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

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Abstract

Octopus vulgaris is a merobenthic octopus with high growth rates, and an opportunistic predator of a wide range of species which adapts the reproductive cycle to the oceanographic characteristics of their habitats. Trophic habitat and gonad quality of two neighbour populations of the Portuguese coast, subject to different oceanographic regimes, one inhabit a productive system with strong seasonality, and the other a oligotrophic system with punctual upwelling events, were compared using the digestive gland and beaks as recorders of trophic and habitat preferences, and gonads as indicators of egg quality. Cholesterol and phospholipids content, fatty acid (FA) profile of the digestive gland and stable isotopes, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, in the flesh and beaks were used as indicators of possible differences in the trophic habitat between populations. For gonad quality, the same bio-indicators were used to identify differences during maturation. The populations present similar trophic positions, with the south population presenting higher $\delta^{15}\text{N}$ signature. The cholesterol content is significantly different between populations as the FA profile of the digestive glands mainly in the EFA 14:0, 16:1n-7, 20:1n-9, ARA, EPA and DHA. The lipid classes' content in the gonad doesn't show a clear influence of environment although the major FA profile of the gonads is dependent of both maturity and region, showing that both timing of maturity and feeding influences the quality of gonads as proxies of eggs.

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

Introduction

The common octopus *Octopus vulgaris* (Cuvier, 1797) is a target species by demersal trawl fleets and in numerous local fisheries in southern Europe and northwestern Africa waters, using hand jigs, pots, traps and trammel nets (Hastie et al. 2009). In the Iberian peninsula, *O. vulgaris* is the most important fisheries resource considering the relationship between first sale price and landings volume, particularly for Portuguese fleet, where the landings volume have increased by 50% in the last 20 years with considerable variation on landing weight in recent years (INE, 2012; ICES, 2012). Here, it is captured mainly with traps (~90%), thus having a major importance in the artisanal fishery, particularly on the south coast, where it accounts for more than 20% of the income of the fishing activity (INE, 2012).

Octopus vulgaris is defined as “r” life-history strategy organism with the production of numerous small eggs that hatch into planktonic free-swimming paralarvae. Those are not truly lecithotrophic, combining endogenous feeding (yolk) with exogenous (prey) feeding (Villanueva & Norman, 2008). As paralarvae, *O. vulgaris* is a specialized predator of crustacean (Roura et al, 2012), whereas as a juvenile and adult, it is an opportunistic predator of crustaceans, mollusks and fish (Quetglas et al, 1998; Smith, 2003; Rosa et al, 2004b). With high food consumption and conversion rates (Semmens et al, 2004), 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), thus having a very variable life cycle within the species. The reproductive season of *O. vulgaris* depends of the local oceanographic regime, presenting a long reproductive season in productive systems, frequently with two reproductive peaks, and with shorter reproductive seasons of one to two months in oligotrophic system (Lourenço et al, 2012). In this system, females direct energy and biomass largely to spawning, presenting significantly higher gonad-somatic indexes during this period in comparison with the females of the more productive areas (Lourenço et al, 2012).

The formation of the yolk is extremely important during maturation and egg development in *O. vulgaris*. Newly hatched paralarvae depend to some extent on the yolk reserves to survive (Villanueva & Norman, 2008). The nutritional content of the yolk is mainly protein, but lipids are also important for the membrane formation and energetic supply (11-14% of dry weight), due to the paralarvae nutritional requirements of polyunsaturated fatty acids (PUFA), phospholipids (phos) and cholesterol (chol) (Navarro and Villanueva, 2000).

The cephalopod hepatopancreas or digestive gland has different functions in the *Octopus* spp. physiology, including the synthesis and secretion of digestive enzymes, the reabsorption and metabolism of nutrients; synthesis and storage of lipids like chol, 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 the essential fatty acids (EFA), particularly polyunsaturated fatty acids (PUFA), or other dietary lipids (e.g. chol and Triacilglycerol, tag) that are deposited in this organ with little or no modification of the lipid content (Philips et al, 2001).

Lipids are important dietary constituents, providing energy, vitamins and essential fatty-acids (EFA). Chol is the predominant sterol in the cephalopods lipids reserves (Sieiro et al, 2006) and proxy of the production of hormones in marine invertebrates (Kanazawa, 2001). The endogenous synthesis of chol seems to be absent in cephalopods, suggesting that it is an essential dietary nutrient (Villanueva & Norman, 2008). The Triacylglycerol (tag) is a neutral lipid involved in the fatty acids (FA) storage and metabolism. The FA are incorporated within the phos, as the building blocks for the membrane lipid bilayer (Dalsgaard et al, 2003, Bergé & Barnathan, 2005, Athenstaedt & Daum, 2006). The lipids, especially the TAG, display wide variability during certain periods of the annual cycle and changed living conditions, and thus possess great potential as “markers” of condition. The phos also characterize the state of the organism, particularly of their cell membrane (Shulman & Love, 2006). The most important EFA are the arachidonic acid 20:4n6 (ARA), the eicosapentaenoic acid 20:5n-3 (EPA) and the docosahexaenoic acid 22:6n-3 (DHA). Requirements of these EFA and the balance between them are important mainly to growth of the individuals, as those EFA are related with the energy channelling, cellular membrane structure and function as integrating the phos in the lipids bilayers (Tocher, 2010). In this study, we are particularly interested in the EFA, tag as reserve and a proxy of the FA metabolism, chol as a proxy of steroid hormones, and phos as a indicator of membrane production in the gonad.

The $\delta^{13}\text{C}$ and the $\delta^{15}\text{N}$ signature in different tissues of a predator like *O. vulgaris* reflects its habitat and trophic position respectively (Stowasser, 2004; Cherel & Hobson, 2005). Consumers or predators are enriched in ^{15}N relatively to their food and consequently $\delta^{15}\text{N}$ measurement is an indicator of the consumer trophic position (Vander Zanden & Rasmussen, 2001; Cherel and Hobson, 2005). ^{13}C varies little with along the food chain and $\delta^{13}\text{C}$ 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 to the food intake (Cherel & Hobson, 2005; Jackson et al, 2007). The study of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope signatures both in the muscle and in the cephalopod beaks, are complementary approaches to the stomach content analysis and EFA profile in studies of trophic dynamic and feeding ecology, permitting the identification of ontogenic migration and feeding shift events (Stowasser, 2004; Jackson et al, 2007). In species like *O. vulgaris*, for which diet studies are difficult to carry out due to the diversity of preys (Smith, 2003) and high prey digestion rate (Boucaud-Camou et al, 1976), a high number of empty stomachs, and the influence of capture methods using bait, this analytic approach combining fatty acids and stable isotopes analyses, provides an average diet information regarding both feeding regime and habitat of a particular population (Stowasser, 2004).

This is the first simultaneous study on the effect of the diet on the nutritional quality of the lipid reserves in the digestive gland and in the mature oocytes of the gonad in two populations of common octopus *Octopus vulgaris* subjected to distinct environmental conditions, one in the northwest Portuguese coast, a productive system integrated in the Western Iberia Upwelling System, and the other one in the south portuguese coast 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. The study aims to use the profile of different lipid classes as EFA, sterols, specifically chol, tag and phos as bio-indicators of variations on diet and eggs quality on immature and fully developed gonads, using as

control the lipid content in the digestive gland. Additionally, from the feeding ecology perspective, it is interesting to identify and quantify the EFA as well as the chol profile in two populations with different diets. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope signatures are determined in the upper and lower beaks of immature and mature individuals of the same populations in order to identify the average feeding regime and habitat of both populations.

