal of Marine Science* **68**: 2059–2063.

HERMOSILLA, C. A., ROCHA, F., FIORITO, G., GONZÁLEZ, A.F. & GUERRA, A. 2010. Age validation in common octopus *Octopus vulgaris* using stylet increment analysis. ICES *Journal of Marine Science* **67**: 1458-1463.

HERWIG, J.N., DEPCZYNSKI, M., ROBERTS, J.D., SEMMENS, J.M., GAGLIANO, M. & HEYWARD, A. J. 2012. Using age-based life history data to investigate the life cycle and vulnerability of *Octopus cyanea PLoS ONE* **7(8)**:e43679.

INE. 2013. Statistics Portugal. www.ine.pt (accessed in 31st August 2013)

JONES, L. 2002. Connective tissues and stains. In: Bancroft, J. D., Gamble, M. (eds). Theory and practice of histological techniques. 139–162.

KATSANEVAKIS, S. & VERRIOPOULOS, G., 2006. Seasonal population dynamics of *Octopus vulgaris* in the eastern Mediterranean. ICES *Journal of Marine Science* **63**: 151–160.

LEPORATI, S. C., PECL, G. T. & SEMMENS, J. M. 2007. Cephalopod hatchling growth: the effects of initial size and seasonal temperatures. *Marine Biology* **151**: 1375–1383.

LEPORATI, S. C., SEMMENS, J. M. & PECL, G. 2008. Determining the age and growth of wild octopus using stylet increment analysis. *Marine Ecology Progress Series* **367**: 213–222.

MANGOLD, K., 1983. *Octopus vulgaris*. In Boyle, P. (ed) Cephalopod Life cycle: Species accounts. Vol 1.Academic press, London, pp. 335-363.

MARQUEZ, F. & RE M. E. 2009. Morphological and chemical description of the stylets of the red octopus, *Enteroctopus megalocyathus* (Mollusca: Cephalopoda). *Molluscan Research* **29**: 27-32.

MOGUEL, C., MASCARÓ, M., AVILA-POVEDA, O., CAAMAL-MONSREAL, C., SANCHEZ, A., PASCUAL, C. & ROSAS, C. 2010 Morphological, physiological and behavioral changes during post-hatching development of *Octopus maya* (Mollusca: Cephalopoda) with special focus on the digestive system. *AquaticBiology* **9**: 35–48.

MORENO, A., PIERCE, G. J., AZEVEDO, M., PEREIRA, J. & SANTOS, A. M. P. 2012. The effect of temperature on growth of early life stages of the common squid *Loligo vulgaris*. *Journal of Marine Biological Association UK* **92**: 1619-1628.

NAPOLEÃO, P., REIS, C. S., ALVES, L. C. & PINHEIRO, T. 2005. Morphologic characterization and elemental distribution of *Octopus vulgaris* Cuvier, 1797 vestigial shell. *Nuclear Instruments Methods in Physic Research Section B* **231**: 345–349.

OTERO, J., ROCHA, F., GONZÁLEZ, A. F., GARCIA, J. & GUERRA, A. 2005. Modelling artisanal

coastal fisheries of Galicia (NW Spain) based on data obtained from fishers: the case of *Octopus vulgaris*. *Scientia Marina* **69**: 577–585.

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.

PANFILI, J., DE PONTUAL, H., TORADEC, H. & WRIGHT, P. J., 2002. Manual of fish sclerochronology. Brest, France: IFREMER - IRD.

PERALES-RAYA, C., BARTOLOMÉ, A., GARCÍA-SANTAMARÍA, M.T., PASCUAL-ALAYÓN, P. & ALMANSA, E. 2010. Age estimation obtained from analysis of octopus (*Octopus vulgaris* Cuvier, 1797) beaks: Improvements and comparisons. *Fisheries Research* **106**: 171–176.

RAYA, C. & HERNÁNDEZ-GONZÁLEZ, C. 1998. Growth lines within the beak microstructure of the octopus *Octopus vulgaris* Cuvier, 1797. *South African. Journal of Marine Science* **20**: 135–142.

ROCHA F., GUERRA, A. & GONZÁLEZ, A. F. 2001. A review of the reproductive strategies in cephalopods. *Biological Reviews* **76**: 291-304.

ROURA, A., 2013. Ecology of planktonic cephalopod paralarvae in coastal upwelling systems. Dissertation, Universidade de Vigo, Vigo, Spain.

ROURA, A., GONZÁLEZ ,A. F., REDD, K. & GUERRA, A., 2012. Molecular prey identification in wild *Octopus vulgaris* paralarvae. *Marine Biology* **159**: 1335–1345.

SEMMENS, J., DOUBLEDAY, Z., HOYLE, K. & PECL, G. 2011.A multilevel approach to examining cephalopod growth using *Octopus pallidus* as a model. *The Journal of Experimental Biology* **214**: 2799 - 2807.

SOUSA REIS C. & FERNANDES, R., 2002. Growth observations on *Octopus vulgaris* Cuvier, 1797 from the Portuguese waters: Growth lines in the vestigial shell as possible tools for age determination. *Bulletin of Marine Science* **71**: 1099-1103.

VECCHIONE, M., 1991. A method for examining the structure and contents of digestive tract in paralarval squids. *Bulletin of Marine Science* **49**: 300–308.

VIDAL, E. A., DI MARCO, F. P., WORMUTH, J. H. & LEE, P. G., 2002. Influence of temperature and food availability on survival, growth and yolk utilization in hatchling squid. *Bulletin of Marine. Science* **71**: 915–931.

VILLANUEVA, R., 1995. Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to setlement. *Canadian Journal of Fisheries and Aquatic Science* **52**: 2639–2650.

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 Fish Biology and Fisheries* **17**: 101–110.

VILLANUEVA, R. & NORMAN, M. D. 2008. Biology of the planktonic stages of benthic octopuses. *Oceanography and Marine Biology - An Annual Review* **46**: 105–202.

4.2. AGE AND GROWTH OF POST-SETTLED OCTOPUS VULGARIS: AGEING METHODOLOGIES AND CHALLENGES[6]

Sílvia Lourenço[1,2,3], Ana Moreno[2], João Pereira[2], Luís Narciso[1], Ángel Gonzalez[3]

[1] Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa,Avenida Nossa Senhora do Cabo 939, 2750-374 Cascais, Portugal

[2] Instituto de Investigaciones Marinas de Vigo, CSIC, C/Eduardo Cabello, 6. Vigo. E-36208, Spain.

[3] Departamento do Mar e Recursos Marinhos, Instituto Português do Mar e da Atmosfera, I.P.Avenida de Brasília, 1449-006, Lisboa, Portugal.

ABSTRACT

Age and growth estimates are essential to understand the life cycle of *Octopus vulgaris* including the time span of paralarvae, juvenile and fully mature individuals. Stylet increment analysis, SIA, is one of the most promising methods to accurately estimate age and growth rate in wild populations of *O. vulgaris*. Despite the recent developments to improve SIA some challenges remain including the definition of protocols to achieve age precision. Here, we estimate the age and growth rates of *O. vulgaris* juveniles hatched along the Portuguese coast based on the daily deposition of growth increments in their stylets. A counting protocol designed to achieve age precision is described. According to our results, *O. vulgaris* juveniles grow following a logarithmic model with an instantaneous growth rate of 6.2 % d^{-1} and take approximately 11 months to attain 750 g, the minimum commercial size established in Portuguese fisheries regulations.

Keywords: *Octopus vulgaris*, age, growth, methods.

[6] To be submitted to the ICES Journal of Marine Science

4.2.1. Introduction

Age and growth estimates are essential to understand the biology and ecology of marine organisms. These estimates are important for stock assessment using population age structured models, longevity and mortality models, productivity and recruitment models, all important tools for sustainable fisheries management. Ecologically, the assessment of age and growth rates permit the determination of the duration of crucial life stages (e.g. paralarva), and the timing of important transitional stages (e.g. settlement). Furthermore, environmental impacts on growth can be assessed based on age and growth estimates throughout the species life cycle.

In cephalopods, estimates of age and growth have been conducted applying both indirect methods based in demographic and modal progression methods and direct methods from hard structures. The direct methods of age estimation are based on counts of growth increments (GI) in the statoliths (González et al., 1996; Moreno et al., 2005), beaks (Perales-Raya *et al.*, 2010; 2014; Canali *et al.*, 2011), internal shell (Ré & Narciso, 1994) and the stylets (vestigial shell) supported by a few studies associated to mark-recapture and daily deposition validation techniques (see Semmens *et al.*, 2004, and Boyle & Rodhouse, 2006 for a review). Additionally, some biochemical and histochemical methods have been used to assess individual age in cephalopods as the RNA/protein ratio in *Eledone cirrhosa* and *Octopus vulgaris* (Pierce *et al.*, 1999) and lipofuscin pigment concentration in the optic gland of *O. pallidus* (Doubleday & Semmens, 2011).

In Incirrate octopods such as *O. vulgaris*, direct ageing methods based on statolith increment analysis are not considered accurate, while approaches using beaks still need accuracy validation, in particular due to erosion by feeding (Perales-Raya *et al.*, 2010, 2014; Canali *et al.*, 2011).

The use of the stylets to assess age has been trialed in the past (e.g. Reis & Fernandes, 2002) with improvements achieved in the preservation and observation techniques in transversal sections of these structures. Today, age estimation by stylet increment analysis (SIA) is considered a successful and promising method to assess age in wild populations of octopus species (e.g. *O. pallidus*, Leporati *et al.*, 2008; *Macroctopus maorum*, Doubleday *et al.*, 2011, *O. cyanea*, Herwig *et al.*, 2012;). Important contributions on validation of daily deposition of GI in *O. pallidus* were made by Doubleday *et al.* (2006) and Leporati *et al.*, (2008) and in *O. vulgaris* by Hermosilla *et al.* (2010). Recent improvements in stylet preparation methods that resulted in the production of good quality

and definitive sections (Barratt & Allcock, 2010) definitively push forward the use of SIA to estimate age in octopus species.

Despite the growing number of studies on age and growth based on the count of growth increments in transverse sections of stylets in incirrate octopods, few efforts have been conducted to follow identical counting protocols between species as is the case of age studies based on fish otoliths (Panfili *et al.*, 2002). The definition of a protocol for ageing purposes allows comparisons to be made between different populations of the same species or between species with different life strategies, e.g. compare growth and lifespan estimates between holobenthic and merobenthic species.

O. vulgaris is a merobenthic species (Mangold, 1983) with a short life cycle of one (Iglesias *et al.*, 2004; Perales-Raya *et al.*, 2014) to two years (Katsanevakis & Verriopoulos, 2006; Otero *et al.*, 2007). The paralarvae are planktonic and settle to the benthic habitat (weighing 173 mg) 32 to 57 days after hatching (Villanueva, 1995; Iglesias *et al.*, 2004; Katsanevakis & Verriopoulos, 2006). Upon settling grow rates are high, at approximately 1.2-1.6% d^{-1}, allowing animals to reach the fisheries legal minimum landing weight (750 g in Portugal, Portaria nº 27/2001) at 9-10 months of age (Domain *et al*., 2000; Katsanevakis & Verriopoulos, 2006). The stylet develops throughout embryogenesis, probably as a single piece (Budelmann *et al.*, 1997; Naef, 1921, 1923 in Bizikov, 2004) and at hatching it is located in the dorso-anterior region of the mantle (Lourenço *et al., unpublished data*).

The present study aims to estimate the age of *O. vulgaris* juveniles (pre-recruits) of the Portuguese northwest coast (western Iberian upwelling system, WIUS) using SIA. Secondly, it aims to provide a protocol for stylet preparation and SIA methods, based on Doubleday *et al.* (2006) and Barratt & Allcock (2010) and the daily deposition validation conducted by Hermosilla *et al.* (2010), such that repeated and comparable age estimates can be made between populations and between various incirrate octopods species to a higher precision than hereto possible, providing greater ageing accuracy.

4.2.2. Material and methods

4.2.2.1. Sampling and storing

Twenty-eight stylets collected from pre-recruits weighing between 99 g and 748 g captured in the small-scale fisheries were stored in a 4% formalin solution. For each

individual (yielding a pair of stylets), dorsal mantle length (DML in mm) and total weight (W in g) were measured to the nearest mm and 0.1 g, respectively. Sex and maturity stage were macroscopically evaluated whenever possible.

Previous studies have highlighted that storing solutions are a key issue in the prevention of stylet degradation and improvement of GI visualization (Sousa-Reis & Fernandes, 2002). To find the best storing solution, two sets of stylets were stored in a 3:1 mixture of 70% ethanol and glycerol (solution A), and in a 4% formalin solution (solution B). The core region of several stylets stored in solution A appeared darkened, layers were easily separated from each other during preparation due to drying, and no improvement in the visualization of GI was observed. Solution B revealed to be a better storing solution: drying was less significant and counting of GI comparatively easier.

4.2.2.2. Stylet preparation

Before resin embedding, the entire stylet was dehydrated in sequential baths of 70%, 80% and 100% ethanol for two hours each. After dehydration, a section of the stylet with roughly 1 cm long (including the stylet bend) was cut towards the post-rostral region. The section was then embedded in a low-viscosity acrylic resin (LR White^{TM}) for 24 h in an Eppendorf after Barratt & Allcock (2010). Following resin infiltration, the stylet section was removed from the solution and placed horizontally in a plastic histology mould. Approximately 50 ml of fresh resin was polymerized using 5 drops of accelerator solution (LR White^{TM} UV accelerator) filling up the mould until the stylet section was completely covered. The section orientation inside the mould was corrected before resin hardening. This operation was conducted over a cold plate at – 8 ºC to prevent resin overheating. The embedded stylets were left overnight to polymerize. After polymerization, the resin blocks were removed from the plastic moulds, lined up over a fresh epoxy resin layer and oriented vertically with the stylet bend facing up. They were then covered with transparent epoxy resin and left to dry overnight. The new epoxy resin block was cut with a variable speed precision cutting machine (Extec's Labcut 250B) blade producing 500 µm transversal sections of the stylets. This new step was introduced in the procedure following the protocols designed by Doubleday *et al.* (2006) and Barratt & Allcock (2010). It facilitates the production of identical 500 µm stylet sections, minimizing the occurrence of variable section thicknesses which would require considerable time in grinding. The 500 µm sections were then removed from the base resin, mounted onto a glass slide with Neo-

Mount^{R} and left to dry for a further 24 h untill completely hard. They were then further ground and polished with a sequence 1200-grade Carborundum fine sandpaper, followed by a sequence of 30, 9 and 3 μm lapping film and finally wet neoprene impregnated with a solution of 0.05 μm aluminum oxide powder and distilled water (after Barratt & Allcock, 2010)**.** During polishing, the sections were checked under a light microscope (100 and 400x magnification) until GI, nucleus and core region were visible, while making sure they would not get polished away.

4.2.2.3. Stylet microstructure analysis and age estimation

Stylet microstructure and GI were observed at 100 and 625x magnifications using a Zeiss Axioplan 2 Imaging light microscope. Sequential digital images of the whole stylet section were captured under 100x magnification with a Zeiss Axiocam MRC digital camera and composed digitally using ImageJ plugin MosaicJ (Thévenaz & Unser, 2007).

The nucleus was identified in 13 stylets and appeared in the center of the stylet as a dark portion limited by the first growth discontinuity or check identified by the a colour change from dark to light grey. The diameter of this portion was on average 5.8 ± 2.2 μm, similar to the diameter of the stylet of a three-day old paralarva (3.99 ± 0.96 μm, Lourenço *et al.*, *unpublished data*). Considering that this was the first growth discontinuity visible after the nucleus, we considered it the hatch check (Lourenço *et al.*, *unpublished data*).

The transversal section of the stylet presented an irregular shape and the nucleus divided the stylet surface in two unequal parts. In order to have reproducible GI counts, two reading segments were defined along the stylet's major axis and called large segment and small segment depending of the length of each.

The core region was identified in all stylets as the region between the hatch check and the second growth discontinuity. The core region was defined as a smooth region where none or few growth increments were visible due to increments low contrast. The core region presented a variable diameter, ending where regular deposition of the GI is visible under the microscope.

Measurements of the area, perimeter, major axis, minor axis, core region (radius) and nucleus (diameter) were taken from the scaled mosaics (Figure 4.8) and compared to individual weight, dorsal mantle length and number of GI. The assumption of normality and homogeneity of the variables was assessed with Shapiro-Wilk's and Bartlett´s tests,

respectively, and the correlation matrix of the dimensions was obtained applying the Pearson correlation index.

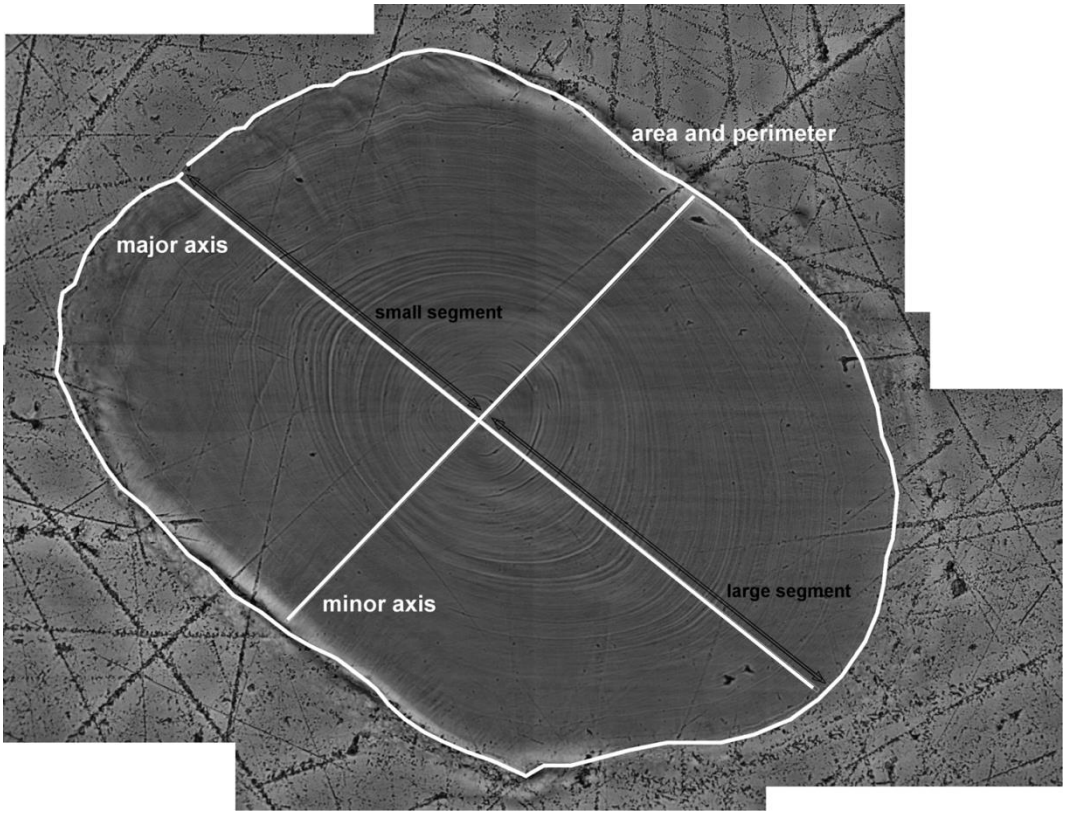


Figure 4.8 – *Octopus vulgaris s*tylet section with the indication of measurements of area, perimeter, minor axis and major axis with arrows indicating of the large and small counting segments along the stylet's major axis (mosaic of images with 100x magnification).

GI were observed under bright light, with the condenser adjusted to optimize brightness and contrast. To count GI in each stylet, a sequential series of 625x magnification grayscale images were captured along the stylet's major axis and composed digitally using the ImageJ plugin MosaicJ (Thévenaz& Unser, 2007).

Based on the validation conducted by Hermosilla *et al.* (2010) of the daily deposition of GI in the stylets of *O. vulgaris*, GI counts were conducted under 625x magnifications along the small and large segments. A daily GI was defined as the set of a light grey (or white) band followed by a dark ring and the increment count was marked in the latter. At this stage, the first increment was defined as the nearest clear dark ring after the nucleus defining the end of the core region (Figure 4.9 A). The last increment was defined as the most marginal dark ring. The light band was always wider than the dark ring, and frequently presented several very fine dark rings or shadows within it, that were considered

sub-daily growth increments (Figure 4.9B).

The distance between consecutive increments was determined by dividing the distance between the first GI and the last GI by the number of GI counted. To asses if the number of increments counted was independent of the axis length, three independent counts were made on both large and small axis. The segment length and correspondent counts were statistically compared by Analysis of Variance (ANOVA) and differences found between segment counts were assessed by a paired t-Student test for differences between group means.

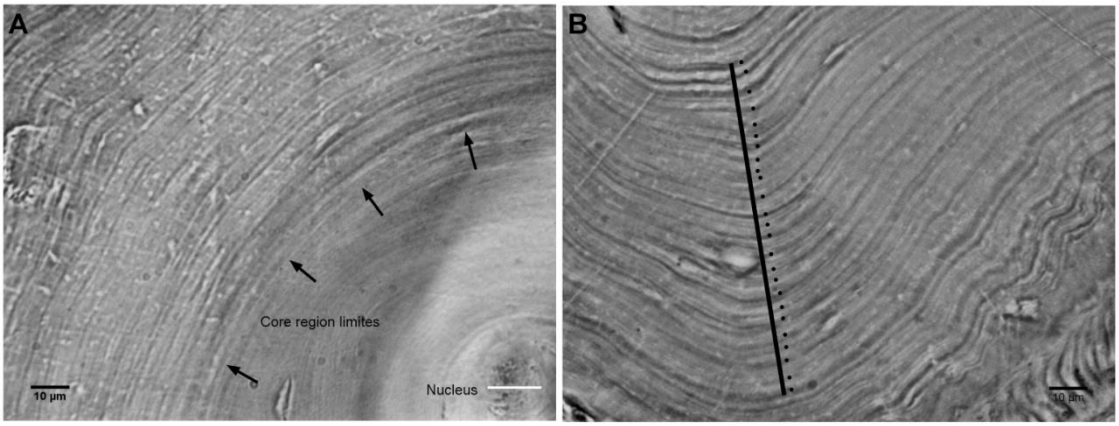


Figure 4.9 – Detail of the cross section microstructure of *Octopus vulgaris* stylets (625x magnification). A - the white line indicates the nucleus position and the black arrows indicate the limits of the core region; B - Black line represents an example of a count segment and the black points represent growth increment positions (GI).

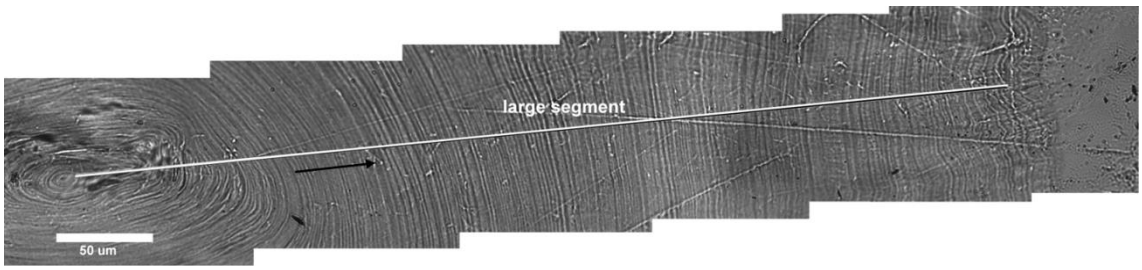


Figure 4.10 – *Octopus vulgaris* stylet microstructure presenting the axis large segment as counting segment. The arrow indicates the counting direction (mosaic of images with 625x magnification).

The precision of age estimates was defined as the reproducibility of repeated counts (Campana, 2001). The precision can be assessed by the average percent error (APE), the index of precision (D) or by the coefficient of variation (CV). The CV tends to be the preferential method used for microstructure studies because it is more robust and more sensitive to differences between observers (Campana, 2001). The CV can be written as

(Campana, 2001) :

$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(x_{ij}-x_j)^2}{R-1}}}{x_j}$, where CV_j is the estimate for age precision of the *j*th stylet. X_{ij} is the *i*th age determination of the *j*th stylet, X_j is the mean age estimate for the *j*th stylet, and *R* is the number of times each stylet is aged. The CV of age estimates was determined per observer based on three independent GI counts made by experienced observers (A) (CV_{WO}) and between observers by comparing the mean GI counts of observer A with an independent GI count of a second experienced observer (B) (CV_{BO}). Additionally, the relative precision between readers was tested by comparing the slopes and intercepts of regressions of number of GI on axis length, as determined by each reader, through an analysis of Covariance (ANCOVA) (Quinn & Keough, 2002).

The GI counts results obtained by observer A over the large segment were used to fit different growth models. To describe the growth of *O. vulgaris* after settlement, four different growth models (linear, exponential, logarithmic and power models) were fitted to the weight-at-age data. The best fit model was selected by checking the model fit residuals for normality with Shapiro-Wilk's test and by calculating the Akaike Information Criterion (AIC) of fitted results (Zuur *et al.*, 2007). Post-settlement instantaneous growth rates (IGR's) were calculated on an individual basis but averaged over the lifetime based on the equation determined by Forsythe & Van Heukelem (1987): $IGR = \frac{\ln W_f - \ln W_i}{\Delta t}$, where Δt is the age determined for each individual of W_f total weight at capture. W_i was assumed to be 173 mg, based on the average weight of 40 day-old *O. vulgaris*, the weight at age of settlement estimated by Villanueva (1995) in laboratory rearing experiments.

4.2.3. **Results**:

The stylet dimensions, minor axis, major axis, perimeter and area are highly correlated with each other and with the juveniles' dml and weight. The core region radius and the nucleus diameter are independent of the other dimensions of both stylets and juveniles. Table 4.3 present the correlation between the stylet dimensions and the juvenile dimensions.

Table 4.3 – Pearson correlation indexes comparing *Octopus vulgaris* juvenile body size (weight and dorsal mantle length, dml) and stylet dimensions (minor and major axes diameter, perimeter, area, core region radius, core_r, and nucleus diameter). Correlation indices in bold indicate a level of significance of $p < 0.05$.

	minor axis	major axis	perimeter	area	core region	nucleus
weight	**0.72**	**0.72**	**0.76**	**0.79**	-0.34	0.02
dml	**0.69**	**0.66**	**0.71**	**0.74**	-0.33	-0.04

Twenty-two sections of the 28 stylets collected were observed and GI counted. This means that 21% of the stylets collected were lost due to processing damage. GI counts were made in 95 % to 99 % of the segments length.

The comparison of counts between large and small segments of the major axis, showed that in 50% of the stylets GI counts are significantly different between the segments (Figure 4.11) with differences found between counts increasing with the increasing differences in the segments size. Considering this, the distance between GI is conservative among segments (D_{LS} = 4.29 ± 0.62 (SD) µm; D_{SS} = 4.56 ± 0.74 (SD) µm). The average CV_{WO} determined was low (5.15%) ranging between 1.57 % and 9.93 %. The average CV_{BO} increased to 8.78 %, ranging between 0 % (total agreement) and 23.08 %.

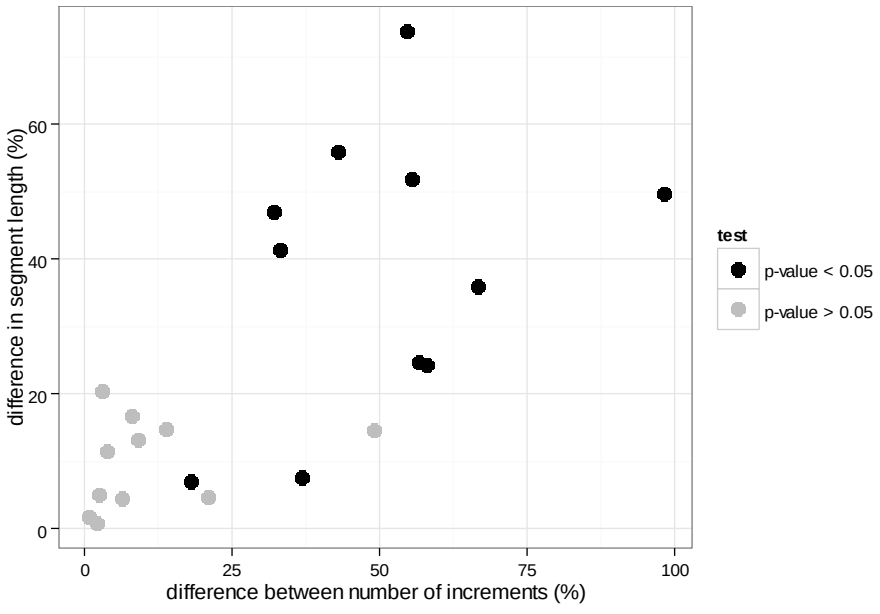


Figure 4.11 - Relationship between differences of the growth increments counted in the two counting axes and the length difference between the axes by stylet. Black bullets indicate significant differences between counts over the two axes; grey bullets indicate no statistical difference between the two counts.

Segment length, GI counts and mean distance between GI were compared by observer with ANOVA and results showed that, although no significant differences were found between observers GI counts (F = 1.914, p-value = 0.174), the mean distance between GI was significantly different (F = 10.86, p-value = 0.002). These results are supported by the ANCOVA analysis (Table 4.3). ANCOVA showed that even if there is no effect of the categorical factor observer in the relationship between segment length and number of GI counted (interaction effect model results: F = 0.013, p-value = 0.90), observer B tends to count more GI than observer A.

Table 4.3 – ANCOVA results comparing growth increments counts made by the two observers from *Octopus vulgaris* juvenile stylets.

Model		DF	Sum Sq	Mean Sq	F	p-value
Interaction	Segment*observer	1	5	5	0.013	0.91
	Residuals	40	14172	14172		
Main factor	Segment	1	46027	46027	133.11	< 0.001
	Observer	1	2581	2581	7.463	0.01
	Residuals	40	14177	346		

Juvenile dorsal mantle measured between 70 mm and 145 mm in length with individual weight ranging between 98.57 g and 748 g (12 females and 10 males). GI counts ranged between 59 and 219 increments. Assuming that the deposition of the growth increments identified occurs on a daily basis, the first GI counted determines the end of the core region and probably matches with settlement. Stylets sections observed therefore correspond to octopus which settled at between two and seven months prior to being caught (Table 4.4).

GIs counted by stylet were compared with major segment axis length, perimeter and core region radius using linear regression. Results show that the number of GI is linearly correlated and increases with increasing size of the stylet (in perimeter, area and major axis length) with the exception of the radius of the core region with which no relationship exists (Figure 4.12).

Table 4.4 – Individual dorsal mantle length (DML, mm), total weight (TW, g), sex and maturity stage (mat), stylet perimeter (SP, mm), stylet major axis radius (SRmax, mm), mean number of growth increments (GI), mean distance between GI (GI_D, µm), coefficient of variation of the observer (CV_{WO}, %), coefficient of variation between observers (CV_{BO}, %).

OBS	Sex/Mat	DML	TW	SP	SRmax	GI	GI_D	CV_{WO}	CV_{BO}
1	M1	90	245.38	3.78	0.512	145	3.54	5.54	9.84
2	F1	105	381.9	3.10	0.572	180	3.18	5.63	3.45
3	F1	95	310.96	2.80	0.547	142	3.74	2.84	1.73
4	F1	90	276.35	2.60	0.429	115	3.73	3.06	4.54
5	F1	130	728	3.50	0.569	142	4.00	1.62	11.53
6	M2	145	748	4.01	0.756	219	3.38	7.63	4.78
7	F1	135	700	3.39	0.548	136	3.96	8.34	7.48
8	M2	135	746	4.14	0.666	165	3.99	4.25	1.78
9	F1	114	711.4	3.80	0.695	160	4.33	1.57	0.00
10	M2	125	682	4.02	0.689	170	4.04	2.37	6.85
11	F1	100	377.59	3.43	0.651	164	3.76	7.78	5.20
12	M2	100	384.34	3.67	0.789	157	5.17	2.57	7.10
13	M2	105	371.27	3.23	0.645	117	5.27	3.08	11.03
14	M1	80	143.24	3.29	0.565	118	4.69	7.85	16.31
15	M1	85	203.48	2.56	0.449	87	5.00	5.14	15.79
16	M2	110	368.68	2.53	0.431	101	4.25	9.93	9.00
17	F2	125	479.4	3.37	0.693	138	4.98	6.44	17.36
18	F1	95	241.28	2.60	0.522	99	5.22	5.07	15.02
19	F1	90	228.41	2.25	0.424	92	4.60	6.16	9.36
20	F1	85	115.65	1.52	0.257	59	4.34	7.39	7.09
21	M1	70	98.57	1.45	0.333	65	5.08	3.18	23.08
22	F1	95	306	2.64	0.471	109	4.32	6.02	4.81

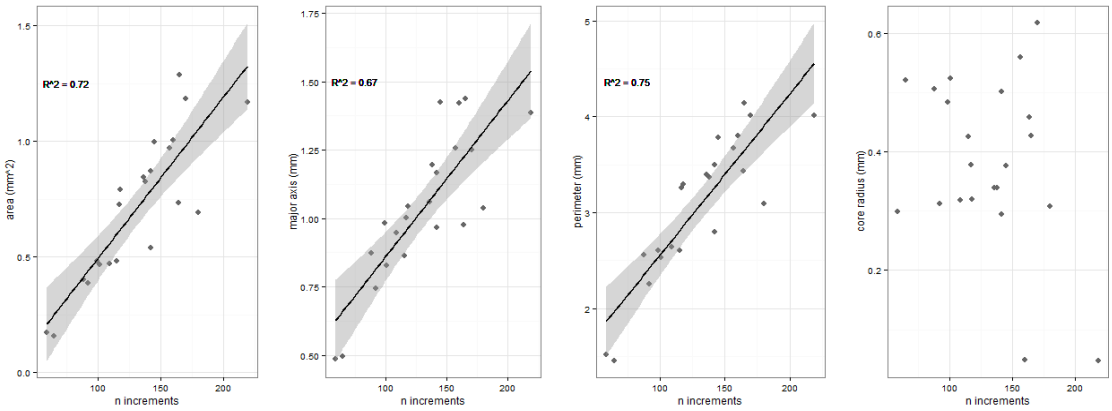


Figure 4.12 - Linear regression results of the comparison of growth increments counts with stylet dimensions. R^2 stands for the correlation coefficient. For relationships that proved to be non-significant, R^2 was not displayed.

Considering that the number of GI counted corresponds to the time in days elapsed between settlement and capture, different growth models were fit to weight-at-age data. Table 4.5 presents the growth model parameters and the AIC obtained. In all cases, the growth parameter *b* estimate was significant, while growth parameter *a* was only significant for the exponential and logarithmic models. Based on the AIC, weight-at age data were best described by a linear model. Based on the fitted growth parameters and on previous knowledge of the biology of the species, the model that appears to better explain growth is the logarithmic model (Figure 4.13 A). Based on the lower AIC result (Table 4.5) and the previously knowledge of the biology of the species, the model that best fits IGR results is the power model (Figure 4.13 B).

