



# Environmental influences on the recruitment process inferred from otolith stable isotopes in *Merluccius merluccius* off the Balearic Islands

Manuel Hidalgo<sup>1,5,\*</sup>, Javier Tomás<sup>1</sup>, Hans Høie<sup>2,3</sup>, Beatriz Morales-Nin<sup>1</sup>,  
Ulysses S. Ninnemann<sup>4</sup>

<sup>1</sup>Institut Mediterrani d'Estudis Avançats (CSIC-UIB), Miquel Marqués 21, 07190 Esporles, Spain

<sup>2</sup>University of Bergen, Department of Biology, PO Box 7800, 5020 Bergen, Norway

<sup>3</sup>Institute of Marine Research, PO Box 1870 Nordnes, 55817 Bergen, Norway

<sup>4</sup>Department of Earth Science, University of Bergen, Allegaten 41, 5007 Bergen, Norway

<sup>5</sup>Present address: Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biology, University of Oslo, PO Box 1066 Blindern, 0316 Oslo, Norway

**ABSTRACT:** Oxygen ( $\delta^{18}\text{O}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotope ratios in sagittal otoliths were analysed in recruits and juveniles of European hake *Merluccius merluccius* L. caught in the northwest (Sóller, SO) and south (Cabrera, CA) of the Island of Mallorca (western Mediterranean) over 2 consecutive years (2003 and 2004). The analytical method used allowed data to be gathered on both environmental and trophic conditions experienced by fish during the pelagic early life stages (registered in the composition of the inner part of the otolith, core area) and the demersal aggregations of recruits (at the otolith edge). Results on the seasonal variation of oxygen isotope signatures at the edge of fish otoliths captured in SO and CA indicated different vertical migration behaviours of hake between the bottom and the thermocline.  $\delta^{13}\text{C}$  showed a clear ontogenetic pattern between the core area and the edge, confirming the differences in diet from the early stages to post-recruits. The seasonal analysis of  $\delta^{13}\text{C}$  data from the otolith edge indicated that fish from the early stages in June 2003 encountered poorer trophic resources, which resulted in poor fish condition and subsequent poorer recruitment. The estimated temperature from the core area of otoliths showed lower temperature regimes in the 2002 hatching season compared to the 2003 hatching season, which could be a possible explanation for the observed differences in success of subsequent recruitment.

**KEY WORDS:** Recruitment · Environmental conditions · Feeding behaviour · *Merluccius merluccius* · Ontogenetic changes · Otoliths · Stable isotopes

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## INTRODUCTION

Recruitment ecology of marine fish populations often assesses the effect of environmental conditions on recruitment by searching for inter-annual environmental and density-dependent driving mechanisms. Reported correlations between environment and recruitment have often been found to break down when more information becomes available. A possible cause lies in the inter-annual changes in seasonal environ-

mental processes that may trigger non-stationary and non-linear effects on the yearly recruitment variability (Stige et al. 2006). It is possible to approach this relationship directly by integrating seasonal and inter-annual variability through the analysis of the chemical composition of the fish otolith (ear bone), as its chemical composition contains a record of changes in environmental conditions. This chemical tag, together with age marks present in the otolith, provides a unique opportunity to directly retrieve from otoliths informa-

\*Email: manuel.hidalgo@bio.uio.no

tion on the environmental conditions experienced by individual fish that can be related to different growth stages, from hatching to capture (Campana 1999).

Of all elements and isotopes included in the otolith carbonate,  $^{18}\text{O}/^{16}\text{O}$  ratios ( $\delta^{18}\text{O}$ ) have been used to estimate past ambient temperatures experienced by fish (Høie et al. 2004a) and applied to corroborate fish age estimation (Weidman & Millner 2000) or to study stock structure (Gao et al. 2001). Studies estimating temperature-dependent fractionation of oxygen isotopes in otoliths have so far concluded that deposition occurs at or near equilibrium; that is, only temperature explains variation in isotopic fractionation between the water and the carbonate of the otolith (Høie et al. 2004b). Moreover,  $^{13}\text{C}/^{12}\text{C}$  ratios ( $\delta^{13}\text{C}$ ) measured in fish otoliths have been used to study metabolic rates and are relevant in the study of ontogenetic changes of trophic levels in the food chain (Schwarcz et al. 1998, Gao & Beamish 2003). The combination of both analyses provides an insight into the changes experienced by fish related to metabolism, food intake and environment.

European hake *Merluccius merluccius* L. is a demersal fish species of great ecological and economic importance in the North Atlantic Ocean and the Mediterranean Sea, where it is widely distributed. In the northwestern Mediterranean Sea, European hake spawns throughout the year, with a main peak in autumn and a secondary peak in spring (Recasens et al. 1998). However, studies have demonstrated a higher than expected seasonal variability in recruitment peaks among neighbouring NW Mediterranean areas (Gulf of Lions: Recasens et al. 1998; Catalan coast: Maynou et al. 2003; other Mediterranean mainland areas: Goñi et al. 2004; Balearic Islands: Hidalgo et al. 2008), suggesting that the timing of the reproduction of hake in the Mediterranean might be an adaptive strategy (Morales-Nin & Moranta 2004). Through this presumed phenotypic plasticity, European hake are able to adapt their spawning to match favourable local conditions and maximise the survival of fish. Hake complete their settlement to the bottom in around the second month of life (ca. 57.5 d and 3.5 cm; Morales-Nin & Moranta 2004, Palomera et al. 2005), and are progressively grouped in nursery areas, where pre-recruits and recruits form patches (Maynou et al. 2006). However, there is still a lack of knowledge on how the environmental conditions affect early life stages of this species and the consequent recruitment success. Here, we studied the hake population off the Balearic Islands—a small population assessed within the framework of the General Fisheries Commission for the Mediterranean (FAO 2006). Recent studies have shown spatial and temporal differences in the occurrence and physiological condition of hake recruited off the Balearic Islands (Hidalgo et al. 2008),

suggesting that these might experience different environmental and trophic conditions (Cartes et al. 2008).

