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Summary: The European hake, Merluccius merluccius, is an important resource for Mediterranean fisheries. This study focuses on juvenile and adult hake feeding ecology in the Gulf of Lions, using information from scientific surveys carried out during two seasons and three years (2004-2006). Stomach content and stable isotope (δ15N, δ13C) analyses were performed, and the main factors explaining variations in δ15N were investigated using GLMs. In the Gulf of Lions, hake mostly fed on crustaceans and fish and reached a dominant piscivorous regime at 15 cm total length. Pelagic fish (sardine, anchovy and small blue whiting) were the main source of prey (40%-80%) and cannibalism was low (<5%). The results confirmed that hake is an opportunistic feeder and also showed that the size and diversity of prey vary among hake size classes, probably as a result of the different spatial distribution and/or foraging migrations. The present study finally postulates that the unbalanced sex ratio (80% female against 20% male) observed at the adult stage could be related to the combination of growth pattern differences, diet and exploitation rate on the continental shelf, where the males spend a longer period of time.

Keywords: foodweb; GLM; habitat; Merluccius merluccius; predator-prey relationships; stable isotopes; stomach contents.

Trophic ecology of the European hake in the Gulf of Lions, northwestern Mediterranean Sea

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Ecología trófica de la merluza europea en el Golfo de León, Mediterráneo noroccidental

Resumen: La merluza europea, Merluccius merluccius, es un importante recurso para las pesquerías mediterráneas. Este estudio se centra en la ecología alimentaria de juveniles y adultos de merluza en el Golfo de León, utilizando información de campañas científicas llevadas a cabo en primavera y otoño durante tres años (2004-2006). Se realizaron análisis de contenidos estomacales e isótopos estable (δ15N, δ13C), mientras que la identificación de los principales factores que explican las variaciones en δ15N se investigaron utilizando GLMs. En el Golfo de León, la merluza se alimenta mayoritariamente de crustáceos y peces y alcanzan un régimen piscívoro dominante a los 15 cm T.L. Los peces pelágicos (sardina, anchoveta y bacaladilla pequeña) constituyen el mayor porcentaje de sus presas (40-80%) y el canibalismo es bajo (<5%). Los resultados confirmaron que la merluza es un alimentador oportunista y también muestran que la talla y la diversidad de las presas varían entre distintas clases de talla de la merluza, probablemente como resultado de una distribución espacial diferente y/o migraciones en busca de alimento. Finalmente, este estudio postula que la proporción de sexos desequilibrada (80% de hembras frente a 20% de machos) que se observa en estado adulto podría estar relacionada con la combinación de diferentes patrones de crecimiento, dieta y tasa de explotación en la plataforma continental, donde los machos pasan un mayor periodo de tiempo.

Palabras clave: red trófica; GLM; hábitat; Merluccius merluccius; relaciones depredador-presa; isótopos estables; contenidos estomacales.


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INTRODUCTION

The Gulf of Lions (GoL) is a rather small area (~15000 km²) of the northwestern Mediterranean that supports an intensive multi-specific fishery. European hake, *Merluccius merluccius* (Linnaeus, 1758), constitutes a significant part of the commercial landings in this area, and the stock is currently overexploited. Today only juvenile and young adult hake remain on the continental shelf after decades of intensive exploitation by bottom trawling (Jadaud et al. 2014). Hake is a permanent inhabitant of the GoL and lives in a wide bathymetric range (30-800 m depth), moving between the continental shelf, the shelf break and the slope during its life cycle (Recasens et al. 1998). The species spawns mainly on the shelf-break, where longline and gillnet fisheries target the largest individuals.

The role of hake in the GoL ecosystem is still poorly understood and the baseline information on its diet remains largely unknown, although this species is considered a key predator of this area. A shift in the main prey occurs from suprabenthic crustaceans to fish in the youngest juvenile stages (Bozzano et al. 1997, Ferraton et al. 2007); spatial and temporal diet variations have only been investigated for juveniles in the GoL (Ferraton et al. 2007). Size relationships between hake and its prey remain unknown in the GoL.

The objective of the present study is to provide new, detailed information on the feeding ecology of hake in the GoL, considering temporal, spatial and ontogenetic variability. To this end, stomach content and stable isotope (δ¹⁵N, δ¹³C) analyses were performed on individuals of hake caught during scientific surveys carried out in the GoL. Dietary studies based on stomach contents reflect the identifiable food ingested during the sampling period depending on the digestion rates of the prey. This approach was supplemented by the stable isotope analysis of nitrogen (¹⁵N/¹⁴N) and carbon (¹³C/¹²C). These indicators give an integrative signal of the food ingested over a longer period (weeks to months). Nitrogen is classically used as an indicator of trophic levels, as consumers are enriched in δ¹⁵N relative to their food by a rather large fractionation factor (Minagawa and Wada 1984, Vander Zanden et al. 1997, Post 2002). In contrast, δ¹³C varies little along the foodweb and is used to identify baseline carbon sources at the base of the food chain (DeNiro and Epstein 1978, Cabana and Rasmussen 1994, Vander Zanden et al. 1997). Therefore, in addition to examining hake diet from stomach contents, we also analysed variations in δ¹⁵N and δ¹³C to identify the main factors that could affect hake trophic ecology during its ontogeny in the GoL.