Material and Methods

Sampling

Digestive gland (DG) and gonad (OV) samples were collected from animals captured in the small-scale *O. vulgaris* fisheries captures landed in the ports of Peniche (northwest coast) and Olhão (south coast) (Figure 1). Considering both area and maturity stage as explanatory factors, sampling was conducted during March and April 2011 in both sampling sites, collecting the digestive gland and the gonad of 5 immature and 5 mature females in each sampling site with a total of $5 \times 2 (\text{mat}) \times 2 (\text{site}) \times 2 (\text{tissue}) = 40$ samples. The DG and OV samples were freeze dried and stored at -20°C . Cleaned upper and lower beaks and bucal mass samples were also collected from frozen animals and kept in 70% ethanol for isotopic analysis. The dorsal mantle length (DML in mm), and individual weight (W in g) were measured to the nearest 5 mm and 0.1 g, respectively. Immature and maturing females were classified as immature, and fully 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 $\delta^{13}\text{C}$ values due to lipids presence, flesh samples rinsed successively in a 2 : 1 chloroform-methanol solution (Cherel et al. 2005). Nitrogen and carbon isotope ratios were determined via Finnigan 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 based on the Pee Dee Belemnite (PDB) for carbon and atmospheric N_2 (AIR) for nitrogen, and expressed as ‰. Replicate measurements of internal laboratory standards (acetanilide) indicate precision $< 0.2\text{‰}$ both for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

The results were analyzed statistically considering the explanatory factors tissue, area and maturity. The assumption of normality for each explanatory factor was tested by the Shapiro-Wilk's test, and sample homogeneity was tested by Bartlett's test. The factorial analysis ANOVA was applied to explore the effects of each factor by itself and the interaction of factors, area and maturity. The hypothesis test for the one-way ANOVA was performed under the assumption of H_0 : no effect of the factor (area or maturity) in the stable isotope ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) mean value. The hypothesis test for the two-way ANOVA was performed under the assumption of H_0 : there is no effect of the interaction between the factors area and maturity in the stable isotope ($\delta^{13}\text{C}$ or $\delta^{15}\text{N}$) mean value.

Trophic position (TP) in the food web of both populations were determined as (Vander Zanden & Rasmussen, 2001): $TP = 2 + (\delta^{15}N_{oct} - \delta^{15}N_{zoopl})/3.4$; where $\delta^{15}N_{oct}$ is the mean $\delta^{15}N$ ratio determined for the flesh by study area, and $\delta^{15}N_{zoopl}$ was determined as the mean $\delta^{15}N$ value for 200-500 μm zooplankton collected in the northwest coast and, as the mean $\delta^{15}N$ value for zooplankton with size $> 200\mu m$ for the south coast. The zooplankton samples and base $\delta^{15}N$ data were collected by Bode et al. (2007) during spring time between of 2002-2004.

Lipid classes 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 DG and OV were used respectively. The results were expressed as g lipid/100 g dry weight. Lipid classes were determined by different spectrophotometric methods. The phos fraction was purified from the total lipid extract according to Auborg et al. (1996). Total phos 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 chol 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 chol/100 g dry weight. FA composition of lipids extracted from the Bligh & Dyer extract, was determined converting total lipids into fatty acid methyl esters (FAME), according to the Lepage & Roy (1984) method. FAME were analysed by gas chromatography. Peaks corresponding to fatty acids were identified by comparison of their retention times with standard mixtures. Peak areas were automatically integrated, 19:0 fatty acid 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.

The results were analysed statistically considering the explanatory factors tissue, area and maturity. The assumption of normality for each explanatory factor was tested by the Shapiro-Wilk's test, and sample homogeneity was tested by Bartlett's test. For the lipid classes analysis, differences between mean content of TL, chol, tag and phos by factor was tested by the Kruskal-Wallis test. The hypothesis for the Kruskal-Wallis test was formulated under the assumption of H_0 : there is no effect of the factor (area or maturity) on the mean lipid class content in the tissue (GD or OV). The interaction effect between factors tissue x area, tissue x maturation and area x maturation were tested applying the Friedman's Two-Way Analysis of Variance by Ranks. The factors tissue and area were used as blocks and the factors area and maturity were used as treatments. The Hypothesis test was defined as H_0 : The levels of each block (tissue or area) are statistically identical, even within the different levels of treatment (area or maturity); H_1 : The levels of each block (tissue or area) are statistically different, beyond the differences found within each level of treatment (area or maturity) (Gibbons & Chakraborti, 2003).

The fatty acid profile was compared by explanatory factor, tissue, area and maturity and the interaction between area and maturity by means of Multivariate ANOVA, followed by the Principal Component Analysis of the majority FA, aiming to identify the FA which drive the differences between areas and between maturity stages (Zuur et al, 2007). For each fatty acid, the assumption of sample normality and homogeneity for each explanatory factor was tested by the Shapiro-Wilk's and Bartlett's

respectively. Differences between mean concentration between area and maturity stage were tested by *t*-test or the non-parametric Mann-whitney test depending on whether the sample was normally distributed or not (Zar, 1999). Major fatty acids content was normalized before PCA.

Results

Diet and Habitat

The mean individual weight of the females collected was 2340.30 ± 979.45 g in the northwest coast and 2637.40 ± 479.00 g for the south coast. The bio-indicators used to assess the nutritional requirements of each population were the total lipid, tag, chol and phos contents in the digestive gland in the two studied areas. Table 1 presents the mean lipid content determined by maturity stage and by area, while Figure 3 displays the mean content of the lipid classes in the digestive gland (DG) and in the gonad (OV) by study area and by maturity stage. The mean content of total lipids in the digestive gland in the northwest coast is 15.00 ± 4.11 % DW, lower than in the south coast, 20.69 ± 6.20 % DW (Table 1). In this tissue, the mean content in chol depends of area with digestive glands collected in the northwest coast present a significantly higher content of chol than in the south coast (Figure 2, Table 2). Considering the effect of maturity, independently of the area of collection, there seems to be no effect in the total lipid content or in the specific lipid classes studied, with the exception of phos (Table 2). However, total lipid, chol and tag content, tend to increase from immature to mature females (Figure 2). The two-way Friedman test for the interaction between the two factors, area and maturity, shows a certain degree of interaction between those, in the case of total lipids content and tag, chol and phos *per se* (Table 2). On the other hand, analyzing the independent effect of area on the lipid classes content, reveals that these vary with area: TAG and Chol content are higher in the northwest coast while the DG of the south coast are richer in Pho than the DG of northwest coast.

The $\delta^{15}\text{N}$ signature is considered a bio-indicator of the consumer trophic position. The $\delta^{15}\text{N}$ value determined in the flesh samples is identical between areas and maturity stages (table 2) ranging between $11.44 \pm 0.67\text{‰}$ in mature females of the northwest coast and $12.09 \pm 0.87 \text{‰}$ in mature females of the south coast (Table 1, Figure 3). This bio-indicator presents values 4‰ higher in the flesh than in both upper and lower beaks. The $\delta^{15}\text{N}$ values in the upper and lower beaks are statistically identical ($t = 1.12$, $df = 72.674$, $p\text{-value} > 0.05$), and are not affected by the maturity stages (Table 2). Despite that, the beaks present different $\delta^{15}\text{N}$ considering the study areas, with the beaks of south coast presenting higher values of this isotope than northwest coast in app 1‰. The trophic position determined based on the flesh $\delta^{15}\text{N}$ signature was 3.40 for the northwest coast and 3.66 for the south coast (Figure 3), slightly higher than expected by the zooplanktonic base $\delta^{15}\text{N}$ signature for the south coast.

The $\delta^{13}\text{C}$ ratio is a bio-indicator of habitat preference. The mean $\delta^{13}\text{C}$ determined for the flesh is identical between areas and maturity stages (Table 2, Figure 3), and significantly different from the mean $\delta^{13}\text{C}$ determined in the beaks. The upper beak presents in average higher $\delta^{13}\text{C}$ values than the lower beak, although these differences are not significative. The factorial analysis shows that there is no statistical differences between beaks of different study areas and different maturity stages for this isotopic

signature ($t = -1.89$, $df = 61.19$, $p\text{-value} > 0.05$). Although, the $\delta^{13}\text{C}$ in lower beaks seems to be affected by maturity stage (Table 2).