Table 4.5 – Model fit results and estimated parameters (*a* and *b*) for the four growth models fitted to weight at age data (growth model) and the three models fitted to the instantaneous growth rate results (IGR models) for the *Octopus vulgaris* juvenile. W – Individual weight; T – days; AIC – Akaike Information criterion. *t* – *t-student* statistic parameter. In bold the best model result.

Model	AIC	**Growth model**					
		a	t	p-value	*b*	t	p-value
Exponential	290.88	122.6±	2.88	0.009	0.01±	4.02	0.001
Linear	288.87	-1331	-1.09	0.288	4.05	4.60	0.002
Log	**289.10**	**-1933.1**	**-3.77**	**0.001**	**483.10**	**4.55**	**< 0.001**
Power	289.18	0.74	0.607	0.55	1.29	3.93	0.008
		InstantaneousGrowth Rate model					
Exponential	81.91	8.77	10.41	< 0.001	-0.001	-3.48	0.002
Linear	83.18	8.17	12.39	< 0.001	-0.005	-3.29	0.004
Log	78.04	18.19	6.59	< 0.001	-2.05	-4.35	< 0.001
Power	**76.31**	**44.18**	**2.60**	**0.02**	**-0.34**	**0.07**	**< 0.001**

4.2.4. Discussion:

The methodology used to prepare the stylets to SIA was time consuming and produced a 21% loss of material. The introduction of the automated 500 µm section was an improvement regarding the previous age studies with SIA (Barrat & Allcock, 2010; Doubleday *et al.*, 2006). However, the high percentage of stylet damage from layer detachment is still high, making this a time and material demanding technique. Reducing the storing time prior to sectioning can probably reduce detachment from layer dehydration, but the discontinued nature of the layered deposition of the stylet matrix will always be a limiting factor for the optimization of the technique.

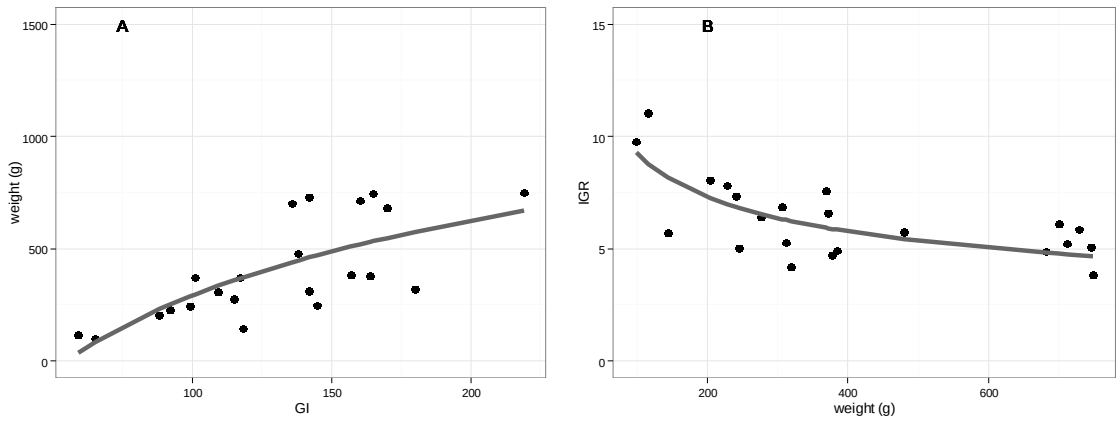


Figure 4.13 - Growth model (A) and instantaneous growth rate model (B) fits to *Octopus vulgaris* juvenile weight – at –age data.

Age estimation based on SIA now appears to be a promising accurate method when used for octopus species. Since the preliminary work conducted by Sousa-Reis & Fernandes (2002), several advances in methodological approaches have been achieved, including the possibility to prepare permanent resin embedded sections (Barratt & Allcock, 2010) and the daily deposition validation in holobenthic (Doubleday *et al.*, 2006; Leporati *et al.*, 2007) and merobenthic (Hermosilla *et al.*, 2010) octopods. In fact, the possibility to have permanent section preparations assures samples will remain available for continued improvements to observation protocols. Here, for the first time, independent GI counts were conducted in different regions of the stylet proving that the choice of the region of interest to conduct GI counts is determining to age assessing particularly in the most irregular stylet sections.

Since the daily deposition of GI in stylets has been validated by Hermosilla *et al.* (2010) for *O. vulgaris*, this is the first study in which wild post-settled juveniles were aged with SIA. According to our results, post-settlement *O. vulgaris* took a minimum of eight months to reach the 750 g minimum landing size required from Portuguese legislation. The average instantaneous growth rate estimated during this period is 6.26% per day, but IGR decreases continuously from settlement, following a power function.

Albeit promising for the accurate and precise assessment of the age of *O. vulgaris*, the described SIA methodologies require the establishment of specific protocols. The prior definition of a region of interest (ROI) in the stylet surface facilitates precise age estimates using SIA because it removes a source of variability, since the distance between GI can vary. Since GI were visible in more than 90 % of the stylet surface, and the major axis

(with the greatest distance between GI) was therefore established *a priori* as the ROI for counts. Stylet section shape is asymmetric and, in most cases, the nucleus is not located at the center of the structure, resulting in counting segments with different lengths depending of their position in relation to the nucleus. Observers tend to use the distance between increments as the main criterion to distinguish daily from sub-daily growth increments, and therefore the counts produced over different length segments tend to be different and the difference increases with increasing length differences between segments. In age studies conducted with squid statoliths, the more frequent ROI defined *a priori* is the region between the nucleus an the rostrum in the lateral dome of the statoliths (Lipinski & Durholtz, 1994; Arkhipkin & Bjorke, 2000; Moreno *et al.*, 2005) for similar reasons.

Despite our best efforts, a mean CV of 9% between observers was the most precise estimate achieved, which is considerably high in comparison with age studies on other octopus species (Herwig *et al.*, 2012; Leporati *et al.*, 2008). The associated error can therefore produce underestimated ages by 3% to 4%, considering a theoretical life span of between 18 months (Domain et al., 2000) and two years (Katsanevakis and Verriopoulos, 2006; Otero et al., 2007).

The use of a magnification of 625x permitted the identification of the nucleus in only 59 % of the stylets observed (13 sections). The nucleus presents characteristics which resemble in size those of the stylet at hatching, as previously defined. However, the nucleus of the stylets observed in the present study, with a mean diameter of 5.80 µm, are statistically different ($t = -2.78$, $df = 15.12$, p-value = 0.01) from the stylet diameter of three-day old paralarvae (3.99 µm, Lourenço *et al., unpublished data*). In comparison with the holobenthic species *O. pallidus* (Doubleday *et al.*, 2006) the nuclear area of the stylets of *O. vulgaris*, which would correspond to hatching size, is proportionately smaller, similarly to the ratio of post-hatching size / adult size of the two species. This extremely small size in conjunction with the existence of the core region, which has not yet been fully explained, may account for the verified difference in size between hatching stylet diameter and nuclear diameter in juvenile and adult specimens.

The stylet nucleus is located at the center of the core region, which is defined as a smooth, mostly unmarked (due to low contrast increments) area of variable size. The core region ends with the deposition of a checkmark which marks the beginning of a regular deposition of rings that extend to the outer edge of the stylet. Number of GI, distance between GI and core region size all seem to be independent of stylet section size and

octopus size suggesting that post-core ring deposition is the only source of a relationship between section size and octopus size (and in some way age). The core region has also been identified in *M. maorum*, another merobenthic octopus (Doubleday *et al.*, 2011b). Based on knowledge of the pre-hatching formation of the stylet and its size in three-day old paralarvae (Lourenço *et al.*, *unpublished data*), it is possible to hypothesize that the core region is formed throughout a relatively long period after hatching, while the paralarva undergoes its pelagic existence, and that probably the first checkmark is layed when the paralarva changes into a juvenile at settlement to the benthic habitat. In fact, the radius of the core region was on average 382 μm. Considering that the mean distance between GI was estimated to be 4.29 μm and assuming that the GI deposition was regular during the entire life of the juveniles, we can estimate that at least 88 GI could be deposited between hatch and settlement marks, corresponding to about 3 months. Although, representing a significantly longer paralarval stage than the results obtained by Villanueva (1995) on captive paralarvae would suggest (they settled 60 days after hatching), it is in line with estimates made by Katsanevakis and Verriopoulos (2006) from density studies, which indicated paralarval stage lengths of three to four months depending on water temperature (colder waters leading to longer paralarval stages).

Support for a conservative nuclear size has been gained from this study, which in turn would support a conservation of the hatchling stylet diameter as the nucleus of the developing structure. It is then possible that the core region somewhat masks nuclear dimensions, making it more difficult to determine nuclear dimensions in larger individuals.

Albeit generally featureless, 5 to 23 low contrasts GI are usually visible in the core region. It would therefore stand to reason that whatever dictates the deposition of GI, either their period or their characteristics vary throughout the life of the individual. Therefore, in complement to the validation of the daily deposition of GI in adults, it is also necessary to attempt to validate it in other life stages. During juvenile and adult life stages, the animals are benthic and the daily deposition of GI is probably related with the circadian activity controlled by the light cycle, even if directly it is dictated by hunting or actual feeding success (Wells *et al.*, 1983; Meisel *et al.*, 2003). If factors such as feeding success play a role, the variability of possible outcomes may also explain the variability observed in the thickness and other characteristics of the individual GI. Paralarvae are pelagic, and other than being subject to external cues, they may also modulate these cues via their own behaviour, collectively the *zeitgeber*, which includes dial migrations, light or temperature

cycles, feeding success, etc., all of which influence their metabolism during this stage. Considering those differences in the external factors that influence behaviour and metabolism, it is essential to take validation methods to another level (Doubleday *et al.*, 2011b) in order to adequately interpret the whole extent of the assumed age record.

Individuals weighing between 99 g and 748 g take 59 to 219 days after settlement to attain those weights. Having in mind that complete age results might be underestimated due to the difficulties in identifying daily GI in the core region, up to 3 months should be added relating to the paralarval stage. We therefore estimate that such juveniles might be conservatively aged at 5 to 10 months. These age estimates are higher than those obtained by Perales-Raya *et al.* (2010, 2014) for the same weight range from beak growth increment analysis. The *O. vulgaris* population of the Central East Atlantic studied by Perales-Raya et al. (2010, 2014) is subject to higher environmental temperatures than those experienced by the population studied here (which is influenced by the Western Iberian upwelling system - WIUS). Different environmental temperatures are known to influence growth rates (Forsythe, 1993), lower temperatures leading to slower weight gains. This could explain the different results obtained here in comparison with Perales-Raya *et al.* (2010). On the other hand, age estimates conducted by Canali *et al.* (2011) in beaks of population of *O. vulgaris* from the Bay of Naples (western Mediterranean Sea) show that animals weighing on average 350 g are aged between 72 days and 371 days, a wide age range for such small animals, even considering that Mediterranean populations attain smaller sizes than Atlantic populations (Quetglas *et al.*, 1998). Studies on beak growth increments, such as those conducted by Perales-Raya *et al.* (2010, 2014) and Canali *et al.* (2011) are however sometimes also questioned due to the unknown level of beak increment erosion induced by feeding.

Despite the high variability in age estimates, the model that best explained growth at this pre-maturity stage was the logarithmic model. This is in accordance with the review conducted by Forsythe & Van Heukelem (1987) for laboratory-based growth in cephalopod species in general. They show that growth in benthic octopods occurs in two phases, the first being exponential, and the second logarithmic. During the logarithmic phase, individuals were never shown to reach an asymptote, contrary to the study of Domain *et al.* (2000), but growth rates decreased progressively. In the present study, the instantaneous growth rate estimated with a power model was on average 6.26 % d^{-1}, which is low when compared to estimates by Iglesias *et al.* (2004) for captive animals and high when

compared to the direct estimates by Domain *et al.* (2000) or the demographic model produced by Katsanevakis & Verriopoulos (2006).

The variability in age estimates obtained through different methods highlights the importance of validation studies, among which those based on wild-caught specimens would be particularly relevant (Perales-Raya *et al.*, 2014). Validations of daily deposition of GI in different stages of the life cycle are then also necessary. In the case of *O. vulgaris* (and possibly other merobenthic species) this validation is essential to understand the differences that are apparent in different regions of the stylet.

Finally, adequate ageing protocols are also essential to allow repeatable and comparable exercises between octopus populations and researchers. The definition *a priori* of count areas (e.g. the larger segment of the major axis) will enhance counts comparison within and between individuals (or populations). Additionally, improving the structures contrast introducing differential interference contrast filters to the observation with the 400x and 625x magnification objectives will improve the GI contrast particularly in the core region, which is, at this stage, the most difficult and demanding stylet region in terms of identification and GI counting. Hand-in-hand with the validation of GI deposition in the paralarval stage, the increasing contrast of the GI laid in the stylet will be the most significant methodological aspect to improve to obtain precise and accurate age estimates to the Incirrate octopuses' species.

ACKNOWLEDGEMENTS

The first author wants to acknowledge Mestre Arsénio for his help with the juveniles collection, António Pedro Mendonça for his assistance with biological sampling and Andreia Silva, Isabel Meneses and Marcos Regueira for their help with stylets observations, growth increment counts and hours of discussion about the best way of conduct SIA on octopus stylets. The first author is particularly grateful to Beatriz Morales-Nin for her precious advice on how to conduct SIA. This study was supported by the Data Collection Framework (DCF)/DG FISH (EC Regulation No. 665/2008 of the 14 July 2008) and PRESPO projects (INTERREG IVB - Atlantic Area Program, co-financed by the European Union ERDF funds). The first author is supported by a PhD fellowship granted by the Portuguese Foundation for Science and Technology (FCT).

BIBLIOGRAPHIC REFERENCES

ARKHIPKIN, A. & BJORKE, H., 2000.Statolith shape and microstructure as indicators of ontogenetic shifts in the squid *Gonatus fabricii* (Oegopsida, Gonatidae) from the Norwegian Sea. *Polar Biol*ogy **23**, 1–10.

BARRATT, I.M. & ALLCOCK, A.L., 2010.Ageing octopods from stylets: development of a technique for permanent preparations. *ICES Journal of Marine Science* **67**, 1452–1457.

BOYLE, P. & RODHOUSE, P., 2006. Cephalopods: Ecology and Fisheries, 2 nd. ed. Blackwell Publishing.

BUDELMANN, B.U., SCHIPP, R. & VON BOLETZKY, S. 1997. Cephalopoda, in: Microscopic Anatomy of Invertebrates. Wiley-Liss, pp. 119–414.

CAMPANA, S., 2001.Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59**, 197–242.

CANALI, E., PONTE, G., BELCARI, P., ROCHA, F. & FIORITO, G., 2011. Evaluating age in *Octopus vulgaris*: estimation, validation and seasonal differences. *Marine Ecology Progress Series* **441**, 141–149.

CHALLIER, L., ROYER, J. & ROBIN, J.-P., 2002. Variability in age-at-recruitment and early growth in English Channel *Sepia officinalis* described with statolith analysis. *Aquatic Living Resources* **15**, 303–311.

DOMAIN, F., JOUFFRE, D. & CAVERIVIÉRE, A., 2000. Growth of *Octopus vulgaris* from tagging in Senegalese waters. *Journal of the Marine Biological Association of the U. K*. **80**, 699–705.

DOUBLEDAY, Z., SEMMENS, J.M., PECL, G. & JACKSON, G., 2006. Assessing the validity of stylets as ageing tools in *Octopus pallidus*. *Journal of Experimental Marine Biology and Ecology* **338**, 35–42.

DOUBLEDAY, Z.A. & SEMMENS, J.M., 2011. Quantification of the age-pigment lipofuscin in known-age octopus (*Octopus pallidus*): A potential tool for age determination. *Journal of Experimental Marine Biology and Ecology* **397**, 8–12.

DOUBLEDAY, Z.A., WHITE, J., PECL, G.T. & SEMMENS, J.M., 2011a. Age determination in merobenthic octopuses using stylet increment analysis: assessing future challenges using *Macroctopus maorum* as a model. ICES Journal of Marine Science **68**, 2059–2063.

DOUBLEDAY, Z.A., WHITE, J., PECL, G.T. & SEMMENS, J.M., 2011b. Age determination in merobenthic octopuses using stylet increment analysis: assessing future challenges using *Macroctopus maorum* as a model. *ICES Journal of Marine Science***68**, 2059–2063.

FORSYTHE, J.W., 1993. A working hypothesis of How Seasonal Temperature change may impact the field growth of young cephalopods, in: Recent Advances in Cephalopod Fisheries Biology, Contributed Papers to 1991 CIAC International Symposium and Proceedings of Workshop on Age, Growth and Population Structure. pp. 133–143.

FORSYTHE, J.W. & VAN HEUKELEM, W.F. 1987. Growth, in: Cephlopod Life Cycles, Vol II: Comparative Reviews. Academoc Press, London U.K, pp. 135–156.

GONZÁLEZ, A.F., CASTRO, B.G. & GUERRA, A. 1996. Age and growth of the short-finned squid *Illex coindetii* in Galician waters (NW Spain) based on statolith analysis. *ICES Journal of Marine Science* **53**, 802–810.

HERMOSILLA, C.A., ROCHA, F., FIORITO, G., GONZÁLEZ, Á.F.& GUERRA, Á. 2010. Age validation in common octopus *Octopus vulgaris* using stylet increment analysis. *ICES Journal of Marine Science* **67**, 1458–1463.

HERWIG, J.N., DEPCZYNSKI, M., ROBERTS, J.D., SEMMENS, J.M., GAGLIANO, M. & HEYWARD, A.J. 2012. Using age-based life history data to investigate the life cycle and vulnerability of *Octopus cyanea*. *PLoS ONE* **7**, e43679

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.

KATSANEVAKIS, S. & VERRIOPOULOS, G., 2006.Seasonal population dynamics of *Octopus vulgaris* in the eastern Mediterranean. *ICES Journal of Marine Science* **63**, 151–160.

LEPORATI, S., SEMMENS, J.& PECL, G. 2008. Determining the age and growth of wild octopus using stylet increment analysis.*Marine Ecology Progress Series***367**, 213–222.

LEPORATI, S.C., PECL, G.T. & SEMMENS, J.M. 2007.Cephalopod hatchling growth: the effects of initial size and seasonal temperatures. *Marine Biology* **151**, 1375–1383.

LIPINSKI, M.R. & DURHOLTZ, M.D. 1994. Problems associated with ageing squid from their statoliths: towards a structured approach. *Antarctic Science***6**, 215–222.

MANGOLD, K., 1983. Octopus vulgaris, in: Cephalopod Life Cycles. Academic Press, London, pp. 335–386.

MEISEL, D., BYRNE, R.., KUBA, M., GRIEBEL, U., MATHER, J.A. 2003.Circadian rhythms in *Octopus vulgaris*. *Berl. Paläobiol Abh* **03**, 171–177.

MORENO, A., PEREIRA, J. & CUNHA, M. 2005. Environmental influences on age and size at maturity of *Loligo vulgaris*. *Aquatic Living Resources* **18**, 377–384.

OTERO, J., GONZÁLEZ, Á.F., SIEIRO, M.P. & GUERRA, Á. 2007. Reproductive cycle and energy allocation of *Octopus vulgaris* in Galician waters, NE Atlantic. *Fisheries Research* **85**, 122–129.

PANFILI, J., DE PONTUAL, H., TROADEC, H., WRIGHT, P.J. 2002.Manual of fish sclerochronology, Panfili, H., de Pontual, H. Toradec and P J Wright.ed. IFREMER - IRD, Brest, France.

PERALES-RAYA, C., BARTOLOMÉ, A., GARCÍA-SANTAMARÍA, M.T., PASCUAL-ALAYÓN, P. & ALMANSA, E. 2010. Age estimation obtained from analysis of octopus (*Octopus vulgaris* Cuvier, 1797) beaks: Improvements and comparisons. *Fisheries Research* **106**, 171–176.

PERALES-RAYA, C., JURADO-RUZAFA, A., BARTOLOMÉ, A., DUQUE, V., CARRASCO, M.N., FRAILE-NUEZ, E. 2014. Age of spent *Octopus vulgaris* and stress mark analysis using beaks of wild individuals.*Hydrobiologia***725**, 105–114.

PIERCE, G.J., KEY, L.., BOYLE, P.R., SIEGERT, K.J., GONÇALVES, J.M., PORTEIRO, F.M. & MARTINS, H. 1999. RNA concentration and the RNA to protein ratio in cephalopod tissues: sources of variation and relationship with growth rate. *Journal of Experimental Marine Biology Ecology* **237**, 185–201.

QUETGLAS, A., ALEMANY, F., CARBONELL, A., MERELLA, P. & SÁNCHEZ, P. 1998. Biology and fishery of *Octopus vulgaris* Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, Western Mediterranean). *Fisheries Research* **36**, 237–249.

QUINN, G. & KEOUGH, M. 2002.Experimental Design and Data Analysis for Biologists.Cambridge University Press.

RÉ, P. & NARCISO, L. 1994.Growth and cuttlebone microstructure of juvenile cuttlefish, *Sepia Officinalis* L., under controlled conditions. *Journal of Experimental Marine Biology and Ecology* **177**, 73–78.

REIS, C.S. & FERNANDES, R. 2002. Growth observations on *Octopus vulgaris* Cuvier, 1797 from the Portuguese waters: growth lines in the vestigial shell as possible tools for age determination. *Bulletin of Marine Science* **71**, 1099–1103.

THÉVENAZ, P. & UNSER, M. 2007.User-friendly semiautomated Assembly of accurate Image Mosaics in Microscopy.*Microscopy Research and Technique* **70**, 135–146.

VILLANUEVA, R. 1995. Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to settlement.*Canadian Journal of Fisheries and Aquatic Science* **52**, 2639–2650.

WELLS, M., O'DOR, R.K., MANGOLD, K. & WELLS, J. 1983. Diurnal changes in activity and metabolic rate in *Octopus vulgaris*. *Marine Behavior & Physiology* **9**, 275–287.

ZUUR, A.F., IENO, E.N. & SMITH, G.M., 2007.Analysing Ecological data.Springer.

CHAPTER 5

GENERAL CONCLUSIONS

5. GENERAL CONCLUSIONS

5.1. GENERAL CONCLUSIONS

The *Octopus vulgaris* population of the northeastern Atlantic is very important from a socioeconomic perspective. The coastal fishing communities of Portugal and Galicia fish an average of 14 911 tons of common octopus each year (FAO 2014; INE 2013) mainly through the small-scale fishing fleet (Freire & García-Allut, 2000; ICES 2012a), and increasingly in the latest years, the bottom-trawling fleet (Fonseca *et al.*, 2008). Characterized by high abundance variability mainly related with the impact of different environmental factors in the paralarval and juvenile stages (González *et al.*, 2005; Otero *et al.*, 2008; 2009; Sobrino *et al.*, 2002; Sonderblohm *et al.*, 2014), the resilience of the species to heavy fishing is, most certainly, related with its plastic life-strategy (Hastie *et al.*, 2009), the highly productive coastal conditions of the Northeastern Atlantic coastal basin (Arístegui *et al.*, 2009; García Lafuente & Ruiz, 2007; Relvas *et al.*, 2007), the opportunistic feeding behaviour of the adults (Quetglas *et al.*, 1998) and the availability of several potential prey assemblages (Sousa *et al.*, 2005).

In the coastal basin of the northeast Atlantic, *Octopus vulgaris* spawn year-round (Hastie *et al.*, 2009). Nevertheless, the northeast Atlantic population presents seasonal trends that differ from north to south. While in Galician waters the population spawns mainly in spring (Otero *et al.*, 2007), we observed that in southern waters near Estremadura Promontory (39º N) the spawning season extends from early spring until the end of summer, with intensity peaks in February-April and June-July, coupled to seasonal upwelling (Lourenço *et al.*, 2012). In the southern section of the Iberian Atlantic basin the spawning season is shorter occurring in summer and peaking in September (Lourenço *et al.*, 2012) similarly to neighbouring eastern waters between the River Guadiana mouth and Cádiz (Silva *et al.*, 2002). In this area, the primary production seasonality is not as pronounced, being mainly driven by the west-east wind pattern, river runoff associated to raining periods and strong water column stratification with significant warming of sea surface waters (García Lafuente & Ruiz, 2007; Ruiz *et al.*, 2006). The *O. vulgaris* population takes advantage of these conditions, channelling more energy for spawning in a shorter period of time (higher Gonad-somatic index attained by females in this area), in the

end of summer, timing paralarval hatching to winter, when the predominant westerly winds favour the transport of nutrients and zooplankton near to the Cape Stª Maria (Ruiz *et al.*, 2006).

On an individual basis, females and males seem to have different reproductive strategies. Males attain maturity at smaller sizes (Lourenço *et al.*, 2012) and maintain spawning capacity for a longer period than females (both to senescence). Females couple their reproductive strategy with environmental conditions (Lourenço *et al.*, 2012) guaranteeing the best survival chances for their offspring. The onset of maturation and energy allocation to maturation depend on individual weight and body condition. However, the lack of a correlation (in the case of males) or the inverse correlation (in the case of females) between total body weight (and digestive gland) and gonad weight (Lourenço *et al.*, 2012), and between the nutritional profiles of the digestive gland and the mature gonad (Lourenço *et al.*, 2014) show that both males and mature females channel energy directly from food to reproduction supporting the hypothesis that *O. vulgaris* is an income breeder (Houston *et al.*, 2006; Quetglas *et al.*, 2011) and whenever the environmental and feeding conditions are favourable (increasing body condition), the maturation process is triggered.

Independently of maturity stage, the lipid content and fatty acids profile in the digestive gland seems to offer a good indication of the nutritional condition of the population (Lourenço *et al.*, 2014). The function of the digestive gland as a reservoir in cephalopods has been under discussion (Blanchier & Boucaud-Camou, 1984; García *et al.*, 2011; García-Garrido *et al.*, 2010; Moguel *et al.*, 2010; Moltschaniwskyj & Johnston, 2006) for long, evidence suggesting that although cephalopods have a protein based metabolism (Lee, 1995), polar lipids play an important role as constituents of the lipoproteins that form the cells membrane (Navarro *et al.*, 2014) and the digestive gland plays an important role in lipid metabolism (Moguel *et al.*, 2010; Moltschaniwskyj & Johnston, 2006). Here, we showed that the FA profile found in the digestive gland of females of populations under different environmental conditions reflects the nutrients available, mainly in the PUFA and MUFA profile but also in the n-3HUFA/n-6HUFA and DHA/EPA ratios (Lourenço *et al.,* 2014).

With a short life cycle (one to two years) and absence of generation overlap, population resilience and the success of recruitment to fisheries are supported by the biological and physical conditions that paralarvae and juveniless find prior to settlement, during and immediately post settlement. The paralarval stage and the transition stage

between paralarva and juvenile are the phases when most significant changes occur in morphology, habitat and behaviour (including feeding behaviour) and also when higher rates of natural mortality occur (see Robin *et al.*, 2014 for a review on the role of habitat, environment, functional morphology and behaviour in the transitions of the life history of cephalopods). It is therefore important to understand which of the environmental parameters (physical and nutritional) contribute to survival and recruitment success. Local factors that most correlate with the higher abundances of *O. vulgaris* paralarvae in the spawning area of the Ría de Vigo were investigated, showing that shallow waters protected from physical disturbance offer the best brooding grounds, as attested by the relatively higher abundance of newly-hatched paralarvae.

The nutritional conditions found by the paralarva are determinant to their survival and later recruitment success. The nutritional requirements of the paralarval stages in the wild and in captivity have been under discussion for several years and are perceived as the bottleneck factor preventing commercial scale aquaculture production of *O. vulgaris* (Iglesias *et al.*, 2007; Seixas, 2009; Vaz-Pires *et al.*, 2004). The identification of the preferential preys of *O. vulgaris* paralarvae in the Ría de Vigo (Roura *et al.*, 2012) open the door to a definition of the nutritional profile of the potential preys and *O. vulgaris* paralarval requirements in their natural conditions. Here, for the first time, it was possible to identify macro-nutrients and lipid classes including the fatty acid (FA) profile in newly hatched and recently-feeding *O. vulgaris* paralarvae and their natural preys, following the changes in the FA trophic markers that occur between preys and paralarval predator. Potential prey (specific zooplankton communities) and *O. vulgaris* paralarval stages present an identical Protein:Lipid ratio while small but significant differences in the ratios of n-3HUFA/n-6HUFA, DHA/ARA and DHA/EPA and in the ARA content. The results obtained here confirm the importance of maintaining the Protein:Lipid ratio between preys and paralarvae to guarantee the nutritional requirements of the latter, but at the same time maintain high levels of n-3 HUFA that can be used by the paralarva to biosynthesize ARA, an essential component to growth.

Few days after hatching in the Ría de Vigo, the pelagic paralarvae disperse, first across-shore and then along-shore by action of the coastal upwelling/downwelling mechanism (Moreno *et al.*, 2009; Roura, 2013), and eventually (two to four months later) find suitable grounds to settle and grow to maturation. Those grounds are located preferentially in the vicinity of estuaries or lagoons systems at short distances from the

coast line (10-15 km) at mean depths of approximately 80 m over grounds with a mixture of coarse sand and rocky outcrops that permit adequate sheltering in. Despite the short distance between estuarine and lagoon systems where changes in salinity can occur more frequently, we found that *O. vulgaris* juvenlies (sub-adults or pre-recruit) aggregations are particularly vulnerable to changes in bottom salinity withstanding salinity variations ranging between 35.60 and 36.30 (with a mean optimum salinity of 36.0) (Moreno *et al.*, 2014). Sea temperature does not play a similarly limiting role, and animals present in northwest and south grounds seem to present different optimal sea bottom and surface temperatures (Moreno *et al.*, 2014) as would be expected from the different reproductive strategies followed on each coasts.

The environmental characteristics of the southern pre-recruit grounds, and particularly the Ría Formosa ground are probably the most persistent in time and the most important to support the fisheries exploitation of the entire Portuguese coast and possibly the neighbouring communities of the Gulf of Cádiz. Considering the division in oceanographic features between the seasonal upwelled northeast coast and the south coast, which Cape St. Vincent provides, and the coastal orientation shift from north-south to west-east, it is believed that this region acts as barrier to biological exchanges between the two adjacent areas (Relvas *et al.*, 2007; Pires et al., 2013). It is therefore most likely that the spawning ground identified in the Ría de Vigo and the nurseries found along the Portuguese northwestern coast do not support the south coast pre-recruitment grounds and the latter is entirely supported by the nurseries identified in the south coast.

The age structure of an exploited population is seen as a fundamental piece of information for the application of traditional assessment models, aiming to achieve the sustainable management of a fisheries resource. In the case of *O. vulgaris*, both the application of traditional assessment models and the accurate estimation of age are not trivial. Firstly, recruitment success does not depend on the previous spawning-stock biomass (due to the absence of generation overlap) but on environmental conditions; and secondly research on age and growth for a number of octopods has shown an incredible variability in size between individuals of the same attributed age (e.g. Leporati *et al.*, 2008, 2007).

Accurate age and growth estimates will allow adding a time scale to stressful events in the life cycle of the animals which potentially increase pressure on the individual, affecting population survival and growth rates. Some known stress events can be related to

hatching, settlement and spawning, but potentially others such as fishing patterns and varying environmental conditions play crucial roles as well. How these influences the time population takes to attain a specified weight or how long the paralarvae from a specific seasonal hatchery remain in the pelagic realm or take to settle in pre-recruit grounds are important pieces of information to add to our modelling capabilities. The fact that stylets register the life of an individual from the very first day, is an advantage of the use of this structure to estimate age in relation to the use of beaks. Beaks have been used to estimate the age of the *O. vulgaris* in the Mediterranean (Canali *et al.*, 2011) and in the Sahara bank (Perales-Raya *et al.*, 2010), but the estimation of absolute age using these structures is difficult due to the erosion on the rostral region from feeding activity.

We identified and measure the stylets in newly hatched paralarvae for the first time. This study also allowed the identification of the hatch check in the stylets of adults on which the daily deposition of growth increments (GI) was already validated. Following the hatch check, juvenile stylets present a core region where the deposition of GI appears irregular or GI are less visible. The core region is limited at its end by the beginning of the regular deposition of GI. As no other check or marked transition in the GI deposition pattern is identifiable in the stylets, we assume that this core region corresponds to the paralarval stage prior to settlement, but recognise that further studies are required in order to validate the correspondence between this core region and the paralarval phase.

Using the stylets of pre-recruits and recruits of the Portuguese Northeast coast we investigated the level of precision that this ageing technique allows and made the first direct determination of the approximate age of the individuals recruiting to the fishery in this area, estimating that it takes approximately eleven months for the animals to attain the minimum landing weight of 750 g.

5.1. FUTURE PERSPECTIVES

The growing interest and dependence of the Portuguese fisheries on the commom octopus as a guaranteed revenue source, associated to the new demands raised with the reform of the Common Fisheries Policy which commits Member-States to assess and manage their most important fisheries, thus achieving sustainability by 2020 (EU Regulation 1380/2013) has sprouted new interest in research on the species. Additionally, the national implementation of the Marine Strategy Framework Directive with the

obligation to achieve Good Environmental Status by 2020 and the use of indicators on biodiversity, food web resilience and fisheries sustainability among others, is an additional challenge to the management of the marine environment and the maintenance of fisheries "health".

Achieving sustainable fisheries for *O. vulgaris* stocks or protecting its best habitat are small bricks in the greater framework that ensures GES. However, the characteristics of the species and the level of its interactions with prey and predators, including a strongly dependent Portuguese fishing community afford it a particular importance. According to ICES (2012b), the common octopus stock is identified as a category 4 stock for which only reliable catch data are available, implying that a precautionary approach should be followed in the advice to future exploitation rates. Due to its life cycle characteristics and recruitment dependence of environmental conditions, the future of the stock assessment of the species seems to follow the path of the integration of environmental effects in the assessment models. This work provided indications of what physical and biological factors could have a predominant role in the survival and recruitment success and what characteristics identify the essential habitat of juveniles. The next step is to analyse the abundance time series (e.g. LPUE, CPUE and survey dedicated abundance estimates) comparing those to environmental indicators (e.g. salinity, temperature, fresh water input) aiming to identify cause-effect relationships and predict their impacts on stock availability to fisheries in the short-term (nine to twelve months predictions to couple with the lifespan of the species).

The coupling of the species life cycle with local environmental conditions, its upper predator level in the trophic web and short life span place *O. vulgaris* (and potentially the other cephalopod species) in a good position to be considered a key functional group in the definition and monitoring of the marine environmental status in relation to biodiversity, fisheries and trophic web descriptors. Associating the biologic monitoring of parameters such as the age structure, growth rates and reproductive status of a population under a specific environmental condition, with the application of environmental biomarkers, such as stable isotope signatures and FA trophic markers, is going to allow the identification of specific environmental conditions or status that can be useful as tools to monitor environmental status and steer towards the GES aimed by the Marine Strategy Framework Directive.