MATERIALS AND METHODS

Data acquisition

Sampling was carried out in the GoL, at four sites on the continental shelf selected from a previous work (Ferraton et al. 2007) and two new sites on the shelf-break and upper-slope (Fig. 1). Zones 2 (in front of the Rhône River) and 4 were located at 30-50 m depth, zones 1 (under the influence of the Rhône river plume) and 3 at 70-100 m, and zones 5 and 6 at 150-250 m and 250-600 m, respectively. Sampling was performed during 14 surveys carried out between 2004 and 2006. Hake (stomach and muscle), prey (muscle), water particulate organic matter (POM), phytoplankton and zooplankton were collected on the continental shelf (zones 1 to 4) during spring and autumn 2004 and 2005 by diurnal standardized operations: bottom trawl (GOC 73 trawl, Anon. 2013), suprabenthic sledge (>0.5 mm), high-flow water pump for phytoplankton (63-200 μm) and zooplankton triple nets (>200 μm). On the shelf-break (zone 5) and slope (zone 6), only hake and its main prey were collected from 2004 to 2006 by nocturnal gillnets (30-38 mm) with stretched mesh sizes. Note that no hauls were conducted in the 50-70 m and very few hauls in the 100-150 m depth ranges, because of the risk of damage to the sampling gear. Thus, the samples of hake (Fig. 2) are not representative of the...
actual spatial distribution of individuals, in particular with regard to small hakes.

To study spatial and temporal fish prey variability in stomachs, a total of 154 hauls and 20 gillnet operations were performed. Fish were measured to the nearest mm of total length (T_L) in small hakes (<25 cm) and to the nearest cm T_L for larger specimens. A total of 20 and 10 individuals per cm size class for small and large hakes, respectively, were randomly selected in each season, site and year, when available. A macroscopic maturity stage was attributed to each individual according to the gonad development. Small hakes were frozen at −20°C immediately after the catch. Large hakes were dissected on board, stomachs were removed, and both were frozen at −20°C. Hake with everted or missing stomachs, the result of necrophagic activity, were excluded from the analyses of stomach contents. Small crustaceans living in the benthic boundary water layer were collected with a suprabenthic sledge equipped with zooplankton nets (0.5 mm mesh size) (Sorbe, 1999). All fish and crustacean species susceptible to being preyed upon by hake were collected and stored frozen as a whole at −20°C. Crustaceans were sorted by large taxonomic groups (mysids, amphipods, euphausiids) and frozen at −20°C. Surface water POM was pre-filtered on 250-μm mesh sieves to remove zooplankton and large detritus, and filtered on pre-weighted Whatman GF/F filters pre-combusted for 4 h at 500°C. Phytoplankton was collected by pumping, with a submersible high-flow water pump (320 L min⁻¹), large volumes of seawater at the fluorescence maximum detected with a Seabird CTD fitted with a fluorometer, through three different vertical nets of limited to the prey size range consumed by hake. When a large prey size range was consumed, different length-categories were considered.

Diet and stable isotope analyses

Stomachs were defrosted, weighed and fixed in 70% ethanol. Empty stomachs were counted and only used to calculate vacuity index (empty stomachs / empty stomachs + stomachs with food) or repletion index (1 – vacuity index) (Table 1). Prey in stomachs were sorted, identified to the lowest taxonomic level possible, counted, measured in mm T_L and weighed (dry weight to the nearest 0.01 mg recorded after 24 to 48 h at 60°C, depending on the prey-type) individually or by broader taxonomic groups. Otoliths of fish prey were measured in mm. Fish size vs otolith-size was calculated for each fish prey and used to estimate the T_L of fish prey partly digested, and then a dry weight correction was made. The relative contribution in dry weight (%W, Hyslop 1980) of each dietary category of each size class (using Euclidean distance and Ward’s minimum-variance method). Hake were grouped by 2-cm T_L class until 24 cm and by 5-cm T_L class afterwards. The spatial and temporal variations in hake diet were examined by comparing W% of each prey category in the resulting length groups defined by the cluster analysis.

Stable isotope analyses (SIA) were conducted on two sub-samples of surface POM and the 63- to 200-μm-sized fraction. One sub-sample was analysed without any prior treatment for δ¹³C determination. The other, used for δ¹⁵N analysis, was acidified with 1% HCl solution to remove carbonates, rinsed with distilled water and oven-dried at 40°C for 24 h, as carbonates have a higher δ¹³C than organic carbon (DeNiro and Epstein 1978, Lorrain et al. 2003). SIA on fish and crustaceans were conducted on the same samples as those for stomach analysis between 2004 and 2006. Samples of muscle posterior to the head for fish, of caudal muscle for shrimp and of whole organisms for zooplankton and suprabenthos were used for SIA. Muscle samples were oven-dried and ground into a powder with a mortar and pestle. Crustaceans were decalcified and samples were divided into two sub-samples and treated as surface POM. Samples of large crustaceans and fish were analysed individually, whereas samples of small crustaceans were pooled to obtain enough material for analysis. Powdered samples were weighed (~1 mg) into tin capsules and combusted in a Euro EA 20-20 continuous-flow isotope-ratio mass spectrometer (Mylnefield Research Services-SCRI, Scrimgeour and Robinson 2003). Based on standard deviation of within-run replicate measurements of standards, analytical error was estimated to be ±0.2‰ for δ¹³C and δ¹⁵N measurements. Stable isotope values are expressed in the standard δ notation as parts per thousand (‰) relative to Vienna Pee Dee Belemnite (δ¹³C) and atmospheric air (δ¹⁵N) standards. SIA were limited to the prey size range consumed by hake. When a large prey size range was consumed, different length-categories were considered.

Lipids were not extracted from our samples prior to SIA. The mean level of lipids (%) in muscle measured over 64 individuals (5-71 cm) was estimated to 5.17±2.67%. This mean value close to 5% can be considered as a low lipid concentration inducing no bias on δ¹³C values (Post et al. 2007).

The hake trophic foodweb was established from the mean (±SE) δ¹³N and δ¹⁵N values of hake and its different prey only for the year 2005 because it was the most complete year for isotopic data (Table 2). Two main sources of organic matter, the marine phytoplankton and the Rhône River POM, were identified in this area.