In the DG, the fatty acids (FA) represent $31\mu\text{g}/\text{mg}$ and $46\mu\text{g}/\text{mg}$ of the total lipid content in the northwest coast and the south coast, respectively. The most abundant FA are the saturated fatty acids (SFA), followed by the polyunsaturated fatty acids (PUFA) and the monounsaturated fatty acids (MUFA) (Table 3). Results of the MANOVA analysis of the major FA profile in the digestive gland shows that content levels depend both on area ($F = 7.96$, $p\text{-value} < 0.05$) and maturity ($F = 5.84$, $p\text{-value} < 0.05$), with no effect of the interaction between both factors ($F = 1.02$, $df = 10$, $p\text{-value} > 0.05$). The mean content of the myristic acid, C14:0, the palmitoleic acid, C16:1n7, the eiconenoic acid C20:1n9, arachidonic acid (ARA), C20:4n6 and the Docosahexaenoic acid (DHA) C22:6n3 is significantly statistically 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 3).

The principal component analysis was conducted based on the correlation matrix of the normalized observations of the major fatty acids contents determined in the 20 digestive glands sampled. For the digestive gland, the first component explains 37.86 % of the variation observed and the second component explains 19.89 % of the variation observed, with both axis explaining 57.75 % of the variation associated observed. Nevertheless, the biplot for the digestive gland (Figure 4, Digestive gland) shows that the SFA C16:0 and the MUFA C16:1n7 and C18:1n9 are positively related with the first component and the PUFA ARA, DHA and EPA are negatively related with the same component. The SFA C14:0 and 18:0 are positively related with the second component while the PUFA EPA and DHA present negative signal in relation to this component. The graphical position of observations scores in relation to the first component show that northwest immature digestive glands are related with higher content of the C16:0 and MUFA fatty acids, while south immature digestive glands are related with higher content of PUFA fatty acids. Regarding mature digestive glands, the PCA is not able to reduce the dimension of the variability.

Maturity

In order to study the effect of differences at the nutritional level on the maturation of the oocytes in the gonad, we followed the same bio-indicators used to study the nutritional effect in the digestive gland, total lipid content, the tag content, the chol content and the phos content in the gonad of immature and mature females.

Mature gonads of the northwest coast present higher contents in tag, chol and phos content, although no statistically significant differences were found on the mean content of those bio-indicators between immature and mature individuals (Figure 2, Table 2). In the south coast, the gonads of mature individuals present higher content of tag and phos but only the latter are significant. Mature females of the northwest coast are not significantly different from mature females of south coast in the total lipids mean content ($\chi^2_{\text{Kruskal}} = 0.88$, $p\text{-value} > 0.05$) although the gonads of females in the northwest coast seem to present higher contents of tag ($1.96 \pm 1.09\%$ dw) and chol ($1.00 \pm 0.45\%$ dw) and lower contents of

phos (0.146 ± 0.04 % dw) than the females of the south coast (tag = 1.31 ± 0.93 % dw; chol = 0.74 ± 0.39 % dw and pho = 0.19 ± 0.04 % dw) (Figure 6).

Stable isotope signatures between maturity stages show that the $\delta^{15}\text{N}$ signature is independent of maturity stage but not $\delta^{13}\text{C}$ (Table 2), for which significant differences were found in the this isotope signature between immature and mature females from the south coast ($t = 2.93$ p-value < 0.05).

The FA profile in the gonads is affected by area (MANOVA, $F = 5.62$, p-value < 0.05) and by maturity (MANOVA, $F = 4.09$, p-value < 0.10). Mature gonads are rich in SFA, showing the capacity to produce saturated fatty acids namely palmitic acid C16:0, with a significant increase from immature to mature females in the northwest coast. Another FA that presents significant differences between immature and mature gonads is the stearic acid C18:0 in the northwest coast, decreasing from immature to mature gonads (Table 3). MUFA are the second most abundant fatty acids in the gonads, with the presence vaccenic acid C18:1n7 as minority FA, presenting significant differences between the northwest coast and the south coast namely in the FA C17:1 and the C20:1n9. The FA C16:1n7 is significantly more abundant in immature than in mature gonads of northwest coast. The PUFA represent 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 gonads to mature in both study areas. Particularly the significant decrease in DHA in both regions leads to significant differences between the DHA/EPA ratio of immature and mature females in that area (Table 3).

For the gonad FA profile, components one and two of the principal component analysis explain about 54.98 % of the observed variation. The first component explains 36,80% of the variation and is positively related with C18:0 and the MUFA C16:1n7, the C17:1, 18:1n9 and with the PUFA ARA, DHA and EPA, on the other and the SFA C14:0 and C16:0 correlates negatively with this component. The second component explains 18.18 % of variance and is positively correlated with C18:0 and the short-chain MUFA C17:1 and C18:1n9, while C14:0, C16:1n7 and the PUFA ARA, DHA and EPA correlates negatively with this component. The position of the scores in relation to the component axes shows that the variation explained by component one is related with maturity, immature individuals being related with lower than average content of DHA, ARA and EPA. It is noteworthy that immature gonads from the northwest coast present higher than average content of the MUFA 17:1, 18:1n9 and 16:1n7 and the immature gonads from the south coast are associated with higher content of the PUFA, ARA, DHA and EPA.

Discussion

This simultaneous study on the effect of the diet on the nutritional quality of the lipid reserves in the digestive gland (GD) and in the mature oocytes of the gonad (OV) in two populations of common octopus *Octopus vulgaris* allowed, firstly to understand the trophic role of two populations subject to different environmental conditions and secondly to evaluate which nutritional differences exist between those and how these affect the nutritional quality of gonad as proxy of the eggs.

The stable isotopic signatures of ^{13}C and ^{15}N are particularly good indicators of cephalopods habitat and diet preferences (Jackson et al., 2007). In this study the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures were assessed in the flesh and beaks. *O. vulgaris* is a fast growing organism with a high turnover rate of tissue, with associated high turnover rates for the stable isotopes (Post, 2002), with the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures in the flesh becoming good short term indicators of diet and habitat. On the other hand, the beaks grow by accumulation of material with no turnover, and here the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures are indicators of the life cycle history. The variation $\delta^{13}\text{C}$ signature observed between flesh and beaks is not significant, although the $\delta^{15}\text{N}$ in the flesh is $\approx 4\text{‰}$ higher than in the upper and lower beaks of *Octopus vulgaris*. This variability is also observed elsewhere for *Octopus vulgaris* and by Cherel & Hobson (2005) in the muscle and beaks of *Psychroteuthis glacialis*. Those authors suggested that the difference found in the nitrogen signature between the flesh and beaks is related with the chitin synthesis and consequent N accretion in the squid beaks. Considering this physiologic depletion in the $\delta^{15}\text{N}$ and the short term turnover of muscle tissues in cephalopods, the flesh is the proper tissue to determine the population trophic position. The TP is identical in both populations and places the *Octopus vulgaris* as third order consumer in the benthic food web in those regions. These results are corroborated by a preliminary study on the stomach content of both populations where the more abundant prey species were fish, crustacean and other molluscs, although with some differences in the frequency of occurrence between regions, with the population of northwest coast feeding more on fish and the south coast population of crustacean and bivalves. On the other hand, studies published on trophodynamics of the Atlantic shelf of the Iberian Peninsula also places pelagic and benthic cephalopod species in the third and fourth consumers level (Bode et al., 2007; Mèndez-Fernandez et al., 2007). Mèndez-Fernandez et al. (2007) actually presents a slightly higher trophic level for *Octopus vulgaris* (TP = 3.8) and *Eledone cirrhosa* (TP = 3.6) in Galician waters (northern neighbouring waters to this study), this corroborates the increase observed in the TP and in the $\delta^{15}\text{N}$ with latitude, due to the increasing base $\delta^{15}\text{N}$ signature with latitude observed by Bode et al. (2007).