LITERATURE CITED[7]

ARÍSTEGUI, J., BARTON, E.D., ÁLVAREZ-SALGADO, X.A., SANTOS, A.M.P., FIGUEIRAS, F.G., KIFANI, S., HERNÁNDEZ-LEÓN, S., MASON, E., MACHÚ, E. & DEMARCQ, H. 2009. Sub-regional ecosystem variability in the Canary Current upwelling. *Progress in Oceanography* 83: 33–48.

BERGÉ, J.-P. & BARNATHAN, G., 2013. Fatty Acids from Lipids of Marine Organisms: Molecular Biodiversity, Roles as Biomarkers, Biologically Active Compounds, and Economical Aspects, in: Ulber, R., Gal, Y. (Eds.), Marine Biotechnology I. Springer-Verlag, Berlin/Heidelberg, pp. 49–125.

BERNAL, M., STRATOUDAKIS, Y., COOMBS, S., ANGELICO, M.M., DE LANZÓS, A.L., PORTEIRO, C., SAGARMINAGA, Y., SANTOS, M., URIARTE, A., CUNHA, E., VALDÉS, L. & BORCHERS, D. 2007. Sardine spawning off the European Atlantic coast: Characterization of and spatio-temporal variability in spawning habitat. *Progress in Oceanography* 74: 210–227.

BIZIKOV, V.A. 2004. The shell in Vampyropoda (Cephalopoda): Morphology, functional role and evolution. *Ruthenica* supplement 3: 87.

BODE, A., ALVAREZ-OSSORIO, M.T., CARRERA, P. & LORENZO, J. 2004. Reconstruction of trophic pathways between plankton and the North Iberian sardine (*Sardina pilchardus*) using stable isotopes. *Scientia Marina* 68: 165–178.

BOUCAUD-CAMOU, E. & BOUCHER-RODONI, R. 1983. Feeding and Digestion in Cephalopods, in: The Mollusca. Academic Press, pp. 149–187.

BOYLE, P. & RODHOUSE, P. 2006. Cephalopods: Ecology and Fisheries, 2 nd. ed. Blackwell Publishing.

BUDGE, S.M., IVERSON, S.J. & KOOPMAN, H.N. 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analyisis and interpretation. *Marine Mammal Science* 22: 759–801.

CANALI, E., PONTE, G., BELCARI, P., ROCHA, F. & FIORITO, G., 2011. Evaluating age in *Octopus vulgaris*: estimation, validation and seasonal differences. *Marine Ecology Progress Series* 441: 141–149.

CATALÁN, I.A., RUBÍN, J.P., NAVARRO, G. & PRIETO, L. 2006. Larval fish distribution in two different hydrographic situations in the Gulf of Cádiz. *Deep Sea Research Part II*. 53: 1377–1390.

CHEREL, Y & HOBSON, K. A. & WEIMERSKIRCH, H. 2000. Using stable-isotopes analysis of feathers to distinguish moulting and breeding origins of seabirds. *Oecologia* 122: 155-162.

CHEREL, Y. & HOBSON, K.A. 2005. Stable isotopes, beaks and predators: a new tool to study the trophic ecology of cephalopods, including giant and colossal squids. *Proceedings of the Royal Society B* 272: 1601–1607.

[7] This list of literature cited concerns only to the chapters one and five.

Cherel, Y. & Hobson, K.A. 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Marine Ecology Progress Series* 329: 281–287.

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 (Phd Thesis). Faculdade de Ciências da Universidade de Lisboa.

DALSGAARD, J., ST JOHN, M., KATTNER, G., MÜLLER-NAVARRA, D., HAGEN, W. 2003. Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* 46: 225–340.

DOS SANTOS, A., SANTOS, A., CONWAY, D., 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.

EL-SABAAWI, R., DOWER, J.F., KAINZ, M. & MAZUMDER, A. 2008. Characterizing dietary variability and trophic positions of coastal calanoid copepods: insight from stable isotopes and fatty acids. *Marine Biology* 156: 225–237.

FAO 2011-2014. Fisheries and Aquaculture software Fishstat J - Software for fishery statistical time series. IN: FAO Fisheries and Aquaculture Department [online]. Rome. Updated in 28 November 2013.

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.

FRANCO-SANTOS, R.M., IGLESIAS, J., DOMINGUES, P.M. & VIDAL, E.A.G. 2014. Early beak development in *Argonauta nodosa* and *Octopus vulgaris* (Cephalopoda: Incirrata) paralarvae suggests adaptation to different feeding mechanisms. *Hydrobiologia* 725: 69–83.

FRANCO-SANTOS, R.M. & VIDAL, E.A.G. 2014. Beak development of early squid paralarvae (Cephalopoda: Teuthoidea) may reflect an adaptation to a specialized feeding mode. *Hydrobiologia* 725: 85–103.

FREIRE, J. & GARCÍA-ALLUT, A. 2000. Socioeconomic and biological causes of management failures in European artisanal fisheries: the case of Galicia (NW Spain). *Marine Policy* 24: 375–384.

FUCHS, D. & WEIS, R. 2010. Taxonomy, morphology and phylogeny of lower Jurassic teudopseid coleoids (Cephalopoda). *Neues Jahrbuch für Geologie und Paläontologie - Abhandlungen* 257: 351–366.

GARCÍA LAFUENTE, J. & RUIZ, J. 2007. The Gulf of Cádiz pelagic ecosystem: A review. *Progress in Oceanography* 74: 228–251.

GONÇALVES, A.M.M., AZEITEIRO, U.M., PARDAL, M.A. & DE TROCH, M. 2012. Fatty acid profiling reveals seasonal and spatial shifts in zooplankton diet in a temperate estuary. *Estuarine, Coastal and Shelf Science* 109: 70–80.

GUERRA, Á., ROURA, Á., GONZÁLEZ, Á.F., PASCUAL, S., CHEREL, Y. & PÉREZ-LOSADA, M. 2010. Morphological and genetic evidence that *Octopus vulgaris* Cuvier, 1797 inhabits Amsterdam and Saint Paul Islands (southern Indian Ocean). *ICES Journal of Marine Science* 67: 1401–1407.

GUZIK, M.T., NORMAN, M.D. & CROZIER, R.H. 2005. Molecular phylogeny of the benthic shallow-water octopuses (Cephalopoda: Octopodinae). *Molecular Phylogenetics and Evolution* 37: 235–248.

HASTIE, L.C., PIERCE, G.J., WANG, J., BRUNO, I., MORENO, A., PIATKOWSKI, U. & ROBIN, J.-P. 2009. Cephalopods in the North-Eastern Atlantic: species, biogeography, ecology, exploitation and conservation. *Oceanography and Marine Biology – An Annual Review* 47. 111 pp

HOBSON, K.A. & CHEREL, Y. 2006. Isotopic reconstruction of marine food webs using cephalopod beaks: new insight from captivity raised *Sepia officinalis*. *Canadian Journal of Zoology* 84: 766–770.

HOBSON, K. A. & WELCH, H. E. 1992. Determination of trophic relationships within a high Artic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Marine Ecology Progress Series* 84: 9-18.

HOUSTON, A.I., STEPHENS, P.A., BOYD, I.L., HARDING, K.C. & MCNAMARA, J.M. 2006. Capital or income breeding? A theoretical model of female reproductive strategies. *Behavioral Ecology* 18: 241–250.

IBÁÑEZ, C.M., PENA, F., PARDO-GANDARILLAS, M.C., MENDEZ, M.A., HERNANDEZ, C.E. & POULIN, E. 2014. Evolution of development type in benthic octopuses: holobenthic or pelago-benthic ancestor? *Hydrobiologia* 725: 205–214.

ICES, 2012a. Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH).

ICES. 2012b. Report of the ICES Advisory Committee 2012. ICES Advice, 2012. Book 1. 156 pp.

INE. 2014. Estatísticas de pesca Lisboa: INE: http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_base_dados (assessed on 1st June 2014)

IGLESIAS, J., SÁNCHEZ, F.J., BERSANO, J.G.F., CARRASCO, J.F., DHONT, J., FUENTES, L., LINARES, F., MUÑOZ, J.L., OKUMURA, S., ROO, J., VAN DER MEEREN, T., VIDAL, E.A.G. & VILLANUEVA, R., 2007. Rearing of *Octopus vulgaris* paralarvae: Present status, bottlenecks and trends. *Aquaculture* 266: 1–15.

IVERSON, S., SPRINGER, A. & KITAYSKY, A. 2007. Seabirds as indicators of food web structure and ecosystem variability: qualitative and quantitative diet analyses using fatty acids. *Marine Ecology Progress Series*. 352: 235–244.

JACKSON, G.D., BUSTAMANTE, P., CHEREL, Y., FULTON, E.A., GRIST, E.P.M., JACKSON, C.H., NICHOLS, P.D., PETHYBRIDGE, H., PHILLIPS, K., WARD, R.D. & XAVIER, J.C., 2007. Applying new tools to cephalopod trophic dynamics and ecology: perspectives from the Southern Ocean Cephalopod Workshop, February 2–3, 2006. *Reviews in Fish Biology and Fisheries* 17: 79–99.

KATSANEVAKIS, S. & G. VERRIPOULOS. 2004. Den ecology of *Octopus vulgaris* Cuvier, 1797, on soft sediment: availability and types of shelter. *Scientia Marina* 68: 147–157.

KRÖGER, B., VINTHER, J. & FUCHS, D. 2011. Cephalopod origin and evolution: A congruent picture emerging from fossils, development and molecules: Extant cephalopods are younger than previously realised and were under major selection to become agile, shell-less predators. *BioEssays* 33: 602–613.

LEE, P.G., 1995. Nutrition of cephalopods: Fueling the System. *Marine and Freshwater Behaviour and Physiology* **25**, 35- 51.

LEITE, T.S., HAIMOVICI, M., MOLINA, W. & WARNKE, K., 2008. Morphological and genetic description of Octopus insularis, a new cryptic species in the *Octopus vulgaris* complex (Cephalopoda: Octopodidae) from the tropical southwestern Atlantic. *Journal of Molluscan Studies* 74: 63–74.

LEPORATI, S., SEMMENS, J. & PECL, G. 2008. Determining the age and growth of wild octopus using stylet increment analysis. *Marine Ecology Progress Series* 367: 213–222.

LEPORATI, S.C., PECL, G.T. & SEMMENS, J.M. 2007. Cephalopod hatchling growth: the effects of initial size and seasonal temperatures. Marine Biology 151: 1375–1383.

LOURENÇO, S., MORENO, A., NARCISO, L., GONZÁLEZ, Á.F. & PEREIRA, J., 2012. Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. *Fisheries Research* 127-128: 116–124.

LOURENÇO, S., NARCISO, L., GONZALEZ, Á.F., PEREIRA, J., AUBORG, S. & XAVIER, J.C. 2014. Does the trophic habitat influence the biochemical quality of the gonad of *Octopus vulgaris*? Stable isotopes and lipid class contents as bio-indicators of different life-cycle strategies. *Hydrobiologia* 725: 33-46

MANGOLD, K., 1983. *Octopus vulgaris*. In: *Cephalopod Life Cycles*. *I* (P.R. Boyle, ed.), 335-364. Comparative Reviews, Academic Press, London.

MARTA-ALMEIDA, M., DUBERT, J., PELIZ, Á. & QUEIROGA, H. 2006. Influence of vertical migration pattern on retention of crab larvae in a seasonal upwelling system. *Marine Ecology Progress Series* 307: 1–19.

MATIAS, A. 2013. Applicability of ecolabelling fish products frm two artisanla Portuguese fisheries. Msc Thesis. Universidade de Lisboa.

MOGUEL, C., MASCARÓ, M., AVILA-POVEDA, O., CAAMAL-MONSREAL, C., SANCHEZ, A., PASCUAL, C. & ROSAS, C. 2010. Morphological, physiological and behavioral changes during post-hatching development of *Octopus maya* (Mollusca: Cephalopoda) with special focus on the digestive system. Aquatic Biology 9: 35–48.

MOLTSCHANIWSKYJ, N. & JOHNSTON, D. 2006. Evidence that lipid can be digested by the dumping squid *Euprymna tasmanica,* but is not stored in the digestive gland. *Marine Biology* 149: 565–572.

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.

MORENO, A., LOURENÇO, S., PEREIRA, J., GASPAR, M.B., CABRAL, H.N., PIERCE, G.J. & SANTOS, A.M.P. 2014. Essential habitats for pre-recruit *Octopus vulgaris* along the Portuguese coast. *Fisheries Research* 152: 74-85

MURTA, A.G., PINTO, A.L. & ABAUNZA, P. 2008. Stock identification of horse mackerel (Trachurus trachurus) through the analysis of body shape. *Fisheries Research* 89: 152–158.

NAVARRO, J. C., MONROIG, O. & SYKES, A. 2014. Nutrition as a key factor for cephalopod aquaculture. In: Iglesias, J., Fuentes, L & Villanueva, R (eds). Cephalopode Culture. Springer. 77-95 pp.

NIXON M. & YOUNG J. Z. 2003. The Brains and Lives of Cephalopods. Oxford University Press, Oxford, UK.

JEREB, P. ROPER C.F.E., NORMAN M. D. & FINN, J. K. (Eds) 2014. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. Volume 3. Octopods and Vampire squids. FAO Species Catalogue for fisheries Purposes. Nº 4 Vol. 3 Rome, FAO.

OTERO, J., ROCHA, F., GONZÁLEZ, Á.F. & GARCÍA, J. 2005. Modelling artisanal coastal fisheries of Galicia (NW Spain) based on data obtained from fishers: the case of *Octopus vulgaris*. *Scientia Marina* 69: 577–585.

OTERO, J., GONZÁLEZ, Á.F., SIEIRO, M.P., GUERRA, Á. 2007. Reproductive cycle and energy allocation of *Octopus vulgaris* in Galician waters, NE Atlantic. *Fisheries Research* 85: 122–129.

PELIZ, Á., 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 Part II* 52: 621–646.

PERALES-RAYA, C., BARTOLOMÉ, A., GARCÍA-SANTAMARÍA, M.T., PASCUAL-ALAYÓN, P. & ALMANSA, E. 2010. Age estimation obtained from analysis of octopus (*Octopus vulgaris* Cuvier, 1797) beaks: Improvements and comparisons. *Fisheries Research* 106: 171–176.

PEREIRA, J. 1999. Control of the portuguese artisnal octopus fishery In: *Proceedings of the International Conference on Integrated Fisheries Monitoring Sydney*. FAO, Rome, pp. 369–378.

PHILLIPS, K.L., JACKSON, G.D. & NICHOLS, P.D. 2001. Predation on myctophids by the squid Moroteuthis ingens around Macquarie and Heard Islands: stomach contents and fatty acid analyses. *Marine Ecology Progress Series* 215: 179–189.

PHILLIPS, K.L., NICHOLS, P.D. & JACKSON, G.D. 2002. Lipid and fatty acid composition of the mantle and digestive gland of four Southern Ocean squid species: implications for food-web studies. *Antarctic Science* 14: 212-220.

PHILLIPS, K.L., NICHOLS, P.D. & JACKSON, G.D. 2003a. Dietary variation of the squid *Moroteuthis ingens* at four sites in the Southern Ocean: stomach contents, lipid and fatty acid profiles. *Journal of the Marine Biologic Association UK* 83: 523–534.

PHILLIPS, K.L., NICHOLS, P.D. & JACKSON, G.D. 2003b. Size-related dietary changes observed in the squid Moroteuthis ingens at the Falkland Islands: stomach contents and fatty-acid analyses. *Polar Biology* 26: 474–485.

PICHÉ, J., IVERSON, S.J., PARRISH, F.A. & DOLLAR, R. 2010. Characterization of forage fish and invertebrates in the Northwestern Hawaiian Islands using fatty acid signatures: species and ecological groups. *Marine Ecology Progress Series* 418: 1–15.

PIERCE, G.J., ALLCOCK, A.L., BRUNO, I., BUSTAMANTE, P., GONZALEZ, A.F., GUERRA, Á., JEREB, P., ET AL, 2010. Cephalopod biology and fisheries in Europe, *ICES Cooperative Research Report* No. 303. 175 pp.

PILAR-FONSECA, T., CAMPOS, A., PEREIRA, J., MORENO, A., LOURENÇO, S. & AFONSO-DIAS, M. 2014. Integration of fishery-dependent data sources in support of octopus spatial management. *Marine Policy* 45: 69–75.

PIRES, R.F.T., PAN, M., SANTOS, A.M.P., PELIZ, A., BOUTOV, D. & DOS SANTOS, A., 2013. Modelling the variation in larval dispersal of estuarine and coastal ghost shrimp: *Upogebia* congeneres in the Gulf of Cadiz. *Marine Ecology Progress Series* 492: 153–168.

PITA, C. et al. *In prep.* The traditional small-scale octopus fishery in Portugal: framing its governability. In: Jentoft, S. and Chuenpagdee, R. (eds). Governing the Governance of small-scale fisheries. Springer.

PORTARIA Nº 27/2001. Diário da Republica Nº 12, Série I-B de 2001-01-15.

PORTARIA Nº 230/2012. Diário da República Nº 150, Série I de 2012-08-03.

QUETGLAS, A., ALEMANY, F., CARBONELL, A., MERELLA, P. & SÁNCHEZ, 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., ORDINES, F. & VALLS, M. 2011. What drives seasonal fluctuations of body condition in a semelparous income breeder octopus? *Acta Oecologica* 37: 476–483.

REGULATION (EU) No 1380/2013 of the European Parliament and of the council of 11 December 2013 on the Common Fisheries Policy.

RELVAS, P., BARTON, E.D., DUBERT, J., OLIVEIRA, P.B., PELIZ, Á., DA 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.

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 Iberian using SeaWiFS and in situ data. *Journal of Marine Systems* 53: 87–107.

ROBIN, J.-P., ROBERTS, M., ZEIDBERG, L., BLOOR, I., RODRIGUEZ, A., BRICEÑO, F., DOWNEY, N., MASCARÓ, M., NAVARRO, M., GUERRA, A., HOFMEISTER, J., BARCELLOS, D.D., LOURENÇO, S.A.P., ROPER, C.F.E., MOLTSCHANIWSKYJ, N.A., GREEN, C.P. & MATHER, J. 2014. Chapter Four - Transitions During Cephalopod Life History: The Role of Habitat, Environment,

Functional Morphology and Behaviour, In: Erica A.G. Vidal (Ed.), *Advances in Marine Biology* 67: 361–437.

ROCHA, F., GUERRA, A. & GONZALEZ, A.F. 2001. A review of reproductive strategies in cephalopods. Biological Reviews 76: 291–304.

ROSA, R., COSTA, P.R. & NUNES, M.L. 2004a. Effect of sexual maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca: Cephalopoda). *Marine Biology* 145: 563-574.

ROSA, R., MARQUES, A.M., NUNES, M.L., BANDARRA, N. & SOUSA REIS, C. 2004b. Spatial-temporal changes in dimethyl acetal (octadecanal) levels of *Octopus vulgaris* (Mollusca, Cephalopoda): relation to feeding ecology. *Scientia Marina* 68: 227–236.

ROURA, Á., 2013. Ecology of planktonic cephalopod paralarvae in coastal upwelling systems. Phd Thesis. Universidade de Vigo.

ROURA, Á., GONZÁLEZ, Á.F., REDD, K. & GUERRA, Á. 2012. Molecular prey identification in wild *Octopus vulgaris* paralarvae. *Marine Biology* **159:** 1335–1345.

RUIZ, J., GARCIA-ISARCH, E., EMMA HUERTAS, I., PRIETO, L., JUÁREZ, A., MUÑOZ, J.L., SÁNCHEZ-LAMADRID, A., RODRÍGUEZ-GÁLVEZ, S., NARANJO, J.M. & BALDÓ, F. 2006. Meteorological and oceanographic factors influencing *Engraulis encrasicolus* early life stages and catches in the Gulf of Cádiz. *Deep Sea Research Part II*. 53: 1363–1376.

SÁNCHEZ, R.F., MASON, E., RELVAS, P., DA SILVA, A.J. & PELIZ, Á. 2006. On the inner-shelf circulation in the northern Gulf of Cádiz, southern Portuguese shelf. *Deep Sea Research Part II* 53: 1198–1218.

SÁNCHEZ, P. & OBARTI, R. 1993. The biology and fishery of Octopus vulgaris caught with clay pots on the Spanish Mediterranean coast. In Okutami, T., O'Dor, R., Kubodera, T. (Eds). Recent Advances in Cephalopod Fisheries Biology. Tokai University Press, Tokyo. 477–487

SANTOS, A.M.P., RÉ, P., DOS SANTOS, A. & PELIZ, Á., 2006. Vertical distribution of the European sardine (Sardina pilchardus) larvae and its implications for their survival. *Journal of Plankton Research* 28: 523–532.

SEIXAS, P. 2009. Composición bioquímica y crecimento de paralarvas de pulpo (*Octopus vulgaris* Cuvier, 1797), alimentadas con juveniles de Artemia enriquecidos con microalgas y otros suplementos nutricionales (Memoria que para optar al grado de doctor en Biología). Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

SILVA, L., SOBRINO, I. & RAMOS, F. 2002. Reproductive biology of the common octopus, *Octopus vulgaris* Cuvier, 1797 (Cephalopoda: Octopodidae) in the Gulf of Cádiz (SW Spain). *Bulletin Marine Science* 71: 837–850.

SILVA, A., SKAGEN, D.W., URIARTE, A., MASSÉ, J., SANTOS, M.B., MARQUES, V., CARRERA, P., BEILLOIS, P., PESTANA, G., PORTEIRO, C. & STRATOUDAKIS, Y. 2009. Geographic

variability of sardine dynamics in the Iberian Biscay region. *ICES Journal of Marine Science* 66: 495–508.

SMITH, C.D. 2003. Diet of *Octopus vulgaris* in False Bay, South Africa. *Marine Biology* 143: 1127–1133.

SOBRINO, I., SILVA, L., BELLIDO, J.M. & RAMOS, F. 2002. Rainfall, river discharges and sea temperature as factors affecting abundance of two coastal benthic cephalopod species in the Gulf of Cadiz (SW Spain). *Bulletin Marine Science* 71: 851–865.

SONDERBLOHM, C., PEREIRA, J. & ERZINI, K. 2014. Environmental and fishery-driven dynamics of the common octopus (*Octopus vulgaris*) based on time-series analyses from leeward Algarve, Southern Portugal. ICES Journal of Marine Science.

SOUSA, P., AZEVEDO, M. & GOMES, M.C. 2005. Demersal assemblages off Portugal: Mapping, seasonal, and temporal patterns. *Fisheries Research* 75: 120–137.

SOUSA, P., AZEVEDO, M. & GOMES, M.C. 2006. Species-richness patterns in space, depth, and time (1989-1999) of the Portuguese fauna sampled by bottom trawl. *Aquatic Living Resources* 19: 93–103.

STOWASSER, G. 2004. Squid and their prey: insights from fatty acid and stable isotope analysis (A thesis presented for the degree of doctor of Philosophy at the University of Aberdeen). University of Aberdeen, UK.

TSANGRIDIS, A., SÁNCHEZ, P. & IOANNIDOU, D. 2002. Exploitation patterns of *Octopus vulgaris* in two Mediterranean areas. *Scientia Marina* 66: 59–68.

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.

VANDERKLIFT, M. A. & POUSARD, S. 2003. Sources of variation in consumer-diet $\delta^{15}N$ enrichement: a meta-analysis. *Oecologia* 136:169-182.

VANDER ZANDEN, M.J. & RASMUSSEN, J.B. 2001. Variation in δ15N and δ13C trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* 46: 2061–2066.

VAZ-PIRES, P., SEIXAS, P. & BARBOSA, A. 2004. Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. *Aquaculture* 238: 221–238.

VELASCO, F., OLASO, I. & SÁNCHEZ, F. 2001. The role of cephalopods as forage for the demersal fish community in the Southern bay of Biscay. Fisheries Research 52: 65–77.

VILLANUEVA, R. & NORMAN, M. 2008. Biology of the planktonic stages of benthic octopuses. *Oceanography and Marine Biology - An Annual Review* 46: 105–202.

WARNKE, K., SÖLLER, R., BLOHM, D. & SAINT-PAUL, U. 2004. A new look at geographic and phylogenetic relationships within the species group surrounding *Octopus vulgaris* (Mollusca, Cephalopoda): indications of very wide distribution from mitochondrial DNA sequences. *Journal of Zoological systematics and Evolutionar Research* 42: 306–312.

YOUNG, R. E. & HARMAN, R. F. 1988. “Larva”, “paralarva” and “subadult” in cephalopods terminology. *Malacologia* **29**, 313-321.

YOUNG, R.E., VECCHIONE, M. & DONOVAN, D.T. 1998. The evolution of coleoid cephalopods and their present biodiversity and ecology. *South African Journal of Marine Science* 20: 393–420.

AGRADECIMENTOS

Aos meus orientadores Dr. Ángel González, Dr. João Pereira e Professor Luís Narciso por terem partilhado a orientação deste projecto de doutoramento. Por me acolherem nos seus laboratórios, Laboratório Marítimo da Guia (Centro de Oceanografia, FCUL), Ecobiomar (IIM-CSIC) e Divisão de Recursos Marinhos e Modelação (IPMA). Por disponibilizarem todos os recursos possíveis, por me ouvirem, pela discussão de ideias e por me guiarem num caminho que nem sempre foi fácil.

Um agradecimento especial à Dra. Ana Moreno pela sua inesgotável disponibilidade para me ajudar, escutar e partilhar ideias. Não sendo minha orientadora formal, muito do que está apresentado nesta tese é também resultado do seu *knowhow*, da sua colaboração e generosidade como a sua co-autoria em todos os capítulos demonstra.

Ao Dr. Santiago Auborg e à Dra. Maria José Fernández Reiriz por me terem acolhido nos seus grupos, pela sua generosidade, partilha de conhecimentos e por terem contribuído com ideias e sugestões em dois artigos desta tese.

Ao Álvaro Roura cujo contributo foi essencial para o desenho experimental, desenvolvimento, análise e discussão dos resultados que constituem o artigo dedicado aos requisitos nutricionais das paralarvas do polvo. E claro, por me ter contagiado com a sua paixão pelos "chiquipulpis"!

Ao José Xavier pelo seu contributo com as análises de isótopos estáveis, pela sua generosidade, amizade e contagiante espírito científico.

Quero agradecer à Ana Moreira, Inês Cardoso, Laura Wise e Patrícia Gonçalves pela inesgotável amizade, pela partilha de ideias e a pela (grande) paciência para me ouvirem. Um agradecimento muito especial à Laura Wise e ao Pedro Costa por numa tarde de Setembro me dizerem Avança!

A todos os colegas e amigos, técnicos e investigadores, do Instituto Português do Mar e Atmosfera por me acompanharem num caminho de 11 anos que moldou a minha vida. Em especial ao Pedro da Conceição com quem partilhei muitas horas de amostragem no laboratório e no mar.

À Fiona Read, Isabel Teixeira, Jessica Olcina, Sónia Valladares e Lorena Olmos pelo Casco Vello, o chocolate com churros, a *paella*, as caminhadas, as comidas no monte, os

concertos, as festas medievais. Pela amizade e por porem a Galiza e Vigo no meu coração onde por mais que chova "*Todos los dias sale el sol…*"

Aos colegas do Ecobiomar, José António Durán, Mariana Rivas, Manuel García, Jorge Urcera, Marcos Regueira, María Teresa Regueira, Santiago Pascual e ao Dr. Ángel Guerra pela sua generosa hospitalidade e amizade.

A todos os meus amigos que partilharam esta maravilhosa aventura comigo e que me ajudaram a concretizá-la!

Um agradecimento muito especial à minha família que está sempre presente, que ouve, que aconselha, que se deixa contagiar pelo meu entusiasmo e que o relembra quando me esqueço dele.

ANEXES[8]

[8] The anexes include the articles already published as they appear in the corresponded peer-reviewed journals.

Fisheries Research 127–128 (2012) 116–124

Contents lists available at SciVerse ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas

Sílvia Lourenço[a,b,*], Ana Moreno[b], Luís Narciso[a], Ángel F. González[c], João Pereira[b]

[a] Centro de Oceanografia/Laboratório Maritimo da Guia, Faculdade de Ciências da Universidade de Lisboa, Avenida Nossa Senhora do Cabo, n°939, 2750-374 Cascais, Portugal
[b] Unidade de Recursos Marinhos e Sustentabilidade, INRB I. P./L-IPIMAR, Av. de Brasília 1449-006 Lisboa, Portugal
[c] Ecobiomar, Instituto de Investigaciones Marinas de Vigo, CSIC, C/Eduardo Cabello, 6, Vigo E-36208, Spain

ARTICLE INFO

Article history:
Received 3 July 2011
Received in revised form 18 April 2012
Accepted 22 April 2012

Keywords:
Octopus vulgaris
Ecology
Environment
Reproduction
Condition

ABSTRACT

Octopus vulgaris is an important fisheries resource, particularly in Iberian waters. Species life cycle is short with capacity to adapt to different environmental conditions and, considered a simultaneous terminal spawner. Data on maturation and other biological parameters collected from January 2007 to November 2010 are used to define spawning seasons for octopus landed by the small-scale trap fisheries in two oceanographically distinct Portuguese coastal areas: the northwest coast (western Iberia upwelling system) and the south coast (Gulf of Cadiz system). On a monthly basis, we followed the proportion of mature individuals, and the Gonad-Somatic and Hayashi Indices. Length–weight relationship, weight-at-maturity, body condition and energy allocation were other biological parameters studied. Spawning season was markedly different in both areas. The northwest population spawns from March to July, in synchrony with the northwest coast upwelling season, and the south coast population spawns mainly in summer, between August and September. A less intense spawning peak in early spring is present occasionally in the south coast. Weight-at-maturity is geographically indistinct, but in both areas males mature at smaller sizes than females. Body condition increases significantly during maturation and mass allocation for reproduction results indicate that males and females channel energy to reproduction from several sources.

1. Introduction

The common octopus *Octopus vulgaris* (Cuvier, 1797) is exploited as a target species by demersal trawl fleets and numerous small-scale fisheries, using hand jigs, pots, traps and trammel nets operating in southern Europe and northwestern Africa (Hastie et al., 2009). In 2010, the Portuguese landings of common octopus in national ports were 12,602 tons (ten years average landings of 9050 tons). The small-scale fleet, using traps and pots, is responsible for 95% of the common octopus landings (ICES, 2011). Nevertheless, a growing interest in this species as well as in other commercial cephalopod species is presently noticed for the bottom trawl fleet, with 10% of the landings, in 2007 (Fonseca et al., 2008).

Cephalopods are characterized by a plastic life cycle with a recognized capacity to adapt to different ecosystems, due to a short life span, high growth rate and diverse diets. *O. vulgaris* is a merobenthic incirrate octopod, common in temperate shallow habitats between the coastline and 200 m depth (Roper et al., 1984; Boletzky, 1992). Due to its wide distribution, *O. vulgaris* is one of the best models to investigate the adaptation of reproductive strategies to specific environmental features.

The species is characterized by a life span of one year in Saharan bank waters to two years in Galician waters attaining relatively large sizes, of up to 6000 g in weight and 25 cm mantle length (Domain et al., 2000; Otero et al., 2007). It is a simultaneous terminal spawner, laying on average 350,000 eggs in sheltered places between 20 and 120 m depth (Rocha et al., 2001; Silva et al., 2002). The spawning season is generally extended throughout the year with one or two peaks geographically variable in timing (Hastie et al., 2009).

The Portuguese coast is a highly dynamic oceanographic region in the temperate northeast Atlantic, influenced by two important oceanographic systems the Western Iberia Upwelling System (WIUS) and the Gulf of Cadiz System (GCS). The WIUS is characterized by a strong influence of the spring-summer coastal upwelling (Fiúza, 1983; Mason et al., 2005). The predominantly equatorward winds observed in summer, drive an offshore Ekman transport and force the upwelling of colder, nutrient-rich, subsurface waters along the coast (Relvas et al., 2007). The presence of the poleward current, responsible for the formation of convergence zones over

* Corresponding author at: Unidade de Recursos Marinhos e Sustentabilidade, INRB I. P./L-IPIMAR, Av. de Brasília 1449-006 Lisboa, Portugal. Tel.: +351 213027313; fax: +351 213 015 948.
E-mail address: salourenco@fc.ul.pt (S. Lourenço).

0165-7836/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fishres.2012.04.006

the shelf-break, has an important ecological role for the retention and/or poleward transport of the phytoplankton (Ribeiro et al., 2005) and zooplankton (Santos et al., 2004), with zooplankton peaking in biomass during late spring and early summer near the outer-shelf (Cunha, 2001). All year round the coastal sea water temperature ranges between 14.4 °C and 19.6 °C (Moreno et al., 2009a) with a shallow thermocline developing during spring (Cunha, 2001). The south Portuguese coast on the other hand, is influenced by the GCS. The south coast orientation does not favor upwelling under northerly winds. Upwelling and downwelling events tend to be weak and the circulation is mainly wind forced and influenced by the local orography (García-Lafuente and Ruiz, 2007). The Cape St^{a} Maria (36° 57′N, 7° 53′W) divides the continental shelf in two, supporting different oceanographic processes (García-Lafuente and Ruiz, 2007). East to the Cape St^{a} Maria, the presence of the Huelva front as a warm coastal current flowing along the shore, the significant fresh water runoff forming buoyant river plumes in spring, the occasional occurrence of the Stafford Shear, a cold–warm frontal region associated to local upwelling systems (Mason et al., 2005) and an extended Sea Surface Temperature (SST) range, between the 15.4 °C and the 21.9 °C (Moreno et al., 2009b) are the main characteristics of the GCS.

Studies on the reproductive features of commercially exploited marine species such as the common octopus are an important tool for the assessment of the stock status. Detailed information about the timing and the sites of spawning, size at maturity, and sex ratio at breeding, require a consistent assessment of the state of sexual maturity. These assessments usually involve several aspects of the reproductive development, such as the state of the gonad and the accessory reproductive structures, and are based on descriptive maturity scales and/or maturity condition indices (Boyle and Rodhouse, 2006). Due to the extensive distribution area of *O. vulgaris* and the commercial interest of the species in the Atlantic waters of the Iberian Peninsula, several studies have been conducted on the reproductive features of geographically distinct sub-populations. Based in her experiments in captivity Mangold (1983) reviewed all aspects of the *O. vulgaris* life cycle, and more recently other two reviews addressing *O. vulgaris* were published (Hastie et al., 2009 and Pierce et al., 2010), compiling recent advances on fisheries and life cycle aspects.