Statistical analyses

To evaluate ontogenetic changes in hake diet composition, a hierarchical agglomerative cluster analysis was performed on the dry weight percentages (W%) of each dietary category of each size class (using Euclidean distance and Ward’s minimum-variance method). Hake were grouped by 2-cm T_L class until 24 cm and by 5-cm T_L class afterwards. The spatial and temporal variations in hake diet were examined by comparing W% of each prey category in the resulting length groups defined by the cluster analysis.

To study the relationship between the ontogenetic changes in prey size and hake size, we used simple linear regression analysis, and we used quantile regres-
Table 1. – Diet composition of hake according to size (total length, cm) from the GoL in 2004-2006, expressed as percentage by dry weight of each prey category (W%). Total number of stomachs included stomachs with and without food, stomachs regurgitated, and stomachs not found because they were eaten by necrophages during the catch. (*, values <0.1%; -, prey category not found). Unid., unidentified.

<table>
<thead>
<tr>
<th>Total length (cm)</th>
<th>05-06</th>
<th>07-14</th>
<th>15-24</th>
<th>25-39</th>
<th>40-49</th>
<th>50-74</th>
<th>TOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomachs with food</td>
<td>75.0</td>
<td>65.5</td>
<td>63.1</td>
<td>44.9</td>
<td>16.0</td>
<td>31.0</td>
<td>2001</td>
</tr>
<tr>
<td>Empty stomachs</td>
<td>23.0</td>
<td>30.6</td>
<td>45.0</td>
<td>27.5</td>
<td>17.1</td>
<td>49.5</td>
<td>1274</td>
</tr>
<tr>
<td>Everted/not found stomachs</td>
<td>13.0</td>
<td>101.0</td>
<td>41.0</td>
<td>77.0</td>
<td>144.0</td>
<td>38.0</td>
<td>414.0</td>
</tr>
<tr>
<td>Total number (n)</td>
<td>111.0</td>
<td>1062.0</td>
<td>1122.0</td>
<td>801.0</td>
<td>475.0</td>
<td>118.0</td>
<td>3689</td>
</tr>
<tr>
<td>Vacuity index (%)</td>
<td>7.0</td>
<td>0.0</td>
<td>5.0</td>
<td>15.0</td>
<td>4.0</td>
<td>9.0</td>
<td>36.0</td>
</tr>
<tr>
<td>Feeding intensity (%)</td>
<td>93.0</td>
<td>95.0</td>
<td>95.0</td>
<td>85.0</td>
<td>96.0</td>
<td>91.0</td>
<td>64.0</td>
</tr>
<tr>
<td>Number of food types</td>
<td>10.0</td>
<td>32.0</td>
<td>21.0</td>
<td>16.0</td>
<td>21.0</td>
<td>11.0</td>
<td>44.0</td>
</tr>
</tbody>
</table>

CRUSTACEANS

<table>
<thead>
<tr>
<th></th>
<th>94.5</th>
<th>16.0</th>
<th>0.8</th>
<th>0.6</th>
<th>0.6</th>
<th>0.7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amphipoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampelisca sp.</td>
<td>-</td>
<td>2.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vibilia armata</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vibilia sp.</td>
<td>-</td>
<td>0.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amphipoda Unid.</td>
<td>71.9</td>
<td>1.9</td>
<td>*</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Cucumaceae

|                     | 1.4  | *   | *   | -   | -   | -   |

Euphausiacea

|                     | 2.1  | 1.0  | -   | -   | -   | -   |

Mysidacea

|                     | 6.2  | 0.8  | -   | -   | -   | -   |

Natantia

|                     | 0.2  | *   | *   | *   | *   | -   |
|                     | 0.2  | *   | *   | *   | *   | -   |
|                     | 0.2  | *   | *   | *   | -   | -   |
|                     | 0.2  | -   | *   | *   | -   | -   |