In the present study, we focused on 2 key phases contributing to recruitment success: (1) early life stages before bottom settlement, through analysis of the core of the otolith, and (2) the later period in the life cycle when the recruiting fish are aggregated at the shelf-break, by analysis of the edge of the otolith. We aimed to study the environmental and trophic conditions (using otolith  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ , respectively) experienced by hake from the early stages to post-recruits, in order to better understand the spatial and temporal variability of the recruitment process of this species. We further studied differences in temperature conditions encountered by fish recruiting north and south of the Balearic Islands over 2 consecutive years (2002 and 2003) which showed different annual recruitment indices (Massutí et al. 2008).

## MATERIALS AND METHODS

**Study area.** This study was conducted around the Island of Mallorca (Balearic Islands, NW Mediterranean; Fig. 1), a zone of oceanographic transition between the cooler, more saline waters of the Balearic basin in the north and the warmer, less saline waters of Atlantic ori-

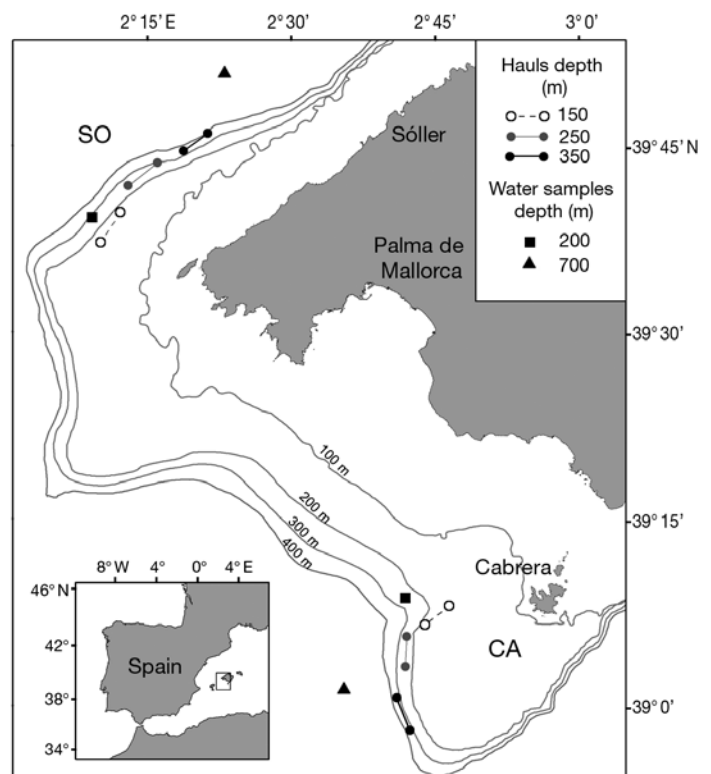


Fig. 1. Locations where both fish sampling hauls and water sampling were conducted. CA: Cabrera; SO: Sóller

gin to the south, in the Algerian basin. These waters are exchanged through the channels between the islands. Sampling locations were northwest of Mallorca, off Sóller (SO) within the Balearic basin, and in the south close to the Cabrera Archipelago (CA), which is located in the Algerian basin (Fig. 1).

**Fish and hydrographical sampling.** European hake *Merluccius merluccius* were caught at the shelf-break at SO and CA during 2 d trawling surveys over 4 seasons (November 2003, and February, April and June 2004) aboard the FV 'Moralti Nou'. Three hauls at 150, 250 and 350 m depth were carried out on each cruise and at each location (Fig. 1). A commercial bottom trawl was used with a cod-end mesh size of 40 mm and a cod-end liner with a mesh size of 10 mm (for additional sampling details see Moranta et al. 2008).

Once on board, fish were measured (total length, cm). They were grouped into 3 size ranges according to both the growth parameters used in the stock assessment for the geographic area of the Balearic Islands by the General Fisheries Commission for the Mediterranean (FAO 2006) and trophic criteria (Bozzano et al. 1997, Carpentieri et al. 2005): (1) the 12 to 15 cm size range grouped recruits of the Age 0 class ( $\leq 18$  cm) that feed mostly on crustaceans; (2) the 18 to 22 cm size range grouped the post-recruits, which have a mixed diet and belong to the Age 1 class (19 to 29 cm); and (3) the 25 to 30 cm size range grouped young adults, which mainly have an ichthyophagous diet. In the laboratory, the total weight ( $\pm 0.01$  g) and total length (mm) of the fish were measured. Left and right sagittal otoliths were extracted and stored dry in individual sealed plastic vials. Individuals from each size range and each area were randomly selected from each of the 4 seasons, giving a total of 63 fish otoliths for analysis (Table 1). The depth of capture was not considered as a factor in our study because of the high vertical mobility of hake (Bozzano et al. 2005).

In parallel with the trawling surveys, hydrographic surveys of the area recorded temperature and salinity, and water samples were collected for analysis for oxygen isotope composition. Temperature and salinity measurements were taken with CTD (SBE911+) casts between the surface and the bottom depth at different stations in each of the 2 areas studied (for details see López-Jurado et al. 2008). Water samples for water oxygen isotope analysis were taken at 200 m, as this is the mean depth of hake recruits and juveniles, and also at 700 m, as this is the mean depth for large adults (Fig. 1). Seawater was collected with a carousel Niskin bottle coupled to a CTD guideline. Once on board, 100 ml plastic bottles previously rinsed 3 times with seawater were filled up to the top ensuring that no air bubbles were trapped inside (Pierre 1999). Water samples were stored cold (4°C) and in the dark until analysis.