Nevertheless, the comparison between the $\delta^{15}\text{N}$ in common octopus beaks of different study areas is possible. The higher $\delta^{15}\text{N}$ ratio in the south coast in relation to the northwest coast can indicate a dominance of crustacea (crabs) in the diet of *O. vulgaris* in this area as indicated by Stowasser (2004) for *Loligo forbesi*, a loliginid common in the northeast Atlantic that is also considered an opportunistic predator. The upper/lower beaks present different $\delta^{15}\text{N}$ signatures between study areas, and $\delta^{13}\text{C}$ between maturity stages (the lower beaks) of the southern population. This could indicate that even if the trophic level does not change, the dietary history between mature and immature females in the south coast is different. Cherel and Hobson (2005) indicate that a variation of 4 ‰ (greater than we have shown) indicates a change in the TP. Nevertheless cephalopod beaks grow by accretion of new molecules of proteins and chitin and there is no turnover after synthesis, suggesting that a certain level of life-history is being trapped. In fact, beaks retain molecules from early development to the time of death, and their isotopic signature therefore integrates the feeding ecology of the animal over its whole life-history (Cherel and Hobson, 2005). Similar signatures have been found for the antarctic giant squids *Mesonychoteuthis hamiltoni* and *Architeuthis dux* (Cherel and Hobson, 2005), and for the squid *Loligo forbesi* for the $\delta^{13}\text{C}$ in the muscle.

Considering that the digestive gland is mainly a lipid reservoir and therefore with high lipid content, it is the ideal organ to trace fatty acids as diet bio-indicators (Phillips et al. 2001; Moltschaniwskyj and Johnston, 2006; García-Garrido et al. 2010). Here the total lipid content ranged between 15 % in northwest coast and 20.69 %, in line with the obtained by Rosa et al. (2004a) for females captured in the Portuguese northwest coast, and lower to have been found in pelagic squids as *Moroteuthis ingens* (Phillips et al., 2001) which accounts for 40% of lipids in the digestive gland (although measured in wet mass). Contrary to what one should expect for a nutrient rich area, the northwest coast population presents relatively low lipid content in comparison to the south coast population. This is probably related with timing. In March and April, the sampling months, the northwest coast is characterized by a prior upwelling conditions, and fish present low fat content as detected for sardine *Sardina pilchardus* (Bandarra et al. 1997) and horse-mackerel *Trachurus trachurus* (Bandarra et al., 2001). On the other hand, in the south Portuguese coast, east to Cape Sta Maria, phytoplankton peaks are common in early spring associated to the warmer and nutrient rich river plumes, which associated to the shallow waters, create good conditions for the development of these earlier phytoplankton blooms (Ruiz et al., 2006). This nutrient richer environment in south coast is corroborated by the relative high content in the essential fatty acids ARA, EPA and DHA, essential fatty acids for carnivorous species produced by the producers community (Dalsgaard et al., 2003).

The higher content in TAG, defined as storage lipid (Lee et al., 2006), in the south coast digestive glands can be related with fito and zooplankton peaks of early spring, but also with the dominance crustacean preys in this coast as detected in the diet preliminarily study and also by Rosa et al. (2004b) which is directly related with the higher content with TAG (Phillips et al., 2003). Nevertheless, the higher content in total lipids, chol, SFA and MUFA in the digestive gland of the northwest coast is likely the result of a dominance in detritivorous species in their diet, where the impact of primary production cycles is attenuated.

The high content of 18:1n-9 (≈ 11 % in both study areas), which is indicative of deeper waters preys, and 16:0 ($\approx 20\%$ in both study areas) indicative of the presence of herbivorous preys, in the digestive gland of *Octopus vulgaris* is indicative of its diverse diet (Piché et al 2010). On the other hand, the DHA/EPA ratio seems to be unbalanced in the northwestern population. For a carnivorous species as this one, the DHA content should be higher relatively to the EPA as it happens in the south coast (Dalsgaard et al., 2003). This is can indicate that probably during this period of time the northwest population is feeding in a detritivores habitat, although the study conducted by Rosa et al (2004a) in the same region didn't found this unbalanced DHA/EPA ratio.

In terms of lipid content, the gonad presents on average 12% dw of total lipids, the tag and cho contents being identical, at around 5% of dry weight. The phos in the gonad represented in this study around 0.5 % dw of total lipids, similarly to what was found by Rosa et al. (2004a). The fatty acid profile shows a decrease in the PUFA content in the gonads with maturity, which can be related with fact that during maturation, these FA are being seized by the phos to form the lipid bilayer of the oocyte walls (Tocher, 2010). As in the squids *Sepioteuthis lessoniana* and *Photololigo* sp. (Semmens, 1998), or the cuttlefish *Sepia officinalis* (Blanchier and Boucaud-Camou, 1984), the lipid class contents in the digestive

gland do not correlate directly with the lipid content of the oocytes in the gonad. Although the females only reach maturity beyond an optimum body mass independently of the gonad size (Lourenço et al. 2012), they are income breeders (Houston et al. 2007; Quetglas et al. 2011) that channel the energy directly from food to the gonad during maturation. So, the different levels in tag and chol noted in the digestive glands between geographical areas should be expected of income breeders. Despite that, the histograms comparing the lipid classes content in the digestive gland and gonads between study areas (Figure 5) show that the lipid classes' content in mature gonads is quite similar between those areas, even though there are differences between tag and chol content in the digestive glands.

On the other hand the fact that the FA profile is variable both with area and maturity is evidence that there are differences between the quality of the oocytes of the northwest and south coasts during the first semester of the year. The gonads of the south coast are richer in PUFA and in MUFA, those of the northwest are richer in SFA. No differences were found in the ratio DHA/EPA in either the gonads or the digestive gland between areas, as also noted by Faria et al. (2011) for the Patagonian red octopus *Enteroctopus megalocyathus*, there being a direct effect of feeding in the quality of the eggs, and the content of FA of the diet seeming to affect the FA profile of the eggs, mainly the DHA content and the DHA/EPA ratio.

The differences found in the $\delta^{13}\text{C}$ signature in lower beaks of immature and mature females in the south coast may be related with the fact that each animal may spend different parts of the life-cycle in different environments. Mature females migrate to rocky substrates to lay and care for the eggs (Boyle and Rodhouse, 2005; Quetglas et al. 2011) and in the south coast this migration represents a major changes in habitat.

In terms of stock management, this study reflects how stocks are coupled with the environment and the habitat that surrounds them, from changes in prey availability to the pattern of physical characteristics of the environment all of which necessarily have variable impacts on the parental generation. What we have been able to show is that at least some differences can be noted in the egg quality, with a potential impact on the offspring.

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Bibliographic References:

Auborg, S., I. Medina & R. Pérez-Martín, 1996. Polyunsaturated fatty acids in tuna Phospholipids: distribution in the sn-2 location and changes during cooking. *Journal of Agricultural and Food Chemistry* 44: 585-589.

410 Athenstaedt, K. & G. Daum, 2006. The life cycle of Neutral lipids: synthesis, storage and degradation.
 411 Cellular and Molecular Life Sciences 63: 1355-1369.

412 Bandarra, N. M., I. Batista, M. L. Nunes, & J. M. Empis, 1997. Seasonal changes in Lipid composition
 413 of sardine (*Sardine pilchardus*). Journal of Food Science. 62: 40-42.