Caridea Unid.       | -    | 0.2  | *   | *   | -   | -   |

Natantia Unid.      | 5.5  | 3.5  | 0.4 | 0.3 | 0.2 | 0.1 |

Pachypleura sivado  | -    | -    | -   | -   | -   | -   |

Penaeidae Unid.     | 0.2  | -    | -   | -   | -   | -   |

Philoprosopis sp.   | 4.8  | 3.0  | *   | -   | -   | -   |

Plesionika sp.      | -    | -    | -   | -   | 0.1 | -   |

Processa canaliculata| 0.7  | 0.2  | *   | *   | *   | -   |

Processa sp.        | 2.1  | 1.3  | *   | -   | -   | -   |

Solenocera membranacea| -   | *    | *   | 0.3 | 0.2 | 0.6 |

Solenoceridae Unid. | -    | *    | *   | *   | *   | -   |

Reptantia

|                     | -    | -    | *   | *   | -   | -   |

Suprabenthos Unid.  | -    | 0.5  | 0.1 | -   | -   | -   |

Crustacea Unid.     | 0.2  | *    | *   | -   | -   | -   |

FISH

|                     | 5.5  | 82.1 | 98.1| 98.8| 98.6| 99.2|

Benthic fish

|                     | 5.5  | 15.0 | 2.1 | 0.8 | 6.8 | 5.7 |

Callionymidae Unid.| -    | 0.3  | -   | -   | -   | -   |

Conger conger       | -    | 0.2  | *   | -   | 0.6 | -   |

Delichotostes quadrimaculatus| - | 0.1 | * | * | * | - |

Gobidae Unid.      | 1.4  | 1.1  | *   | *   | *   | -   |

Gobius niger       | 1.5  | 0.6  | -   | 0.1 | -   | -   |

Lepidopus caudatus  | -    | -    | -   | 4.2 | -   | -   |

Lepidotrigla cavillone| - | 0.2 | -   | -   | -   | -   |

Lescueurbius friesi| 4.1  | 11.1 | 1.1 | *   | *   | -   |

Mullus sp.         | -    | 0.2  | *   | -   | -   | -   |

Phycis blemnoideis | -    | -    | -   | 1.8 | 5.7 | -   |

Phycis sp.         | -    | -    | -   | 0.7 | -   | -   |

Triglidae Unid.    | 1.0  | -    | -   | -   | -   | -   |

Demersal fish

|                     | 18.8 | 2.9  | 3.8 | 12.8 | 21.3 |

Argentina sphyraena | -    | -    | 1.3 | 0.1  | -    |

Capros aper        | -    | -    | 0.7 | 3.5  | -    |

Cepola macrophthalmus| - | 0.9 | 0.6 | 2.8 | 10.0 |

Epigonus denticulatus| - | - | 0.2 | - | - |

Gaidropsaurus bicusayensis| - | 0.7 | - | - | - |

Merluccius merluccius| 3.4 | 0.6 | 0.1 | 1.1 | 4.1 |

Trisopterus minutus| 14.8 | 14 | 1.5 | 8.1 | 3.7 |

Pelagic fish

|                     | 43.6 | 79.1 | 88.2| 75.7| 28.1 |

Engraulis encrasicolus| - 9.8 | 30.4 | 5.7 | 2.8 | - |

Micromesistius poutassou| 2.7 | 1.4 | 2.8 | 29.8 | 25.7 |

Sardina pilchardus| 21.6 | 38.7 | 74.1 | 38.3 | - |

Scomber scombrus| - 0.3 | 0.7 | 0.5 | - | - |

Sprattus sprattus| - 0.2 | - | - | - | - |

Trachurus mediiteraneus| - | 0.1 | 0.6 | 0.1 | - |

Trachurus sp.| - | 0.1 | 0.6 | 0.1 | 1.6 |

Pelagic fish Unid.| 9.5 | 8.0 | 4.9 | 3.8 | 0.8 |

Fish Unid.| 4.6 | 14.0 | 5.9 | 3.3 | 44.1 |

CEPHALOPODS

|                     | 1.0  | 0.5  | -   | 0.4 | -   |

Alloteuthis sp.| 0.8  | 0.1  | -   | 1.7 | -   |

Sepiola sp.| 0.2  | 0.3  | *   | -   | -   |

Teuthida Unid.| -    | -    | *   | *   | -   |

Cephalopods Unid.| *    | *    | *   | 0.4 | -   |
sions to estimate changes in minimum and maximum prey size. Quantiles ranged between the 90th and 99th for upper bound and the 1st and 10th for the lower bound.

We also investigated variations in hake δ\textsuperscript{15}N based on all available isotopic data (2004-2006) and tested the potential influence of different factors (season, depth, east-west gradient, sex) and continuous variables (length, condition, δ\textsuperscript{13}C) as well as potential interactions between these explanatory variables, using generalized linear models (Venables and Dichmont 2004). Because the adults and the juveniles are not sympatric and because juveniles were not collected on the shelf-break and the upper-slope (Fig. 2), we performed one model for the juveniles and another for the adults. Both models included all factors and variables, but sex was added as a supplementary factor for the model on adults. Interactions between factors and variables were also tested and presented only when significant. As δ\textsuperscript{15}N is a continuous positive variable that is not normally distributed, we chose a Gamma error distribution with an identity link function. Diagnostic plots including the predicted values of mean of response and the standardized Pearson residuals, as well as the correlation among parameters, were examined for each analysis performed. The Akaike information criterion (AIC) was used to select the final model.

Hake maturity was identified according to two categories: juvenile or adult. Fish were considered as juvenile when the attributed maturity stage was virgin or at the beginning of maturation. Male adult stages were defined from the maturity scale used during the MED-ITS surveys (Anon., 2013). Female adult stages were defined from gonadosomatic index (GSI) calculated as GSI=100(GW/W), where GW is the gonad wet weight and W is the observed individual eviscerated weight.

Fish condition was estimated by the relative condition factor, Kn, proposed by Le Cren (1951, see also Froese 2006) computed as Kn=W/aLn, where W is the observed individual eviscerated weight, L is the TL in cm, and aLn is the estimated eviscerated weight from the log W-log L relationship, where: aLn=10\textsuperscript{-intercept} TL\textsuperscript{slope}. In the original formula, W was the total weight of the fish, but we considered the eviscerated weight for two reasons: (i) the weight of stomach content is highly variable and can therefore bias the estimation of the relation aLn; and (ii) the condition is here used as an explanatory variable of δ\textsuperscript{15}N in hake muscle.

Statistical analyses were performed with the support of SPSS 17 (SPSS 2008) and R Softwares (R Development Core Team 2009).