Table 1. *Merluccius merluccius*. Number of European hake analysed for  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  isotope ratios in otoliths by size range (total fish length), survey (date) and sampling site

| Size range (cm) | Date     | Sampling site |        |
|-----------------|----------|---------------|--------|
|                 |          | Cabrera       | Sóller |
| 12–15           | Nov 2003 | 2             | 3      |
|                 | Feb 2004 | 2             | 3      |
|                 | Apr 2004 | 2             | 3      |
|                 | Jun 2004 | 3             | 2      |
| 18–22           | Nov 2003 | 2             | 2      |
|                 | Feb 2004 | 3             | 3      |
|                 | Apr 2004 | 3             | 3      |
|                 | Jun 2004 | 3             | 3      |
| 25–30           | Nov 2003 | 2             | 3      |
|                 | Feb 2004 | 3             | 3      |
|                 | Apr 2004 | 3             | 1      |
|                 | Jun 2004 | 3             | 3      |

**Otolith preparation.** Right otoliths were embedded in epoxy resin (EpoThin Buehler) and cut into 500  $\mu\text{m}$  thick sections along the transverse plane through the core area with a slow-speed saw using a double-diamond blade. These sections were affixed to glass slides with thermoplastic glue (Crystalbond), and ground and polished consecutively with P-400, P-1000 and P-4000 grade paper (Federation of European Producers of Abrasives, FEPA standards).

**Carbonate sampling and stable isotopes.** A high-resolution sampling technique using a computer-controlled micromill (NewWave Research) was used to collect the carbonate in the hake otolith sections. Sampling was designed to meet the requirements of the Finnigan MAT 251 mass spectrometer, which has an optimal minimum sample size requirement ranging between 40 and 50  $\mu\text{g}$  of carbonate mass. Assuming an average otolith density of 2.7  $\text{g cm}^{-3}$  (Hoff & Fuiman 1993), the necessary volume for a single analysis ranged between 0.014 and 0.018  $\text{mm}^3$ . About 0.025  $\text{mm}^3$  was extracted from each sample to ensure enough carbonate mass. Hake otoliths show a complex growth increment pattern deposited around a central area. Carbonate was sampled in the core area, defined as the inner region between the nucleus or primordium and the accessory growth centres that correspond to an age of 40 to 55 d old, depending on the season (Fig. 2) (pelagic pre-settlement period; Morales-Nin & Moranta 2004) and at the edge, along the ventro-proximal side (Fig. 2).

Prior to otolith sampling, each otolith section was photographed with an Olympus C5050 camera at  $\times 2.5$  magnification in a stereomicroscope using transmitted light. From that digital image of the otolith section, the region to be sampled was outlined along the edge and data were imported to the micromill software. Drilling depth was set to 200  $\mu\text{m}$  and was performed by 2 passes of 100  $\mu\text{m}$  each (for detailed micromill proce-

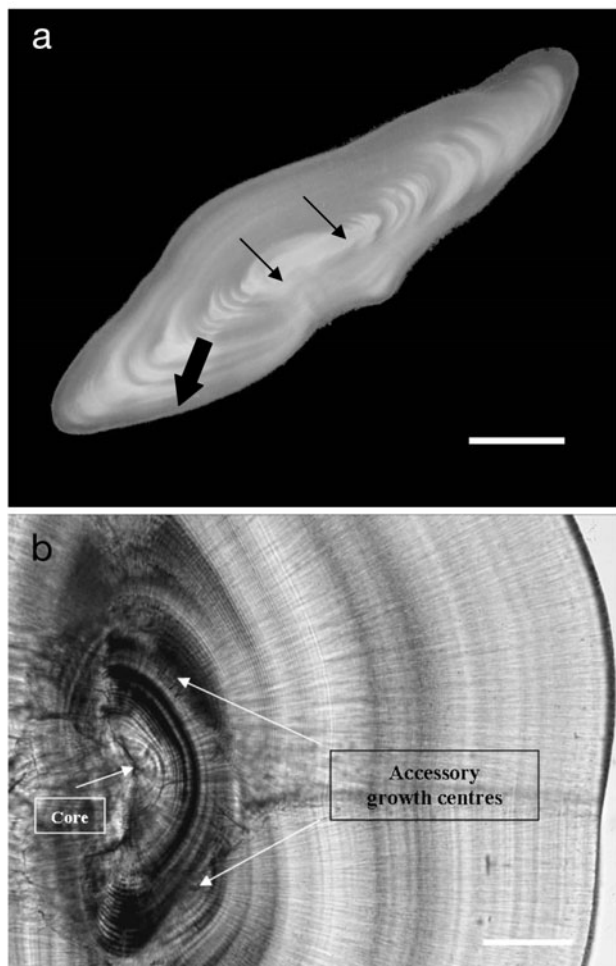


Fig. 2. *Merluccius merluccius*. (a) European hake (170 mm total length [TL] caught in April 2004 at Cabrera [CA]) transverse section of a sagittal otolith showing the core area (thin arrows) and the edge area (thick arrow) where the sampled carbonate was taken. (b) European hake (174 mm TL, caught in June 2004 at CA) transverse section of a sagittal otolith showing the core area. Arrows indicate the core and accessory growth centres. Scale bars = (a) 500  $\mu\text{m}$  and (b) 100  $\mu\text{m}$

ture see Høie et al. 2004a). External and internal calibrations of the micromill and the software were also carried out.

Isotope analyses were performed with a Finnegan MAT (M-251) mass spectrometer at the Department of Earth Science, University of Bergen, Norway. Measurements were taken from the  $\text{CO}_2$  gas released from the reaction of the otolith carbonate with orthophosphoric acid at 70°C in an automated 'Kiel' on-line preparation device, with acid added to the sample in individual reaction chambers. Seawater samples were equilibrated with  $\text{CO}_2$  at 20°C in an automated preparation line, and were measured for  $\delta^{18}\text{O}$  with a Finnegan DELTA-E mass spectrometer, with an analytical precision of 0.1‰. Carbonate isotope results are

reported relative to the VPDB (Vienna Pee Dee Belemnite) standard through calibration against NBS-19 and NBS-18 standards. Oxygen isotopes from the water were reported relative to VSMOW (Vienna Standard Mean Ocean Water). The long-term reproducibility of the system was  $\pm 0.07\text{‰}$  for  $\delta^{18}\text{O}$  and  $\pm 0.06\text{‰}$  for  $\delta^{13}\text{C}$  and was based on replicate measurements of an internal carbonate standard.