414 Bandarra, N.M., I. Batista, M. L. Nunes & J. M. Empis, 2001. Seasonal variation in the chemical
 415 composition of horse-mackerel (*Trachurus trachurus*). European Food Research and Technology
 416 212:535-539.

417 Bergé, J-P. & G. Barnathan, 2005. Fatty acids from lipids of marine organisms: molecular biodiversity,
 418 roles as biomarkers, biologically active compounds, and economical aspects. Advances in Biochemical
 419 Engineering/Biotechnology 96: 49-125.

420 Blanchier B & E. Boucaud-Camou, 1984. Lipids in the digestive gland and the gonad of immature and
 421 mature *Sepia officinalis* (Mollusca: Cephalopoda). Marine Biology 80: 39-43.

422 Bligh, E. & W. Dyer, 1959. A rapid method of total extraction and purification. Canadian Journal
 423 Biochemical Physiology 37: 911-917.

424 Bode, A., M. T. Alvarez-Ossorio, M. E. Cunha, S. Garrido, J. B. Peleteiro, C. Porteiro, L. Valdés, Manuel
 425 Varela, 2007. Stable nitrogen studies of the pelagic food web on the Atlantic shelf of the Iberian
 426 Peninsula. Progress in Oceanography 74: 115-131.

427 Boyle, P. & P. Rodhouse, 2006. Cephalopods: Ecology and Fisheries. Second Ed. Blackwell Publishing,
 428 452 p.

429 Budelmann B. U., R. Schipp & Boletzky S von-, 1997. Cephalopoda. In Harrison F W. Microscopic
 430 anatomy of invertebrates Vol. 6A Mollusca II, Wiley-Liss, pp. 119-414.

431 Cherel, Y. & K. Hobson, 2005. Stable isotopes, beaks and predators: a new toll to study the trophic
 432 ecology of cephalopods, including giant and colossal squids. Proceedings of Royal Society B 272: 1601-
 433 1607.

434 Cortés, E., 1996. A critical review of methods of studying fish feeding based on analysis of stomach
 435 contents: application to elasmobranch fishes. Canadian Journal Fish Aquatic Science 54: 726-738.

436 Cunha, M. E., 2001. Physical Control of Biological Processes in a Coastal Upwelling System:
 437 Comparison of the Effects of Coastal Topography, River, Run-off and Physical Oceanography in the
 438 Northern and Southern Parts of Western Portuguese Coastal Waters. A thesis submitted to the Faculdade
 439 de Ciências da Universidade de Lisboa for the degree of Doutor em Biologia (ramo Ciências do Mar). 307
 440 p.

441 Dalsgaard, J., M. St. John, G. Kattner, D. Müller-Navarra, & W. Hagen, 2003. Fatty acid trophic
 442 markers in the pelagic marine environment. Advances in Marine Biology 46:225-340.

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

445 García-Garrido, S., I. Hachero_Cruzado, D. Garrido, C. Rosas, & P. Domingues, 2010. Lipid composition
446 of the mantle and digestive gland of *Octopus vulgaris* juveniles (Cuvier, 1797) exposed to prolonged
447 starvation. Aquaculture International 18:1223-1241.

448 Garcia-Lafuente, J. & J. Ruiz, 2007. The Gulf of Cádiz pelagic ecosystem: A review. Progress In
449 Oceanography. 74:228-251.

450 Gibbons, J.D. & S. Chakraborti, 2003. Nonparametric statistical inference. Fourth Edition, Revised and
451 Expanded. Marcel Dekker. New York, 645 pp.

452 Guerra, A., 1975. Determinación de las diferentes fases del desarrollo sexual de *Octopus vulgaris* Lamarck,
453 mediante un índice de madurez. Investigacion Pesquera. 39: 397-416.

454 Hastie, L.C., G. J. Pierce, J. Wang, I. Bruno, A. Moreno, U. Piatkowski, & J.P. Robin, 2009.
455 Cephalopods in the North-eastern Atlantic: species, biogeography, ecology, exploitation and
456 conservation. Oceanography and Marine Biology: An Annual Review. 47:11-190.

457 Holme D & H. Peck, 1998. Analytical biochemistry (3 rd edition ed.). Essex: Pearson Education Limited.

458 Houston, A.I., P.A. Stephens, I. L. Boyd, K. C. Harding & J. M. M. McNamarab, 2007. Capital or
459 income breeding? A theoretical model of female reproductive strategies. Behavioral Ecology 18: 241-250.

460 Huang, T., C. Chen, A. Wefler & Raftery, 1961. A stable reagent for the Liebermann-Buchardt reaction.
461 Analytical Chemistry 33: 1405-1407.

462 ICES, 2010. Report of the workshop on sexual maturity staging of cephalopods, 8-11 November 2010.
463 ICES CM2010/ACOM: 49.

464 ICES, 2012. Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH), 27–30
465 March 2012, Cadiz, Spain. ICES CM 2012/SSGEF:04. 241 pp.

466 Jackson, G.D., P. Bustamante, P., Y. Cherel, Fulton E. A., E. P. M. Grist, C. H. Jackson, P. D. Nicholls,
467 H. Pethybridge, K. Phillips, D. R. Ward, & J.C. Xavier, 2007. Applying new tools to cephalopod trophic
468 dynamics and ecology: perspectives from the Southern Ocean Cephalopod Workshop, February 2–3,
469 2006. Reviews in Fish Biology and Fisheries 17: 79-99.

470 Kanazawa, A., 2001. Sterols in marine invertebrates. Fisheries Science 67:997-1007.

471 Lee, R. F., W. Hagen & G. Kattner, 2006. Lipid storage in marine zooplankton. Marine Ecology Progress
472 Series 307:233-306.

473 Lourenço, S., A. Moreno, L. Narciso, Á. F. González & J. Pereira, 2012. Seasonal trends of the
474 reproductive cycle of *Octopus vulgaris* in two environmentally distinct coastal areas. Fisheries Research
475 127-128: 116-124.

476 Lowry, R. & I. Tinsley, 1976. Rapid Colorimetric determination of free fatty acids. Journal of the
477 American Oil Chemists' Society 53: 470-472.

478 Mead J F, R.B. Alfin-slater, D. R. Howton, & G. Popják, 1985. Lipids: Chemistry, Biochemistry and
479 Nutrition. New York. Plenum Press.

480 Mèndez-Fernandez, P., P. Bustamante, A. Bode, T. Chouvelon, M. Ferreira, A. López, G. J. Pierce, M. B.
481 Santos, J. Spitz, J. V. Vingada & F. Caurant, 2012. Foraging ecology of five toothed whale species in the
482 Northwest Iberian Peninsula, inferred using carbon and nitrogen isotope ratios. Journal of Experimental
483 Marine Biology and Ecology 413:150-158.

484 Moltschaniwskyj, N. & D. Johnston, 2006. Evidence that lipid can be digested by the dumpling squid
485 *Euprymna tasmanica*, but is not stored in the digestive gland. Marine Biology 149:565-572.

486 Navarro J. C. & R. Villanueva, 2000. Lipid and fatty acid composition of early stages of cephalopods: an
487 approach to their lipid requirements. Aquaculture 183:161-177.

488 Nixon, M., 1985. Capture of Prey, Diet and feeding of *Sepia officinalis* and *Octopus vulgaris* (Mollusca:
489 Cephalopoda) from hatchling to adult. Vie et Milieu, 35:255-261.

490 Philips K L, G. D. Jackson & P. D. Nichols, 2001. Predation on myctophids by the squid *Moroteuthis*
491 *ingens* around Macquarie and Herad Islands: Stomach contents and fatty acid analyses. Marine Ecology –
492 Progress Series 215:179-189.

493 Phillips K L, P. D. Nichols & G. D. Jackson, 2003. Dietary variation of the squid *Moroteuthis ingens* at
494 four sites in the Southern Ocean: stomach contents, lipid and fatty acid profiles. Journal of the Marine
495 Biological Association of the United Kingdom 83:523-534.