Regarding specifically *O. vulgaris* reproductive aspects, several papers targeting Mediterranean, Iberian and other regional sub-populations have been published since the late 70s, with diversified sampling designs and methodology used to identify spawning season mainly based on proportions of mature individuals and several condition indices, such as Gonad-Somatic index (GSI) or Hayashi index (HI). Such studies rely mostly on landings from trawl fleets (e.g. Quetglas et al., 1998) and small-scale fleets (e.g. Sánchez and Obarti, 1993; Hernández-García et al., 2002; Fernández-Rueda and García-Flórez, 2007). A few studies were conducted with sampling designed specifically to *O. vulgaris* (e.g. Smith and Griffiths, 2002; Oosthuizen and Smale, 2003; Rodríguez-Rua et al., 2005; Katsanevakis and Verripoulos, 2006).

Considering its regional proximity and the methodology used, the studies of Silva et al. (2002) and Otero et al. (2007) are particularly relevant for the present study. In the Northwest Galician waters, Otero et al. (2007) define a single spawning season during spring (May–June) related with the seasonal coastal upwelling in Galician waters, despite the presence of mature females all year round. In those waters, males mature at smaller sizes than females and the mass transfer during the maturation process occurs directly from feeding sources. According to Silva et al. (2002), in waters of the Gulf of Cadiz, the reproductive season covers most of the year, with two distinct peaks in April/May and August/September. As in the previous study, males mature at smaller sizes than females.

The present study aims to identify different reproductive strategies adopted by different populations, considering the distinct oceanographic features of the northwest coast within the WIUS and the South coast within the GCS. The reproductive strategies identified are described in terms of sex ratio, proportion of mature individuals and monthly mean Gonad-Somatic and Hayashi indices. At the individual level, length–weight relationships are determined by sex and area, as well as maturity ogives and the size effect at maturity. Additionally the effect of size and body condition is analyzed for each sex in both regions in order to identify sexual and regional differences regarding the use of storage mass for reproduction.

2. Materials and methods

2.1. Sampling

Samples were collected monthly from January 2007 to November 2010 from the small-scale trap fishery (Table 1) and frozen. Sampling sites were chosen considering the two different oceanographic regions, the Northwest coast, with Peniche as the landing port, and the South coast with Olhão as the landing port (Fig. 1). For all individuals, the dorsal mantle length (ML in mm), individual weight (W in g) and digestive gland weight (DGW in g) were measured to the nearest 5 mm and 0.1 g, respectively. To assess maturation, the following data were collected: testis and ovary weight (TW and OW), Needham's complex weight (NCW), oviduct weight (ODW) and oviducal complex weight (OCW), all weighed to the nearest 0.01 g. A maturity scale of four stages for males (I: immature; II: maturing; III: mature; IV: post-spawning) and five stages for females (I: immature; II: maturing; III: pre-spawning; IV: mature; V: post-spawning) was used (adapted from Guerra, 1975).

2.2. Data analysis

2.2.1. Sex ratio and spawning season

Individual weight data aggregated in 200 g weight classes by study area were analyzed using RMIX, an algorithm designed to identify and separate normal distributions in size frequency distributions (Macdonald and Green, 1988; Macdonald, 2010). RMIX identifies a set of overlapping component distributions that give the best fit to the histogram. The modal weight classes, identified by visual inspection of the weight distribution, were used as the initial weight parameters. Constraints were applied in the analysis, such as a fixed variance coefficient for each component. Only components with $n > 50$ were considered and all components were assumed to be lognormal.

The sex ratio by month was estimated as the ratio between males and females. Significant deviations from 1:1 were tested by a χ^2 test with $\alpha = 0.05$. In order to define the spawning season by study area, the proportion of mature individuals (MI) was determined monthly as: $MI_{fi} = (\Sigma M_i/N_i)_f$, with i as month, and M as the frequency of mature females, f (maturity stage III and IV) in each month; $MI_{mi} = (\Sigma M_i/N_i)_m$, with i as month, and M as the frequency of mature males, m (maturity stage III) in each month. The spawning season was defined empirically as the sampling months in which $MI_{fi} > 0.1$ and $MI_{mi} > 0.1$.

Both the Gonad Somatic index (GSI) and the Hayashi index (HI) are important tools to assess the reproductive and maturation condition both at the individual and population level. The GSI, most indicated to assess the maturation condition in females (Guerra, 1975), was determined individually for all females in the form $GSI = OW \times 100/(BW - OW)$ (Otero et al., 2007). The monthly GSI was determined as the monthly mean of the individual GSI and

Table 1
Summary of sample collection between January 2007 and November 2010.

Month	Northwest region		South region		*N* total
	Females	Males	Females	Males	
Jan-07	7	12	42	41	102
Feb-07	13	10	16	12	51
Mar-07	10	20	–	–	30
Apr-07	17	10	–	–	27
May-07	12	19	33	27	91
Jun-07	44	38	–	–	82
Jul-07	12	15	15	18	60
Aug-07	8	9	–	–	17
Sep-07	19	12	13	19	63
Oct-07	16	16	11	25	68
Nov-07	13	9	–	–	22
Dec-07	–	–	–	–	–
Jan-08	12	22	42	32	108
Feb-08	12	10	39	26	87
Mar-08	15	14	15	11	55
Apr-08	14	12	12	16	54
May-08	13	9	11	12	45
Jun-08	9	26	16	4	55
Jul-08	15	15	24	15	69
Aug-08	6	11	–	–	17
Sep-08	12	23	25	7	67
Oct-08	18	7	18	35	78
Nov-08	16	14	18	36	84
Dec-08	–	–	8	20	28
Feb-09	7	20	16	14	57
Mar-09	15	16	28	20	79
Apr-09	16	10	32	21	79
May-09	4	21	18	24	67
Jun-09	5	4	22	20	52
Jul-09	11	12	17	11	51
Aug-09	10	21	23	28	82
Sep-09	12	17	60	38	127
Oct-09	21	13	39	55	128
Nov-09	19	18	40	42	119
Dec-09	–	–	20	35	55
Jan-10	10	12	44	52	118
Feb-10	17	13	–	–	30
Mar-10	12	22	34	25	47
Apr-10	10	22	–	–	32
May-10	15	23	–	–	38
Jun-10	18	22	13	25	78
Jul-10	13	10	–	–	23
Aug-10	16	19	–	–	35
Sep-10	23	24	26	29	102
Oct-10	24	18	20	29	91
Nov-10	17	19	16	21	73
N total	607	689	835	836	2967

analyzed separately for each study area. The HI, most indicated to assess the maturation condition in males (Guerra, 1975) was determined individually for all males in the form HI = NCW/(NCW + TW) (Otero et al., 2007). The monthly HI was determined as the monthly mean of the individual HI_m and analyzed separately for each study area. The standard error was adopted as the measure of variability for the determination of the monthly mean of GSI and HI, and determined as $SE = \sqrt{(Var(GSI_i)/n_i)}$ or $SE = \sqrt{(Var(HI_i)/n_i)}$ with i as month (Zar, 1999).

2.2.2. Length–weight relationship and weight-at-maturity

The effect of both study area and sex in the length–weight relationship and maturity proportion was investigated with Scheffé *F*-tests at a significance level $\alpha = 0.05$. In order to normalize the data, ML, W and MI by 200 g weight class were log transformed and compared with a two-way ANOVA test at a significance level $\alpha = 0.05$.

Length–weight relationships for combined sexes as well as for all females and all males were determined by means of non-linear estimates by maximum likelihood of the form $\log(W) = a + b \times \log(ML)$. Goodness of fit was expressed by r^2 and the significance level of the parameters estimated by Student's *t*-test.

A maturity curve, for each sex in each study area, was fitted to the weight frequency distribution of the proportion (P_i) of mature individuals by 200 g weight class, with a logistic model: $P_i = 1/(1 + \exp(\alpha + \beta W_i))$ by weight class, *i*. Differences in the maturity curves between areas and between sexes were tested by means of the non-parametric Kruskal–Wallis test for successive comparisons. The weight-at-maturity was defined as the weight class where 50% of individuals are mature and determined from $W_{50\%} = -\alpha/\beta$.

2.2.3. Body condition and energy allocation

The body condition was determined for each study area for females and males separately, as the value of the residuals obtained from the geometric mean regression of log(BW) by log(BW) – log(OW) for the females, replacing log(OW) by log(TW) for the males. The residuals resulting from this analysis allow a comparison of individuals independently of body weight: positive residuals indicate animals that are heavier than predicted by the

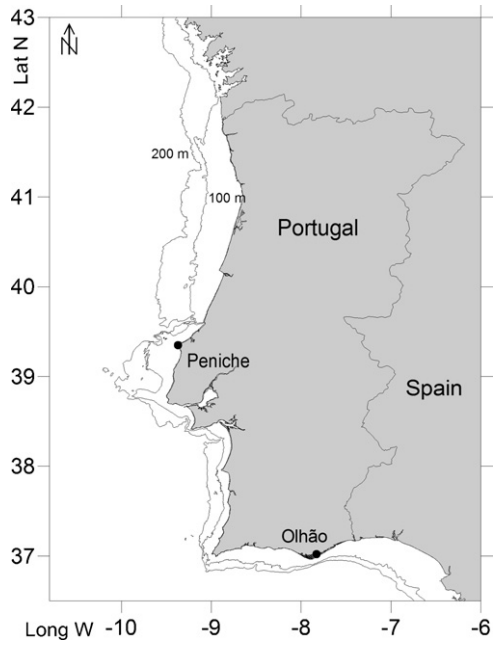


Fig. 1. Sampling ports and coastal characteristics.

model, and negative residuals indicate individuals that weight less than predicted. Variations in body condition were compared by maturity stage using a one-way ANOVA and Tukey's honesty significant difference HSD post hoc test (McGrath Steer and Jackson, 2004; Zar, 1999).

To study the relative energy allocation, the variation in weight between organs in mature animals was assessed. Two geometric regressions were performed on mature individuals of each study area, for each sex separately: (A) the body weight vs. gonad weight and (B) the body weight vs. digestive gland weight, after log transformation. The residuals obtained from these two regressions, provide a size-independent estimate of the relative weight of the gonads and digestive gland. To determine if there is a mass transfer between organs during the reproductive process, the residuals derived from both regressions were compared to the body weight (W), the reproductive complex weight (OW + OCW for females and TW + NCW for males) and the digestive gland weight using the Pearson correlation test procedure (Zar, 1999).

3. Results

The weight of females ranged between 625 g (135 mm ML, maturity stage II, northwest coast) and 6189 g (247 mm ML, maturity stage IV, south coast). The weight of males ranged between 635 g (100 mm ML, maturity stage II, south coast) and 5612 g (248 mm ML, maturity stage III, south coast). The weight distributions were significantly different between areas ($\chi^2_{\text{Kruskal–Wallis}} = 19.62$, df = 6, $p < 0.05$) and within each area between sexes (Northwest coast, $\chi^2_{\text{Kruskal–Wallis}} = 28.55$, df = 6, $p < 0.05$; South coast, $\chi^2_{\text{Kruskal–Wallis}} = 42.07$, df = 6, $p < 0.05$) (Fig. 2). Weight frequency analysis indicates that the weight distribution was bimodal in both areas. On the Northwest coast the weight distribution presents two modal peaks in the 1100 g weight class (modal weight, 1147 g) and in the 2200 g weight class (modal weight, 2216 g). On the South coast the weight distribution presents a mode in the 900 g weight class (modal weight of 979 g) and another in the 1700 g weight class (modal weight of 1724 g) (Table 2).

3.1. Sex ratio and spawning season

The overall sex ratio (F:M) showed that in the Northwest coast sample, males are significantly more abundant than females, 0.88:1. In these samples, more males occurred in all months with the exception of April, October and November when the sex ratio was balanced towards females. On the other hand, despite the fact that the overall sex ratio on the South coast was 1:1, females were more abundant than males except for the months of August, October, November and December, where the sex ratio was balanced towards males (Table 3).

Table 4 presents the proportion of mature individuals found by month in both sampling sites. In the Northwest coast, mature males were present every month with the exception of November 2007. For females, it is possible to identify a consistent pattern of a higher proportion of mature individuals (proportion mature > 0.10) between early spring and late summer. In the south coast, no mature males were found in the samples in September 2008 and October 2008, between March 2009 and May 2009, and in September 2010 and November 2010. In this region, there was a non-negligible presence of mature females in late winter and earlier spring months of 2007, 2008 and 2010, although the major proportion of mature females occurs in a single month in summer or late summer in each year.

Considering the overall results, females in maturity stage I present a mean GSI of 0.198 (±0.004, SE), in stage II of 0.507 (±0.022), in stage III of 3.947 (±0.215), and for fully mature females (stage IV) of 9.134 (±0.566), revealing a clear separation between maturing females and pre-spawning females. In males, the HI maturity index also presents increasing values from immature to mature males, with HI values of 0.357 (±0.004) for stage II males, and 0.429 (±0.005) for stage III males, although in this case with overlapping extremes. For both maturity stages I and IV, not enough data were available for the determination of HI by maturity stage ($n = 2$ for maturity stage I, and $n = 1$ for maturity stage IV).

In the northwest coast (Fig. 3) females present higher GSI between early spring and summer, with two GSI peaks, during the studied period. After the second GSI peak, values decrease to near 0.1, comparable to the mean GSI for stage I. Regarding males in the northwest coast, HI present higher values roughly between late winter and late summer, comparable to those of stage III males.

In the south coast (Fig. 4), the plot of mean monthly GSI for females shows that there is a spawning peak in the summer or late summer months, although in adjacent months values are always high and near the average for mature animals, indicating the occurrence of mature females throughout the year. A secondary peak occurs in some spring months as is evident in March 2008 and June to July 2009. As for the HI of males, no specific season stands out for a higher proportion of mature specimens, although peaks are found in January 2007, from March 2008 to July 2008, and from April 2009 to March 2010, indicating that mature males are present independently of the season.

3.2. Length–weight relationship and weight-at-maturity

Length–weight relationship parameters by area and by sex as well as the statistical tests to the parameters estimated are summarized in Table 5. The ANCOVA analysis of the influence of geographic location and sex in length–weight relationships shows that the combined effect of both factors is significant (area: sex, $F = 11.83$, p-value < 0.05), with the effect of the single factor area also being significant ($F = 46.93$, p-value < 0.05) but not the effect of the single factor sex ($F = 2.47$, p-value > 0.05), resulting in different length–weight relationships in each area, and for males and females in the northwest coast. In every case the increase in weight with length may be characterized as positively allometric ($b > 1$).

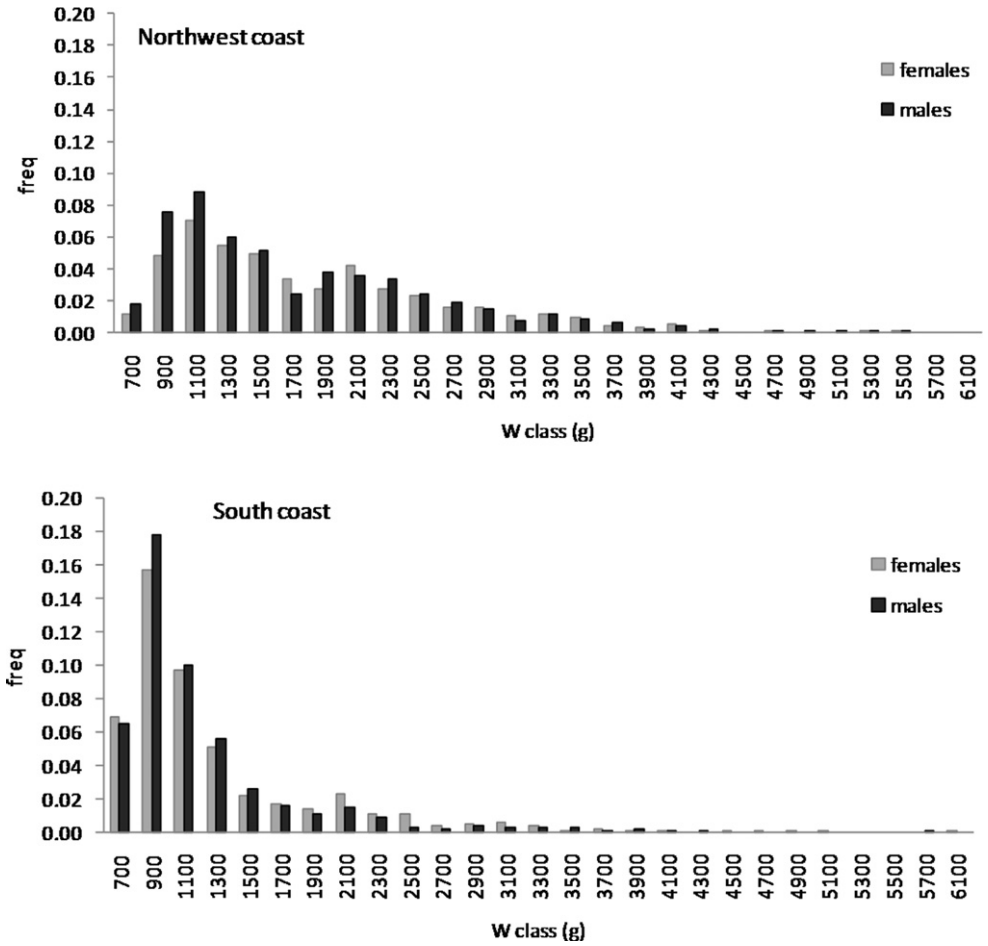


Fig. 2. Weight distribution of the females and the males sampled in the northwest coast and the south coast.

Table 2
Weight frequency analysis results for the Northwest coast and for the South coast: A, B: distribution components, SE: standard error, χ^2: chi-square test value, df: degrees of freedom.

	Northwest coast			South coast		
	Mean weight (±SE)	Probability (±SE)	Standard deviation (±SE)	Mean weight (±SE)	Probability (±SE)	Standard deviation (±SE)
A	1147 (±15)	0.40 (±0.03)	221.1 (±14.0)	978.8 (±6)	0.66 (±0.03)	179.6 (±9.1)
B	2216 (±47)	0.60 (±0.03)	866.1 (±25.6)	1724.1 (±80)	0.33 (±0.03)	981.0 (±41.4)
Significance test	$\chi^2 = 114.29$, df = 19, p-value < 0.05			$\chi^2 = 115.83$, df = 20, p-value < 0.05		

Table 3
Sex ratio and χ^2 test for deviance from 1:1 sex ratio for Northwest and South coast by month sampled.

Month	Northwest coast			South coast		
	F:M	χ^2	p-value	F:M	χ^2	p-value
Jan	0.63:1	71.49	<0.01	1.16:1	248.87	<0.01
Feb	0.92:1	98.12	<0.01	1.37:1	118.37	<0.01
Mar	0.72:1	120.35	<0.01	1.38:1	128.72	<0.01
Apr	1.05:1	106.98	<0.05	1.19:1	76.88	<0.01
May	0.61:1	112.51	<0.01	0.98:1	121.05	<0.01
Jun	0.84:1	162.19	<0.01	1.06:1	96.98	<0.01
Jul	0.96:1	98.08	<0.01	1.27:1	95.80	<0.01
Aug	0.67:1	96.43	<0.01	0.82:1	47.27	<0.01
Sep	0.86:1	138.17	<0.01	1.35:1	211.73	<0.01
Oct	1.46:1	248.87	<0.01	0.61:1	228.50	<0.01
Nov	1:1	158.02	<0.01	0.74:1	132.32	<0.01
Dec				0.51:1	79.68	<0.01
Overall	0.88:1	1329.12	<0.01	1:1	1630.00	<0.01

Table 4
Monthly proportion of mature females and males between January 2007 and November 2010 in the Northwest region and the South region.

Month	Northwest region		South region		Month	Northwest region		South region	
	Females	Males	Females	Males		Females	Males	Females	Males
Jan-07	0.000	0.250	0.048	0.512	Jan-09	–	–	–	–
Feb-07	0.077	0.500	0.125	0.667	Feb-09	0.000	0.400	0.063	0.143
Mar-07	0.500	0.350	–	–	Mar-09	0.333	0.313	0.000	0.000
Apr-07	0.294	0.800	–	–	Apr-09	0.688	0.600	0.000	0.000
May-07	0.250	0.368	0.030	0.333	May-09	0.250	0.524	0.000	0.000
Jun-07	0.341	0.500	–	–	Jun-09	1.000	1.000	0.348	0.200
Jul-07	0.667	0.533	0.400	0.333	Jul-09	1.000	0.667	0.176	0.273
Aug-07	0.625	0.555	–	–	Aug-09	0.800	0.238	0.130	0.286
Sep-07	0.053	0.167	0.000	0.210	Sep-09	0.000	0.176	0.450	0.108
Oct-07	0.000	0.125	0.000	0.160	Oct-09	0.000	0.385	0.026	0.164
Nov-07	0.000	0.000	–	–	Nov-09	0.105	0.556	0.000	0.190
Dec-07	–	–	–	–	Dec-09	–	–	0.000	0.314
Jan-08	0.083	1.000	0.024	0.312	Jan-10	0.200	0.500	0.077	0.227
Feb-08	0.083	0.700	0.077	0.154	Feb-10	0.650	0.789	–	–
Mar-08	0.333	0.857	0.200	0.545	Mar-10	0.583	0.579	0.206	0.160
Apr-08	0.429	0.750	0.000	0.375	Apr-10	0.500	0.636	–	–
May-08	0.231	0.333	0.000	0.417	May-10	0.400	0.870	–	–
Jun-08	0.444	0.731	0.000	0.500	Jun-10	0.333	0.737	0.077	0.240
Jul-08	0.333	0.333	0.000	0.200	Jul-10	0.308	0.700	–	–
Aug-08	0.833	0.545	–	–	Aug-10	0.688	0.368	–	–
Sep-08	0.000	0.130	0.440	0.000	Sep-10	0.130	0.292	0.154	0.000
Oct-08	0.000	0.286	0.000	0.000	Oct-10	0.000	0.222	0.150	0.034
Nov-08	0.000	0.286	0.000	0.055	Nov-10	0.059	0.526	0.000	0.000
Dec-08	–	–	0.000	0.050					

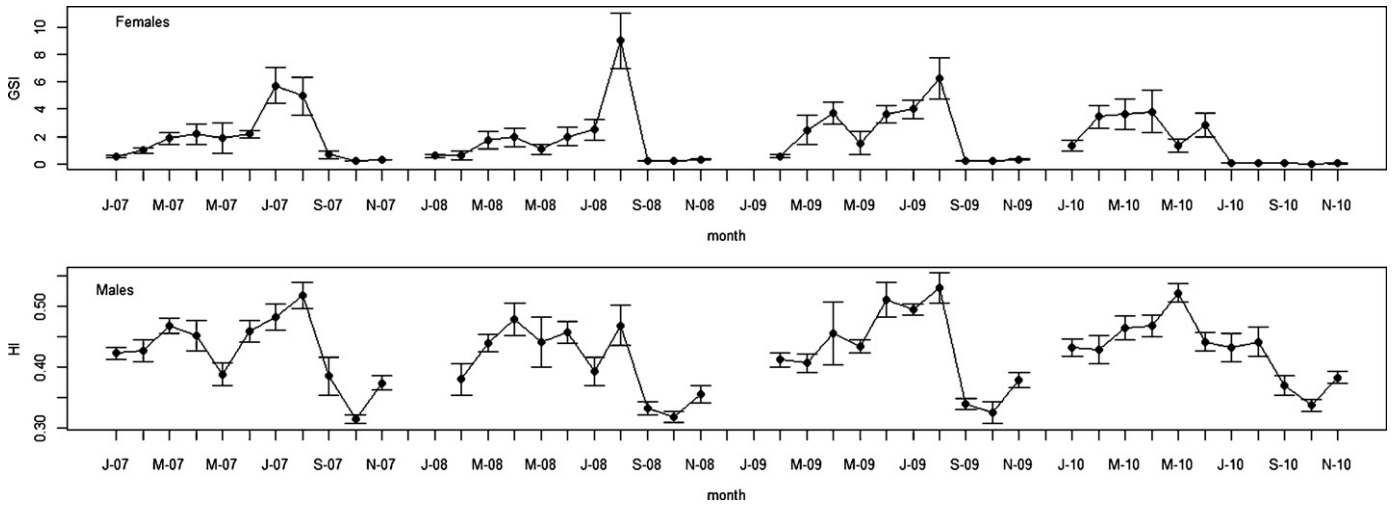


Fig. 3. Monthly evolution of the mean Gonad-Somatic index (±SE) for females (upper plot) and Hayashi index (±SE) for males (lower plot) in the northwest coast.

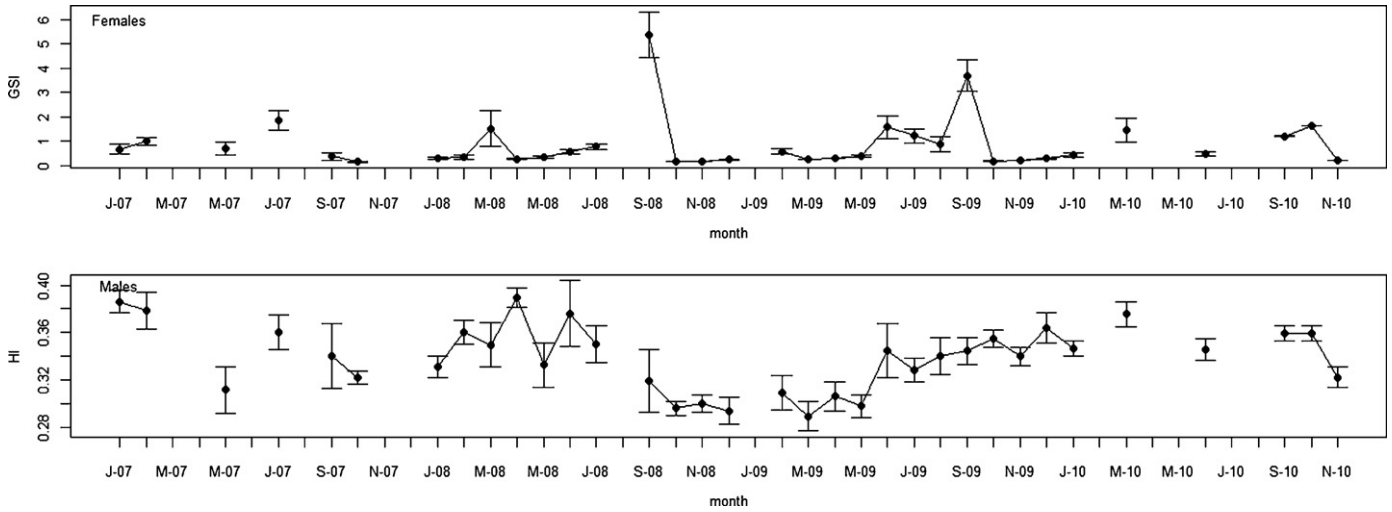


Fig. 4. Monthly evolution of the mean Gonad-Somatic index (±SE) for females (upper plot) and Hayashi index (±SE) for males (lower plot) in the south coast.

Table 5
Length–weight relationships determined for northwest coast and south coast considering both sexes (M + F), males and females separately: a and b as parameters of the length–weight relationships; df – degrees of freedom; r^2 – linear regression coefficient; t_a and t_b – t-test value for the regression parameters; and p-value.

		a	b	df	r^2	Parameter significance level
Northwest coast	Males (M)	2.47	2.77	687	0.83	$t_a = 29.04$, p-value < 0.05 $t_b = 60.33$, p-value < 0.05
	Females (F)	2.28	2.57	605	0.84	$t_a = 24.86$, p-value < 0.05 $t_b = 56.88$, p-value < 0.05
	M + F	2.37	2.66	1294	0.83	$t_a = 37.55$, p-value < 0.05 $t_b = 81.66$, p-value < 0.05
South coast	Males (M)	1.96	2.34	834	0.78	$t_a = 21.37$, p-value < 0.05 $t_b = 53.97$, p-value < 0.05
	Females (F)	1.83	2.24	833	0.83	$t_a = 23.65$, p-value < 0.05 $t_b = 64.49$, p-value < 0.05
	M + F	1.89	2.34	1669	0.83	$t_a = 31.86$, p-value < 0.05 $t_b = 83.89$, p-value < 0.05

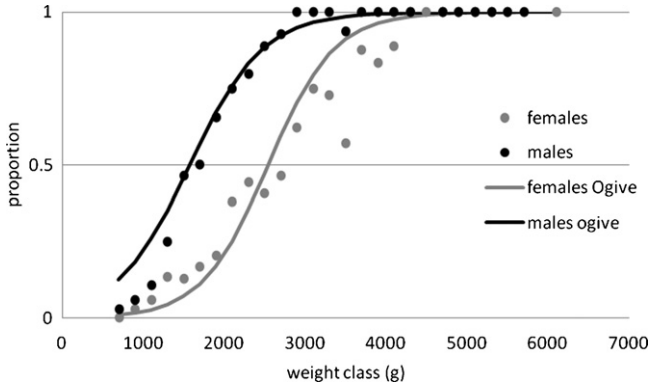


Fig. 5. Mature proportion by weight class and maturity ogives for females and males. The weight-at-maturity is the weight at which 50% of the individuals in the population are mature.

Regarding weight-at-maturity, the Kruskal–Wallis one-factor analysis of variance shows no effect of geographic area of origin ($\chi^2_{\text{Kruskal–Wallis}} = 0.20$, $p > 0.05$) but a significant difference between sexes ($\chi^2_{\text{Kruskal–Wallis}} = 8.34$, $p < 0.05$). Considering each sex separately, the effect of geographic area was tested and a no significant effect of area was shown for both females ($\chi^2_{\text{Kruskal–Wallis}} = 0.11$, $p > 0.05$) and males ($\chi^2_{\text{Kruskal–Wallis}} = 0.003$, $p > 0.05$). Therefore there are only distinct maturity ogives for males and females with different values of weight at maturity, 2548.01 g for females and 1577.54 g for males (Fig. 5).

3.3. Body condition and energy allocation

In females, body condition increases significantly between the early maturation (maturity stage II) and late maturation (maturity stage III) ($\mu_{\text{stageII}} \neq \mu_{\text{stageIII}}$, $p < 0.05$). Males also present a considerable increase in body condition between maturity stage II and stage III ($\mu_{\text{stageII}} \neq \mu_{\text{stageIII}}$, $p < 0.05$) (Fig. 6).

The Pearson correlation index for female energy allocation shown by the size-independent digestive gland weight, evidences a negative correlation to individual total weight (−0.109, df = 160, $p < 0.05$) and to the weight of reproductive structures (−0.226, df = 160, $p < 0.05$). In the case of males, no significant correlation was found between the size-independent digestive gland weight and total weight or reproductive complex weight. Energy allocation shown by the size-independent gonad weight evidences a negative correlation with total weight for both females (−0.428, df = 261, $p < 0.05$) and males (−0.256, df = 492, $p < 0.05$), and similarly with the digestive gland weight (females: −0.256, df = 261, $p < 0.05$; males: −0.412, df = 492, $p < 0.05$).

4. Discussion

The definition of different spawning seasons in octopus populations within relatively short distances off the Portuguese coast confirms the plasticity of environmental responses of the *O. vulgaris* life cycle, as observed in other cephalopods such as *Loligo forbesi* and *Loligo vulgaris* (Boyle et al., 1995; Moreno et al., 2005). In the northwest coast, females mature preferentially between early spring and summer in a long spawning season that encompasses two spawning peaks, one in February–April and another in June–July. In this region, mature males are found all year round and are generally more abundant in the fishery than females, due to the spawning behavior of the latter, which decreases their availability at spawning. In autumn, the proportion of immature females in the population increases with a decrease of mature individuals, both males and females.

In the south coast, the reproductive season occurs during summer peaking in September, despite the non-negligible occurrence of mature females during late winter or early spring, which would indicate that more favorable conditions could generate a secondary peak. Similar situations have been described for other populations in oligotrophic conditions as in the eastern Mediterranean waters and south-eastern coast of South Africa (Sánchez and Obarti, 1993; Oosthuizen and Smale, 2003; Katsanevakis and Verripoulos, 2006). Here, the overall sex ratio is balanced towards males, which seems particularly common in commercially exploited *O. vulgaris* populations (Hernández-García et al., 2002; Fernández-Rueda and García-Flórez, 2007; Otero et al., 2007). This variation in sex ratio, other than purely derived from the behavioral unavailability of females to commercial fisheries, has also been explained by a combination of different factors, such as different growth rates, migrations, feeding or post-spawning behavior (Mangold, 1983) although we have not found any study that indisputably proves the existence of ontogenic or reproductive migrations in populations of the common octopus.

Our results seem to indicate that it is mainly the females that display environmentally adaptive reproductive strategies. In the

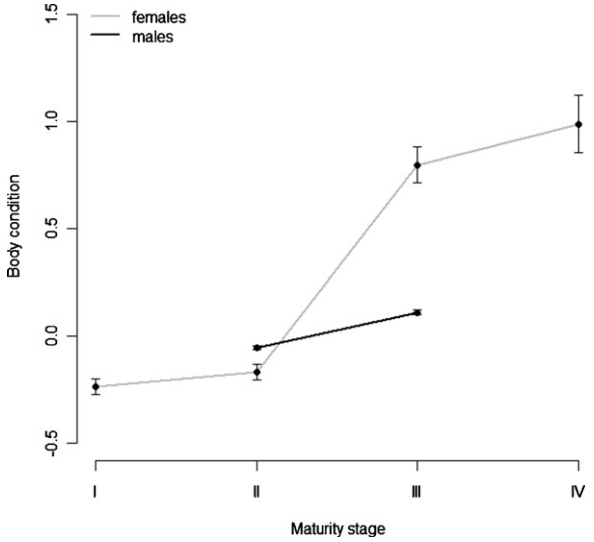


Fig. 6. Mean body condition (±SE) with maturity stages.

Northwest coast where the ocean is more dynamic, the spawning season is extended over more than 6 months usually with two reproductive peaks similarly to the results of Hernández-García et al. (2002) and Fernández-Rueda and García-Flórez (2007) and Otero et al. (2007). In this area, the reproductive cycle of *O. vulgaris*, therefore appears to be highly connected with the long upwelling season as in other regions, such as the adjacent Galician waters and the Sahara Bank (Hatanaka, 1979; Demarcq and Faure, 2000; Otero et al., 2007), synchronizing hatching with ideal conditions for food supply (Otero et al., 2008; Moreno et al., 2009a) combined with reduced exchanges with offshore areas (Demarcq and Faure, 2000). Ecologically, the WIUS is characterized by a persistent nutrient and zooplankton shoreward transport (Almeida and Queiroga, 2003; Santos et al., 2004; Queiroga et al., 2007), favored by the westerly winds in late winter and spring, that create a continuous supply of food and potentiate the survival of two cohorts of paralarvae, hatching approximately four months apart, in July and November (Mangold and Boletzky, 1987; Otero et al., 2008; Moreno et al., 2009b).