**Temperature estimations.** Since no oxygen isotope fractionation relationship has been established for the European hake, temperature was estimated using the equation resulting from a robust study on another gadoid, Atlantic cod *Gadus morhua* L. (Høie et al. 2004b), with a 0.95‰ prediction limit range:

$$\delta_c - \delta_w = 3.90(\pm 0.24) - 0.2(\pm 0.19)T, \quad r^2 = 0.94, n = 163 \quad (1)$$

$\delta_c$  refers to the oxygen isotopic composition of  $\text{CO}_2$  from carbonate in otoliths relative to the international standard VPDB calcite, and  $\delta_w$  refers to the isotopic composition of the  $\text{CO}_2$  gas in seawater equilibrated with the seawater sample relative to the standard VPDB.  $T$  is temperature in °C. The relationship between  $\delta^{18}\text{O}_{\text{VSMOW}}$  and  $\delta_w$  from Friedman & O'Neill (1977) converts oxygen isotopic values of seawater relative to VSMOW ( $\delta^{18}\text{O}_{\text{VSMOW}}$ ) to  $\delta_w$ :

$$\delta_w = 0.99978(\delta^{18}\text{O}_{\text{VSMOW}}) - 0.22 \quad (2)$$

The  $\delta^{18}\text{O}$  of seawater ( $\delta_w$ ) and the temperature at 200 m from the CTD profiles were used as independent inputs in the estimation of the temperature-dependent fractionation in the oxygen isotopic composition at the edge of hake otoliths. When temperature was estimated from the core area, the average  $\delta^{18}\text{O}$  of seawater at 200 m for each area was used. The depths of trawling hauls (150, 250 and 350 m), which correspond to the depth distribution range of hake juveniles and recruits, were well above 700 m, and therefore the 200 m water data are more appropriate to use in calculations of temperature from otolith isotopic composition.

Two fish growth rates were used to back-calculate the hatching date of fish (the median point of the fish length range was used as the actual size). For recruits (12 to 15 cm) and post-recruits (18 to 22 cm), we used the growth equation obtained from the same sampling in a parallel study (Hidalgo 2007), in which daily micro-increments in hake otoliths between 7 and 18 cm were analysed. Differences in growth rate were not observed between hake recruiting north and south of the Island of Mallorca. Consequently, we used the following equation obtained for all individuals analysed to back-calculate the hatching date:

$$L_t = 0.457(\pm 0.78)t^{1.007(\pm 0.26)} + 24.04(\pm 20.65), \quad r^2 = 0.94, n = 168 \quad (3)$$

where  $L_t$  is the length (mm) at time  $t$ , and  $t$  is the age (d). Larger hake presumably have lower growth rates than the juveniles, and reading microstructure of hake otoliths has proven to be an impossible task after the first year of life (ca. 18 cm; Morales-Nin & Moranta 2004). Therefore, for hakes in the size range between 25 and 30 cm, the von Bertalanffy growth function estimated in previous studies for hake from the Balearic Island area was used ( $L_\infty = 126.9$ ,  $k = 0.184$ ,  $t_0 = 0.035$ ; Alemany & Oliver 1995).

As shown in previous studies around the Balearic Islands (Hidalgo et al. 2008) and at other locations in the NW Mediterranean (Recasens et al. 1998), the months between February and April are when abundance of recruits is maximal, thus contributing a large percentage to the annual recruitment. Assuming that the estimated fish growth rate from Hidalgo (2007) is valid for approximately the first year of life, the hatching season must be between about May and September of the previous year. Differences in the  $\delta^{13}\text{C}$  and temperature experienced by fish in their early life stages were compared between the 2 consecutive hatching years 2002 and 2003 and between CA and SO using isotopic values of the core areas.

**Statistics.** A 1-way analysis of variance (ANOVA) was used to segregate  $\delta^{18}\text{O}$  data between the core area and the edge of the otolith, while a heterogeneity of slopes model was used to segregate  $\delta^{13}\text{C}$  and to compare the relationship between  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  for the sampled regions in the otoliths. At the otolith edge, factorial ANOVA was used to test for seasonal and size differences in oxygen isotopic fractionation ( $\delta_c - \delta_w$ ), and analysis of covariance (ANCOVA) was used to test for seasonal and size interval differences in  $\delta^{13}\text{C}$  values, with  $\delta_c - \delta_w$  as the continuous predicted variable removing the temperature effect in  $\delta^{13}\text{C}$  variation. Size ranges of 18 to 22 cm and 25 to 30 cm were pooled when the area of capture (SO and CA) was introduced as a factor in the factorial ANOVA and ANCOVA to avoid losing degrees of freedom.

For the otolith core, individuals belonging to the main recruitment season (February to June, hatching season from May to September of the previous year) were analysed to test for differences in conditions experienced in the early life stages. In this case, differences between areas of capture (CA and SO) and hatching year (2002 and 2003) for both the estimated temperatures and the  $\delta^{13}\text{C}$  composition were also analysed with a factorial ANOVA and ANCOVA, respectively. A Tukey test of honestly significant differences (HSD) was used as a post hoc test of

comparison of means. A significance level of  $\alpha = 0.05$  was used in all tests. Unless otherwise stated, all means are reported with their standard error (SE).