RESULTS

Hake diet

Among the 3689 hake sampled, 2001 showed stomachs with food, 1274 empty stomachs (39%) and 414 stomachs regurgitated or not found because they were eaten by necrophages. The vacuity index increased with length (23% to 61%, Table 1). Larger individuals that displayed empty stomachs more frequently (61%) were caught in deeper waters (down to 600 m, Fig. 2). Fish contribution in hake diet increased with hake length from 5.5% to 99.2% (Table 1). Crustaceans were the dominant food in the smallest individuals and cephalopods never exceeded 1%. The results of cluster analysis clearly differentiated six groups of diet size classes (Fig. 3), confirming the changes in hake diet during its growth. The smallest size class (5-6 cm TL, a singleton) included a low number of prey types (10), mostly amphipods (71.9%). By contrast, the second smallest group in size (7-14 cm TL) displayed the most diversified diet (32 prey types). Benthic and demersal fish species were nearly as important (33.8%) as pelagic fishes (43.6%), while crustaceans were clearly less important (16.6%). The following groups (hake of 15-24, 25-39 and 40-49 cm TL) showed an increasing relative importance of crustaceans (16.6-21.9%), while benthic and demersal fish species were increasingly less important (23.8-15.2%). The last size class (50-74 cm TL) displayed the highest diversity of diet (75 prey types), including crustaceans (29.8%), cephalopods (25.6%) and benthic and demersal fish species (30.1%).
cm T₁) exhibited 16 to 21 prey types, including mostly fish (>98%), mostly pelagic species (75.7-88.2%). In the largest group (50-74 cm), the number of prey types decreased to 11 (similar to the smallest fish group) and the diet was mostly composed of fish (99.2%), among which 28.1% were pelagic, 21.3% demersal and 5.7% benthic (the remaining part of the fish diet could not be determined).

Only three pelagic fish, sardine (*Sardina pilchardus* Walbaum, 1792), European anchovy (*Engraulis encrasicolus* Linnaeus, 1758) and small blue whiting (*Micromesistius poutassou* Risso 1827), two demersal fish, poor cod (*Trisopterus minutus* Linnaeus, 1758) and red bandfish (*Cepola macrophthalma* Linnaeus, 1758), and two benthic fish, *Lesueurigobius friesii* (Malm, 1874), and the greater forkbeard (*Phycis blennoides* Brünich, 1768), exceeded individually more than 5% of dry weight of hake diet (Table 1). The contribution of the three main pelagic fish species varied with hake length (Table 1). Sardine predominated in hake ranging from 7 to 49 cm T₁ (21-74%), while anchovy only prevailed in the 15-24 cm T₁ size class (30%) and small blue whiting in fish larger than 39 cm T₁ (26-30%). The other groups never exceeded 15%. Cannibalism was observed in all size classes displaying a piscivorous diet, but remained low (0.1 to 4.1%). It was higher in the smallest size class (3.4%) and in the largest hake (4.1%), with a maximum rate on the slope (6%).

**Spatio-temporal feeding variations**

Because all hake size classes did not occur in all depth ranges, seasons and years, the influence of these factors on hake diet were examined with separate data sets (Fig. 4A-D).

The effect of depth (continental shelf vs shelf-break/slope) on the diet was analysed on 25-49 cm T₁ (2 size classes) (Fig. 4A). In the first size class (25-39 cm T₁), pelagic fish was the main prey (90%) on the continental shelf, while hake consumed 2/3 pelagic fish and 1/3 demersal or benthic fish on the shelf-break/slope. Sardine was the main prey on the continental shelf (77%), while small blue whiting dominated on the shelf-break/slope (42%). In the second size class (40-49 cm T₁), there was no difference in the proportion of pelagic fish or in the proportion of demersal or benthic fish between the two areas. Nonetheless, sardine also dominated hake diet on the continental shelf (63%), while small blue whiting dominated in deeper waters (74%).

The analysis of dietary changes with season (spring vs autumn) was only possible on the continental shelf for 7-49 cm T₁ (4 size classes) (Fig. 4B). Considering pelagic fish, there was no significant difference between seasons in the first three size classes (7-14 cm, 15-24 cm and 25-39 cm T₁), but the proportion of pelagic fish was 30% lower in spring than in autumn in the largest size class (40-49 cm T₁). Crustaceans were only preyed on by the 7-14 cm T₁ class, especially in spring. Total fish prey represented 73% in spring and 87% in autumn in this size class. Demersal or benthic fish dominated more in spring (37%) than in autumn (13%) in the 40-49 cm T₁ class.

Year-to-year variations were analysed separately on the shelf-break/slope in only one size class (40-49 cm T₁) and on the continental shelf (5-49 cm T₁) in 5 size classes (Fig. 4C-D). On the shelf-break/slope...
European Hake trophic ecology

(Fig. 4C), where small blue whiting largely dominated (>70% the pelagic fish group in the diet of the 40-49 cm TL class, there was no difference between 2004 and 2006 in the proportion of pelagic fish (72%) or demersal/benthic fish (20%). On the continental shelf (Fig. 4D), there were no major differences between years in the respective proportion of pelagic fish, demersal fish and crustaceans, except in the 7-14 cm and the 25-39 cm TL classes. In the 7-14 cm TL class, the proportion of small pelagics (54%) dominated in 2004 and the proportion of crustaceans (33%) increased in 2005. In the 25-39 cm TL class, the proportions of small pelagics represented more than 80% in 2004 and 2005, but only 50% in 2006. Yearly diet changes were mainly observed at the level of the dominant crustaceans in the smallest size classes (5-6 cm TL): amphipods dominated in 2004 (78%), whereas natantia decapods (55%) and mysids (45%) prevailed in 2005.

Prey-predator length relationships

The range of prey sizes consumed by hake increased significantly with increasing fish size (R²=0.653, N=1702, P<0.0001, Fig. 5). The slopes of the extreme bounds (5th-95th quantiles) of prey size distribution were also significant (P<0.0001), but of a different amplitude (the slope was higher for the 95th quantile than for the 5th one, Fig. 5). Hake 5-6 cm TL class fed on a mean length prey of 0.7±0.05 cm TL and the following size classes fed on 2.6±0.1 cm, 6.6±0.4 cm, 9.9±0.15 cm, 11.4±0.4 cm and 15.2±1.7 cm TL, respectively. However, hake was able to ingest larger prey, ≥2/3 of its body size in the case of the red bandfish (C. macrophantalma). Hake size classes fed on different mean lengths of the main fish prey such as gobids, anchovy, sardine, blue whiting and poor cod (Table 3).