In the South coast, where conditions are more stable but less productive, females concentrate spawning over a shorter period of time. The factors that trigger the onset of the reproduction seem to be related with the seasonal occurrence of favorable oceanographic conditions, such as higher temperatures and salinities, favored by the easterly winds which are common during summer in the coastal areas close to Cape St[a] Maria (Sánchez and Relvas, 2003; García-Lafuente et al., 2006). Ecologically, the GCS is a less dynamic and oligotrophic system, in which species tend to direct all of their energy onto a single spawning peak, such as in the case of the sardine *Sardina pilchardus*, the anchovy *Engraulis encrasicolus* and the wedge sole *Dicologlossa cuneata* (Baldó et al., 2006). In the case of the common octopus, individuals spawn in late summer, with the next generation hatching in winter, when the predominant westerly winds favor the transport of nutrients and zooplankton near to the Cape St[a] Maria (García-Lafuente and Ruiz, 2007).

Actually, these differences of timing and spawning strategies adopted by neighboring *O. vulgaris* populations is also observed on the South African coast, where the southwest coast is bathed by the cold-water northward flowing Benguela Current producing long and strong upwelling events, and the southeastern coast is influenced by the Agulhas Current, a western boundary current that transports warm equatorial water pole wards (Gibbons et al., 2010). On the west coast, *O. vulgaris* females spawn throughout the year but mainly between spring and summer (Smith and Griffiths, 2002), and in the southeast coast, females spawn in summer, in a shorter and more intense spawning season (Oosthuizen and Smale, 2003).

The length–weight relationship in the present study varies not only with sex but also with region, apparently reflecting environmental conditions. For the northwest region we determined different length–weight relationships for females and males, both positively allometric, but showing that males increase in weight faster than females ($b_{\text{males}} > b_{\text{females}}$). In the south coast, no differences were found in the length–weight relationship between sexes and both present lower increases in weight with length than either sex in the northwest coast.

It is particularly interesting to note that in this study, geographic area is not reflected in maturity ogives, which may suggest that biological constraints could be imposing limits to minimum maturation body size, as occurs in other cephalopod species (see e.g. Boavida-Portugal et al., 2010; Moreno et al., 2005). The weight-at-maturity for females is 2550 g and 1577 g for males. The differences found between sexes are probably linked with the simultaneous terminal spawning strategy, with females having to store reserves that are then mobilized to produce yolk, and for behavioral traits related to egg laying and protection. Additionally males probably reach maturity earlier in the life cycle, followed by an earlier decrease in growth rate (Mangold, 1983; Smith et al., 2005). However, weight-at-maturity registered for both sexes in our samples is higher than previously published for adjacent waters. In the northerly neighboring waters of Galicia, females mature at 1788.3 g and males at 903.4 g (Otero et al., 2007), and in the southeastern neighboring waters of the Gulf of Cadiz females mature at 2023 g and males at 671 g (Silva et al., 2002). The differences found in the weight-at-maturity in different regions may be related with the sampling strategy followed by each author, since weight-at-maturity decreases at peak population maturity (Moreno, 2008) and thus the proportion of samples taken from peak reproduction seasons in relation to those obtained throughout the year affects the size-composition of the sample of mature females. It can also indirectly result from the gears with which the samples were collected, since some gears are less likely to collect fully mature females (Fernández-Rueda and García-Flórez, 2007). It is therefore likely that there is high variability between individuals in any population and that sample characteristics, among which size and timing, may strongly influence the results. It seems reasonable to assume, and has been demonstrated, that maturity is first of all driven by a minimum weight limit and subsequently modulated by biological and environmental conditions. In our samples, there is a significant increase in body condition for both females and males between stage II (immature) and stage III (pre-spawning), indicating that the relative success of somatic growth in this pre-spawning stage may be crucial to determine subsequent events (McGrath Steer and Jackson, 2004; Otero et al., 2007).

Mass allocation data show that reserves are heading for reproduction in both sexes, although some differences can be found between males and females, in accordance with the studies conducted by Otero et al. (2007) and Rosa et al. (2004), and similarly to *Loligo forbesi* (Collins et al., 1995; Smith et al., 2005). Considering that males mature at smaller sizes than females, and can maintain this stage for several months until a female is available to breed, there seems to be a differentiated breeding strategy between males and females. Other studies, such as Gonçalves et al. (2002) and Rodríguez-Rua et al. (2005), additionally suggest that mature males are able to breed with immature females which receive and store the mature spermatophores inside the oviducal glands until the oocytes mature. However, only the mass transfer between organs was considered here, and this should be complemented by a study of the types of energetic nutrients that are transferred between organs.

The present study has shown the potential influence and relative importance of environmental factors on the reproduction cycle of *O. vulgaris*, modulating timing, intensity and synchronism. Other studies have been constantly disclosing interactions that may improve our understanding of the main mechanisms. Nevertheless, in terms of population segregation, it seems evident that even under conditions of apparent genetic homogeneity (Cabranes et al., 2008), populations subjected to fisheries in different geographic areas must be managed differently. And if we agree on the extent of its distribution pattern, *O. vulgaris* is one of the most diversely impacted cephalopods in terms of environmental exposure.

Acknowledgements

The authors wish to express their acknowledgment to Pedro Mendonça for assistance with the biological sampling. The authors also thank four anonymous referees for their comments, which greatly improved the final version of this paper. Data collection was conducted under the CEPHVAR (FAIR-CT96-1520), the Data Collection Framework (DCF)/DG FISH (EC Regulation No. 665/2008 of the 14 July 2008) and PRESPO projects (INTERREG IVB – Atlantic Area Program, co-financed by the European Union ERDF funds). The first

author is supported by a PhD fellowship granted by the Portuguese Foundation for Science and Technology (FCT).

References

Almeida, M.J., Queiroga, H., 2003. Physical forcing of onshore transport of crab megalopae in the northern Portuguese upwelling system. Estuarine Coastal Shelf. Sci. 57, 1091–1102.

Baldó, F., García-Isarch, E., Jiménez, M.P., Romero, Z., Sánchez-Lamadrid, A., Catalán, I.A., 2006. Spatial and temporal distribution of the early life stages of three commercial fish species in the northeastern shelf of the Gulf of Cádiz. Deep-Sea Res. Part II 53, 1391–1401.

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

Boletzky, S.v, 1992. Evolutionary aspects of development, life style, and reproductive mode in incirrate octopods (Mollusca, Cephalopoda). Rev. Suisse Zool. 99 (4), 755–770.

Boyle, P., Rodhouse, P., 2006. Cephalopods: Ecology and Fisheries, second ed. Blackwell Publishing, 452 p.

Boyle, P.R., Pierce, G.J., Hastie, L.C., 1995. Flexible reproductive strategies in the squid *Loligo forbesi*. Mar. Biol. 121, 501–508.

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 J. Mar. Sci. 65 (1), 12–16.

Collins, M.A., Burnell, G.M., Rodhouse, P.G., 1995. Reproductive strategies of male and females *Loligo forbesi* (Cephalopoda: Loliginidae). J. Mar. Biol. Assoc. UK 75, 621–634.

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. A thesis submitted to the Faculdade de Ciências da Universidade de Lisboa forthe degree of Doutor em Biologia (ramo Ciências do Mar). 307 p.

Demarcq, H., Faure, V., 2000. Coastal upwelling associated retention indices derived from satellite SST. Application to *Octopus vulgaris* recruitment. Oceanol. Acta 23 (4), 391–408.

Domain, F., Jouffre, D., Caverivière, A., 2000. Growth of *Octopus vulgaris* from tagging in Senegalese waters. J. Mar. Biol. Assoc. UK 80, 699–705.

Fernández-Rueda, P., García-Flórez, L., 2007. *Octopus vulgaris* (Mollusca: Cephalopoda) fishery management assessment in Asturias (North-west Spain). Fish. Res. 83, 351–354.

Fiúza, A.F.G., 1983. Upwelling patterns off Portugal. In: Suess, E., Thiede, J. (Eds.), Coastal Upwelling: Its Sediment Records, Part A. Plenum, New York, pp. 85–98.

Fonseca, T., Campos, A., Afonso-Dias, M., Fonseca, P., Pereira, J., 2008. Trawling for cephalopods off the Portuguese coast-fleet dynamics and landings composition. Fish. Res. 92, 180–188.

García-Lafuente, J., Ruiz, J., 2007. The Gulf of Cádiz pelagic ecosystem: a review. Prog. Oceanogr. 74, 228–251.

García-Lafuente, J., Delgado, J., Criado-Aldeanueva, F., Bruno, M., Río, J., Vargas, J.M., 2006. Water mass circulation on the continental shelf of the Gulf of Cádiz. Deep-Sea Res. Part II 53, 1182–1197.

Gibbons, M.J., Buecher, E., Thibault-Botha, D., Helm, R., 2010. Patterns in marine hydrozoan richness and biogeography around southern Africa: implications of life cycle strategy. J. Biogeogr. 37, 606–616.

Gonçalves, I., Sendão, J., Borges, T.C., 2002. *Octopus vulgaris* (Cephalopoda: Octopodidae) Gametogenesis: a histological approach to the verification of the macroscopic maturity scales. Abh. Geol. B. –A. 57, 79–88.

Guerra, A., 1975. Determinación de las diferentes fases del desarollo sexual de *Octopus vulgaris* Lamarck, mediante unindice de madurez. Invest. Pesq. 39 (2), 397–416.

Hastie, L.C., Pierce, G.J., Wang, J., Bruno, I., Moreno, A., Piatkowski, U., Robin, J.P., 2009. Cephalopods in the North-eastern Atlantic: species, biogeography, ecology, exploitation and conservation. Oceanogr. Mar. Biol. 47, 111–190.

Hernández-García, V., Hernández-López, J.L., Castro-Hernández, J.L., 2002. On the reproduction of *Octopus vulgaris* off the coast of the Canary Islands. Fish. Res. 57, 197–203.

ICES, 2011. Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH), 28 February–03 March 2011, Lisbon, Portugal. ICES CM 2011/SSGEF: 03. 118 pp.

Katsanevakis, S., Verripoulos, G., 2006. Seasonal population dynamics of *Octopus vulgaris* in the eastern Mediterranean. ICES J. Mar Sci. 63, 151–160.

Macdonald, P., 2010. Finite Mixture Distribution Models, 34 pp. Accessed on: 20-11-2011 http://www.r-project.org.

Macdonald, P.D.M., Green, P.E.J., 1988. User's Guide to Program MIX: An Interactive Program for Fitting Mixtures of Distributions. Ichthus Data Systems, 58 pp.

Mangold, K., 1983. Octopus vulgaris. In: Boyle, P. (Ed.), Cephalopod Life cycle: Species Accounts, vol. 1. Academic Press, London, pp. 335–363.

Mangold, K., Boletzky, S.v, 1987. Cephalopods. In: Fischer, W., et al. (Eds.), Fiches FAO d'Identification des Espèces pour les Besoins de la Pèche (Rev. 1) Méditerranée et mer Noire. Zone de Pêche 37. FAO, Rome, pp. 633–714.

Mason, E., Coombs, S., Oliveira, P.B., 2005. An overview of the literature concerning the oceanography of the eastern north Atlantic region. Relatórios científicos e técnicos do IPIMAR – Série Digital, 33, p. 58. (http://www.inrb.pt/fotos/editor2/Reln33final.pdf, 20-11-2011).

McGrath Steer, B.L., Jackson, G.D., 2004. Temporal shifts in the allocation of energy in the arrow squid, *Nototodarus gouldi*: sex-specific responses. Mar. Biol. 144, 1141–1149.

Moreno, A., 2008. Geographic variation in cephalopod life history traits. Dissertação original apresentada para acesso à categoria de Investigador auxiliary. Unidade de Recursos Marinhos e Sustentabilidade. INRB/L-IPIMAR. 181 pp.

Moreno, A., Pereira, J., Cunha, M., 2005. Environmental influences on age and size at maturity of *Loligo vulgaris*. Aquat. Living Resour. 18, 377–384.

Moreno, A., Dos Santos, A., Piatkowski, U., Santos, A.M.P., Cabral, H., 2009a. Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia. J. Plankton Res. 31, 73–91.

Moreno, A., Santos, A., Dos, Piatkowski, U., Santos, A.M.P., Cabral, H., 2009b. Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia. J. Plankton Res. 31 (1), 73–91.

Oosthuizen, A., Smale, M.J., 2003. Population biology of *Octopus vulgaris* on the temperate south-eastern coast of South Africa. J. Mar. Biol. Assoc. UK 83, 535–541.

Otero, J., González, A.F., Sieiro, M.P., Guerra, A., 2007. Reproductive cycle and energy allocation of *Octopus vulgaris* in Galician waters, NE Atlantic. Fish. Res. 85, 122–129.

Otero, J., Álvarez-Salgado, X.A., González, A.F., Miranda, A., Groom, S.B., Cabanas, J.M., Casas, G., Weatley, B., Guerra, A., 2008. Bottom-up control of common *Octopus vulgaris* in the Galician upweeling system, Northwest Atlantic Ocean. Mar. Ecol. Prog. Ser. 362, 181–192.

Pierce, G.J., Allcock, L., Bruno, I., Bustamante, P., González, A., Guerra, A., Jereb, P. et al., 2010. Cephalopod biology and fisheries in Europe. ICES Cooperative Research Report No. 303. 175 pp.

Queiroga, H., Cruz, T., dos Santos, A., Dubert, J., González-Gordillo, J.I., Paula, J., Peliz, A., Santos, A.M.P., 2007. Oceanographic and behavioral processes affecting invertebrate larval dispersal and supply in the western Iberia upwelling ecosystem. Prog. Oceanogr. 74, 174–191.

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

Relvas, P., Barton, E.D., Dubert, J., Oliveira, P.B., Peliz, A., da Silva, J.C.B., Santos, A.M.P., 2007. Physical oceanography of the western Iberia ecosystem: latest views and challenges. Prog. Oceanogr. 74 (2–3), 149–173.

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. J. Mar. Syst. 53 (1–4), 87–107.

Rocha, F., Guerra, A., González, A.F., 2001. A review of the reproductive strategies in cephalopods. Biol. Rev. 76, 291–304.

Rodríguez-Rua, A., Pozuelo, I., Prado, M.A., Gómez, M.J., Bruzón, M.A.<ET AL>, 2005. The gametogenic cycle of *Octopus vulgaris* (Mollusca: Cephalopoda) as observed on the Atlantic coast of Andalusia (south of Spain). Mar. Biol. 147, 927–933.

Roper, C.F.E., Sweeney, M.J., Nauen, C.E., 1984. Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. FAO Fish. Synop. 3, 227.

Rosa, R., Costa, P.R., Nunes, M.L., 2004. Effect of the sexual maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca: Cephalopoda). Mar. Biol. 145, 563–574.

Sánchez, P., Obarti, R., 1993. The biology and fishery of *Octopus vulgaris* caught with clay pots on the Spanish Mediterranean coast. In: Okutami, T., O'Dor, R., Kubodera, T. (Eds.), Recent Advances in Cephalopod Fisheries Biology. Tokai University Press, Tokyo, pp. 477–487.

Sánchez, R.F., Relvas, P., 2003. Spring-summer climatological circulation in the upper layer in the region of Cape St Vincent, Southwest Portugal. ICES J. Mar. Sci. 60, 1232–1250.

Santos, A.M.P., Peliz, A., Dubert, J., Oliveira, P.B., Angelico, M.M., Ré, P., 2004. Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. Cont. Shelf. Res. 24, 149–165.

Silva, L., Sobrino, I., Ramos, F., 2002. Reproductive biology of the common octopus, *Octopus vulgaris* Cuvier, 1797 (Cephalopoda: Octopodidae) in the gulf of Cádiz (SW Spain). Bull. Mar. Sci. 71 (2), 837–850.

Smith, C.D., Griffiths, C.L., 2002. Aspects of the population biology of *Octopus vulgaris* in False bay, South Africa. S. Afr. J. Mar. Sci. 24, 185–192.

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

Zar, J., 1999. Biostatistical Analysis, 4th ed. Prentice Hall, 663 pp.

Hydrobiologia
DOI 10.1007/s10750-013-1717-0

CEPHALOPOD BIOLOGY AND EVOLUTION

Does the trophic habitat influence the biochemical quality of the gonad of *Octopus vulgaris*? Stable isotopes and lipid class contents as bio-indicators of different life-cycle strategies

Sílvia Lourenço · Luís Narciso · Ángel F. Gonzalez · João Pereira · Santiago Auborg · José C. Xavier

Received: 14 December 2012 / Accepted: 19 October 2013

Abstract This study aims to test whether environmental conditions including the trophic habitat and diet impact the biochemical composition of storage organs and affect the nutritional quality of eggs of *Octopus vulgaris*. Trophic habitat and gonad quality of neighbouring populations off the Portuguese coast, subject to different oceanographic regimes, were compared using the digestive gland and beaks as recorders of trophic and habitat preferences, and gonads as indicators of egg quality. Cholesterol, phospholipids and triacylglycerol content, essential fatty acid (EFA) profile of the digestive gland and stable isotopes, $\delta^{15}N$ and $\delta^{13}C$, in the buccal mass flesh and beaks were indicators of the differences in the trophic habitat between populations. For gonad quality, the same bio-indicators were used to identify differences with maturation. The study shows that, although diet influences the EFA profile of the gonads to a certain degree, the main lipid content, phospholipids and cholesterol content in the gonads are not influenced by habitat conditions. This, therefore, suggests that *O. vulgaris* is able to influence the quality of egg content independent of diet. The species is believed to be an income breeder which attains maturity upon reaching a sufficient condition level, then channelling energy directly from food to gonad development.

Keywords *Octopus vulgaris* · Trophic habitat · Gonad quality · Bio-indicators

Guest editors: Erica A. G. Vidal, Mike Vecchione & Sigurd von Boletzky / Cephalopod Life History, Ecology and Evolution

S. Lourenço (✉) · L. Narciso
Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Avenida Nossa Senhora do Cabo 939, 2750-374 Cascais, Portugal
e-mail: salourenco@fc.ul.pt

S. Lourenço · Á. F. Gonzalez · S. Auborg
Instituto de Investigaciones Marinas de Vigo CSIC, C/Eduardo Cabello, 6, 36208 Vigo, Spain

S. Lourenço · J. Pereira
Departamento do Mar e Recursos Marinhos, Instituto Português do Mar e da Atmosfera, I.P.Avenida de Brasília, 1449-006 Lisbon, Portugal

J. C. Xavier
Department of Life Sciences, Institute of Marine Research, University of Coimbra, 3001-401 Coimbra, Portugal

Introduction

The common octopus, *Octopus vulgaris* Cuvier (1797), is an important fisheries resource and a key component of coastal food webs as a prey and generalist predator (Smith, 2003; Katsanevakis & Verripoulos, 2004). The species is believed to channel energy to growth and reproduction directly from food (Quetglas et al., 2011; Smith et al., 2011). It is a species with high feeding and turnover rates (Semmens et al., 2004), short life cycle

Published online: 07 November 2013

and high plasticity to environmental conditions. Studies on the molecular and nutritional composition of reserve and reproductive organs reflect the benthic characteristics of the species. The habitat biodiversity and energy flow to which the populations are exposed are mirrored in the molecular and energetic composition of the tissues (Rosa et al., 2004a; Bandarra et al., 2006; Cherel et al., 2009; García-Garrido et al., 2010).

The diet of *O. vulgaris* depends on the life-cycle stage, size, depth of occurrence, habitat and seasonal availability of their prey (Nixon, 1985; Smith, 2003). The cephalopod hepatopancreas or digestive gland has different functions in the physiology of *Octopus* spp., including the synthesis and secretion of digestive enzymes, the reabsorption and metabolism of nutrients; synthesis and storage of lipids like cholesterol, lipoproteins, glycogen, pigments, vitamins and protein-bound Fe, Cu, Ca and non-physiological heavy metals; and excretion and rejection of waste products of the digestion and cell metabolism (Blanchier & Boucaud-Camou, 1984; Budelmann et al., 1997; Moltschaniwskyj & Johnston, 2006). Its function as a storage organ indicates its utility as the ideal source of dietary tracers such as essential fatty acids (EFA) (Phillips et al., 2001) and dietary lipids (e.g., triacylglycerol and cholesterol) that are deposited in this organ with little or no modification of the lipid content (Boucaud-Camou & Boucher-Rodoni, 1983; Phillips et al., 2003).

Lipids are important dietary constituents, providing energy, vitamins and EFA. Cholesterol is the predominant sterol in the cephalopod's lipid reserves (Sieiro et al., 2006) and proxy of the production of hormones in marine invertebrates (Kanazawa, 2002). The endogenous synthesis of cholesterol seems to be absent in cephalopods, suggesting that it is an essential dietary nutrient (Villanueva & Norman, 2008). Triacylglycerol is a neutral lipid involved in fatty acid (FA) storage and metabolism (Lee et al., 2006). The FAs incorporated within the phospholipids act as building blocks for the membrane lipid bilayer (Dalsgaard et al., 2003; Bergé & Barnathan, 2005; Athenstaedt & Daum, 2006). The seasonal variability of triacylglycerols and phospholipids is related with cellular mechanisms under different environmental conditions and because of that, they are good indicators of the condition (Shulman & Love, 2006). The most important EFA are arachidonic acid C20:4n6 (ARA), eicosapentaenoic acid C20:5n3 (EPA) and docosahexaenoic acid C22:6n3 (DHA). The requirements on ARA, EPA and DHA and the balance between them are important for growth (Navarro & Villanueva, 2000; Shulman & Love, 2006). These EFA are related with the energy channelling, the cellular membrane structure and function, and are integral elements of phospholipids as components of lipid bilayers (Tocher, 2010).

Reproduction timing in *O. vulgaris* depends on the local oceanographic regime: a long season in productive systems, frequently with two reproductive peaks; and a shorter season (1–2 months) in oligotrophic systems (Lourenço et al., 2012). The formation of the yolk is extremely important during maturation and egg development, because the newly hatched paralarvae are not truly lecithotrophic depending to some extent on these reserves to survive (Boletzky, 1975; Villanueva & Norman, 2008). The nutritional content of the yolk is mainly protein, but lipids are also important for membrane formation and energetic supply (11–14% of dry weight) particularly that of polyunsaturated fatty acids (PUFA), phospholipids and cholesterol (Navarro & Villanueva, 2000).

The $\delta^{13}C$ and $\delta^{15}N$ signatures in different tissues of a predator like *O. vulgaris* reflect its habitat and trophic position, respectively (Cherel & Hobson, 2005). Consumers or predators are enriched in ^{15}N relative to their food and consequently the $\delta^{15}N$ measurement is an indicator of the consumer trophic position (Vander Zanden & Rasmussen, 2001; Hobson & Cherel, 2006). With little variation along the food chain, the $\delta^{13}C$ is used to determine primary sources in the trophic web indicating the habitat of the organism, and the inshore versus offshore, or pelagic versus benthic contribution of food intake (Cherel & Hobson, 2007; Jackson et al., 2007). The determination of stable isotope $\delta^{13}C$ and $\delta^{15}N$ signatures both in muscle and in cephalopod beaks are complementary approaches to stomach content and fatty acid analysis in studies of trophic dynamics and feeding ecology, allowing the identification of ontogenic migration and feeding shift events (Stowasser, 2004; Jackson et al., 2007). In species like *O. vulgaris*, diet studies are difficult to carry out due to the diversity of preys (Smith, 2003); the fast digestion rate (Boucaud-Camou & Boucher-Rodoni, 1983); and the large number of empty stomachs. For these cases, an analytic approach combining fatty acids and stable

isotopes analyses provides overall information on the average diet regarding both trophic level and habitat of a particular population.

Here we hypothesise that environmental conditions, including the trophic habitat, influence the biochemical composition of storage organs and the nutritional quality of the eggs (indirectly potentially affecting the next generation). The Portuguese coast presents an advantageous geographical setting where it is possible to follow *O. vulgaris* populations which are subjected to distinct environmental conditions: in the northwest coast, one of the populations is in a productive system integrated in the Western Iberia Upwelling System (Relvas et al., 2007); in the south, the other is integrated in the Gulf of Cadiz System influenced by the oligotrophic and warmer waters of the Huelva front where downwelling and upwelling events are weaker and not seasonal (García-Lafuente et al., 2006). To test our hypothesis, we followed the EFA profile and the content of total lipids, cholesterol, triacylglycerol and phospholipids as bio-indicators of variations in the digestive gland and in immature and fully developed gonads of females of both populations. We also followed the stable isotope $\delta^{13}C$ and $\delta^{15}N$ signatures in the upper and lower beaks and buccal mass tissue of immature and mature individuals to identify the possible differences in the overall diet between population and maturity stages.

Materials and methods

Sampling

All tissue samples were obtained from females captured in the small-scale *O. vulgaris* fisheries from March to April 2011 within the two study areas represented by landings in ports of Peniche (northwest coast area; lat: 39°21.40 N, long: 9°20.38 W) and Olhão (south coast area; lat: 36°59.34 N, long: 7°50.44 W). Within each area, five immature females and five mature females were selected and digestive gland tissue and gonads were collected, freeze-dried and stored at −20°C.

Cleaned upper and lower beaks and buccal mass muscular tissue (referred as flesh hereafter) samples were collected from frozen animals and kept in 70% ethanol for isotopic analysis. Sampling was conducted considering the same explanatory factors, area and maturity in an unbalanced sampling design, collecting 27 flesh samples (4 mature and 6 immature samples in the northwest coast and 5 mature and 12 immature samples in the south coast) and 33 beak samples (8 mature and 9 immature in the northwest coast and 5 mature and 12 immature in the south coast). The dorsal mantle length and individual weight were measured to the nearest 5 mm and 0.1 g, respectively. Immature and maturing females were classified as immature whereas mature and spawning females were classified as mature (according to Guerra, 1975).

Stable isotope analyses

Beaks and flesh samples were freeze-dried and homogenized prior to analysis. To avoid the depletion of $\delta^{13}C$ values due to the presence of lipids, flesh samples were rinsed successively in a 2:1 chloroform–methanol solution (Cherel et al., 2005). Nitrogen and carbon isotope ratios were determined via Finningan conflo II interface to a Thermo Delta V S mass spectrometer coupled to a Flash EA1112 Series elemental analyser. Approximately 0.3 mg of each sample was combusted in a tin cup for the simultaneous determination of nitrogen and carbon isotope ratios. Isotope ratios are presented in the usual δ notation relative to the PeeDee Belemnite (PDB) for carbon and atmospheric N_2 (AIR) for nitrogen, and expressed as ‰. Replicate measurements of internal laboratory standards (acetanilide) indicate a precision of <0.2‰ both for $\delta^{13}C$ and $\delta^{15}N$. The C/N mass ratio was used to check the effectiveness of the lipid extraction in the flesh and in the beaks (Post et al., 2007; Cherel et al., 2009).

Prior to the statistical analysis, the assumptions of normality and sample variance homogeneity were assessed by Shapiro–Wilk's test and Bartlett's test, respectively. A paired *t* test was performed to assess possible differences in stable isotope signatures between upper and lower beaks. A two-way analysis of variance (ANOVA) was performed to assess the effect of area, maturity and the interaction between the two factors in the ratios $\delta^{13}C$ and $\delta^{15}N$ in the upper and lower beaks and flesh. The hypothesis for the two-way ANOVA was formulated under the assumption of: H_{0A}: there is no main effect of the factor area on the stable isotope ($\delta^{13}C$ or $\delta^{15}N$) mean value in the beaks upper and lower beaks and flesh; H_{0B}: there is no main effect of the factor maturity on the stable isotope mean

value in the upper and lower beaks and flesh; H_{0C}: there is no additive effect of the interaction between area and maturity on the stable isotope mean value in the upper and lower beaks and flesh. If H_{0C} was rejected, a post-hoc Tukey test for multiple comparisons was performed to determine which combination of factors were significantly different (P value < 0.05).

Lipid class analyses

The total lipid (TL) fraction was extracted by the Bligh & Dyer (1959) method. Samples of ≈ 1 and ≈ 2 g of dry tissue of digestive glands and gonads were used, respectively. The results were expressed as g lipid/100 g dry weight. Lipid classes were determined by different spectrophotometric methods. The phospholipids fraction was purified from the total lipid extract according to Auborg et al. (1996). Total phospholipids were quantified by measuring the organic phosphorus in total lipid extracts according to the Raheja et al. (1973) method based on a complex formation with ammonium molybdate. Results are expressed as g PL/100 g dry weight. Total cholesterol was determined in the total lipids extracts by the method of Huang et al. (1961) based on the Liebermann–Buchardt reaction. Results are expressed in g cholesterol/100 g dry weight. FA composition of lipids present in the Bligh & Dyer extract was determined by converting total lipids into fatty acid methyl esters (FAME), according to the method described by Lepage & Roy (1984). FAME were analysed by gas chromatography. Peaks corresponding to FAs were identified by comparison of their retention times with standard mixtures. Peak areas were automatically integrated with C19:0 being used as an internal standard for quantitative analysis. The concentration of each fatty acid or fatty acid group was expressed as g/100 g total FAME.

Prior to the statistical analysis, the assumptions of normality and sample variance homogeneity were assessed with Shapiro–Wilk's and Bartlett's tests, respectively. Whenever these assumptions failed the data were log-transformed to guarantee normality and variance homogeneity. Two-way ANOVA was performed to assess the effect of area, maturity and the interaction between the two factors in the lipid classes in the digestive gland and in the gonad. The hypothesis for the two-way ANOVA was formulated under the assumption of: H_{0A}: there is no main effect of the factor area on the lipid class content in the tissue; H_{0B}: there is no main effect of the factor maturity on the lipid class content in the tissue; H_{0C}: there is no additive effect of the interaction between area and maturity on the mean lipid class content in the tissue. If H_{0C} was rejected, a post hoc Tukey test for multiple comparisons was performed to determine which factor combinations were significantly different.

Major FAs were defined as those presenting concentration by area and by maturity stage higher than 1 g/100 g of FAME. For each FA, the assumption of sample normality and homogeneity was tested by the Shapiro–Wilk's and Bartlett's, respectively, by tissue and factor (area and maturity), when these assumptions failed, the variable was log-transformed. Differences in mean concentration of each FA between area and maturity stage were tested with t test (Zar, 1999). The major fatty acids profile was compared by explanatory factor, area and maturity and the interaction between area and maturity by means of Multivariate ANOVA (MANOVA), followed by Discriminant Function Analysis (DFA). The MANOVA and DFA are complementary approaches based in the separation of observation groups represented by their centroids obtained under the effect of two or more factors levels (Quinn & Keough, 2002). DFA is particularly useful to detect the variables that better discriminates between different observation groups (Zuur et al., 2007).

Results

Diet and habitat

According to the mean C:N mass ratio, lipids were effectively removed from flesh (C:N $= 3.13 \pm 0.06$, mean $\pm$ standard deviation, SD) and from beaks (upper beak: 3.38 ± 0.07; lower beak: 3.33 ± 0.08). The flesh of *O. vulgaris* females presents a mean $\delta^{15}N$ of 11.86 ± 0.66 (SD) ‰, and the mean $\delta^{13}C$ value of -16.77 ± 0.74 (SD) ‰ (Fig. 1) independently of area, maturity stage or the interaction of both factors (Table 1). Upper and lower beaks present different $\delta^{15}N$ and $\delta^{13}C$ signatures between them (for $\delta^{15}N$: paired $t = -2.12$; df $= 32$, P value < 0.01; for $\delta^{13}C$: paired $t = -4.14$, df $= 32$, P value < 0.01). The factorial ANOVA shows that area has a significant effect in $\delta^{15}N$ signature (Table 1), with both beaks presenting higher values in south coast (Fig. 1). The

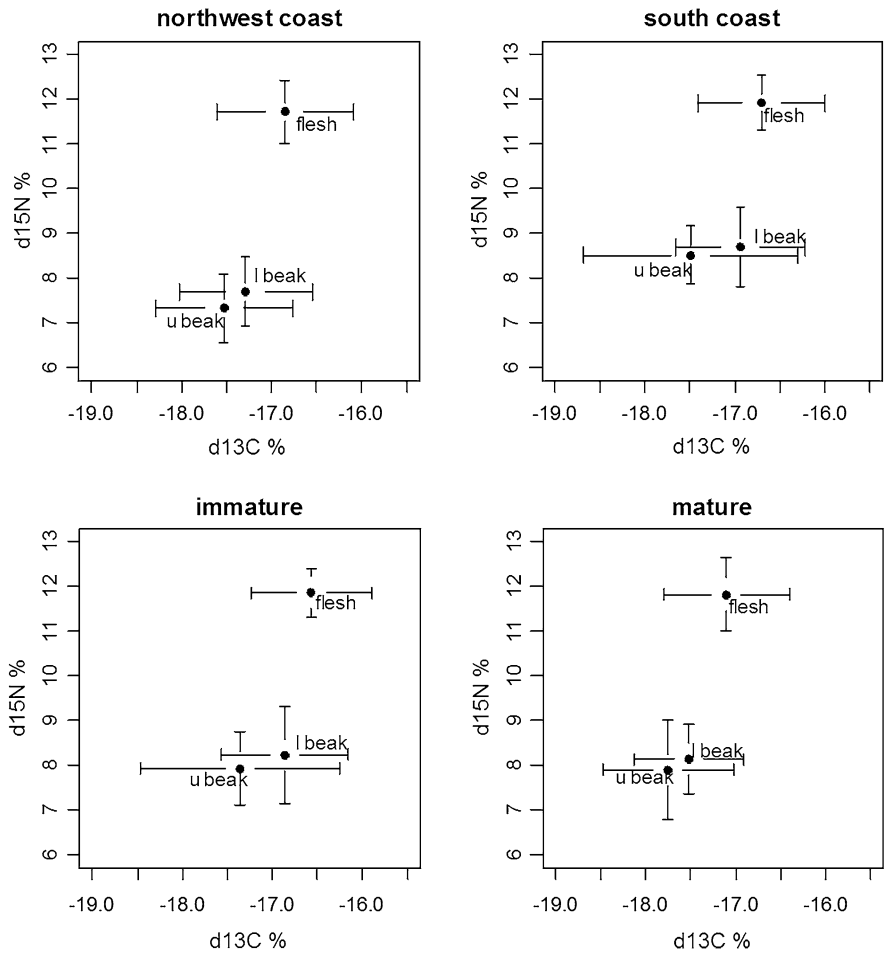


Fig. 1 *Octopus vulgaris* flesh and upper and lower beaks stable isotope $\delta^{15}N$ and $\delta^{13}C$ signatures by explanatory factors area and maturity. The upper right and left graphics represent the stable isotope signatures for the northwest and south coast, and the lower left and right graphics represent the stable isotope signatures for immature and mature individuals. The dot indicates the mean value for the $\delta^{15}N$ and $\delta^{13}C$ for each tissue studied; the error bars indicate the magnitude of standard deviation on either axis

$\delta^{13}C$ signature is not affected by area in both beaks (Table 1).

The mean individual weight of the females collected was 2340.30 ± 979.45 (SD) g in the northwest coast and 2637.40 ± 479.00 (SD) g in the south coast. Digestive gland and gonad presented different mean contents in total lipids and lipid classes, with the digestive gland presenting higher mean content of total lipids, cholesterol and triacylglycerol (Fig. 2). Despite that, the gonads were richer in phospholipids than the digestive gland (Fig. 2).