## RESULTS

### Water $\delta^{18}\text{O}$

The oxygen isotope composition of seawater ( $\delta^{18}\text{O}_{\text{VSMOW}}$ ) showed low variation at 200 m depth (mean  $\pm$  SE:  $1.08 \pm 0.04\text{‰}$  in CA and  $1.27 \pm 0.09\text{‰}$  in SO; Table 2) and at 700 m depth ( $1.03 \pm 0.04\text{‰}$  in CA and  $1.11 \pm 0.05\text{‰}$  in SO; Table 2), consistent with the low variation of salinity (see temperature and salinity profiles in Fig. 3). The  $\delta^{18}\text{O}_{\text{VSMOW}}$ /salinity relationship in both areas was positive, as expected. SO values were significantly correlated at 200 m ( $\delta^{18}\text{O}_{\text{VSMOW}} = -103.89 + S2.744$ ,  $r^2 = 0.86$ ,  $p = 0.035$ ; where  $S$  = salinity) and marginally insignificantly correlated at 700 m ( $\delta^{18}\text{O}_{\text{VSMOW}} = -315.41 + S8.21$ ,  $r^2 = 0.85$ ,  $p = 0.076$ ). However, in CA, the positive correlation was not significant at 200 m ( $\delta^{18}\text{O}_{\text{VSMOW}} = -15.91 + S0.443$ ,  $r^2 = 0.28$ ,  $p = 0.71$ ). At 700 m, salinity and  $\delta^{18}\text{O}_{\text{VSMOW}}$  remained almost constant at around 38.52 and 1.26‰, respectively.

### $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values in the core area and edge of hake otoliths

$\delta^{18}\text{O}$  values at the edge of the otoliths of European hake *Merluccius merluccius* were significantly higher than those in the core area (ANOVA,  $F_{1,122} = 94.81$ ,  $p < 0.01$ ; Table 3), indicating that early life stages experience cooler temperatures than later in life.  $\delta^{13}\text{C}$  values followed the same pattern (heterogeneity of slopes model,  $F_{1,122} = 33.21$ ,  $p < 0.01$ ; Table 3).  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were significantly correlated in both the otolith core area ( $r^2 = 0.69$ ,  $p < 0.0001$ ; Fig. 4) and at the edge ( $r^2 = 0.12$ ,  $p = 0.007$ ; Fig. 4), yet the slopes of the rela-

Table 2. Results of  $\delta^{18}\text{O}_{\text{VSMOW}}$  (‰) and salinity values from the stations where water was sampled (see Fig. 1). VSMOW: Vienna Standard Mean Ocean Water

| Depth (m) | Survey date | Cabrera                              |          | Sóller                               |          |
|-----------|-------------|--------------------------------------|----------|--------------------------------------|----------|
|           |             | $\delta^{18}\text{O}_{\text{VSMOW}}$ | Salinity | $\delta^{18}\text{O}_{\text{VSMOW}}$ | Salinity |
| 200       | Nov 2003    | 1.06                                 | 38.37    | 1.53                                 | 38.4     |
|           | Feb 2004    | 0.97                                 | 38.25    | 1.07                                 | 38.24    |
|           | Apr 2004    | 1.2                                  | 38.31    | 1.17                                 | 38.29    |
|           | Jun 2004    | 1.08                                 | 38.37    | 1.29                                 | 38.35    |
| 700       | Nov 2003    | 1.28                                 | 38.52    | 1.04                                 | 38.51    |
|           | Feb 2004    | 1.11                                 | 38.52    | 1.02                                 | 38.5     |
|           | Apr 2004    | 1.28                                 | 38.52    | 1.16                                 | 38.51    |
|           | Jun 2004    | 1.24                                 | 38.51    | 1.23                                 | 38.52    |

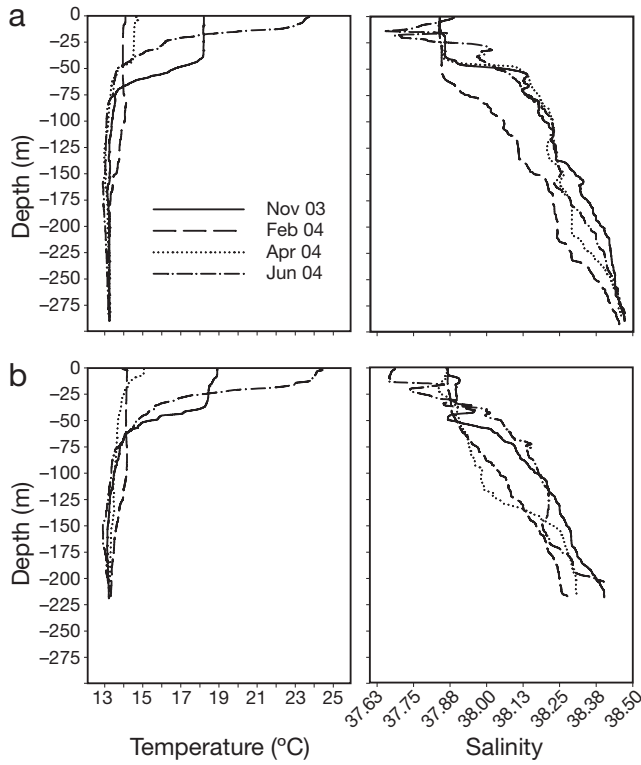


Fig. 3. Vertical temperature and salinity CTD (SBE911+) profiles at the 2 regions studied during the different cruises. (a) Soller and (b) Cabrera

tionships were different (homogeneity slopes model,  $F_{1,122} = 18.48$ ,  $p < 0.01$ ). These results indicate that the environmental and trophic conditions experienced by fish before metamorphosis are different from those experienced by older stages.