496 Piché, J., S. J. Iverson, F. A. Parrish, & R. Dollar, 2010. Characterization of forage fish and invertebrates
497 in the Northwestern Hawaiian Islands using fatty acid signatures: species and ecological groups. Marine
498 Ecology Progress Series Vol 418 :15. Post, D. 2002. Using stable isotopes to estimate trophic position:
499 Models, methods, and assumptions. Ecology 83:703-718.

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

503 Quetglas, A., A. Ordines, & M. Valls, 2011. What drives seasonal fluctuations of body condition in a
504 semelparous income breeder *Octopus*? Acta Oecologica 37: 476-483.

505 Raheja, R., C. Kaur, A. Singh, & A. Bhatia, 1973. New colorimetric method for the quantitative
506 determination of Phopholipids without acid digestion. Journal of Lipid Research 14: 695-697.

507 Ribeiro A.C., A. Peliz, & A. M. P. Santos, 2005. A study of the response of chlorophyll-a biomass to a
508 winter upwelling event off Western Iberia using SeaWiFS and in situ data. *Journal of Marine Systems* 53:
509 87-107.

510 Rocha, F., A. Guerra & A. F González, 2001. A review of the reproductive strategies in cephalopods.
511 *Biological Reviews*. 76:291-304. Rosa R, P. R. Costa P R & M. L. Nunes, 2004a. Effect of the sexual
512 maturation on the tissue biochemical composition of *Octopus vulgaris* and *O. defilippi* (Mollusca:
513 Cephalopoda). *Marine Biology* 145:563-574. Rosa R, A. M. Marques, M. L. Nunes, N. Bandarra & C. S.
514 Sousa Reis, 2004b. Spatial-temporal changes in dimethyl acetal (octadecanal) levels of *Octopus vulgaris*
515 (Mollusca: Cephalopoda): Relation to feeding ecology. *Scientia Marina* 68:227-236.

516 Rosa, R., P. R. Costa, J. Pereira & M. L. Nunes, 2004c. Biochemical dynamics of spermatogenesis and
517 oogenesis in *Eledone cirrhosa* and *Eledone moschata* (Cephalopoda: Octopoda). *Comp. Biochem.*
518 *Physiol B* 139:299-310.

519 Roura, A., Á. F. González, , K. Reid, & Á. Guerra, 2012. Molecular prey identification in wild *Octopus*
520 *vulgaris* paralarvae. *Marine Biology* 159:1335-1345.

521 Ruiz, J., E. García-Isarch, I. E. Huertas, L. Prieto, A. Juárez,, J. L. Muñoz,, A. Sánchez-Lamadrid, S.
522 Rodríguez-Gálvez, J. M. Naranjo, & F. Baldó, 2006. Meteorological and oceanographic factors
523 influencing *Engraulis encrasicolus* early life stages and catches in the Gulf of Cádiz. *Deep Sea research II*
524 53:1363-1376.

525 Santos, A.M.P, A. Peliz, J. Dubert, P. B. Oliveira, M. M. Angelico, & P. Ré, 2004. Impact of a winter
526 upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off
527 western Iberia: a retention mechanism. *Continental Shelf Research* 24:149–165.

528 Semmens, J.M. 1998. An examination of the role of the digestive gland of two loliginid squids, with
529 respect to lipid: storage or excretion? *Proceedings of Royal Society of London B*. 265:1685-1690.

530 Semmens, J.M., G. T. Pecl, R. Villanueva, D. Jouffre, I. Sobrino, J. B. Wood & P. R. Rigby, 2004.
531 Understanding *Octopus* growth: Patterns, variability and physiology . *Marine and freshwater Research* ,
532 55:367-377.

533 Shulman, G.E., & R. M. Love, 2006. The Biochemical Ecology of Marine Fishes. *Advances in Marine*
534 *Biology*, 36, 351 pp. Sieiro, M P, S. Auborg, & F. Rocha, 2006. Seasonal study of the lipid composition
535 in different tissues of the common octopus (*Octopus vulgaris*). *European Food Research and Technology*
536 108:479-487.

537 Silva, L., I. Sobrino & F. Ramos, 2002. Reproductive biology of the common octopus, *Octopus vulgaris*
538 *cuvier*, 1797 (cephalopda: Octopodidae) in the gulf of Cádiz (SW Spain). *Bulletin of Marine. Science*
539 71:837-850.

540 Smith, C. 2003. Diet of *Octopus vulgaris* in False Bay, South Africa. *Marine Biology* , 143:1127-1133.

541

542 Stowasser, G., 2004. Squid and their prey: insights from fatty acid and stable isotope analysis. Thesis
543 presented for the degree of doctor of Philosophy at the University of Aberdeen. Aberdeen, UK. 271pp.

544 Tocher, D. R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquaculture
545 Research 41: 717-732.

546 Vander Zanden M. J. & J. B. Rasmussen, 2001. Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation:
547 Implications for aquatic food web studies. Limnology and Oceanography 46:2061-2066.

548 Villanueva R & M. D. Norman, 2008. Biology of the planktonic stages of Benthic octopuses.
549 Oceanography and Marine biology: An Annual Review 46:105-202.

550 Zar, J. 1999. Biostatistical Analysis. 4th Edition. Prentice Hall. 663p.

551 Zuur, A.F., E. N, Ieno & G.M. Smith, 2007. Analysing Ecological Data. Springer. 672 pp.

552

553 **Figure legends:**

554 Figure 1 - Sampling ports and coastal characteristics.

555 Figure 2 – Mean lipid classes content in % dw ($\pm$ SD) aggregated by tissue (digestive gland; gonad), by
556 area (nw – Northwest coast; south – South coast) and by maturity stage (i - immature, m - mature).
557 Different superscript letters indicate significant statistical differences ($p < 0.05$).

558 Figure3 – *Octopus vulgaris* stable isotope $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures in the flesh and upper and lower beaks
559 by explanatory factors area and maturity. The upper right and left graphics represent the stable isotope
560 signatures for the northwest and south coast, and the lower left and right graphics represent the stable
561 isotope signatures for immature and mature individuals.

562 Figure 4 – Principal component analysis byplots of the fatty acid profiles of the digestive gland (on the
563 left) and gonad (on the right). The loadings correspond to the FA variables analysed and the scores in
564 grey correspond to each one of the sample analysed.

565 Figure 5 – Comparison of the Lipid classes content in the digestive gland and gonad of mature females in
566 the northwest coast and in the south coast. tag – Triacylglycerol, chol – Cholesterol and phos –
567 Phospholipids in the digestive gland and in the gonad.

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Table 1 – Carbon ($\delta^{13}\text{C}$), Nitrogen ($\delta^{15}\text{N}$) isotope ration (mean $\pm$ SD, ‰) in the flesh and in the upper and lower beak, trophic level ($\pm$ SD) and total lipid content (mean $\pm$ SD, %dw) in the digestive gland and gonad by study area (northwest coast and south coast), and maturity stage (immature and mature). The superscript * indicates significant statistical difference (p-value < 0.05) between maturity stages, and the superscript ** indicates significant statistical difference between study areas.