In the digestive gland the mean total lipid content and mean cholesterol content depended on area. The digestive glands collected in the northwest coast presented a significantly lower content of total lipids ($t = -2.42$, df = 18, P value < 0.01), and higher mean content in cholesterol ($t = 7.91$, df = 18, P value < 0.01; see Fig. 2) when compared with south coast. The two-way ANOVA for the interaction between the factors area and maturity showed significant differences in the mean content of cholesterol and triacylglycerol due to the additive effect of the two factors (Table 1).

In the digestive gland, the fatty acids (FA) represented 31 and 46 µg/mg of the total lipid content in the northwest coast and the south coast, respectively. The most abundant FA were the saturated fatty acids (SFA), followed by the polyunsaturated fatty acids (PUFA) and the monounsaturated fatty acids (MUFA) (Table 2). The mean content of myristic acid, C14:0; palmitoleic acid, C16:1n7; gondoic acid, C20:1n9; ARA, C20:4n6 and DHA were significantly different between areas, as well as the ratio DHA/EPA mainly due to the increase in DHA content in digestive glands of the south coast (Table 2). Results of the MANOVA analysis showed that content levels depend both on area and maturity, with no effect on the interaction between both factors (Table 3). In the DFA, the first

Table 1 Two-way analysis of variance and hypothesis testing considering the applied bio-indicators, $\delta^{15}N$ and $\delta^{13}C$, total lipids content (TL), Triacylglycerol content (tag), Cholesterol content (cho) , Phospholipids content (phosp) determined for the upper and the lower beaks and the flesh, digestive gland and gonad by area and maturity stage (Mat)

Tissue	Indicator	Between groups DF	Residual DF	ANOVA					
				Area		Mat		Area × mat	
				F	*P* value	*F*	*P* value	*F*	*P* value
Upper beak	$\delta^{13}C$	1	29	1.16	0.29	**5.68**	**0.02**	0.02	0.88
	$\delta^{15}N$	1	29	**25.99**	**<0.01**	0.03	0.87	0.06	0.80
Lower Beak	$\delta^{13}C$	1	29	2.03	0.16	**7.68**	**<0.01**	0.02	0.89
	$\delta^{15}N$	1	29	**11.98**	**<0.01**	0.08	0.78	0.06	0.80
Flesh	$\delta^{13}C$	1	23	0.28	0.60	4.00	0.06	0.44	0.51
	$\delta^{15}N$	1	23	0.62	0.44	0.02	0.88	1.83	0.19
Digestive gland	TL	1	16	**5.88**	**0.03**	**5.88**	**0.03**	2.48	0.13
	tag	1	16	1.85	0.29	3.62	0.07	**4.68**	**0.05**
	cho	1	16	**62.54**	**<0.01**	0.13	0.72	**6.44**	**0.02**
	phosp	1	16	3.49	0.08	4.15	0.06	2.26	0.15
Gonad	TL	1	16	3.48	0.08	2.35	0.14	0.49	0.49
	tag	1	16	1.87	0.19	1.72	0.21	0.04	0.84
	cho	1	16	1.22	0.29	0.01	0.92	0.13	0.72
	phosp	1	16	0.94	0.34	**14.68**	**<0.01**	1.44	0.25

Values in bold indicate statistical significance

discriminant function maximizes the differences between groups. The DFA for region groups show that the first function explained 84% of the variability, with a good separation between the northwest coast group (class score = 0.92) and the south coast group (class score = −0.92). The FA that contributed the most to the group separation was the DHA which was positively correlated with the south coast and negatively correlated with northwest coast. The DFA for maturity groups showed a good separation between immature (class score = −0.85) and mature digestive glands (class score = 1.02) with 86% of explained variation. The FAs that most contributed to the group separation were EPA and DHA and C18:0. The DFA for area x maturity groups (Fig. 3, digestive gland) showed a poor separation between groups with an explained variance of 51% in accordance with MANOVA results. Nevertheless, the FAs that contributed the most to the group separation were: DHA, positively related with immature females of the south coast area and negatively related with the immature and mature females from northwest coast: EPA, positively related with the immature females, and negatively related with the northwest coast mature females.

Maturity

Stable isotope signatures showed that the $\delta^{13}C$ signature is statistically significant different with the maturity stage (Table 1), with the beaks of immature females presenting significant higher $\delta^{13}C$ signature (Fig. 1).

The effect of different nutritional levels on maturity was studied by assessing differences in the digestive gland and gonads with maturity. For the digestive gland, there was no effect of this factor in the lipid classes content studied (Table 1). Total lipid, cholesterol and triacylglycerol contents tended to increase from immature to mature females although this increase is not statistically significant. In relation to the phospholipid content, the digestive glands of mature females of south coast were significantly poor in phospholipids in relation to the immature ones ($t = 3.50$, df = 18, P value < 0.01) (Fig. 2). Nevertheless, when comparing the digestive glands lipid content between mature females of northern coast with mature females of south coast, some differences arose. Particularly, the total lipid content ($t = -2.82$, df = 18, P value = 0.01), the cholesterol content

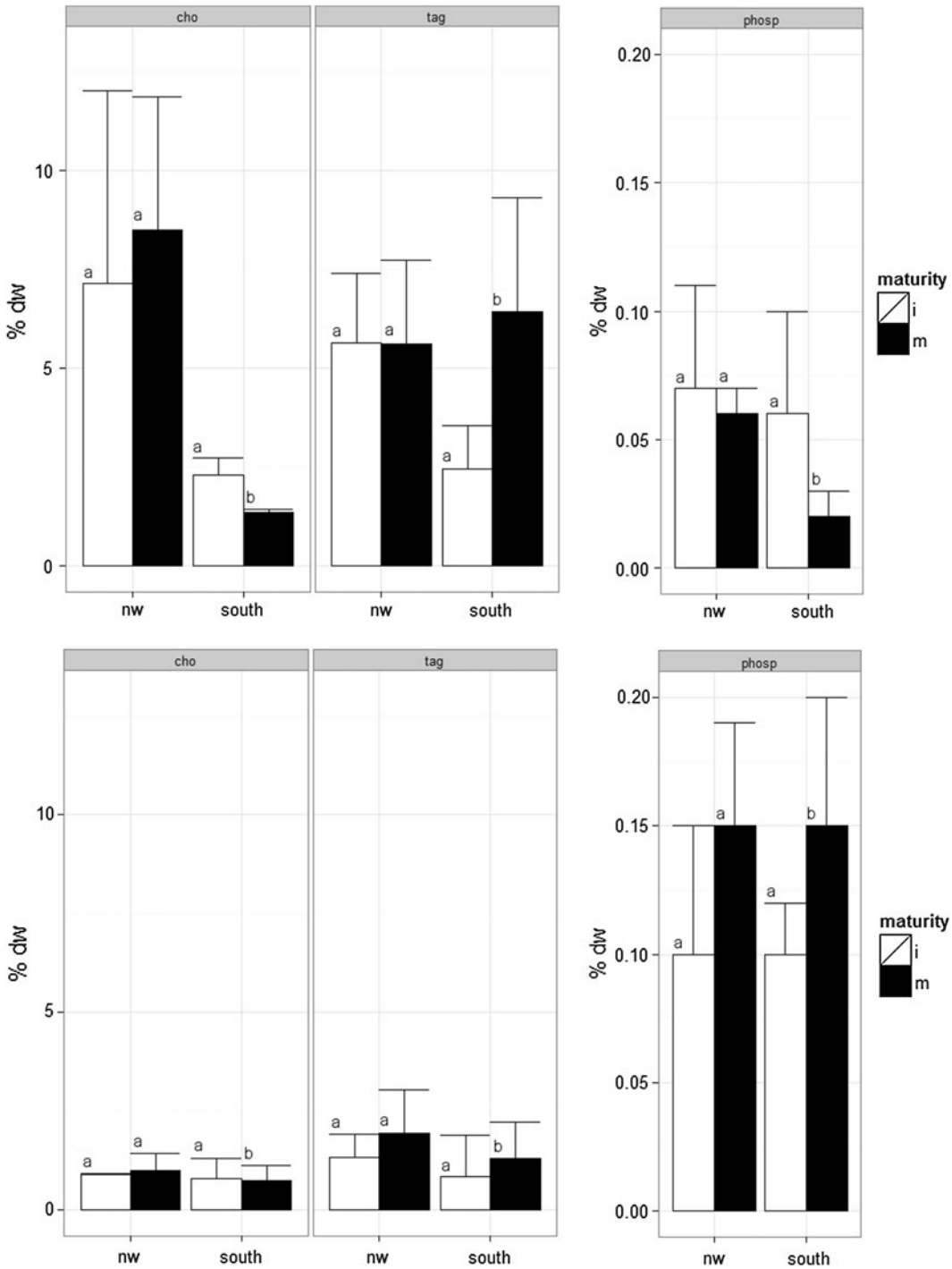


Fig. 2 Mean lipid classes' content in % dw (±SD) aggregated by tissue (digestive gland; gonad), by area (nw—Northwest coast; south—South coast) and by maturity stage (maturity; i—immature, m—mature). Different superscript letters indicate significant statistical differences ($P < 0.05$). The acronym cho stands for cholesterol, tag for triacylglycerol and phosp for phospholipids

Table 2 Total lipid content (mean ± SD, % dw) and major fatty acid (mean ± SD, g/100 g FAME) profiles in the digestive gland and gonad of immature and mature females collected in the northwest and south Portuguese coast

	Digestive gland					
	Northwest coast			South coast		
	Immature	Mature	Mean	Immature	Mature	Mean
Total lipid (TL)	14.60 ± 4.44	15.39 ± 4.22	15.00 ± 4.11**	16.97 ± 4.30*	24.42 ± 5.76*	20.69 ± 6.20**
Saturated fatty acids (SFA)						
C 14:0	4.99 ± 1.58	4.89 ± 2.05	4.94 ± 1.75**	1.88 ± 0.96	3.25 ± 1.62	2.51 ± 1.42**
C 16:0	22.62 ± 4.35	18.34 ± 5.71	20.48 ± 5.33	22.08 ± 14.61	18.48 ± 9.58	20.44 ± 12.14
C 18:0	13.73 ± 4.27	11.63 ± 0.32	12.68 ± 3.09	12.88 ± 1.58	11.21 ± 1.78	12.12 ± 1.80
Other SFA[a]	6.21 ± 2.98	6.60 ± 2.66	6.40 ± 2.70**	3.85 ± 1.08	3.58 ± 0.62	3.72 ± 0.87**
Σ SFA	47.55 ± 8.68	41.46 ± 8.25	44.51 ± 8.68	40.68 ± 13.32	36.52 ± 13.50	38.79 ± 12.90
Monounsaturated fatty acids (MUFA)						
C 16:1n7	6.61 ± 1.51	6.78 ± 2.84	6.69 ± 2.17**	3.05 ± 0.52	4.11 ± 1.34	3.53 ± 1.08**
C 17:1	1.77 ± 0.62	1.15 ± 0.10	1.46 ± 0.53	1.56 ± 0.22*	1.25 ± 0.26*	1.42 ± 0.28
C 18:1n9	11.49 ± 2.85	10.49 ± 6.77	10.99 ± 4.98	8.54 ± 3.46	14.51 ± 3.22	11.26 ± 4.45
C 20:1n9	2.25 ± 0.60	2.72 ± 0.94	2.49 ± 0.79**	3.33 ± 1.08	3.98 ± 0.96	3.34 ± 1.08**
Other MUFA[b]	2.19 ± 0.98	1.78 ± 0.86	1.98 ± 0.90	2.23 ± 0.78	3.04 ± 1.08	2.60 ± 0.97
ΣMUFA	24.30 ± 3.76	22.92 ± 9.26	23.61 ± 6.78	18.19 ± 3.22*	26.88 ± 4.86*	22.14 ± 5.94
Polyunsaturated fatty acids (PUFA)						
C: 20:4n6[c]	4.34 ± 1.55	5.18 ± 2.62	4.76 ± 2.09**	7.64 ± 3.22	6.06 ± 2.06	6.92 ± 2.75**
C 20:5n3	8.54 ± 4.06	17.45 ± 11.53	13.00 ± 9.46	10.82 ± 9.46	11.00 ± 6.08	10.90 ± 5.02
C 22:6n3	11.43 ± 7.92	8.42 ± 6.23	9.92 ± 6.97**	19.54 ± 8.70	16.28 ± 8.97	18.06 ± 8.54**
Other PUFA[d]	2.32 ± 0.77	3.15 ± 1.75	2.73 ± 1.36**	1.92 ± 0.30	1.88 ± 0.41	1.91 ± 0.34**
ΣPUFA	26.63 ± 11.34	34.19 ± 16.02	30.41 ± 13.81	39.92 ± 15.68	35.23 ± 16.73	37.79 ± 15.52
Σ FAME (μg/mg PS)	31.35 ± 9.55	33.14 ± 8.30	31.25 ± 8.53**	48.79 ± 11.56	43.40 ± 9.59	46.34 ± 10.56**
Σn−3/Σn−6	2.03 ± 1.46	1.11 ± 0.55	1.57 ± 1.15	2.11 ± 0.83	2.07 ± 0.98	2.09 ± 0.85
DHA/EPA	1.31 ± 0.53	0.63 ± 0.37*	0.97 ± 0.56**	1.76 ± 0.34	1.57 ± 0.31	1.68 ± 0.36**
	Gonad					
	Northwest coast			South coast		
	Immature	Mature	Mean	Immature	Mature	Mean
Total lipid (TL)	15.39 ± 2.36	12.53 ± 3.27	13.69 ± 3.08	12.16 ± 3.62	11.03 ± 0.99	11.59 ± 2.57
Saturated fatty acids (SFA)						

Table 2 continued

	Gonad					
	Northwest coast			South coast		
	Immature	Mature	Mean	Immature	Mature	Mean
C 14:0	4.19 ± 0.48	4.09 ± 0.36	4.14 ± 0.40	4.06 ± 0.72	3.94 ± 0.52	3.99 ± 0.58
C 16:0	19.25 ± 1.98*	23.85 ± 1.18*	21.73 ± 2.83	18.78 ± 2.44	22.31 ± 3.50	20.83 ± 3.48
C 18:0	8.68 ± 1.33*	6.36 ± 0.93*	7.43 ± 1.62	7.47 ± 1.26	5.90 ± 1.00	6.56 ± 1.33
Other SFA[a]	2.19 ± 0.22	2.25 ± 0.45	2.22 ± 0.35**	2.04 ± 0.14	1.93 ± 0.38	1.98 ± 0.30**
Σ SFA	34.32 ± 1.33*	36.55 ± 0.85*	35.52 ± 1.56**	32.36 ± 1.58	34.08 ± 3.41	33.37 ± 2.83**
Monounsaturated fatty acids (MUFA)						
C 16:1n7	2.68 ± 0.34*	2.05 ± 0.42*	2.34 ± 0.49	2.79 ± 0.51	2.63 ± 0.73	2.70 ± 0.63
C 17:1	2.53 ± 0.40	2.62 ± 0.55	2.58 ± 0.47**	2.26 ± 0.42	1.90 ± 0.42	2.05 ± 0.44**
C 18:1n9	6.23 ± 1.33	5.37 ± 0.56	5.76 ± 1.05	5.34 ± 1.32	4.41 ± 1.74	4.80 ± 1.59
C 20:1n9	5.50 ± 0.63*	4.48 ± 0.21*	4.95 ± 0.69**	6.11 ± 1.18	7.90 ± 6.51	7.15 ± 4.95**
Other MUFA[b]	3.50 ± 0.40*	2.46 ± 0.36*	2.94 ± 0.65**	2.88 ± 0.38	2.13 ± 0.65	2.44 ± 0.66**
ΣMUFA	20.45 ± 1.21*	16.99 ± 1.17*	18.59 ± 2.13	19.39 ± 1.45	18.97 ± 7.69	19.15 ± 5.31
Polyunsaturated fatty acids (PUFA)						
C: 20:4n6[c]	9.86 ± 1.71*	7.51 ± 0.96*	8.59 ± 1.78	9.80 ± 1.00	9.18 ± 0.68	9.48 ± 0.87
C 20:5n3	1.64 ± 0.32	1.47 ± 0.28	1.55 ± 0.30	2.58 ± 2.20*	1.14 ± 0.20*	1.74 ± 1.53
C 22:6n3	1.09 ± 0.30*	0.27 ± 0.22*	0.65 ± 0.49	1.27 ± 0.44*	0.67 ± 0.31*	0.92 ± 0.47
Other PUFA[d]	n.d	n.d	n.d	n.d	n.d	n.d
ΣPUFA	12.60 ± 1.64	9.25 ± 1.04	10.79 ± 2.16	13.75 ± 3.26	10.99 ± 0.82	12.14 ± 2.50
Σ FAME (μg/mg PS)	63.74 ± 15.67	67.99 ± 9.53	66.03 ± 12.35	58.27 ± 16.11	67.56 ± 15.02	63.69 ± 15.50
Σn−3/Σn−6	3.52 ± 0.67*	5.07 ± 0.76*	4.35 ± 1.06	3.66 ± 0.53	4.01 ± 0.36	3.86 ± 0.52
DHA/EPA	0.66 ± 0.10*	0.18 ± 0.15*	0.40 ± 0.28	0.65 ± 0.03	0.57 ± 0.05	0.60 ± 0.23

* indicates significant statistical difference (P value < 0.05) between immature and mature individuals; ** indicates significant statistical difference (P value < 0.05) in the fatty acid content between sampling areas

[a] Other minor SFA C15:0, C17:0 and C24:0

[b] Other minor MUFA C18:1n7, C22:1n9 and C24:1n9

[c] As the FA C20:3n3 and the FA C20:4n6 have the same retention time, and the concentration of the FA C20:4n6 is dominant in marine products, the concentration presented here is representative of C20:4n6

[d] Other minor FA C18:2n6 and C20:2n6

Table 3 Multivariate analysis of variance (MANOVA) and hypothesis testing to the major fatty acids profile in the digestive gland and in the gonads grouped by the factors area and maturity (mat)

Tissue	Num Df	Denom DF	MANOVA								
			Area			Maturity			Area × mat		
			λ_{Wilks}	F_{Pillai}	P value	λ_{Wilks}	F_{Pillai}	P value	λ_{Wilks}	F_{Pillai}	P value
Digestive Gland	10	12	0.13	7.96	0.001	0.17	5.84	0.003	0.36	1.79	0.186
Gonad	10	14	0.20	5.62	0.002	0.26	4.09	0.008	0.24	3.74	0.017

P value < 0.05 indicates significant statistical difference

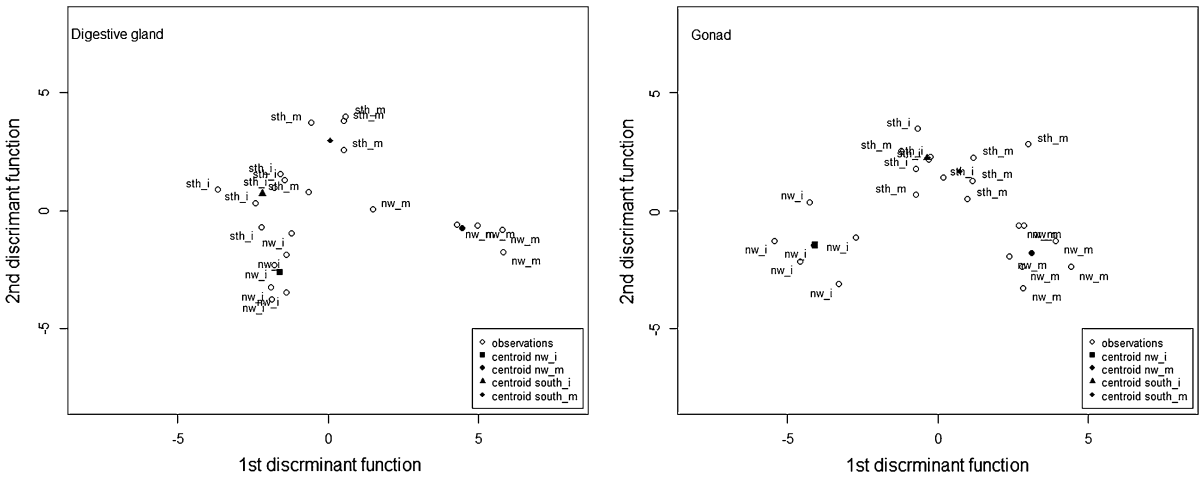


Fig. 3 Discriminant function analysis biplots of the fatty acid profiles of the digestive gland (on the left) and gonad (on the right). The acronym nw_i stands for the group immature females of northwest coast, nw_m stands for the group mature females of northwest coast, sth_i stands for the immature individuals of the south coast and sth_m stands for the mature individuals of south coast

($t = 10.52$, df $= 18$, P value < 0.01) and the phospholipids content ($t = 6.04$, df $= 18$, P value < 0.01) were significantly higher in the mature digestive glands of south coast (Fig. 2).

Mature gonads of the northwest coast present higher contents in triacylglycerol, cholesterol and phospholipids, although no significant differences were found for the mean content of those bio-indicators between immature and mature individuals (Fig. 2; Table 1). In the south coast, the gonads of mature individuals are significantly higher than immature individuals in triacylglycerol ($t = -2.86$, df $= 18$, P value < 0.01) and phospholipids ($t = 3.50$, df $= 18$, P value < 0.01).

The FA profile in the gonads was affected by area, maturity, and by the interaction of both factors (Table 3). Mature gonads were rich in SFA, showing the capacity to produce saturated fatty acids namely palmitic acid C16:0, increasing significantly from immature to mature females in the northwest coast. In opposition, the stearic acid C18:0 in the northwest coast decreased from immature to mature gonads (Table 2). MUFA were the second most abundant fatty acids in the gonads, with the presence of vaccenic acid C18:1n7 as a minor FA, presenting significant differences between the northwest coast and the south coast namely in the FA C17:1 and C20:1n9. The FA C16:1n7 was significantly more abundant in immature than in mature gonads from the northwest coast. The PUFA represented between 9 and 13% of the FAME present in the total lipid content of the gonads, with the content in ARA, DHA and EPA decreasing significantly from immature to mature gonads in both study areas. In particular, the significant decrease in DHA in the northwest coast led to significant differences between the DHA/EPA ratio of immature and mature females in that area (Table 1).

The DFA on the FA profile of the females' gonads between areas showed that the first function explained 79% of the variability, with a good separation between the northwest coast group (class score = 0.82) and the south coast group (class score = −0.97). The FA that contributed the most to the group separation is the C18:0 which is positively correlated with the northwest coast. The DFA for maturity groups showed a good separation between immature (class score = −0.94) and mature individuals (class score = 0.79) with 74% of the variability explained. The FA that contributed the most to the group separation was the C18:0, positively correlated to the immature individuals. The DFA for area x maturity groups (Fig. 3, Gonad) showed a good separation between groups with an explained variability of 95% in concordance with MANOVA results. The FAs that contributed the most to the group separation were C16:0, 18:0 and ARA that were positively correlated with immature gonads from both areas.

Discussion

The $\delta^{15}N$ and $\delta^{13}C$ signatures in the flesh seem to be adequate short-term indicators of diet and habitat. Cherel & Hobson (2005), working on *Psychroteuthis glacialis*, suggested that the difference between the $\delta^{15}N$ of the flesh relative to the beaks relates to the chitin synthesis and consequent N accretion in the beaks. In the more recently formed regions of the beaks, such as the wing, the $\delta^{13}C$ signature of the predator closely matches that of the prey (Hobson & Cherel, 2006), also reflecting to an extent the recent dietary composition. The $\delta^{15}N$ and $\delta^{13}C$ of beaks are, therefore, used as indicators of habitat and diet preference (Post, 2002; Jackson et al., 2007). In our southern population of *O. vulgaris*, beak $\delta^{13}C$ signature is different between maturity stages, probably a reflection of the recent trophic history of the different maturation stages. The higher $\delta^{15}N$ ratio in the beaks from the south coast in relation to the northwest coast is probably related to the higher frequency of occurrence of crustacean prey (e.g., decapod crabs) found in the diet of *O. vulgaris* sampled (Lourenço, *unpublished data*). Rosa et al. (2004b) show that the decapod crabs are important prey of the *O. vulgaris* populations along the Portuguese coast and the presence of crustacean prey in the diet of *Loligo forbesi* is known to increase the $\delta^{15}N$ content of the beaks and flesh of that species (Stowasser, 2004). Darnaude et al. (2004) relate increased $\delta^{15}N$ to a significant input from river plumes, through the added input of $\delta^{15}N$-rich particulate organic matter. This, however, does not appear to be the case of our study, since river input is more important on the west than on the south coast.

Considering that the digestive gland is mainly a lipid reservoir and, therefore, has a high lipid content, it is the ideal organ to trace fatty acids as diet bioindicators (Phillips et al., 2001; Moltschaniwskyj & Johnston, 2006; García-Garrido et al., 2010). In our study the total lipid content ranged between 14.60% in northwest coast immature females, in line with values obtained by Rosa et al. (2004a), and 24.42% in south coast mature females. Contrary to what might be expected for a nutrient-rich area, the digestive glands of the northwest coast population present relatively low lipid content in comparison to the south coast population, in particular to the storage lipid triacylglycerol. This is probably related to nutrient seasonality. In March and April the pre-upwelling conditions in the northeast coast determine a low-fat content in sardine *Sardina pilchardus* (Bandarra et al., 2006) and horse-mackerel *Trachurus trachurus* (Bandarra et al., 2001), while on the other hand phytoplankton peaks in the south coast are common in early spring associated with the warmer and nitrogen-rich river plumes. Conditions in the south are then relatively favourable to the development of earlier phytoplankton blooms dominated by dinoflagellates (Navarro & Ruiz, 2006; Crespo et al., 2012), which is corroborated by the higher relative content in ARA, EPA and DHA produced by the community of primary producers (Dalsgaard et al., 2003).

The high digestive gland content of C18:1n9 ($\approx$11% in both study areas), which is indicative of deep waters prey species, and C16:0 ($\approx$20% in both study areas) which is indicative of the presence of herbivorous preys, actually suggest a diverse diet (Piché et al., 2010). Comparing both areas, the DHA/EPA ratio is exceptionally low in the north-western population. According to Dalsgaard et al. (2003), the DHA content should be higher relative to the EPA for a carnivorous species. In their study Rosa et al. (2004b) observed the possibility of a carnivorous preys on the northwest coast, while this study highlights a dominance of a carnivorous preys towards the south coast. A higher triacylglycerol content in the

South can in addition be related with the dominance of crustacean preys (Phillips et al., 2003) in the diet of the population of this area, which is also suggested by the $\delta^{15}N$ and $\delta^{13}C$ of the beaks.

It is, therefore, likely that during the period in analysis the diet of the south coast population had a relatively higher dominance of crustacean prey, those in turn recently fed on lipid-rich plankton.

As in the squids *Sepioteuthis lessoniana* and *Photololigo* sp. (Semmens, 1998), or the cuttlefish *Sepia officinalis* (Blanchier & Boucaud-Camou, 1984), the lipid class contents in the digestive gland do not correlate directly with the lipid contents of the oocytes in the gonad, especially for total lipid content, triacylglycerol and phospholipids (see Fig. 2). The lipid class contents of the digestive gland of mature females show significant differences between regions but this regional difference is not found in the gonads of the same females, which seems to indicate that the factors triggering oocytes maturity are independent of the nutritional value of reserves, even though females generally reach maturity beyond the optimum body mass and independently of gonad size (Lourenço et al., 2012). As income breeders that channel the energy directly from food to the gonad during maturation (Houston et al., 2006; Quetglas et al., 2011), the different levels in triacylglycerol and cholesterol noted in the *O. vulgaris* digestive glands between geographical areas should be expected to show in the gonads as a reflection of diet. Despite that, only the gonad fatty acid profile presents significant differences related with both area and maturity stage evidencing not only that the composition changes as maturation progresses but also that there are differences between areas at every stage of the process. The gonads of the south coast are richer in PUFA and in MUFA and the gonads of the northwest coast are richer in SFA especially C16:0 and C18:0. Nevertheless, the differences found in the ratio DHA/EPA between areas are marginally insignificant ($t = -1.86$, $df = 18$, P value $= 0.08$), indicating a low impact of trophic habitat in the quality of oocytes. These results partially aggree with the results obtained by Farías et al. (2011). In that study, the authors found a direct effect of diet in the DHA/EPA ratio for Patagonian red octopus *Enteroctopus megalocyathus*; here, although FA profile in the gonads revealed different between areas, the contents in DHA, EPA or DHA/EPA ratio are identical between the studied areas.

In conclusion, this study shows that, despite the variability observed in the stable isotopic signatures studied, $\delta^{15}N$ signature in beaks is a valuable bio-indicator to follow the diet history of different *O. vulgaris* populations, and the $\delta^{13}C$ signature has potential to assess feeding behaviour changes related with maturity in *O. vulgaris* females. Furthermore, specific lipid classes as the triacylglycerol and the cholesterol and its content in the digestive gland can be used as indicators of their nutritional availability in the local habitat. The fatty acid profile is a good indicator of both nutritional availability in the environment and also of gonads quality as shown by the main EFA content and the EPA/DHA ratio. The environmental availability of these EFA depends on seasonal cycles. Upwelling/downwelling events for example determine changes in the lipidic contents of the plankton and in turn of the prey of octopus, as evidenced by the higher total lipids and cholesterol content in the digestive glands of the animals from the south coast. In spite of their characteristics as income breeders, it is difficult to observe a direct relationship between the lipid class contents in the digestive gland and in the gonad, suggesting that a measure of regulation is exerted between food intake and oocyte formation.

Acknowledgments The authors wish to express their acknowledgment to the anonymous reviewers for their comments, which greatly improved the final version of this paper. The authors also want to thank to António Pedro Mendonça for assistance with the biological sampling and to Cristina Vidal for teaching and supervising the biochemical analyses. Data collection and analytical analysis were conducted under the Data Collection Framework (DCF/DG FISH (EC Regulation No. 665/2008 of the 14 July 2008)) and LARECO project (CTM2011-25929), supported partially by FEDER funds. The first author is supported by a PhD fellowship granted by the Portuguese Foundation for Science and Technology (FCT).

References

Athenstaedt, K. & G. Daum, 2006. The life cycle of neutral lipids: synthesis, storage and degradation. Cellular and Molecular Life Sciences 63: 1355–1369.

Auborg, S., I. Medina & R. Pérez-Martin, 1996. Polyunsaturated fatty acids in tuna phospholipids: distribution in Sn – 2 location and changes during cooking. Journal of Agricultural and Food Chemistry 44: 585–589.

Bandarra, N., I. Batista, M. Nunes & J. Empis, 2001. Seasonal variation in the chemical composition of horse-mackerel

(*Trachurus trachurus*). European Food Research and Technology 212: 535–539.

Bandarra, N. M., I. Batista, M. L. Nunes, J. M. Empis & W. W. Christie, 2006. Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). Journal of Food Science 62: 40–42.

Bergé, J.-P. & G. Barnathan, 2005. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. In Ulber, R. & Y. Gal (eds), Marine Biotechnology I. Springer-Verlag, Heidelberg: 49–125.

Blanchier, B. & E. Boucaud-Camou, 1984. Lipids in the digestive gland and the gonad of immature and mature *Sepia officinalis* (Mollusca: Cephalopoda). Marine Biology 80: 39–43.

Bligh, E. & W. Dyer, 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911–917

Boletzky, S. V., 1975. A contribution to the study of yolk absorption in the Cephalopoda. Zeitschrift für Morphologie der Tiere 80: 229–246.

Boucaud-Camou, E. & R. Boucher-Rodoni, 1983. Feeding and digestion in cephalopods. In Wilburn, K. M. (ed.), The Mollusca. Academic Press, New York: 149–187.

Budelmann, B. U., R. Schipp, & S. V. Boletzky, 1997. Cephalopoda. In Harrison, F. W. (ed.), Microscopic Anatomy of Invertebrates. Wiley-Liss, New York: 119–414.

Cherel, Y. & K. A. Hobson, 2005. Stable isotopes, beaks and predators: a new tool to study the trophic ecology of cephalopods, including giant and colossal squids. Proceedings of the Royal Society B: Biological Sciences 272: 1601–1607.

Cherel, Y. & K. A. Hobson, 2007. Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. Marine Ecology Progress Series 329: 281–287.

Cherel, Y., K. A. Hobson & H. Weimerskirch, 2005. Using stable isotopes to study resource acquisition and allocation in Procellariiform seabirds. Oecologia 145: 533–540.

Cherel, Y., V. Ridoux, J. Spitz & P. Richard, 2009. Stable isotopes document the trophic structure of a deep-sea cephalopod assemblage including giant octopod and giant squid. Biology Letters 5: 364–367.

Crespo, B. G., O. Espinoza-González, I. G. Teixeira, C. G. Castro & F. G. Figueiras, 2012. Structure of the microbial plankton community in the NW Iberian margin at the end of the upwelling season. Journal of Marine Systems 95: 50–60.

Dalsgaard, J., M. St John, G. Kattner, D. Müller-Navarra & W. Hagen, 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in marine biology 46: 225–340.

Darnaude, A. M., C. Salen-Picard, N. V. C. Polunin & M. L. Harmelin-Vivien, 2004. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138: 325–332.

Farías, A., J. C. Navarro, V. Cerna, S. Pino & I. Uriarte, 2011. Effect of broodstock diet on the fecundity and biochemical composition of eggs of the Patagonian red octopus (*Enteroctopus megalocyathus* Gould 1852). Ciencias Marinas 37: 11–21.

García-Garrido, S., I. Hachero-Cruzado, D. Garrido, C. Rosas & P. Domingues, 2010. Lipid composition of the mantle and digestive gland of *Octopus vulgaris* juveniles (Cuvier, 1797) exposed to prolonged starvation. Aquaculture International 18: 1223–1241.

García-Lafuente, J., J. Delgado, F. Criado-Aldeanueva, M. Bruno, J. Del Río & J. Miguel Vargas, 2006. Water mass circulation on the continental shelf of the Gulf of Cádiz. Deep Sea Research Part II: Topical Studies in Oceanography 53: 1182–1197.

Guerra, A., 1975. Determinación de las diferentes fases del desarollo sexual de *Octopus vulgaris* Lamarck, mediante unínidice de madurez. Investigacion. Pesquera 39: 397–416.

Hobson, K. A. & Y. Cherel, 2006. Isotopic reconstruction of marine food webs using cephalopod beaks: new insight from captively raised *Sepia officinalis*. Canadian Journal of Zoology 84: 766–770.

Houston, A. I., P. A. Stephens, I. L. Boyd, K. C. Harding & J. M. McNamara, 2006. Capital or income breeding? A theoretical model of female reproductive strategies. Behavioral Ecology 18: 241–250.