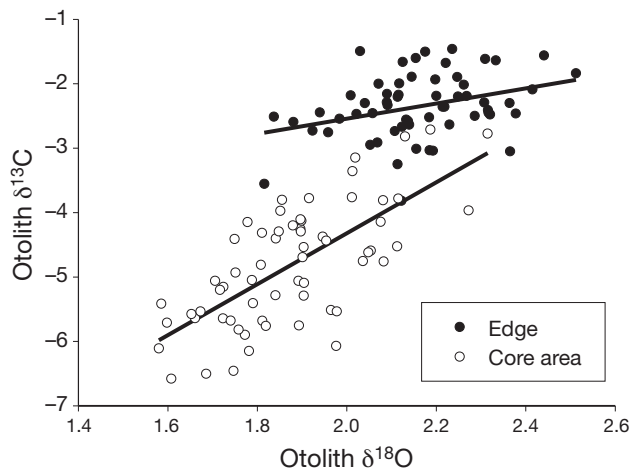


Fig. 4. *Merluccius merluccius*. Relationship between  $\delta^{13}C$  and  $\delta^{18}O$  for the 2 otolith regions sampled. Both sampling sites were significantly correlated: in core area  $\delta^{13}C = -12.22 + 3.95 \delta^{18}O$  ( $r^2 = 0.69$ ,  $p < 0.0001$ ) and at the edge  $\delta^{13}C = -4.89 + 1.17 \delta^{18}O$  ( $r^2 = 0.12$ ,  $p = 0.007$ )

Table 3. *Merluccius merluccius*. Summary of  $\delta^{13}C$  and  $\delta^{18}O$  results from analyses conducted in the core area and at the edge of otoliths of European hake of the 3 size ranges studied

| Size range (cm) | Survey date | Estimated month of hatching | N  | Core area          |                |       | Edge               |       |                |      |              |
|-----------------|-------------|-----------------------------|----|--------------------|----------------|-------|--------------------|-------|----------------|------|--------------|
|                 |             |                             |    | $\delta^{13}C$ (‰) | Mean           | Range | $\delta^{13}C$ (‰) | Mean  | Range          |      |              |
| 12–15           | Nov 2003    | Apr 2003                    | 5  | -4.75              | -5.89 to -4.14 | 1.95  | 1.77 to 2.08       | -2.68 | -2.91 to -2.49 | 2.08 | 1.92 to 2.29 |
|                 | Feb 2004    | Jul 2004                    | 5  | -4.89              | -5.67 to -3.77 | 1.82  | 1.66 to 1.91       | -2.71 | -3.03 to -2.44 | 2.07 | 1.93 to 2.18 |
|                 | Apr 2004    | Aug 2004                    | 7  | -4.39              | -5.06 to -3.35 | 1.84  | 1.70 to 2.01       | -2.46 | -2.73 to -2.18 | 1.97 | 1.83 to 2.11 |
|                 | Jun 2004    | Oct 2004                    | 5  | -3.43              | -4.29 to -2.77 | 2.04  | 1.84 to 2.31       | -3.24 | -3.81 to -2.58 | 2.07 | 1.81 to 2.16 |
| 18–22           | Nov 2003    | Dec 2002                    | 3  | -5.00              | -5.15 to -4.81 | 1.77  | 1.72 to 1.80       | -1.70 | -1.99 to -1.46 | 2.15 | 2.02 to 2.31 |
|                 | Feb 2004    | Mar 2003                    | 6  | -5.07              | -6.50 to -3.78 | 1.86  | 1.58 to 2.11       | -2.04 | -3.04 to -1.49 | 2.18 | 2.09 to 2.36 |
|                 | Apr 2004    | May 2003                    | 6  | -4.98              | -6.14 to -2.71 | 1.85  | 1.65 to 2.18       | -2.26 | -2.55 to -1.89 | 2.12 | 1.78 to 2.26 |
|                 | Jun 2004    | Jul 2003                    | 6  | -5.71              | -6.45 to -5.28 | 1.85  | 1.72 to 1.97       | -2.39 | -3.03 to -1.93 | 2.18 | 2.08 to 2.25 |
| 25–30           | Nov 2003    | May 2002                    | 4  | -4.83              | -6.07 to -3.96 | 2.1   | 1.97 to 2.27       | -2.36 | -2.46 to -2.19 | 2.29 | 2.21 to 2.38 |
|                 | Feb 2004    | Aug 2002                    | 6  | -4.42              | -6.10 to -3.76 | 1.9   | 1.57 to 2.08       | -2.03 | -2.40 to -1.56 | 2.33 | 2.11 to 2.51 |
|                 | Apr 2004    | Oct 2002                    | 4  | -4.99              | -5.70 to -3.97 | 1.77  | 1.59 to 1.90       | -2.19 | -2.47 to -1.64 | 2.22 | 2.02 to 2.33 |
|                 | Jun 2004    | Dec 2002                    | 6  | -5.25              | -6.57 to -3.80 | 1.79  | 1.60 to 2.04       | -2.05 | -2.63 to -1.60 | 2.23 | 2.07 to 2.41 |
| All individuals |             |                             | 63 | -4.81              | -6.58 to -2.71 | 1.88  | 1.58 to 2.31       | -2.35 | -3.81 to -1.46 | 2.16 | 1.78 to 2.51 |

### Effect of season, area of capture and fish size on $\delta_c - \delta_w$ at the edge

Seawater temperature at locations and depths where the fish were caught ranged between 13.02 and 13.35°C. Within this range of temperatures the  $\delta_c - \delta_w$  at the edge varied between 0.61 and 1.66‰ (mean  $\pm$  SE:  $1.20 \pm 0.03$ ‰). Significant differences existed between otoliths from different fish size ranges (factorial ANOVA,  $F_{2,48} = 5.29$ ,  $p < 0.05$ ) with lower  $\delta_c - \delta_w$  (i.e. higher temperature) for the 12 to 15 cm range. A Tukey test showed that significant differences did not exist between the 18 to 22 cm and the 25 to 30 cm size ranges ( $p = 0.47$ ), and these were higher than values measured in the 12 to 15 cm fish range. Data from the 18 to 22 cm and 25 to 30 cm size ranges were thus pooled to keep the highest degrees of freedom when testing for the effect of area of capture. Resulting  $\delta_c - \delta_w$  still showed significant differences related to the smallest fish size range (factorial ANOVA,  $F_{1,44} = 20.73$ ,  $p < 0.05$ ) and to the season (factorial ANOVA,  $F_{3,44} = 14.94$ ,  $p < 0.05$ ). February had the highest values, and November, the lowest. The individuals captured in CA showed significantly higher  $\delta_c - \delta_w$  values at the edge of the otoliths than those captured in SO (factorial ANOVA,  $F_{1,44} = 47.09$ ,  $p < 0.05$ ). Moreover, interaction between the area of capture and season was significant (factorial ANOVA,  $F_{3,44} = 11.27$ ,  $p < 0.05$ ), showing that the seasonal pattern was different between SO and CA.