Hake trophic web

The hake trophic web was based on a marine phytoplankton source and represented a continuum of feeding types ranging from crustaceans to pelagic, benthic and demersal fish (Fig. 6). As expected, phytoplankton and suprabenthic crustaceans (amphipods, euphausiids, and mysids) displayed the lowest δ¹⁵N and δ¹³C values. Stable isotope ratios of the main prey types of hake ranged from 5‰ to 11.2‰ for δ¹⁵N and from –20.3‰ to –16.9‰ for δ¹³C (Table 4). The smallest size classes of fish prey displayed a lower δ¹⁵N value than the largest ones, except for sardine. The largest sardine (≥13.5 cm)
The lowest δ¹⁵N values (7.2‰) were found in the largest sardine (≥ 13.5 cm T L), in the smallest blue whiting (< 15 cm T L), poor cod (≥ 28 cm T L), hake (≥ 25 cm T L) and the greater forbeard exhibited the highest δ¹⁵N values (≥ 10‰). A continuous increase in δ¹⁵N with length was thus observed in hake, from 7.2‰ to 11.8‰. A slight increase (0.4‰) in hake δ¹³C with length occurred from the smallest (– 17.7‰) to the largest individuals (– 17.3‰).

**Hake δ¹⁵N variation**

This analysis was based on 892 isotope samples. All factors (season, depth, E-W gradient), two continuous variables (size and δ¹³C) and one interaction (season-length) were highly significant (P < 0.001) in the selected model for juveniles, but δ¹³C was finally removed because of high correlation with the intercept (Table 5A). The length factor showed the highest contribution to the total explained deviance (50%), followed by season (13%), season-length interaction (11%), depth (2%) and E-W gradient (1%). The model selected for adults was similar to that of juveniles, but it also included the condition and sex as significant covariates, while the length-season interaction did not appear to be significant (Table 5B). Length had again the greatest contribution to hake δ¹⁵N variation.

### Table 4. Mean isotopic signature with standard error (SE) by size class (SC) in the GoL in 2005.

<table>
<thead>
<tr>
<th>SC (cm)</th>
<th>Code</th>
<th>n</th>
<th>δ¹⁵N (‰)</th>
<th>δ¹³C (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mean SE</td>
<td>mean SE</td>
</tr>
<tr>
<td>Surf Particulate Organic Matter</td>
<td>Surf POM</td>
<td>55</td>
<td>5.13 0.15</td>
<td>–22.07 0.14</td>
</tr>
<tr>
<td>Phytoplankton.</td>
<td>Phytopk</td>
<td>24</td>
<td>3.80 0.08</td>
<td>–20.86 0.16</td>
</tr>
<tr>
<td>Zooplankton.</td>
<td>Zoopk</td>
<td>6</td>
<td>6.71 0.63</td>
<td>–21.41 0.31</td>
</tr>
<tr>
<td>Amphipods</td>
<td>Amp</td>
<td>9</td>
<td>6.28 0.38</td>
<td>–20.34 0.11</td>
</tr>
<tr>
<td>Euphausiids</td>
<td>Eup</td>
<td>3</td>
<td>4.99 0.03</td>
<td>–19.96 0.02</td>
</tr>
<tr>
<td>Mysids</td>
<td>Mys</td>
<td>9</td>
<td>6.68 0.11</td>
<td>–20.34 0.19</td>
</tr>
<tr>
<td>Natantia</td>
<td>Nat</td>
<td>41</td>
<td>8.16 0.19</td>
<td>–17.90 0.20</td>
</tr>
</tbody>
</table>

**Table 5. Stepwise generalized linear model for factors and continuous variables controlling δ¹⁵N variability in juvenile (A) and adult (B) hakes in the GoL. Parameter estimates (c) and standard error for the best fitting GLMs model.**