		Flesh		Upper beak		Lower beak		Trophic Level	DG Total Lipid (% dw)	OV Total Lipid (% dw)
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)			
Northwest coast	Immature	-16.55 $\pm$ 0.82	11.91 $\pm$ 0.71	-17.25 $\pm$ 0.69	7.29 $\pm$ 0.64	-17.01 $\pm$ 0.76	7.64 $\pm$ 0.94		14.60 $\pm$ 4.44	15.39 $\pm$ 2.36
	Mature	-17.31 $\pm$ 0.37	11.44 $\pm$ 0.67	-17.84 $\pm$ 0.76	7.38 $\pm$ 0.91	-17.61 $\pm$ 0.60	7.78 $\pm$ 0.61	3.40 $\pm$ 0.21**	15.39 $\pm$ 4.22	12.53 $\pm$ 3.27
	Mean	-16.85 $\pm$ 0.76	11.72 $\pm$ 0.70	-17.53 $\pm$ 0.76	7.33 $\pm$ 0.75**	-17.29 $\pm$ 0.74	7.70 $\pm$ 0.78**		15.00 $\pm$ 4.11	13.96 $\pm$ 3.08
South coast	Immature	-16.57 $\pm$ 0.62	11.84 $\pm$ 0.46	-17.45 $\pm$ 1.36	8.43 $\pm$ 0.56	-16.76 $\pm$ 0.67	8.69 $\pm$ 0.98		16.97 $\pm$ 4.30	12.16 $\pm$ 3.62
	Mature	-16.96 $\pm$ 0.86	12.09 $\pm$ 0.87	-17.60 $\pm$ 0.72	8.74 $\pm$ 0.89	-17.38 $\pm$ 0.67	8.71 $\pm$ 0.70	3.66 $\pm$ 0.18**	24.42 $\pm$ 5.76	11.03 $\pm$ 0.99
	Mean	-16.70 $\pm$ 0.71	11.92 $\pm$ 0.61	-17.49 $\pm$ 1.19	8.52 $\pm$ 0.66**	-16.94 $\pm$ 0.71	8.70 $\pm$ 0.89**		20.69 $\pm$ 6.20	11.59 $\pm$ 2.57

Table 2 - One-way, two-way analysis of variance and hypothesis testing considering the applied bio-indicators, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, total lipids content % dw TL), Triacilglycerol content (% dw tag), Cholesterol content (% dw cho) and Phospholipids content (% dw phos) determined for flesh, beaks, digestive gland and gonad by studied region and maturity stage.

Tissue	Indicator	Test	Factor	df	Statistics	p-value	Conclusion
Flesh	$\delta^{13}\text{C}$	One-way ANOVA	area	26	F=0.28	>0.05	Accept H_0
			mat	26	F=4.00	>0.05	Accept H_0
	$\delta^{15}\text{N}$	Two-way ANOVA	area*mat	24	F=0.44	>0.05	Accept H_0
		One way ANOVA	area	26	F=0.62	>0.05	Accept H_0
			mat	26	F=0.02	>0.05	Accept H_0
		Two-way ANOVA	area*mat	24	F=1.83	>0.05	Accept H_0
Beaks	$\delta^{13}\text{C}$	One-way ANOVA	area	66	F=0.80	>0.05	Accept H_0
			mat	66	F=5.93	<0.05	Reject H_0
	$\delta^{15}\text{N}$	Two-way ANOVA	area*mat	64	F=0.21	>0.10	Accept H_0
		One-way ANOVA	area	66	F=33.46	<0.05	Reject H_0
			mat	66	F=5.93	<0.05	Reject H_0
		Two-way ANOVA	area*mat	64	F=0.21	>0.05	Accept H_0
Digestive gland	TL	One-way ANOVA	area	1	$\chi^2_{\text{K}}=3.57$	>0.05	Accept H_0
			mat	1	$\chi^2_{\text{K}}=1.28$	>0.05	Accept H_0
	tag	Two-way ANOVA	area*mat	1	$\chi^2_{\text{F}}=34.00$	<0.05	Reject H_0
		One-way ANOVA	area	1	$\chi^2_{\text{K}}=1.66$	>0.05	Accept H_0
			mat	1	$\chi^2_{\text{K}}=3.57$	>0.05	Accept H_0
		Two-way ANOVA	area*mat	1	$\chi^2_{\text{F}}=34.00$	<0.05	Reject H_0
	cho	One-way ANOVA	area	1	$\chi^2_{\text{K}}=14.28$	<0.05	Reject H_0
			mat	1	$\chi^2_{\text{K}}=0.21$	>0.05	Accept H_0
	phos	Two-way ANOVA	area*mat	1	$\chi^2_{\text{F}}=32.00$	<0.05	Reject H_0
		One-way ANOVA	area	1	$\chi^2_{\text{K}}=3.35$	>0.05	Accept H_0
			mat	1	$\chi^2_{\text{K}}=3.93$	=0.05	Reject H_0
		Two-way ANOVA	area*mat	1	$\chi^2_{\text{F}}=40.00$	<0.05	Reject H_0
Gonad	TL	One-way ANOVA	area	1	$\chi^2_{\text{K}}=3.29$	>0.05	Accept H_0
			mat	1	$\chi^2_{\text{K}}=2.28$	>0.05	Accept H_0
	tag	Two-way ANOVA	area*mat	1	$\chi^2_{\text{F}}=40.00$	<0.05	Reject H_0
		One-way ANOVA	area	1	$\chi^2_{\text{K}}=2.29$	>0.05	Accept H_0
			mat	1	$\chi^2_{\text{K}}=1.85$	>0.05	Accept H_0
		Two-way ANOVA	area*mat	1	$\chi^2_{\text{F}}=40.00$	<0.05	Reject H_0
	cho	One-way ANOVA	area	1	$\chi^2_{\text{K}}=1.65$	>0.05	Accept H_0
			mat	1	$\chi^2_{\text{K}}=0.02$	>0.05	Accept H_0
	phos	Two-way ANOVA	area*mat	1	$\chi^2_{\text{F}}=40.00$	<0.05	Reject H_0
		One-way ANOVA	area	1	$\chi^2_{\text{K}}=0.76$	>0.05	Accept H_0
			mat	1	$\chi^2_{\text{K}}=8.73$	<0.05	Reject H_0
		Two-way ANOVA	area*mat	1	$\chi^2_{\text{F}}=40.00$	<0.05	Reject H_0

Table 3 - Fatty acids (mean $\pm$ SD, g/100 g FAME) profile 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 area.