These results indicate that temperatures experienced by fish captured in SO were higher than for fish from CA for each survey (Fig. 5a, Table 4). The only exception was April, when no significant differences were detected (Fig. 5a, Table 4). The comparison of the estimated temperatures (Fig. 5a) with the measured temperatures (Fig. 5b, see also temperature and salinity profiles in Fig. 3) showed that fish caught in SO had experienced temperatures closer to those measured in the 25 to 75 m depth range. However, in CA, fish had experienced significantly lower temperatures, closer to those existing below the thermocline, with the exception of April (Fig. 5b).

### $\delta^{18}\text{O}$ -derived temperature estimates for the early stages: seasonal, inter-annual and spatial effects

Estimated temperature values experienced by fish in the early development stages (from hatching to larvae) varied from 12.2 to 16.7°C (mean  $\pm$  SE:  $14.9 \pm 0.1$ °C; Table 4). Of all the fish analysed, up to 50% showed an oxygen isotope composition associated with temperatures  $>15$ °C, and nearly 90% were associated with temperatures  $>14$ °C. Estimated temperature for early

life stages of fish 12 to 15 cm, caught in different seasons, reflected the natural seasonal variation of temperatures (Fig. 6a). Estimated temperature from otolith core areas of fish 18 to 22 cm in size showed a decrease in temperature at the beginning of winter and a slight increase during the first months of summer. The 25 to 30 cm sized fish showed that estimated temperature from otolith core areas increased from April 2002 throughout the summer and autumn 2002 (Fig. 6a).

The comparison of temperatures experienced by individuals belonging to the main recruitment season, which may have hatched from May to September (see grey bars at Fig. 6a); showed significantly lower values in summer 2002 (mean  $\pm$  SE:  $14.45 \pm 0.27$ °C) compared to summer 2003 ( $15.05 \pm 0.14$ °C) (factorial ANOVA,  $F_{1,31} = 5.93$ ,  $p = 0.020$ ; Fig. 6b). Significant differences also existed between SO and CA (factorial ANOVA,  $F_{1,31} = 4.85$ ,  $p = 0.035$ ; Fig. 6c), with higher temperatures in SO ( $15.1 \pm 0.1$ °C) than in CA ( $14.6 \pm 0.2$ °C; Fig. 6c). The interactions were not significant ( $p > 0.05$ ).

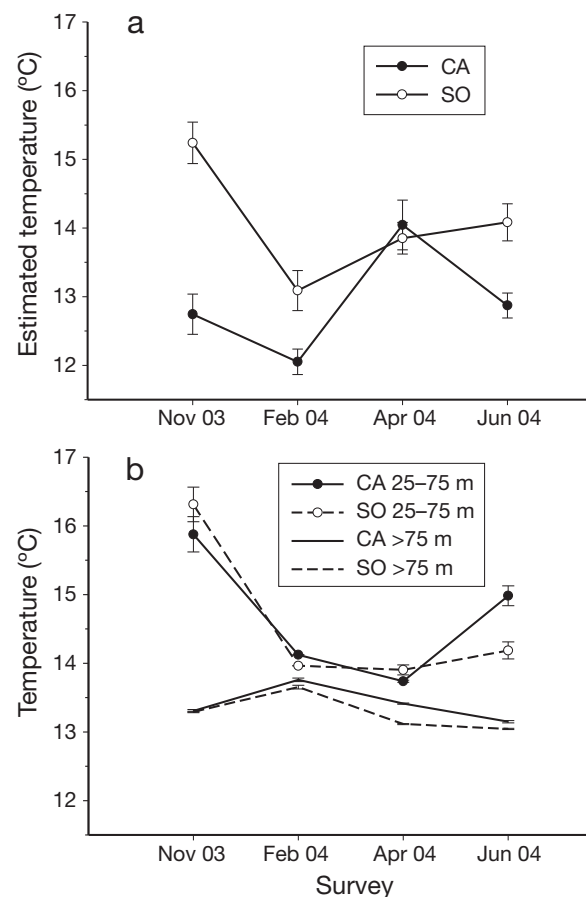


Fig. 5. *Merluccius merluccius*. (a) Temperature estimated from oxygen isotopes at the edge of otoliths; 2 zones and 4 surveys are plotted. (b) Mean temperatures from CTD profiles (Fig. 3) at seawater sampling stations

Table 4. *Merluccius merluccius*. Estimated temperatures from oxygen isotopes using the equation proposed by Høie et al. (2004b). SE: standard error