<table>
<thead>
<tr>
<th>Factors and continuous variables added</th>
<th>c</th>
<th>SE</th>
<th>Residual df</th>
<th>Deviance</th>
<th>Df</th>
<th>Deviance decrement</th>
<th>Cumulative deviance explained</th>
<th>% of total deviance explained</th>
<th>P-value (chisquared)</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Juvenile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>5.056</td>
<td>.1893</td>
<td>497</td>
<td>12,407</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1798</td>
</tr>
<tr>
<td>+ Length</td>
<td>.127</td>
<td>.0035</td>
<td>496</td>
<td>6.194</td>
<td>1</td>
<td>6.213</td>
<td>50.077</td>
<td>65%</td>
<td>0.000</td>
<td>1453</td>
</tr>
<tr>
<td>+ Season</td>
<td>2.678</td>
<td>.1387</td>
<td>495</td>
<td>4.579</td>
<td>1</td>
<td>1.615</td>
<td>63.093</td>
<td>17%</td>
<td>0.000</td>
<td>1305</td>
</tr>
<tr>
<td>+ Season*Length</td>
<td>-.073</td>
<td>.0060</td>
<td>494</td>
<td>3.160</td>
<td>1</td>
<td>1.419</td>
<td>74.351</td>
<td>15%</td>
<td>0.000</td>
<td>1122</td>
</tr>
<tr>
<td>+ Depth</td>
<td>-.049</td>
<td>.0058</td>
<td>330</td>
<td>0.692</td>
<td>1</td>
<td>0.084</td>
<td>42.105</td>
<td>14%</td>
<td>0.000</td>
<td>471</td>
</tr>
<tr>
<td>+ E-W gradient</td>
<td>-.045</td>
<td>.0053</td>
<td>328</td>
<td>0.587</td>
<td>1</td>
<td>0.014</td>
<td>49.791</td>
<td>3%</td>
<td>0.000</td>
<td>427</td>
</tr>
<tr>
<td>B: Adult</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>7.132</td>
<td>.3442</td>
<td>334</td>
<td>1.197</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>648</td>
</tr>
<tr>
<td>+ Length</td>
<td>.061</td>
<td>.0042</td>
<td>333</td>
<td>0.931</td>
<td>1</td>
<td>0.266</td>
<td>22.222</td>
<td>44%</td>
<td>0.000</td>
<td>566</td>
</tr>
<tr>
<td>+ Season</td>
<td>.461</td>
<td>.0551</td>
<td>332</td>
<td>0.777</td>
<td>1</td>
<td>0.154</td>
<td>35.067</td>
<td>25%</td>
<td>0.000</td>
<td>507</td>
</tr>
<tr>
<td>+ Depth</td>
<td>.449</td>
<td>.0790</td>
<td>331</td>
<td>0.692</td>
<td>1</td>
<td>0.084</td>
<td>42.105</td>
<td>14%</td>
<td>0.000</td>
<td>471</td>
</tr>
<tr>
<td>+ Sex</td>
<td>.404</td>
<td>.0654</td>
<td>330</td>
<td>0.621</td>
<td>1</td>
<td>0.072</td>
<td>48.120</td>
<td>12%</td>
<td>0.000</td>
<td>436</td>
</tr>
<tr>
<td>+ Condition</td>
<td>1.086</td>
<td>.3214</td>
<td>329</td>
<td>0.601</td>
<td>1</td>
<td>0.02</td>
<td>49.791</td>
<td>3%</td>
<td>0.000</td>
<td>427</td>
</tr>
<tr>
<td>+ E-W gradient</td>
<td>.143</td>
<td>.0511</td>
<td>328</td>
<td>0.587</td>
<td>1</td>
<td>0.014</td>
<td>50.960</td>
<td>2%</td>
<td>0.000</td>
<td>422</td>
</tr>
</tbody>
</table>
ability (22%), while season remained important (13%) and was followed by depth (7%), sex (6%), condition (2%) and E-W gradient (1%).

Juvenile and adult final models explained 77% and 51% of the total deviance, respectively. The diagnostic plots were satisfactory (Fig. 7A-D). Note that Figure 7B tends to show a lower number of data in the range of 8.0-10‰, which actually corresponds to a low sampling of individuals from 20 to 24 cm in comparison with the other size classes.

Both sexes exhibited a similar δ¹⁵N trend with age but females had a higher δ¹⁵N than males at the same age (Fig. 8A). The three smallest hake size classes (i.e. up to 24 cm T₁) displayed a lower δ¹⁵N in spring than in autumn (Fig. 8B). This partially matched the seasonal variation of euphausiids δ¹⁵N used as the primary consumer baseline, with higher values in autumn. From 25 cm T₁ there were no such large seasonal differences in the δ¹⁵N. In spring, the first two size classes showed similar δ¹⁵N, whereas the third size class (15-24 cm T₁) displayed an intermediate δ¹⁵N value between juveniles (<15 cm T₁) and specimens larger than 24 cm T₁. In autumn, δ¹⁵N increased regularly between the different size classes. In the largest hakes (25-75 cm T₁), the trends of δ¹⁵N were similar in both seasons. Higher δ¹⁵N values in hake ≥25 cm T₁ were observed on the continental shelf than on the shelf-break/slope (Fig. 8C).

Fig. 7. – Results of the GLM with a Gamma error and an identity link for juvenile (A, B) and adult (C, D) hakes: plots of the residuals and the fitted values versus δ¹⁵N.

Fig. 8. – Ontogenetic δ¹⁵N changes of hake with (A) sex (F, female; M, male), (B) season (δ¹⁵N Euphausiids = baseline) and (C) depth.
DISCUSSION

Hake diet and feeding strategy

The results of this study confirm that hake is a carnivorous predator that feeds on a variety of benthic, demersal and pelagic prey on the continental shelf and slope. Suprabenthic crustaceans and pelagic fish, i.e. sardine, anchovy and small blue whiting, were the main components of the diet and cephalopods were of minor importance. Diet changes with increasing body length from crustaceans to piscivorous regimes, as previously described in the GoL (Bozzano et al. 1997, Ferraton et al. 2007) and in different hake populations world-wide (e.g. Papaconstantinou and Caragiotis 1987, Payne et al. 1987, Du Buit 1996). The shift occurred progressively between 7 and 14 cm, reaching the piscivorous regime at 15 cm TL before the end of the first year of life (Mellon-Duval et al. 2010). The main difference between regions is related to the dominant pelagic fish preyed on by hake: herring in the Pacific (Tanasichuk et al. 1991), anchovy and pilchard in South Africa (Pillar and Wilkinson 1995), horse mackerel and anchovy in the northern Bay of Biscay (Guichet 1995), and blue whiting and horse mackerel in the Cantabrian Sea (Velasco and Olaso 1998).

Cannibalism has been commonly observed in hake populations (Guichet 1995, Garrison and Link 2000, Cartes et al. 2004). It also occurred in the GoL, mostly on the shelf-break but at low levels (<5%), confirming previous findings by Bozzano et al. (1997) and similar results in the southern Bay of Biscay and Portuguese coasts (Velasco and Olaso 1998, Cabral and Murta 2002). This contrasts with the central Tyrrenian Sea, the northern Bay of Biscay and the Celtic Sea, where cannibalism can exceed 15% (Mahe et al. 2007, Carpentieri et al. 2005). The low level of cannibalism in the GoL could be related to the weak overlap of small and large hakes, which mainly occurred during the reproductive period on the shelf-break.