Huang, T., C. Chen, V. Wefler, A. Raftery, 1961. A stable reagent for the Liebermann-Buchardt reaction. Application to rapid serum cholesterol determination. Analytical Chemistry 33: 1405–1407

Jackson, G. D., P. Bustamante, Y. Cherel, E. A. Fulton, E. P. M. Grist, C. H. Jackson, P. D. Nichols, H. Pethybridge, K. Phillips, R. D. Ward & J. C. Xavier, 2007. Applying new tools to cephalopod trophic dynamics and ecology: perspectives from the Southern Ocean Cephalopod Workshop, February 2–3, 2006. Reviews in Fish Biology and Fisheries 17: 79–99.

Kanazawa, A., 2002. Sterols in marine invertebrates. Fisheries Science 67: 997–1007.

Katsanevakis, S. & G. Verripoulos, 2004. Den ecology of *Octopus vulgaris* Cuvier, 1797, on soft sediment: availability and types of shelter. Scientia Marina 68: 147–157.

Lee, R. F., W. Hagen & G. Kattner, 2006. Lipid storage in marine zooplankton. Marine Ecology Progress Series 307: 273–306.

Lepage, G & C. Roy, 1986. Direct transesterification of all classes of lipids in a one step reaction. Journal of Lipid Research 27: 114–120

Lourenço, S., A. Moreno, L. Narciso, Á. Gonzalez & J. Pereira, 2012. Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. Fisheries Research 127–128: 116–124.

Moltschaniwskyj, N. & D. Johnston, 2006. Evidence that lipid can be digested by the dumping squid *Euprymna tasmanica*, but is not stored in the digestive gland. Marine Biology 149: 565–572.

Navarro, G. & J. Ruiz, 2006. Spatial and temporal variability of phytoplankton in the Gulf of Cádiz through remote sensing images. Deep Sea Research Part II: Topical Studies in Oceanography 53: 1241–1260.

Navarro, J. C. & R. Villanueva, 2000. Lipid and fatty acid composition of early stages of cephalopods: an approach to their lipid requirements. Aquaculture 183: 161–177.

Nixon, M., 1985. Capture of prey, diet and feeding of *Sepia officinalis* and *Octopus vulgaris* (Mollusca: Cephalopoda) from hatchling to adult. Vie et Milieu 35: 255–261.

Phillips, K. L., G. D. Jackson & P. D. Nichols, 2001. Predation on myctophids by the squid *Moroteuthis ingens* around Macquarie and Heard Islands: stomach contents and fatty acid analyses. Marine Ecology Progress Series 215: 179–189.

Phillips, K. L., P. D. Nichols & G. D. Jackson, 2003. Dietary variation of the squid *Moroteuthis ingens* at four sites in the Southern Ocean: stomach contents, lipid and fatty acid profiles. Journal of the Marine Biological Association of the UK 83: 523–534.

Piché, J., S. J. Iverson, F. A. Parrish & R. Dollar, 2010. Characterization of forage fish and invertebrates in the Northwestern Hawaiian Islands using fatty acid signatures: species and ecological groups. Marine Ecology Progress Series 418: 1–15.

Post, D., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi & C. G. Montaña, 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotopes analyses. Oecologia 152: 179–189.

Quetglas, A., F. Ordines & M. Valls, 2011. What drives seasonal fluctuations of body condition in a semelparous income breeder octopus? Acta Oecologica 37: 476–483.

Quinn, G. & M. Keough, 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press.

Raheja, R., C. Kaur, A. Sing & A. Bhatia, 1973. New colorimetric method for the quantitative determination of phospholipids without acid digestion. Journal of Lipid Research 14: 695–697

Relvas, P., E. D. Barton, J. Dubert, P. B. Oliveira, Á. Peliz, J. C. B. Da Silva & A. M. P. Santos, 2007. Physical oceanography of the western Iberia ecosystem: latest views and challenges. Progress in Oceanography 74: 149–173.

Rosa, R., P. R. Costa & M. L. Nunes, 2004a. Effect of sexual maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca: Cephalopoda). Marine Biology 14: 563–574.

Rosa, R., A. M. Marques, M. L. Nunes, N. Bandarra & C. Sousa Reis, 2004b. Spatial-temporal changes in dimethyl acetal (octadecanal) levels of *Octopus vulgaris* (Mollusca, Cephalopoda): relation to feeding ecology. Scientia Marina 68: 227–236.

Semmens, J., 1998. An examination of the role of the digestive glands of two loliginid squids, with respect to lipid: storage or excretion? Proceedings of the Royal Society B: Biological Sciences 265: 1685–1690.

Semmens, J., G. Pecl, R. Villanueva, D. Jouffre, I. Sobrino, I. Sobrino, J. B. Wood & P. R. Rigby, 2004. Understanding octopus growth: patterns, variability and physiology. Marine & Freshwater Research 55: 367–377.

Shulman, G. E. & R. M. Love, 2006. The biochemical ecology of marine fishes. Advances in Marine Biology 36: 351 pp.

Sieiro, M. P., S. P. Aubourg & F. Rocha, 2006. Seasonal study of the lipid composition in different tissues of the common octopus (*Octopus vulgaris*). European Journal of Lipid Science and Technology 108: 479–487.

Smith, C. D., 2003. Diet of *Octopus vulgaris* in False Bay, South Africa. Marine Biology 143: 1127–1133.

Smith, J. M., G. J. Pierce, A. F. Zuur, H. Martins, M. C. Martins, F. Porteiro & F. Porteiro, 2011. Patterns of investiment in reproductive and somatic tissues in the loliginid squid Loligo forbesii and Loligo vulgaris in Iberian and Azorean waters. Hydrobiologia 670: 201–221

Stowasser, G., 2004. Squid and their prey: insights from fatty acid and stable isotope analysis. Thesis presented for the degree of doctor of Philosophy at the University of Aberdeen. University of Aberdeen. Aberdeen.

Tocher, D. R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquaculture Research 41: 717–732.

Vander Zanden, M. J. & J. B. Rasmussen, 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography 46: 2061–2066.

Villanueva, R. & M. D. Norman, 2008. Biology of the planktonic stages of benthic octopuses. Oceanography and Marine Biology: An Annual Review 46: 105–202.

Zar, J., 1999. Biostatistical Analysis, 4th ed. Prentice Hall, Upper Saddle River.

Zuur, A. F., E. N. Ieno & G. M. Smith, 2007. Analysing Ecological Data. Springer, New York.

Hydrobiologia (2014) 725:47
DOI 10.1007/s10750-013-1750-z

ERRATUM

Erratum to: Does the trophic habitat influence the biochemical quality of the gonad of *Octopus vulgaris*? Stable isotopes and lipid class contents as bio-indicators of different life-cycle strategies

Sílvia Lourenço · Luís Narciso · Ángel F. Gonzalez · João Pereira · Santiago Aubourg · José C. Xavier

Published online: 22 November 2013

Erratum to: Hydrobiologia
DOI 10.1007/s10750-013-1717-0

Due to an unfortunate turn of events, the penultimate author's surname appeared incorrectly in the original publication and should have read Aubourg. The correct representation of the authors' names is listed above and should be treated as definitive by the reader.

The online version of the original article can be found under doi:10.1007/s10750-013-1717-0.

S. Lourenço (✉) · L. Narciso
Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Avenida Nossa Senhora do Cabo 939, 2750-374 Cascais, Portugal
e-mail: salourenco@fc.ul.pt

S. Lourenço · Á. F. Gonzalez · S. Aubourg
Instituto de Investigaciones Marinas de Vigo CSIC, C/Eduardo Cabello, 6, 36208 Vigo, Spain

S. Lourenço · J. Pereira
Departamento do Mar e Recursos Marinhos, Instituto Português do Mar e da Atmosfera, I.P.Avenida de Brasília, 1449-006 Lisbon, Portugal

J. C. Xavier
Department of Life Sciences, Institute of Marine Research, University of Coimbra, 3001-401 Coimbra, Portugal

Fisheries Research 152 (2014) 74–85

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Essential habitats for pre-recruit *Octopus vulgaris* along the Portuguese coast

A. Moreno[a,*], S. Lourenço[a,b], J. Pereira[a], M.B. Gaspar[a], H.N. Cabral[c], G.J. Pierce[d,e], A.M.P. Santos[a]

[a] Instituto Português do Mar e da Atmosfera (IPMA), Av. Brasília, 1449-006 Lisboa, Portugal
[b] Centro de Oceanografia, Laboratório Marítimo da Guia, Faculdade de Ciências, Universidade de Lisboa, Avenida Nossa Senhora do Cabo 939, 2750-374 Cascais, Portugal
[c] Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[d] Oceanlab, University of Aberdeen, Newburgh, Aberdeenshire, AB41 6AA, UK
[e] CESAM & Departamento de Biologia, Universidade de Aveiro 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form 19 April 2013
Accepted 10 August 2013

Keywords:
Octopus vulgaris
Pre-recruits
Recruitment grounds
Habitat
EFH
Portugal

ABSTRACT

The exploitation of the common octopus *Octopus vulgaris* in Portugal increased 50% in the last 20 years, largely motivated by the depletion of many fish stocks. Recently, the biomass of this fishery resource sharply decreased in some areas causing serious concern among local fishermen and an effort is underway to advise on novel and sustainable management measures. In this context, the octopus pre-recruit aggregations along the Portuguese coast are identified using georeferenced fishery-independent data, from autumn and winter sampling between 1996 and 2008. The relationships between pre-recruit aggregations and several environmental variables are analyzed to characterize their essential habitats (EFH). Pre-recruits are distributed throughout the Portuguese coast aggregated in 8 distinct recruitment grounds located on the middle-shelf at 11–19 km from the coastline, which are characterized by average bottom depths of 65–110 m and are associated to major rivers and lagoon systems. Within each season pre-recruit abundance is much higher in the south region, while pre-recruit aggregations on the northwest coast showed high inter-annual and seasonal variation driven by environmental variability. The western zone adjacent to Ria Formosa lagoon (southern coast) was identified as the main recruitment ground for *O. vulgaris* along the Portuguese coast. This is supported by the higher abundance of pre-recruits and by the recurrence of their presence in this area over the years analyzed, both in autumn and winter. The effects of physical variables on pre-recruit abundance modelled with generalized additive models (GAM) showed important regional differences. Bottom salinity and river runoff are the environmental variables that have most impact on pre-recruit distribution and abundance on the west coast, regardless of any seasonal effects. On the other hand, temperature imposes distinct seasonal and regional limitations on pre-recruit distribution, both on the NW and S regions. Pre-recruit preferential habitat is characterized by bottom temperatures of 14 °C, salinity values around 36.0, low precipitation (average <200 mm), and coarse sediments (in which they find shelters to escape predation). Some of the octopus recruitment grounds identified are located in areas under intense fishing pressure, both by artisanal fisheries using traps and by bottom trawling. Thus, their value for the sustainability of the octopus fishery should be taken into consideration in future marine management strategies.

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 (1986–2011). It is captured mainly with traps (~90%), thus having a major importance in the Portuguese artisanal fishery, especially in the south region (Algarve), where it accounts for more than 20% of the fishing activity income (DGRM, 2012). The exploitation of octopus in Portugal increased 50% in the last 20 years, largely motivated by the depletion of many finfish stocks. Recently, the landed biomass of this fishery resource has sharply decreased in some areas causing serious concern among local fishermen and an effort is

* Corresponding author. Tel.: +351 213027113.
E-mail addresses: amoreno@ipma.pt, amorenomarques@gmail.com (A. Moreno).

0165-7836/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.fishres.2013.08.005

underway by scientists to advise on novel and sustainable management measures.

O. vulgaris has a short life cycle of 12–18 months (Iglesias et al., 2004; Katsanevakis and Verriopoulos, 2006). The spawning season extends, in general, throughout the year (Hastie *et al.*, 2009). Over the NW Portuguese shelf spawning occurs in two main peaks (April and August) and on the south shelf a single peak is generally detected in summer (Lourenço et al., 2012). Paralarvae are planktonic and settle in the benthic habitat of adults at ca 173 mg total weight (Mangold, 1983; Villanueva, 1995). After settlement animals grow fast at ca. 1.2–1.6% d^{-1}, reaching the fisheries minimum landing weight (750 g) at 9–10 months old (Domain et al., 2000; Katsanevakis and Verriopoulos, 2006). *O. vulgaris* is mainly a coastal species (0–100 m depth) with a wide geographic distribution. Density is low between 100 and 200 m and only few specimens have been found beyond the continental shelf break (Belcari and Sartor, 1999; Quetglas et al., 2000; Silva et al., 2002; Garofalo et al., 2010). Sub-adults and spawners generally have a distinct depth range distribution (Sánchez and Obarti, 1993; Hernández-Garcia et al., 1997; Faraj and Bez, 2007). Of the environmental variables that may affect the spatial distribution and abundance of sub-adults, seawater temperature is thought to be the most important one (Garofalo et al., 2010).

Habitat modelling for marine animals is increasingly used as a management tool, to support conservation and sustainable exploitation. "Essential Fish Habitat" (EFH) is the habitat identified as essential to the ecological and biological requirements for critical life history stages of exploited species, and which may require special protection to improve stock status and long term sustainability (e.g. Ardizzone, 2006; Valavanis and Smith, 2007). EFH may be modelled based on the abundance of animals at specific life cycle stages (e.g. sub-adults) and the habitat identified as the aggregation of abiotic and biotic parameters where individuals of that specific life cycle stage are concentrated (e.g. recruitment grounds). Successful modelling depends on knowledge of species biology and ecology, thus permitting selection of appropriate variables, measured at an appropriate scale (see Valavanis et al., 2008).

The *O. vulgaris* life cycle characteristics increase resilience of this species to high fishing pressure. At the same time *O. vulgaris* populations are vulnerable to overfishing because of non-overlapping generations (Boyle and Rodhouse, 2005), and the sub-adult phase (the pre-recruits) is therefore a key life stage on which we may concentrate fishery management to ensure sustainability of this heavily exploited species. The present study aims to, firstly identify areas along the Portuguese coast where pre-recruits of *O. vulgaris* aggregate, using georeferenced fishery-independent data, that may be protected in future management options; and secondly, to analyze the relationships between pre-recruit abundance and several abiotic environmental variables to delineate their optimum habitat.

2. Materials and methods

2.1. Survey sampling

Two sets of bottom trawl surveys carried out on the Portuguese continental coast onboard R/V Noruega and R/V Capricórnio were selected for the analysis of *O. vulgaris* pre-recruit aggregations, the autumn and winter surveys. The main objective of these research surveys is to estimate indices of abundance and biomass of the most commercially important fish and crustacean species. For our study we selected only the surveys which used a bottom trawling net suitable to sample benthic species, the "FGAV019", 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 sampling area covered latitudes 36.7° to 41.8° N and longitudes 7.47° to 10.0° W in the NE Atlantic. Both datasets were obtained following a similar

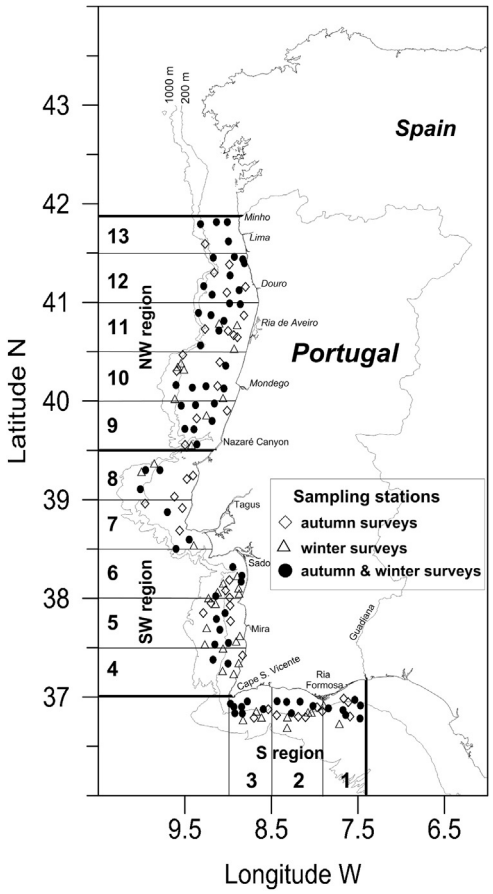


Fig. 1. Sampling stations in autumn and winter surveys. Location of survey sampling areas (1–13), regions (NW, SW, S) and main rivers and coastal lagoons.

depth-stratified sampling design, with ca. 70–80 hauls distributed along the Portuguese continental shelf and slope (Fig. 1). The tow duration varied between 20 and 60 min, but 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 1. In each sampling station, all of the *O. vulgaris* captured were weighted (individual body weight, to the nearest g) and measured (mantle length, to the nearest 0.5 mm).

2.2. Environmental data

The following environmental variables were selected and assigned to each sampling station. Satellite-derived sea surface temperature data (SST) were obtained for each sampling station from the AVHRR Pathfinder V.5 provided by NASA-JPL-Physical Oceanography Distributed Active Archive Center (PO.DAAC). Sea bottom temperature (SBT), sea surface salinity (SSS), and sea bottom salinity (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 winter 2005 cruise. SBT data to fill in these gaps were obtained from CTD casts extracted from NODC and ICES databases. Bottom sediment type (BS) in each sampling station was classified based on the fishery charts from the Instituto Hidrográfico as gravel (gv), coarse sand (cs), sand (s), mud (md) and rocky mud (rm). River runoff (RR) from the main rivers and rainfall (RA) were extracted from the Sistema Nacional de Informação de Recursos Hídricos (http://snirh.pt).

Table 1
Octopus vulgaris sampling details for each research survey: number of fishing hauls, number of fishing hauls with *O. vulgaris* catches (Fishing hauls +), in situ temperature and salinity data (CTD), number of individuals captured and sampled (N), percentage of pre-recruits and mean body weight of the pre-recruits (BWJ).

Cruise	Start date	End date	Fishing hauls	Fishing hauls +	CTD	*N*	% Pre-recruits	BWJ (g)
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
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

2.3. Data analysis

Octopus with body weight below the fisheries minimum landing weight (750 g) captured during the survey cruises listed in Table 1, were classified as pre-recruits and their distribution and abundance used to identify *O. vulgaris* recruitment grounds. Catches were converted into an abundance index as number of pre-recruits per hour trawling.

The spatial distribution of pre-recruits was analyzed and mapped with the geostatistical interpolation method, Krigging (Cressie, 1991), implemented in the software SURFER 9.0. Pre-recruit abundance (PR) data were interpolated separately by Krigging, using a linear variogram with no nugget effect, for each cruise and also averaged for autumn and winter cruises. Distinct recruitment grounds were identified as those areas centred at pre-recruit distribution centroids with a mean PR ≥ 5 ind h^{-1} (in at least one season). Additionally, to assess the relevance of each recruitment ground for the common octopus pre-recruits, an index of exclusiveness, EI was calculated as the ratio between PR and the total species abundance. To verify whether the recruitment grounds were located in the same area consistently through time, an index of persistence, PI (adapted from Garofalo et al., 2010) was also estimated as the proportion of cruises within each season during which a given recruitment ground was actually used.

To analyze the spatial distribution, the sampled area was divided into 3 regions, which are environmentally different, and 13 areas. To analyze distribution with depth (DepthZ), the sampling stations were also assigned to the inner-shelf (is, <40 m), middle-shelf (ms, 40–90 m), outer-shelf (os, 90–200 m), and slope (s, >200 m). The distance of each sampling station to the coast line (Dcoast) was also estimated. Total pressure (PRS) in each sampling station was estimated from bottom depth. A measure of RR by cruise and area was calculated as the total runoff in each of the 13 areas. A measure of RA by cruise and area was calculated as the total rainfall for the period of each survey cruise recorded in the main coastal city in each of the 13 areas. These values were replicated and assigned to each sampling station within each area. Mean pre-recruit abundance was calculated in relation to categorized temporal, spatial and physical variables. The effects of these explanatory variables on pre-recruit abundances 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 Region and the other variables and the interactions between Season and the other variables, were also tested by two-way ANOVA. The relationships between log-transformed pre-recruit abundance (log PR) and the continuous physical explanatory variables: Dcoast, PRES, SST, SBT, SSS, SBS, RA, and log-transformed RR (log RR) were further investigated using Generalized Additive Models (GAMs), thereby allowing non-linearity in the relationships to be taken into account. Since log PR was normally distributed we used a Gaussian GAM with identity link. The nominal variables Year and BS were also included in models. For all continuous explanatory variables, degrees of freedom were constrained to be less than 5 to avoid overfitting. Models were fitted using a backwards selection starting with full models and removing explanatory variables with non-significant partial effects. The Akaike Information Criterion (AIC) was used to choose the best fitting model (lowest AIC). More information about these techniques can be found in Zuur et al. (2007). Preferred habitat (highest mean PR) and habitat limits (PR < 1 ind h^{-1}) for *O. vulgaris* pre-recruits within the sampled area were estimated as proxies of EFH for this key life stage.

3. Results

3.1. Environmental variation along the Portuguese continental shelf

Over the Portuguese continental shelf SST is much higher in autumn than in winter and was ca. 2–4 °C lower in the NW region than in the S region. Within the period analysed autumn SST ranged between 14.0 °C and 21.7 °C and winter SST ranged between 11.6 °C and 16.6 °C (Fig. 2a). There was little seasonal variation in the mean SBT (13.9 ± 0.9 °C), but SBT was on average ~1.5 °C lower in the NW region than in the S region. Autumn SBT ranged between 11.4 °C and 17.3 °C, and winter SBT ranged between 11.7 °C and 15.8 °C (Fig. 2b). In autumn the water column was still stratified and important differences existed between SST and SBT, especially in the SW and S regions (~5 °C). On the contrary SST was only slightly higher than SBT during the winter period of water column mixing, namely in the NW region (~0.5 °C).

The expected positive correlation between temperature and salinity may be observed in the spatial variation of salinity which is similar to that of temperature, decreasing with increasing latitude and also away from the influence of the highly saline Mediterranean waters. Despite this fact, both mean SSS and mean SBS along the Portuguese shelf were lower in autumn (when temperature was higher) than in winter (Fig. 2c and d). Rainfall levels during autumn and winter were similar between areas 1 and 10, but towards the north rainfall increased substantially, especially during autumn (Fig. 3a). The highest river runoff levels occurred in autumn, located in areas 7, 10, 12 and 13 (Fig. 3b), associated mainly with the rivers Tagus, Mondego, Douro and Lima, respectively. The low saline waters from these river discharges generally spread along the west coast in a poleward direction, therefore with no influence in the areas south of area 7. The seasonal differences in salinity derive most likely from the higher levels of autumn rainfall and consequent river discharges, which contributed to a decrease in salinity of coastal waters which is more significant in autumn than in winter.

3.2. Recruitment grounds

O. vulgaris pre-recruits were quite frequent (55–83% of total octopus catches) both during the autumn and winter research surveys and were represented by specimens with a mean body weight of 300–450 g (Table 1). They were widely distributed

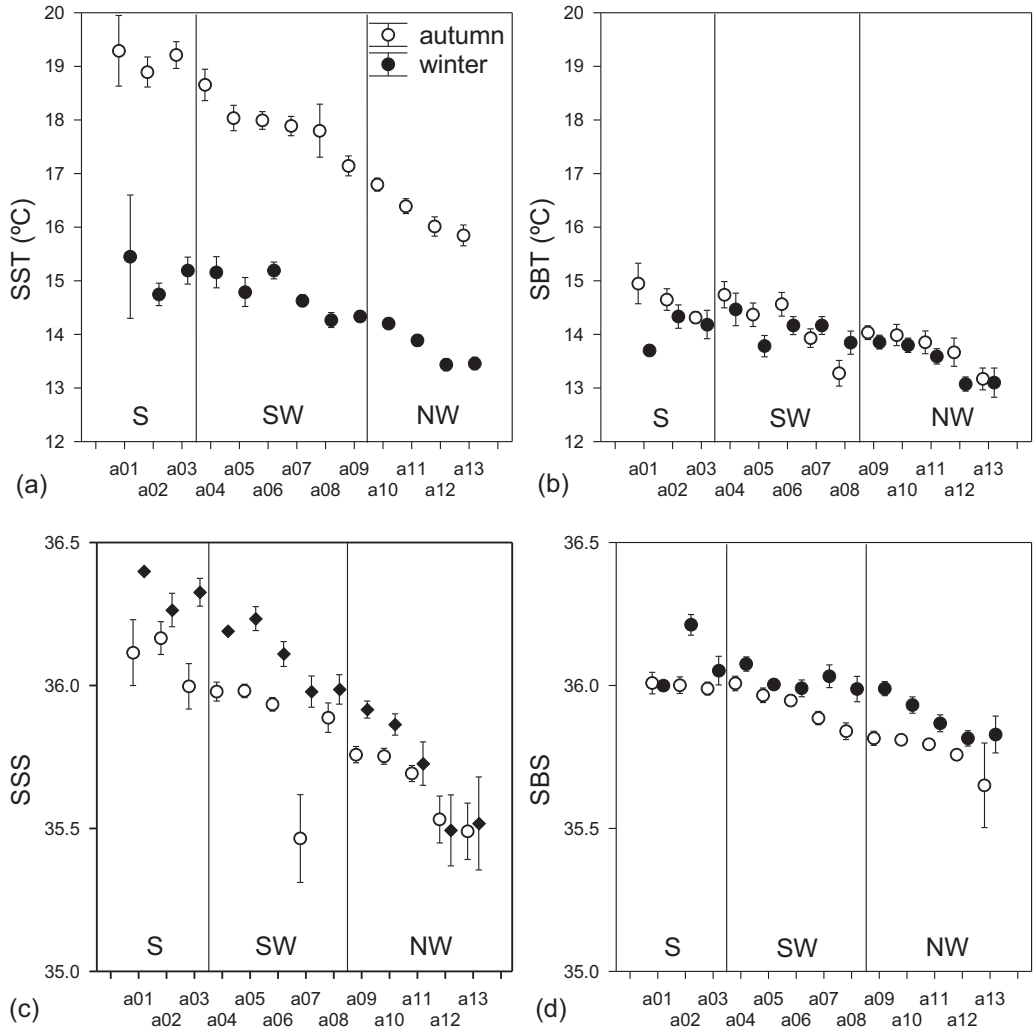


Fig. 2. Spatial variation of (a) mean sea surface temperature (SST), (b) mean sea bottom temperature (SBT), (c) mean sea surface salinity (SSS), and (d) mean sea bottom salinity (SBS) during the autumn and winter surveys in areas a01 to a13 within the northwest (NW), southwest (SW) and south (S) regions. Vertical bars denote standard error.

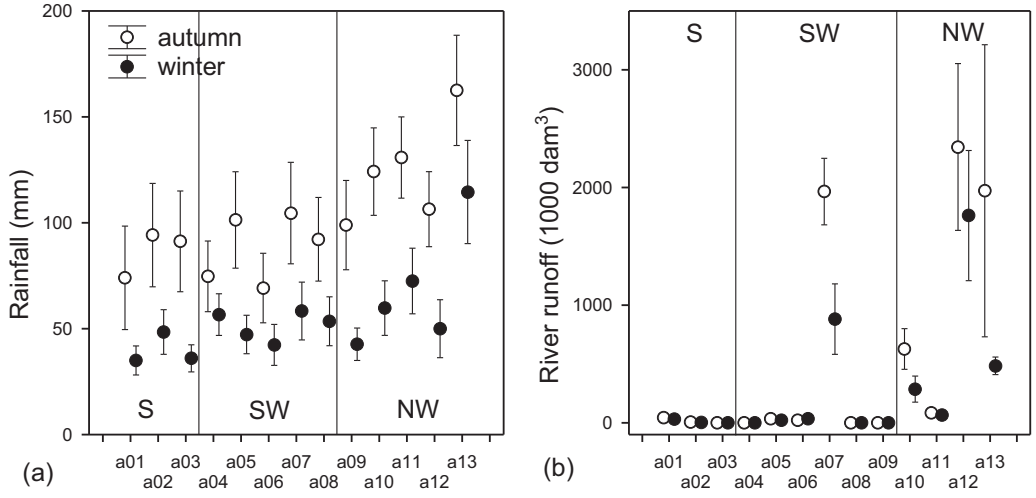


Fig. 3. Spatial variation of (a) mean rainfall and (b) mean river runoff in autumn and winter surveys in areas a01 to a13 within the northwest (NW), southwest (SW) and south (S) regions. Vertical bars denote standard error.

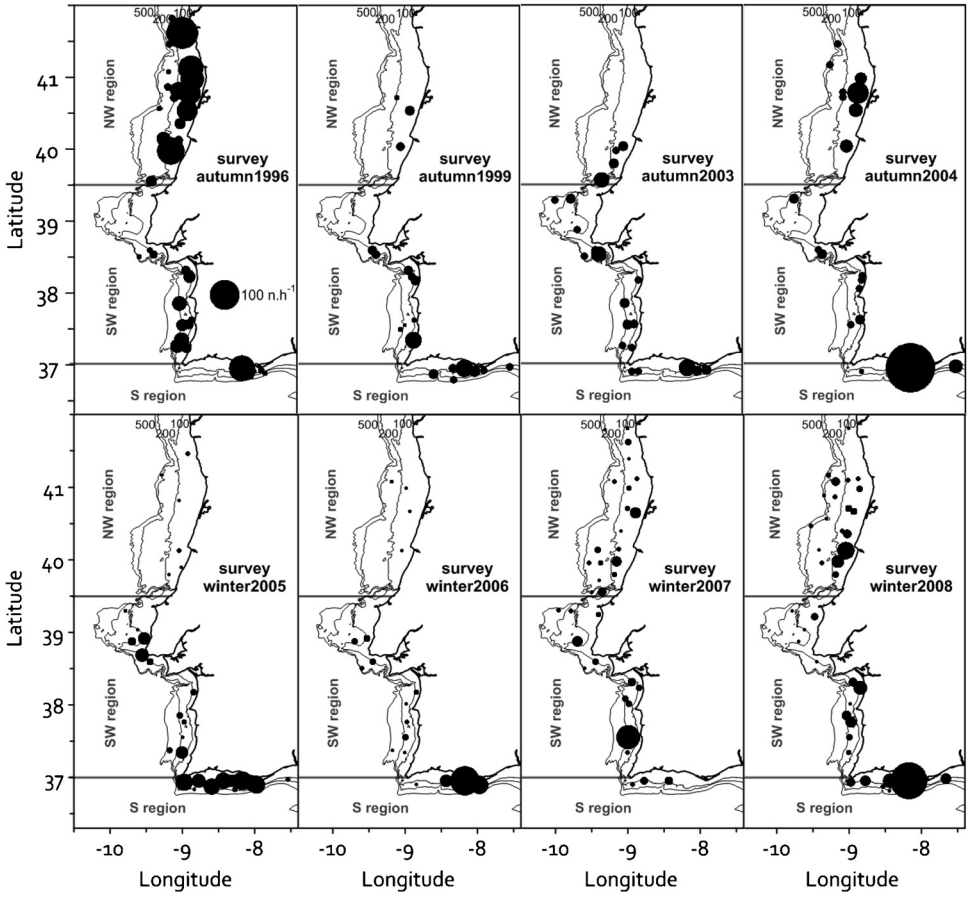


Fig. 4. Distribution and abundance of *O. vulgaris* pre-recruits in (a) the autumn and (b) the winter survey cruises.

along the Portuguese coast, but an important seasonality in their distribution and abundance was apparent: in winter, octopus pre-recruits were more abundant and their distribution shifted towards the south (Fig. 4). In winter abundance was twice as high (mean PR ≈ 6 ind h^{-1}) as in autumn (mean PR ≈ 3 ind h^{-1}), in particular during the winter 2005 and winter 2008 surveys. However, in the NW region, pre-recruit abundance was higher in autumn, especially in the autumn1996 survey. Within each season abundance was much higher in the S region, especially in area 2, where the concentration of pre-recruits was recurrent each year.

Pre-recruits were found aggregated in eight distinct grounds, A to H. In the NW region three recruitment grounds were identified during autumn (Fig. 5a), located close to the mouth of river Lima (A, only present in autumn1996 survey); close to river Douro and the lagoon system of Ria de Aveiro (B); and south of river Mondego (C). During winter (Fig. 5b), the recruitment ground B shifted to the southern part of Ria de Aveiro, partially merging with C. In the SW region, three recruitment grounds were also identified: D located at ≈140 m in autumn in the vicinity of the Nazaré canyon and shifted southwards closer to the mouth of river Tagus in winter; E in area 6, close to the estuary of the river Sado with higher PR in winter; and the recruitment ground F in areas 4 and 5 extending from the coastline to offshore of the 130 m isobath in winter. In the S region the highest concentration of pre-recruits was located west of the lagoon system of Ria Formosa (H) in autumn (mean above 30 ind h^{-1}). The whole S region could be considered a single recruitment ground in winter. Despite this, based on spatial differences in abundance within this region, two distinct areas of concentration of pre-recruits may be identified: the recruitment ground G centred in area 3; and H with the highest concentration of pre-recruits (winter mean above 80 ind h^{-1}), centred in area 2 west of the lagoon system of Ria Formosa, as was in autumn, but spreading eastwards into area 1.

The location of each recruitment ground is summarized in Table 2. Overall, the most persistent recruitment grounds, with PI = 1, were the recruitment grounds F and G in winter and H both in autumn and winter. Additionally, these grounds were important habitats in particular for pre-recruits, showing high levels of exclusiveness (EI). The recruitment grounds in the NW region showed high EI but low PI, giving evidence of higher inter-annual variation in pre-recruit distribution within this region.

3.3. Environmental effects and essential habitat

ANOVA results indicated that the individual effects of all temporal, spatial and physical variables analyzed on pre-recruit abundance were significant. The effects of the explanatory variables Year, DepthZ, Dcoast, BS, SST and SBT were significantly dependent on Region, but independent from any seasonal effects (except for SST) (Table 3). The effects of the physical variables related to water salinity: SSS, SBS, RA, and RR, were independent both of Region and Season.

To further investigate the relationships between physical variables and pre-recruit abundance, GAMs were fitted separately for the NW, SW and S regions using the above mentioned "explanatory variables": Region interactions. The optimum models are listed in Table 4. The best-fit GAM for pre-recruit abundance in the NW region explains about 47% of deviance and has the form: log PR ~ 1 + factor(Year) + factor(Season) + factor(BS) + s(Dcoast) + s(SST) + s(SBS) + s(log RR). The best-fit GAM for the SW

Table 2
Recruitment grounds identified by krigging of pre-recruit abundance (PR). PI = Persistence Index and EI = Exclusiveness Index.

Region	Recruitment ground	Season	Centroid PR (n h^{-1})	Centroid lat°N/long°W	Centroid depth (m)	PI	EI
NW	A	Autumn	12.5	41.624/8.997	51	0.33	0.93
		Winter	–	–	–	0	–
	B	Autumn	14.4	40.798/8.736	68	0.5	0.64
		Winter	12.5	40.617/8.871	30	0.5	0.83
	C	Autumn	7.8	39.983/9.164	84	0.5	0.95
		Winter	11.2	40.101/9.028	51	0.5	0.93
SW	D	Autumn	5.1	39.520/9.373	82	0.25	0.78
		Winter	9.3	38.716/9.566	55	0.75	0.72
	E	Autumn	–	–	–	0	–
		Winter	12.3	38.258/8.808	5	0.75	0.63
	F	Autumn	–	–		0	–
		Winter	16.3	37.566/8.998	181	1	0.86
S	G	Autumn	–	–	–	0	–
		Winter	26.5	36.878/8.591	185	1	0.90
	H	Autumn	33.9	36.991/8.152	36	1	0.64
		Winter	87.0	36.990/8.152	39	1	0.83

region explains ca. 44% of deviance and has the form: log PR ~ 1 + factor(Year) + s(Dcoast) + s(SBS) + s(log RR). Finally, the best-fit GAM for the S region explains ca. 69% of deviance and has the form: log PR ~ 1 + factor(BS) + s(PRS) + s(SST).