Fatty acid	Digestive Gland						Gonad					
	northwest coast			south coast			northwest coast			South coast		
	immature	mature	mean	immature	mature	mean	immature	mature	mean	immature	mature	mean
Saturated fatty acids (SFA)												
C 14:0	4.99 $\pm$ 1.58	4.89 $\pm$ 2.05	4.94 $\pm$ 1.75**	1.88 $\pm$ 0.96	3.25 $\pm$ 1.62	2.51 $\pm$ 1.42**	4.19 $\pm$ 0.48	4.09 $\pm$ 0.36	4.14 $\pm$ 0.40	4.06 $\pm$ 0.72	3.94 $\pm$ 0.52	3.99 $\pm$ 0.58
C 16:0	22.62 $\pm$ 4.35	18.34 $\pm$ 5.71	20.48 $\pm$ 5.33	22.08 $\pm$ 14.61	18.48 $\pm$ 9.58	20.44 $\pm$ 12.14	19.25 $\pm$ 1.98*	23.85 $\pm$ 1.18*	21.73 $\pm$ 2.83	18.78 $\pm$ 2.44	22.31 $\pm$ 3.50	20.83 $\pm$ 3.48
C 18:0	13.73 $\pm$ 4.27	11.63 $\pm$ 0.32	12.68 $\pm$ 3.09	12.88 $\pm$ 1.58	11.21 $\pm$ 1.78	12.12 $\pm$ 1.80	8.68 $\pm$ 1.33*	6.36 $\pm$ 0.93*	7.43 $\pm$ 1.62	7.47 $\pm$ 1.26	5.90 $\pm$ 1.00	6.56 $\pm$ 1.33
Other SFA	6.21 $\pm$ 2.98	6.60 $\pm$ 2.66	6.40 $\pm$ 2.70**	3.85 $\pm$ 1.08	3.58 $\pm$ 0.62	3.72 $\pm$ 0.87**	2.19 $\pm$ 0.22	2.25 $\pm$ 0.45	2.22 $\pm$ 0.35**	2.04 $\pm$ 0.14	1.93 $\pm$ 0.38	1.98 $\pm$ 0.30**
Σ SFA	47.55 $\pm$ 8.68	41.46 $\pm$ 8.25	44.51 $\pm$ 8.68	40.68 $\pm$ 13.32	36.52 $\pm$ 13.50	38.79 $\pm$ 12.90	34.32 $\pm$ 1.33*	36.55 $\pm$ 0.85*	35.52 $\pm$ 1.56**	32.36 $\pm$ 1.58	34.08 $\pm$ 3.41	33.37 $\pm$ 2.83**
Monounsaturated fatty acids (MUFA)												
C 16:1n-7	6.61 $\pm$ 1.51	6.78 $\pm$ 2.84	6.69 $\pm$ 2.17**	3.05 $\pm$ 0.52	4.11 $\pm$ 1.34	3.53 $\pm$ 1.08**	2.68 $\pm$ 0.34*	2.05 $\pm$ 0.42*	2.34 $\pm$ 0.49	2.79 $\pm$ 0.51	2.63 $\pm$ 0.73	2.70 $\pm$ 0.63
C 17:1	1.77 $\pm$ 0.62	1.15 $\pm$ 0.10	1.46 $\pm$ 0.53	1.56 $\pm$ 0.22*	1.25 $\pm$ 0.26*	1.42 $\pm$ 0.28	2.53 $\pm$ 0.40	2.62 $\pm$ 0.55	2.58 $\pm$ 0.47**	2.26 $\pm$ 0.42	1.90 $\pm$ 0.42	2.05 $\pm$ 0.44**
C 18:1n-9	11.49 $\pm$ 2.85	10.49 $\pm$ 6.77	10.99 $\pm$ 4.98	8.54 $\pm$ 3.46	14.51 $\pm$ 3.22	11.26 $\pm$ 4.45	6.23 $\pm$ 1.33	5.37 $\pm$ 0.56	5.76 $\pm$ 1.05	5.34 $\pm$ 1.32	4.41 $\pm$ 1.74	4.80 $\pm$ 1.59
C 20:1n9	2.25 $\pm$ 0.60	2.72 $\pm$ 0.94	2.49 $\pm$ 0.79**	3.33 $\pm$ 1.08	3.98 $\pm$ 0.96	3.34 $\pm$ 1.08**	5.50 $\pm$ 0.63*	4.48 $\pm$ 0.21*	4.95 $\pm$ 0.69**	6.11 $\pm$ 1.18	7.90 $\pm$ 6.51	7.15 $\pm$ 4.95**
other MUFA ²	2.19 $\pm$ 0.98	1.78 $\pm$ 0.86	1.98 $\pm$ 0.90	2.23 $\pm$ 0.78	3.04 $\pm$ 1.08	2.60 $\pm$ 0.97	3.50 $\pm$ 0.40*	2.46 $\pm$ 0.36*	2.94 $\pm$ 0.65**	2.88 $\pm$ 0.38	2.13 $\pm$ 0.65	2.44 $\pm$ 0.66**
Σ MUFA	24.30 $\pm$ 3.76	22.92 $\pm$ 9.26	23.61 $\pm$ 6.78	18.19 $\pm$ 3.22*	26.88 $\pm$ 4.86*	22.14 $\pm$ 5.94	20.45 $\pm$ 1.21*	16.99 $\pm$ 1.17*	18.59 $\pm$ 2.13	19.39 $\pm$ 1.45	18.97 $\pm$ 7.69	19.15 $\pm$ 5.31
Polyunsaturated fatty acids (PUFA)												
C: 20:4n-6 ³	4.34 $\pm$ 1.55	5.18 $\pm$ 2.62	4.76 $\pm$ 2.09**	7.64 $\pm$ 3.22	6.06 $\pm$ 2.06	6.92 $\pm$ 2.75**	9.86 $\pm$ 1.71*	7.51 $\pm$ 0.96*	8.59 $\pm$ 1.78	9.80 $\pm$ 1.00	9.18 $\pm$ 0.68	9.48 $\pm$ 0.87
C 20:5n-3	8.54 $\pm$ 4.06	17.45 $\pm$ 11.53	13.00 $\pm$ 9.46	10.82 $\pm$ 9.46	11.00 $\pm$ 6.08	10.90 $\pm$ 5.02	1.64 $\pm$ 0.32	1.47 $\pm$ 0.28	1.55 $\pm$ 0.30	2.58 $\pm$ 2.20*	1.14 $\pm$ 0.20*	1.74 $\pm$ 1.53
C 22:6n-3	11.43 $\pm$ 7.92	8.42 $\pm$ 6.23	9.92 $\pm$ 6.97**	19.54 $\pm$ 8.70	16.28 $\pm$ 8.97	18.06 $\pm$ 8.54**	1.09 $\pm$ 0.30*	0.27 $\pm$ 0.22*	0.65 $\pm$ 0.49	1.27 $\pm$ 0.44*	0.67 $\pm$ 0.31*	0.92 $\pm$ 0.47
Other PUFA ⁴	2.32 $\pm$ 0.77	3.15 $\pm$ 1.75	2.73 $\pm$ 1.36**	1.92 $\pm$ 0.30	1.88 $\pm$ 0.41	1.91 $\pm$ 0.34**	n.d	n.d	n.d	n.d	n.d	n.d
Σ PUFA	26.63 $\pm$ 11.34	34.19 $\pm$ 16.02	30.41 $\pm$ 13.81	39.92 $\pm$ 15.68	35.23 $\pm$ 16.73	37.79 $\pm$ 15.52	12.60 $\pm$ 1.64	9.25 $\pm$ 1.04	10.79 $\pm$ 2.16	13.75 $\pm$ 3.26	10.99 $\pm$ 0.82	12.14 $\pm$ 2.50
Σ FAME (ug/mg PS)	31.35 $\pm$ 9.55	33.14 $\pm$ 8.30	31.25 $\pm$ 8.53*	48.79 $\pm$ 11.56	43.40 $\pm$ 9.59	46.34 $\pm$ 10.56**	63.74 $\pm$ 15.67	67.99 $\pm$ 9.53	66.03 $\pm$ 12.35	58.27 $\pm$ 16.11	67.56 $\pm$ 15.02	63.69 $\pm$ 15.50
Σ n-3/ Σ n-6	2.03 $\pm$ 1.46	1.11 $\pm$ 0.55	1.57 $\pm$ 1.15	2.11 $\pm$ 0.83	2.07 $\pm$ 0.98	2.09 $\pm$ 0.85	3.52 $\pm$ 0.67*	5.07 $\pm$ 0.76*	4.35 $\pm$ 1.06	3.66 $\pm$ 0.53	4.01 $\pm$ 0.36	3.86 $\pm$ 0.52
DHA/EPA	1.31 $\pm$ 0.53	0.63 $\pm$ 0.37*	0.97 $\pm$ 0.56**	1.76 $\pm$ 0.34	1.57 $\pm$ 0.31	1.68 $\pm$ 0.36**	0.66 $\pm$ 0.10*	0.18 $\pm$ 0.15*	0.40 $\pm$ 0.28	0.65 $\pm$ 0.03	0.57 $\pm$ 0.05	0.60 $\pm$ 0.23

¹ – other minor SFA C15:0, C17:0 and C24:0;

² – other minor MUFA C18:1n-7, C22:1n-9 and C24:1n-9;

³ – as the FA C20:3n-3 and the FA C20:4n-6 have the same retention time, and the concentration of the FA C20:4n-6 is dominant in marine products, the concentration presented here is representative of C20:4n-6.

⁴ – other minor FA C18:2n-6 and C20:2n-6.

Digestive Gland

Gonad

northwest coast

south coast

immature

mature

Digestive Gland

Gonad