Year-to-year (like seasonal) variations in diet were of low magnitude, except for the types of suprabenthic crustaceans preyed on by the smallest hake (5-6 cm). Amphipods were dominant in 2004 while mysids and shrimps were dominant in 2005. Similar variations were observed with a dominance of euphausiids and mysids in 2002 and of shrimps and amphipods in 2003 (Ferraton et al. 2007).

The present study confirms that hake is an opportunistic feeder, as already demonstrated (Bozzano et al. 1997, Hidalgo et al. 2008), but also shows that the length and the diversity of prey vary with hake size, probably as a result of different spatial distributions and/or foraging migration. Bozzano et al. (2005) pointed out that 2.5–4.5 cm TL hake have not yet moved to the bottom and the 5–6 cm group represents the first recruits that settle. At this stage, juvenile hake has low mobility (Recasens et al. 1998, Arneri and Morales-Nin 2000) and our results indicate that their diet is restricted by mean prey size <1 cm TL and low prey diversity. The maximum prey diversity observed in the following hake size-group (7-14 cm) is likely to be linked to the mixed diet of crustaceans and fish and to the increasing mean length of prey to 2.6 cm TL. This wider spectrum of prey leads us to postulate that foraging migrations could start at this size class, as corresponds to previous findings in the Tyrrenian Sea (Bartolino et al. 2008). Increasing mobility is related to the development of sensory organs allowing hake to undertake vertical nocturnal migration in water columns in response to similar movement of their prey (Bozzano et al. 2005, Mas-Riera 1991, Lombarte and Popper 2004). The decrease in diversity and the increase in the range of prey size in the diet of the three following hake size classes, from 15 to 49 cm, is probably the result of a restrictive piscivorous regime and could suggest more mobility in the water column to catch larger fish prey.

Foodweb structure

The δ13C values of different size classes exhibit little variation, indicating that the hake trophic web in the GoL is based mainly on a marine phytoplankton source (and not on a Rhône river discharge-related foodweb). By contrast, δ15N values changed with body size and season for both juvenile and adult hake. Size and season interaction influenced δ15N in juveniles, and depth and sex also affect δ15N in adults. Because small crustaceans, mainly eaten by the smallest hake, were pooled to obtain enough material for analysis, the detail of isotopic results concerning this hake size may not be very fine.

The δ15N variations in a consumer can be related to changes in δ15N of the baseline or changes in its diet or growth (Olive et al. 2003, Trueeman et al. 2005, Sweeting et al. 2007). In the present study, ontogenetic shift in the composition and size of the prey probably explains the bulk of variations in δ15N observed. Because our work is based on only two sampling periods, it is not possible to determine a direct relationship between the composition of the diet and the isotopic signature. There is indeed a time lag between the ingestion of prey and its incorporation in the tissue of the predator, and a minimum of 4 to 6 sampling periods would have been necessary. Nonetheless, the increase of δ15N with body length is commonly observed in many predatory fish species (Overman and Parrish 2001, Badalamenti et al. 2002, Jennings et al. 2002), including hake (Le Loc’h and Hily 2005). In our dataset, crustaceans had a lower δ15N than fish, while large blue whiting, large demersal and benthic fish had a higher δ15N than the main fish prey: sardine, anchovy and small blue whiting. Most fish prey, except sardine, increased in δ15N with body length.

The differences observed between spring and autumn in the δ15N baseline (euphausiads) may partially explain the δ15N differences observed in juvenile hakes. A lower growth rate leads to lower consumption and higher excretion of nitrogen compounds, resulting in a δ15N increase in a consumer (Olive et al. 2003, Trueeman et al. 2005, Sweeting et al. 2007). Morales-Nin and Moranta (2004) showed that the growth rate of juvenile hakes is lower in autumn than in spring. This finding suggests that growth could be a contributory
factor in explaining the seasonal effect on δ15N observed in juvenile hake. An increase of δ15N inversely with depth was observed in hake ≥25 cm in the GoL, a finding which is consistent with the work of Sherwood and Rose (2005). Influence of sex on δ15N could be due to an indirect effect of growth dimorphism, male eating smaller prey sizes than females of the same age (lower δ15N for males).

**Stock status considerations with regard to diet**

Hake and its main pelagic prey, except blue whiting, are important fishery resources in the GoL. Sardine and anchovy dominated on the continental shelf and more generally in the northwestern Mediterranean (Palomera et al. 2007), while blue whiting dominated offshore from the shelf-break. All the pelagic fish make up 40% to 80% of hake diet in the GoL, indicating their key trophic role. Since the late 2000s, the pelagic ecosystem of the GoL has shifted to a different regime, characterized by a low biomass of anchovy and sardine related to a slowdown in growth and a bad body condition (Van Beveren et al. 2014, Brosset et al. 2015). Therefore, the population fluctuations and the general “state of health” of these two species may adversely affect the diet, body condition and reproduction of their predators, and consequently reinforce the decline of hake, a species that is already severely overexploited (Jadaud et al. 2014).

Because of growth dimorphism observed in hake of the GoL (males are indeed significantly smaller than females from 2 to 3 years old, Mellon-Duval et al. 2010), males feed longer than females on smaller prey (i.e. sardine, anchovy), which they find in abundance on the continental shelf. For this reason, we hypothesize that the long and intensive exploitation on the continental shelf by trawlers, which led to the reduction of the abundance of large individuals (80% of hake total catch in weight and >97% in number composed of specimens ≤38 cm TL, Jadaud et al. 2014) could affect males and females differently. An increase in the mortality of males could then explain the unbalanced sex ratio observed (80% female against 20% male at 38 cm TL, Jadaud et al. 2014) in the hake population of the GoL.

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