Preferred habitat and habitat limits of *O. vulgaris* pre-recruits are summarized in Table 5. The variation in pre-recruit abundance with the categorical variables Year, Season, DepthZ, Dcoast, and BS in each region is depicted in Fig. 6. Pre-recruit abundance presented an important inter-annual variation within each region (Fig. 6a), particularly high in autumn 1996 on the NW region and in autumn 2004 on the S region. During winter, the abundance was very high in 2005, 2006 and 2008 in the S region and in 2007 in the SW region. In the west regions the Year effect explained some variability not accounted by the other explanatory variables considered (Table 4). On the other hand, the Year effect was not retained in the optimum GAM for the S region.

Pre-recruit' habitat includes a variety of bottom sediments, but they seemed to concentrate preferentially over large grain sediments in relation to fine sand or mud (Fig. 6b). Their abundance was the highest over gravel in the NW region and coarse sand in the S region (gravel not sampled). The effect of BS in the SW region was not significant (Table 4), but the overlay of the recruitment grounds on a bottom sediments type map (online figure) showed that those in the SW region were located over several BS, all neighbouring rock outcrops.

Pre-recruits were concentrated mainly in the middle-shelf at a mean depth of 79 m (±44 SD) on the NW region, 108 m (±45 SD) in the SW region, and 66 m (±31 SD) in the S region, with insignificant seasonal variation in the preferred depth zone (Fig. 6c). The highest abundances were located at 19 km (±9 SD) from the coastline in the NW region, 11 km (±6 SD) in the SW region, and 13 km (±3 SD) in the S region (Fig. 6d). The distribution limit was slightly deeper in winter (360 m) than in autumn (332 m), but abundances on the slope deeper than 350 m (PRS > 35.7 atm) were consistently rather low (Table 5, PR < 1 ind h^{-1}). In the SW region, because the continental shelf is narrow and steep, pre-recruits aggregated on the middle-shelf deeper than in the other regions (see also Table 2). Nevertheless, distribution on the west coast was mainly affected by the distance to the coast line (Dcoast) rather than by total pressure (PRS). Abundance decreased linearly with increasing distance to the coast line; gradually on the wider continental shelf of the NW region (Fig. 7a), and more obviously on the narrow and steeper continental shelf of the SW region (Fig. 7e). On the other hand, pre-recruit distribution in the S region was mainly affected by PRS, with abundance

Table 3
Effects of temporal, spatial and environmental variables on pre-recruit abundance.

Pre-recruits	Mean Square	df	Residual mean square	Residuals df	*F* value	*p*
Year	1.078	7	0.204	378	5.293	***
Season	1.884	1	0.215	384	8.753	**
Region	4.747	2	0.196	383	24.230	***
Area	1.192	12	0.188	373	6.328	***
DepthZ	2.109	2	0.210	383	10.058	***
Dcoast	1.242	10	0.192	375	6.459	***
BS	1.649	4	0.205	381	8.064	***
SST	0.582	7	0.213	378	2.734	**
SBT	0.967	4	0.212	380	4.563	**
SSS	1.136	3	0.190	324	5.980	***
SBS	3.543	2	0.177	323	19.994	***
RA	1.125	4	0.210	381	5.357	***
RR	0.965	3	0.215	377	4.498	**
Region * Year	0.860	14	0.151	362	5.709	***
Region * Season	3.148	2	0.174	380	18.134	***
Region * DepthZ	1.615	5	0.122	564	13.247	***
Region * Dcoast	0.281	17	0.144	545	1.955	*
Region * BS	0.727	7	0.173	372	4.198	***
Region * SST	0.572	13	0.166	363	3.170	***
Season * SST	1.241	2	0.202	375	6.134	**
Region * SBT	1.041	7	0.179	371	5.822	***

* Summary of ANOVA results for significant effects (<0.05).
** Summary of ANOVA results for significant effects (<0.01).
*** Summary of ANOVA results for significant effects (<0.001).

Table 4
Results for the optimum GAMs fitted to log PR (log transformed pre-recruit abundance) on the northwest (NW), southwest (SW) and south (S) regions. Estimates, SE, *t*-ratio and associated parameters are given for the nominal variables. The reference year is 1996, the reference season is winter, and the reference bottom sediment types are gravel (NW and SW) or coarse sand (S). Edf, *F*-statistic and associated probabilities are given for smoothers and the parametric terms.

Region	GAM variables	Estimate	SE	*t*-Ratio	*p*-Value	Edf	*F*-statistic	*p*-Value	Deviance explained	AIC
NW	Year					7	7.61	***	46.5%	104.5
	1999	−0.368	0.082	−4.48	***					
	2003	−0.312	0.077	−4.07	***					
	2004	−0.220	0.066	−3.32	**					
	2005	0.072	0.075	0.96	ns					
	2006	−0.132	0.048	−2.74	**					
	2007	0.276	0.062	4.46	***					
	2008	0.043	0.058	0.75	ns					
	Season					1	4.76	*		
	Autumn	0.201	0.092	2.18	*					
	BS					4	4.37	***		
	Coarse sand	−0.193	0.088	−2.18	*					
	Sand	−0.325	0.089	−3.65	***					
	Mud	−0.301	0.088	−3.42	***					
	Rocky mud	−0.042	0.216	−0.19	ns					
	Dcoast					1.00	21.3	***		
	SST					3.32	3.81	**		
	SBS					2.24	5.43	**		
	log RR					2.09	8.95	***		
SW	Year					7	3.73	***	44.3%	91.3
	1999	−0.173	0.107	−1.62	ns					
	2003	−0.056	0.114	−0.49	ns					
	2004	−0.287	0.107	−2.68	**					
	2005	0.128	0.115	1.11	ns					
	2006	−0.151	0.116	−1.30	ns					
	2007	0.010	0.114	0.08	ns					
	2008	0.082	0.118	0.70	ns					
	Dcoast					1	24.80	***		
	SBS					3.61	4.28	**		
	log RR					1.50	3.85	*		
S	BS					3	4.39	***	68.7%	83.4
	Sand	−0.559	0.175	−3.20	**					
	Mud	−0.357	0.115	−3.12	**					
	Rocky mud	−0.228	0.141	−1.62	ns					
	PRS					3.77	22.50	***		
	SST					3.08	9.83	***		

SE, standard error; Edf, estimated degrees of freedom; AIC, Akaike Information Criterion.
* $p < 0.05$
** $p < 0.01$.
*** $p < 0.001$.
ns: $p > 0.05$.

abruptly decreasing until depths of 25 atm and with no further effect in deeper waters (Fig. 7h).

Over the continental shelf pre-recruits were found associated preferentially with SST of 15.3 °C (±1.3 SD) in the NW region and with SST of 16.0 °C (±1.6 SD) on the SW (Table 5). In the NW region, the effect of SST was non-linear showing some seasonal dependence: abundance increases with increasing local winter SST and with increasing local autumn SST, i.e. the main recruitment grounds are located under the warmest temperatures available and the general SST effect shows a more complex nonlinear relationship (Fig. 7b). On the other hand, abundance decreases with increasing SST in the S region (Fig. 7i). In this region, over the continental shelf, pre-recruits were found associated preferentially with SST of 16.0 °C (±1.8 SD). The effect of SBT showed some regional dependence, but regional SBT differences were of small magnitude and the effect of this explanatory

Table 5
Preferred habitat and habitat limits of *O. vulgaris* pre-recruits. Regional and/or seasonal differences reported only when significant.

Variables	Sampled habitat	Preferred habitat (mean ± SD)	Limits (PR < 1 ind h^{-1})
Region	NW, SW, S	S	–
Area	1–13	2	–
DepthZ	NW & SW: is, ms, os, s; S: ms, os, s	ms	–
Depth	NW: 20–650 m; SW: 35–650 m; S: 45–675 m	NW: 79 ± 44 m; SW: 108 ± 45 m; S: 66 ± 31 m	>350 m
Dcoast	NW: 2–60 km; SW: 2–45 km; S: 4–43 km	NW: 19 ± 9 km; SW: 11 ± 6 km; S: 13 ± 3 km	None
BS	gv, cs, s, md, rm	NW: gv; S:cs	None
SST	NW: 11.6–19.6 °C; SW: 13.1–20.6 °C; S: 13.7–22.0 °C Autumn: 14.0–22.0 °C; winter:11.6–16.9 °C	NW: 15.3 ± 1.3 °C; SW: 16.0 ± 1.6 °C; S: 16.0 ± 1.8 °C Autumn: 17.3 ± 1.5 °C; winter: 14.8 ± 0.8 °C	<13 °C <13 °C
SBT	NW: 11.4–17.3 °C; SW: 10.3–16.7 °C; S: 11.7–17.0 °C	NW: 14.0 ± 0.7 °C; SW: 14.1 ± 0.7 °C; S: 14.6 ± 0.9 °C	<12 °C
SSS	33.26–36.90	35.99 ± 0.31	<34.70 & >36.47
SBS	33.90–36.60	36.03 ± 0.16	<35.60 & >36.30
RA	36–730 mm	166 ± 82 mm	>438 mm
RR	0–3,357,835 dam^3	155,904 ± 394,096 dam^3	None

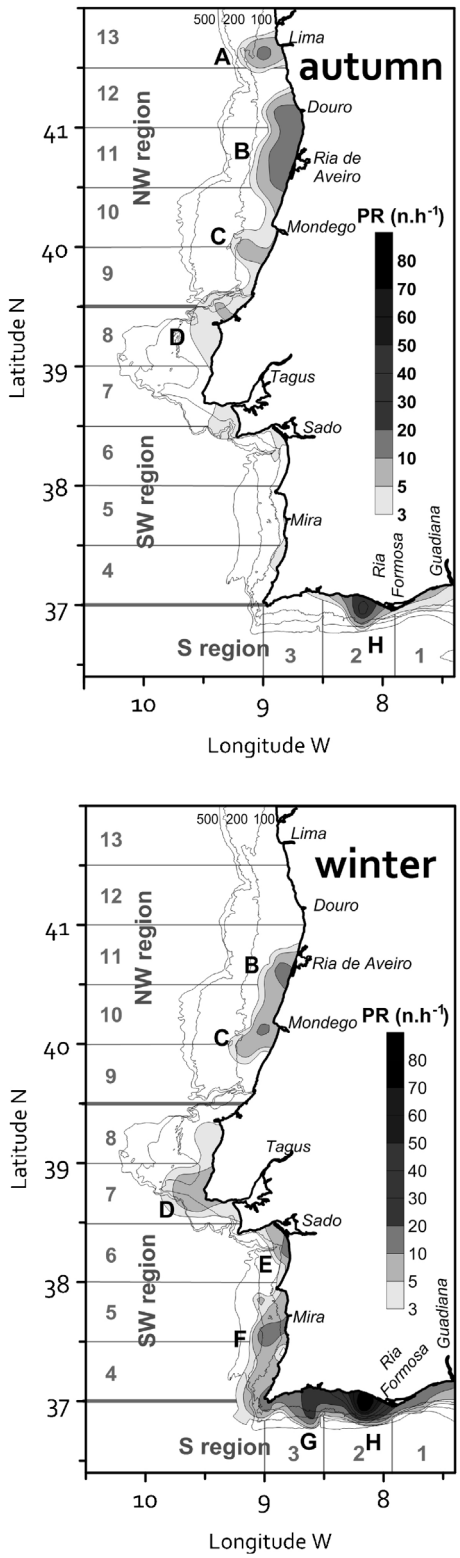


Fig. 5. Recruitment grounds of *O. vulgaris* identified by mapping of pre-recruit mean abundances in (a) autumn and (b) winter survey cruises.

variable on GAMs for each region was negligible (Table 4). Pre-recruits were found associated preferentially with SBT of 14.0 °C (±0.7 SD) on the NW region, 14.1 °C (±0.7 SD) on the SW region and 14.6 °C (±0.9 SD) in the S region. In general, pre-recruit habitat seems to be limited to temperatures above 12.0 °C, SST > 13 °C and SBT > 12 °C (Table 5).

Pre-recruit abundance was consistently higher in the more saline waters, with salinity ≈36.0, independently of any regional or seasonal effects. The effect of SSS was not relevant to explain pre-recruit distribution, but in the NW region abundance increased with SBS between 35.5 and 36.2 where most data were obtained. Moreover, the exceptional occurrence of recruitment ground A was probably related to the unusual high salinities in area 13 verified in the autumn1996 survey. Episodic lower salinities also introduced some complexity to the SBS effect on this region (Fig. 7c). SBS also explained also the location of recruitment grounds in the SW region: pre-recruit abundance increased with increasing SBS until a maximum of ca. 36.1, decreasing towards the saltiest waters (Fig. 7f). In general, pre-recruit habitat was limited by SBS below 35.60 and above 36.30 (Table 5).

Higher concentrations of pre-recruits were also related to areas with relatively low rainfall (preferred RA = 166 ± 82 mm), such as in the S region (Table 5). However, rainfall was not relevant to explain pre-recruit distribution within each region (Table 4). Similarly, the highest concentrations of pre-recruits were found in the S region, where there is considerably less river runoff than on the west coast (Fig. 3b). Both in the NW and SW regions, distribution and abundance were significantly affected by RR. Although the main recruitment grounds were located close to the main rivers and lagoon systems, i.e. associated with areas with important river runoff, abundance decreased under the highest runoff levels of the NW region (Fig. 7d). This negative effect was not observed in the SW region (Fig. 7g), despite the important RR levels which occurred in area 7 (Fig. 3b).

4. Discussion

O. vulgaris pre-recruits were widely distributed along the Portuguese coast. The abundance was substantially higher in the south coastal waters than on the west coast as expected, considering that the general distribution of this species in the Eastern Atlantic have the highest densities towards the south, namely on the north-western coasts of Africa (Balguerías et al., 2000). Pre-recruit distribution in Portuguese waters was patchy and animals aggregated in several recruitment grounds. The area surrounding the lagoon system "Ria de Aveiro" seems to be the most important recruitment ground for *O. vulgaris* in the NW region, and in the SW region pre-recruits aggregated in recruitment grounds close to the Tagus and Sado estuaries. Moreover, in the S region, despite the whole continental shelf showed a high abundance of small octopus, they were aggregated more abundantly close to the lagoon system "Ria Formosa". Consequently, some of the most important recruitment grounds of *O. vulgaris* are located in the vicinity of estuarine or lagoon systems. These are highly productive ecosystems with high abundance and diversity of marine fish and invertebrate species, providing excellent feeding conditions to the young 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 may take advantage of these productive ecosystems by living in their vicinity.

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 Portuguese waters, as in other areas of the *O. vulgaris* occurrence, juveniles and sub-adults generally aggregate in coastal waters at a relatively short distance from the coastline. This is the case in several Mediterranean areas, where the highest abundances of *O. vulgaris* captured by bottom trawl surveys (mostly pre-recruits) were found within the depth range of 10–50 m by Belcari et al. (2002). Similarly, sub-adult densities are higher in

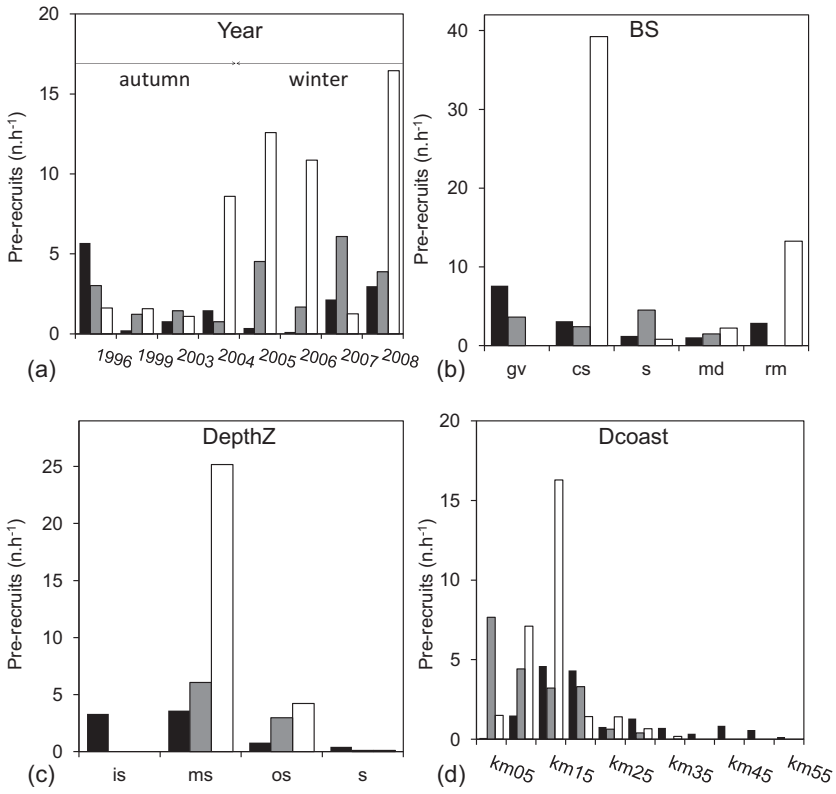


Fig. 6. Variation of mean abundance of *O. vulgaris* pre-recruits on the NW (black bar), SW (grey bar) and S (white bar) regions by (a) in each research cruise (Year), (b) bottom sediment (BS), (c) depth zone (DepthZ), and (d) distance to coast line (Dcoast).

shallow waters (20–40 m) than further offshore on the NW African coasts (Faraj and Bez, 2007). Even so, these authors located the preferred distribution of small octopus further inshore than the main recruitment grounds identified along the Portuguese coast. In contrast, a different demographic distribution was described by Belcari et al. (2002), Quetglas et al. (1998) and Sánchez and Obarti (1993) for the Western Mediterranean and by Hernández-Garcia et al. (1997) for the Canary Islands, as they all observed a wider bathymetric distribution of the small animals. In Portuguese waters we also found a wide distribution of *O. vulgaris* pre-recruits, with aggregations centred at mean depths of ~80 m, and at approximately 10–15 km from the coast. However, the surveyed area of the cruises analyzed provides poor coverage of the inner-shelf, especially in the S region (see Fig. 1), the region where the abundance was the highest, which may have contributed to an overestimation of the pre-recruit preferred depth or distance to the coast line. Overcoming this sampling constraint, both the spatial distribution predicted by geostatistical interpolation of abundance and the GAM modelling of the Depth or Dcoast effects on PR for each region indicated that PR increases towards shallow waters, and for that reason the main recruitment grounds should in general be located in even more coastal areas.

O. vulgaris adults are equally abundant over a variety of sediment types (Mangold, 1983). Nevertheless, we observed that sub-adults have a preference for large grain sediments, namely gravel and coarse sand. Despite the lack of sampling over the rocky ocean floor, we found also the pre-recruit distribution to be closely related to the proximity of rocky bottoms and abundance over mud with rock outcrops 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 shelters ("dens") in the substratum (Mather, 1988).

The relationships between pre-recruit abundance and several abiotic environmental variables were analyzed to delineate pre-recruit optimum habitat and eventual environmental limitations to their distribution. Using presence–absence data, Hermosilla et al. (2011) modelled the distribution and large-scale 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.

In Portuguese waters salinity proved to be an important determinant for the aggregation of *O. vulgaris* pre-recruits. Their distribution was limited by SBS below 35.60 and above 36.30 and the preferred habitat was characterized by salinities of ≈36.0. The highest abundance was hence observed in the S region, where both rainfall and river runoff are generally low and mean seasonal SBS has a narrow range (36.01–36.21, Fig. 2d). Within its global distribution this species prefers habitats with bottom salinity between 30 and 45 (Hermosilla et al., 2011), and is fairly abundant under salinities at least up to 39 (e.g. in the Greek Seas, Katsanevakis and Verriopoulos, 2004a). Thus, the reason why we observed a negative effect of salinities above 36.03 in Portuguese waters may be partially related to the low availability of more saline waters in the study area, or that the negative effect relates to regional environmental tolerances. At the same time, low salinity is a limitation for octopus survival (Chapela et al., 2006; Vaz-Pires et al., 2004) and hence constrains its distribution. The negative effect of SBS was mainly observed on the west coast. Here, salinities generally fall below 36.0 in autumn and winter, especially in the northern area, caused by heavy rainfall and subsequent important river discharges. Despite this, as mentioned before, several recruitment grounds were identified close to large rivers and lagoon systems (although this

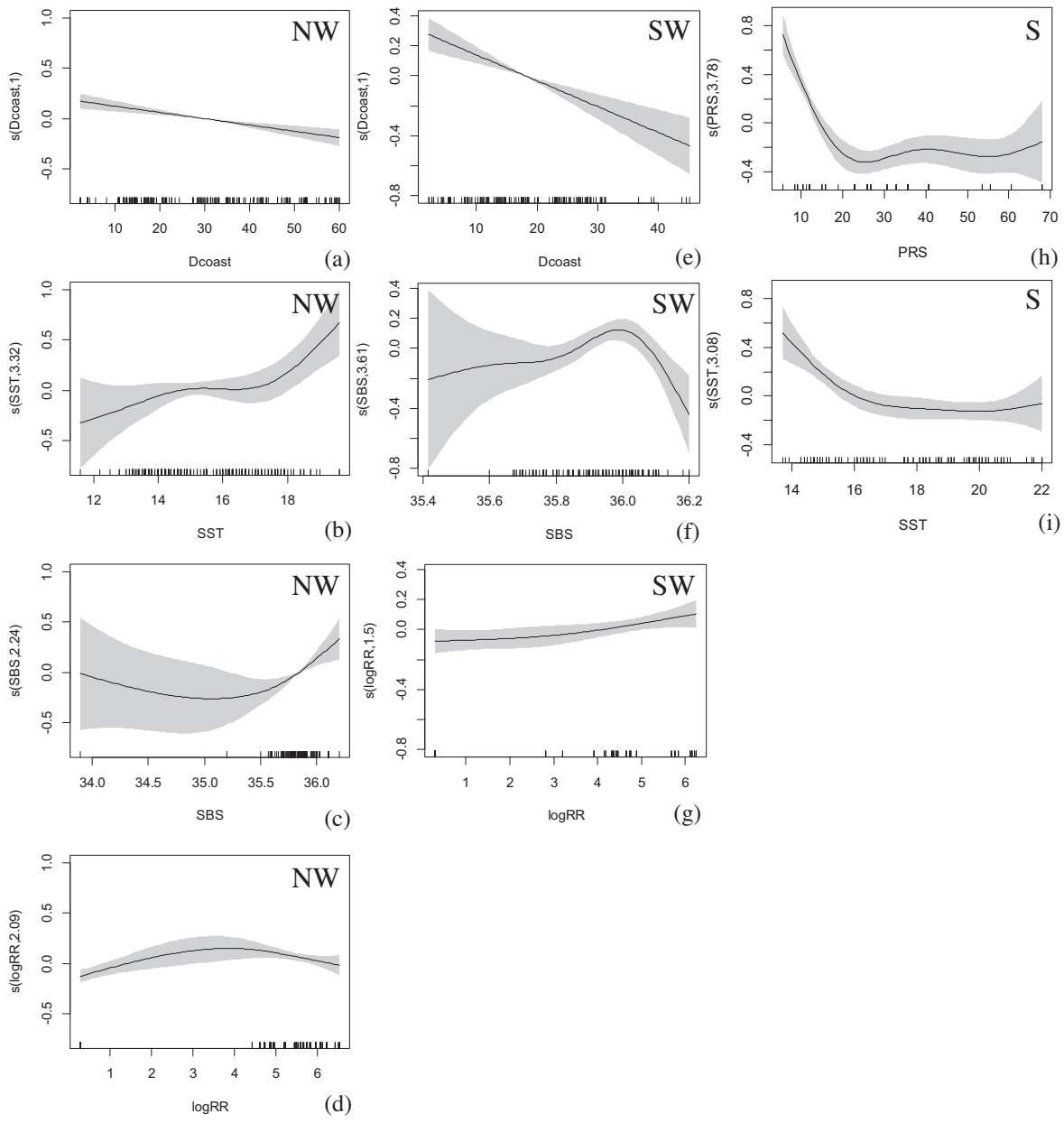


Fig. 7. GAMs results: smoothing curve for partial effects of (a, e) distance to the coastline (Dcoast), (b, i) sea surface temperature (SST), (c, f) sea bottom salinity (SBS), (d, g) log transformed river runoff (log RR), and (h) total pressure (PRS) on pre-recruit abundance (PR) on the northwest (NW), southwest (SW) and south (S) coasts. Shaded area indicates 95% confidence intervals around the main effects. The density of tick marks on the *x*-axis indicates the data points available for different values of *x*.

does not equate to low salinity water). The proximity to estuarine systems provides an increased availability of prey, for example bivalves, but greatly enhances the risk of low salinity episodes. In fact, mass mortality episodes 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 effect of temperature on pre-recruits distribution was also important, even if less significant than salinity. It was observed that SST affects 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 temperature near the bottom. Pre-recruits were found associated prefererentially with SBT between 15 and 16 °C and the main recruitment grounds were limited to mean SBT > 13.6 °C. Similarly to the general distribution of the species in relation to the available environment described by Hermosilla and co-workers (2011), we found that the pre-recruits of *O. vulgaris* concentrate also under the warmest temperatures. On the other hand, if compared with the habitat modelling in Hermosilla et al. (2011), cold water (SBT < 12 °C) seems to impose a more pronounced limitation on pre-recruit distribution than to adult distribution. During the surveys analyzed, SBT was quite stable within each region and no significant effect of this variable on PR was retained by the GAMs. Even so, in the NW region we observed important seasonal and inter-annual variation in distribution and abundance of pre-recruits which may stand for seasonal and yearly differences in SBT (e.g. unusual low SBT in autumn 2004). Even if temperature at the surface supposedly has less effect on the life stages living on the bottom, we found a significant effect of SST on pre-recruit distribution (positive in the west regions and negative

in the S region). Pre-recruit preferred habitat was characterized by SSTs of 15.3–16.0 °C and limited by SST < 13 °C. Furthermore, SSTs above 21 °C seem to have constrained the eastwards spread of the recruitment ground H in the S region in 2003. In this region the Year effect was not retained in the optimum GAM for the S region and consequently the inter-annual variation in pre-recruit abundance in this region must be explained essentially by SST (the only variable in the model affected by yearly changes).

Other studies which modelled *O. vulgaris* abundance in relation to environmental variables found that oceanographic conditions play a very significant role in determining the distribution and abundance during the paralarval phase (e.g. Moreno et al., 2009; Otero et al., 2009). Inter-relationships between paralarval distribution, settlement and recruitment ground locations are still largely unknown. This is a field in which much work is still required and that we expect will be evolving in the near future.

Based on pre-recruit abundance, PI and EI together we may consider the winter recruitment grounds D and F in the SW region, and G and H in the S region, as the main pre-recruit hot spots on the Portuguese continental shelf, where these animals meet their optimum habitat. The Ria Formosa recruitment ground (H) is probably the most important *O. vulgaris* pre-recruit hot spot along the Portuguese coast, showing the highest abundances both in autumn and in winter. Important recruitment grounds were also observed in the NW region close to Ria the Aveiro (B) and south of the mouth of the river Mondego (C), but pre-recruit abundance showed larger inter-annual variation, probably associated with the environmental dynamics of the local upwelling ecosystem.

Overall, the distribution pattern of pre-recruits predicted by the spatial interpolation might have missed the identification of some important aggregation areas. Indeed, the model predicted a low concentration of small octopuses in the eastern part of the south coast. Notwithstanding, according to fishermen surveys, in this part of the coast aggregations occur in very shallow waters (6–12 m depth) between October and December, with higher concentrations in front of the inlets of the Ria Formosa lagoon and near the estuary of the river Guadiana. In these areas, there is high abundance of prey, mainly bivalves (Rufino et al., 2010), and the substrate is coarse (Rosa et al., 2013) with a high fraction of bioclasts constituted by large molluscan shells fragments where small octopuses can hide. Altogether, food availability and habitat characteristics might explain the high concentration of pre-recruits in this part of the coast. Similarly, fishermen also reported that small-sized octopuses are numerous during winter in the westernmost part of the south coast (between Lagos and Sagres), especially near the rocky outcrops in inshore waters, where they find refuge from predators.

The recruitment grounds 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 recruitment grounds (Fonseca et al., 2008). In principle, Portuguese fishery regulations determine that small animals (<750 g) are to be returned to the water if caught, which if respected would suffice to maintain the safety of octopus recruitment grounds, regardless of the type of activity in the area. Nevertheless, the identification of a spatially restricted critical area could be useful to reinforce the legislation.

This study was an essential first step towards the understanding of *O. vulgaris* pre-recruits essential habitat. 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 Verriopoulos, 2004b). Additionally, it would be desirable to include 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. More input derived from "Fishers' ecological knowledge" (FEK) would be desirable in future studies.

Acknowledgements

We are very grateful to the survey cruise teams for all the octopus sampling. We also thank Corina Chaves for her help with the survey database CRUZDEM. The work was undertaken within the scope of the Project "Desarrollo Sostenible de las Pesquerías Artesanales del Arco Atlántico – PRESPO" (Programme INTERREG IV B, co-financed by European Union, ERDF funds), was a contribution to the project LongUp (PTDC/AAC-CLI/105296/2008) funded by FCT, and was partially financed by the "Data Collection for Fisheries Sector Programme" (DCF-EC/DG Maritime Affairs & Fisheries/Joint Research Center). GJP thanks Caixa Geral de Depósitos (Portugal) for financial support.

References

Ardizzone, G.D., 2006 (Inédito). Sensitive and Essential Fish Habitats in the Mediterranean Sea. Working document to the STECF/SGMED-06-01 sub-group meeting on sensitive and essential fish habitats in the Mediterranean. Rome, p. 17.

Balguerías, E., Quintero, M.E., Hernández-González, C.L., 2000. The origin of the Saharan Bank cephalopod fishery. ICES J. Mar. Sci. 57, 15–23.

Belcari, P., Sartor, P., 1999. *Octopus vulgaris*. In: Relini, G., Bertrand, J.A., Zamboni, A. (Eds.), Synthesis of the knowledge on bottom fishery resources in Central Mediterranean (Italy and Corsica). Biol. Mar. Medit. 6, 757–766.

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. Sci. Mar. 66, 157–166.

Boyle, P.R., Rodhouse, P., 2005. Cephalopods: Ecology and Fisheries. John Wiley & Sons, New York.

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. J. Sea Res. 57, 209–217.

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. Sci. Mar. 70, 121–129.

Cressie, N., 1991. Statistics for Spatial Data. J. Wiley, New York.

DGRM, 2012. Recursos da pesca, Ano 2011. Série Estatística 24A-B (online in http://www.dgrm.min-agricultura.pt/).

Domain, F., Jouffre, D., Caverivière, A., 2000. Growth of *Octopus vulgaris* from tagging in Senegalese waters. J. Mar. Biol. Ass. U.K. 80, 699–705.

Faraj, A., Bez, N., 2007. Spatial considerations for the Dakhla stock of *Octopus vulgaris*: indicators, patterns, and fisheries interactions. ICES J. Mar. Sci. 64, 1820–1828.

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

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 J. Mar. Sci. 67, 1363–1371.

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). J. Plank. Res. 27, 271–277.

Guerra, A., 1981. Spatial distribution pattern of Octopus vulgaris. J. Zool. 195, 133–146.

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. Oceanogr. Mar. Biol. Ann. Rev. 47, 119–190.

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). Fish. Res. 35, 183–189.

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. Aquac. Int. 12, 481–487.

Katsanevakis, S., Verriopoulos, G., 2004a. Abundance of *Octopus vulgaris* on soft sediment. Sci. Mar. 68, 553–560.

Katsanevakis, S., Verriopoulos, G., 2004b. Den ecology of *Octopus vulgaris* Cuvier, 1797, on soft sediment: availability and types of shelter. Sci. Mar. 68, 147–157.

Katsanevakis, S., Verriopoulos, G., 2006. Modelling the effect of temperature on hatching and settlement patterns of meroplanktonic organisms: the case of octopus. Sci. Mar. 70, 699–708.

Lourenço, S., Moreno, A., Narciso, L., González, A.F., Pereira, J., 2012. Seasonal trends of the reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. Fish Res. 127–128, 116–124.

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

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

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. J. Plankt. Res. 31, 73–91.

Otero, J., Álvarez-Salgado, X.A., González, Á.F., Gilcoto, M., Guerra, Á., 2009. High-frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. Mar. Ecol. Prog. Ser. 386, 123–132.

Quetglas, A., Alemany, F., Carbonell, A., Merella, P., Sánchez, P., 1998. Biology and fishery of *Octopus vulgaris* Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, Western Mediterranean). Fish. Res. 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. Estuar. Coast. Shelf Sci. 50, 739–749.

Rosa, F., Rufino, M., Ferreira, Ó., Matias, A., Brito, A.C., Gaspar, M.B., 2013. The influence of coastal processes on inner shelf sediment distribution: the Eastern Algarve Shelf (Southern Portugal). Geol. Acta 11, 59–73.

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).

Rufino, M.M., Gaspar, M.B., Pereira, A.M., Maynou, F., Monteiro, C.C., 2010. Ecology of megabenthic bivalve communities from sandy beaches on the south coast of Portugal. Sci. Mar. 74 (1), 163–177.

Sánchez, P., Obarti, R., 1993. The biology and fishery of *Octopus vulgaris* caught with clay pots on the Spanish Mediterranean coast. In: Okutani, T., O'Dor, R.K., Kubodera, T. (Eds.), Recent Advances in Fisheries Biology. Tokai University Press, Tokyo.

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). Bull. Mar. Sci. 71, 837–850.

Valavanis, V.D., Smith, C., 2007. Essential fish habitats. In: Papaconstantinou, C., Zenetos, A., Tserpes, G., Vassilopoulou, V. (Eds.), State of the Hellenic Fisheries. HCMR Publ., Athens.

Valavanis, D.V., Pierce, G.J., Zuur, A.F., Palialexis, A., Saveliev, A., Katara, I., Wang, J., 2008. Modelling of essential fish habitat based on remote sensing, spatial analysis and GIS. Hydrobiologia 612, 5–20.

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

Villanueva, R., 1995. Experimental rearing and growth of planktonic *Octopus vulgaris* from hatching to settlement. Can. J. Fish. Aquat. Sci. 52, 2639–2650.

Zuur, A.F., Ieno, E.N., Smith, G.M., 2007. Analysing Ecological Data. Springer, New York.