| Size range (cm) | Survey date | Estimated month of hatching | N | Estimated temperature (°C) |           |     |      |           |     |
|-----------------|-------------|-----------------------------|---|----------------------------|-----------|-----|------|-----------|-----|
|                 |             |                             |   | Core area                  |           |     | Edge |           |     |
|                 |             |                             |   | Mean                       | Range     | SE  | Mean | Range     | SE  |
| 12–15           | Nov 2003    | Apr 2003                    | 5 | 14.6                       | 13.4–15.9 | 0.5 | 14.7 | 12.3–16.4 | 0.9 |
|                 | Feb 2004    | Jul 2004                    | 5 | 15.3                       | 14.3–15.8 | 0.2 | 13.2 | 12.3–14.1 | 0.3 |
|                 | Apr 2004    | Aug 2004                    | 7 | 15.0                       | 14.4–16.0 | 0.2 | 14.4 | 13.7–15.2 | 0.3 |
|                 | Jun 2004    | Oct 2004                    | 5 | 14.0                       | 12.2–15.5 | 0.5 | 13.9 | 13.1–15.8 | 0.5 |
| 18–22           | Nov 2003    | Dec 2002                    | 3 | 15.5                       | 14.7–16.1 | 0.4 | 14.5 | 13.5–15.5 | 0.5 |
|                 | Feb 2004    | Mar 2003                    | 6 | 15.0                       | 14.0–16.3 | 0.4 | 12.6 | 11.4–13.3 | 0.3 |
|                 | Apr 2004    | May 2003                    | 6 | 15.0                       | 12.8–15.8 | 0.4 | 14.0 | 12.9–15.5 | 0.4 |
|                 | Jun 2004    | Jul 2003                    | 6 | 15.0                       | 14.0–16.1 | 0.3 | 13.4 | 12.8–13.9 | 0.1 |
| 25–30           | Nov 2003    | May 2002                    | 4 | 14.0                       | 12.4–14.8 | 0.5 | 14.0 | 12.4–14.9 | 0.6 |
|                 | Feb 2004    | Aug 2002                    | 6 | 14.8                       | 14.3–15.9 | 0.2 | 11.8 | 11.2–13.2 | 0.3 |
|                 | Apr 2004    | Oct 2002                    | 4 | 15.2                       | 14.3–16.7 | 0.5 | 13.3 | 12.7–14.1 | 0.3 |
|                 | Jun 2004    | Dec 2002                    | 6 | 15.3                       | 13.5–16.7 | 0.5 | 13.2 | 11.7–14.5 | 0.4 |

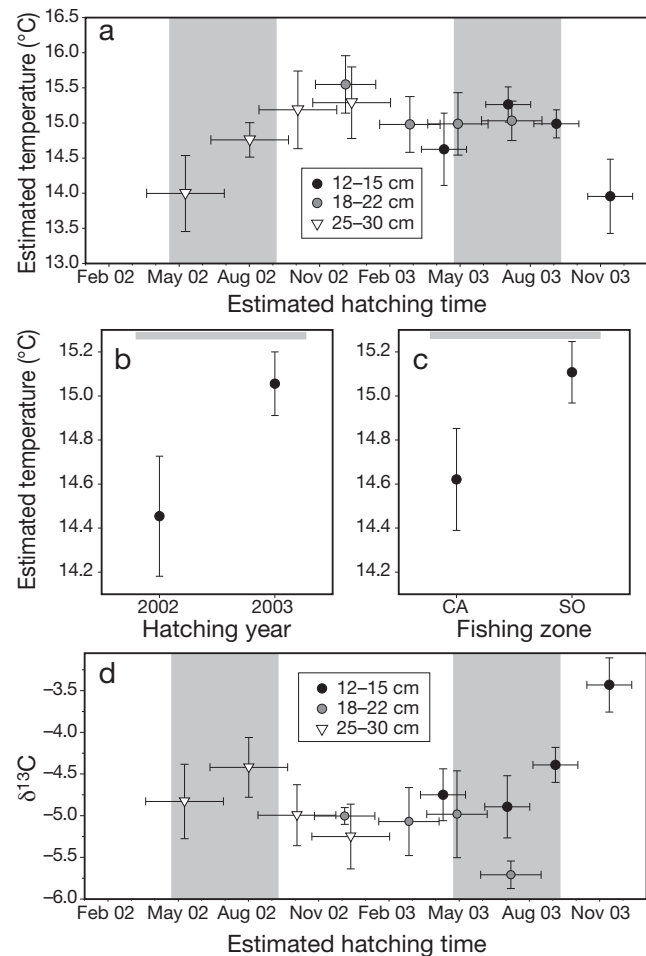
### $\delta^{13}\text{C}$ variation in hake otoliths

Otolith core areas of fish 12 to 15 cm in size reflected a decrease in  $\delta^{13}\text{C}$  during summer, with values increasing in autumn and at the beginning of winter (Fig. 6d). Otolith core areas in fish 18 to 22 cm in size showed a decrease in  $\delta^{13}\text{C}$  in summer, but no variability between winter and spring. The 25 to 30 cm sized fish had otolith core areas showing a decrease throughout the summer and autumn, and  $\delta^{13}\text{C}$  was lower in spring 2002 (Fig. 6d). However, significant differences were not found between factors (area of capture and hatching year) at the core areas related to the hatching season between May to September (ANCOVA, for all the factors  $p > 0.05$ ).

$\delta^{13}\text{C}$  increased in otoliths between the early life stages and post-recruits (Fig. 7a). At the edge, the  $\delta_c - \delta_w$  increment was similar in  $\delta^{13}\text{C}$  between the groups of factors (season and fish size) (homogeneity slopes, for all the factors,  $p > 0.05$ ), and thus the temperature effect was consistent among them.  $\delta^{13}\text{C}$  was consistently lower for smaller fish (12 to 15 cm) than for larger ones (ANCOVA,  $F_{2,47} = 15.37$ ,  $p < 0.05$ ; Fig. 7a; all interactions  $p > 0.05$ ). However, the Tukey test showed no significant differences between the 18 to 22 and 25 to 30 cm size range groups ( $p = 0.99$ ; Fig. 7a).

Fig. 6. *Merluccius merluccius*. (a) Estimated temperature at the core area of the otolith  $\delta^{18}\text{O}$  versus season of birth; 12 to 15, 18 to 22 and 25 to 30 cm size range intervals are shown. Grey sections define the estimated period when spring recruits could have hatched (see 'Materials and methods' for details). (b) Average estimated temperature for this period (spring recruits) in 2002 and 2003 and (c) in zones (CA: Cabrera; SO: Sóller). (d)  $\delta^{13}\text{C}$  of the core area for different size classes of fish hatched at different times. Vertical bars represent standard error and horizontal bars represent the hatching date estimated for the entire size range

Both size ranges were also pooled to test for the effect of area of capture. The  $\delta_c - \delta_w$  increment was also similar in  $\delta^{13}\text{C}$  between CA and SO (homogeneity slopes,  $p > 0.05$ ), although there were no significant differences between the 2 areas (ANCOVA,  $F_{1,43} = 0.36$ ,





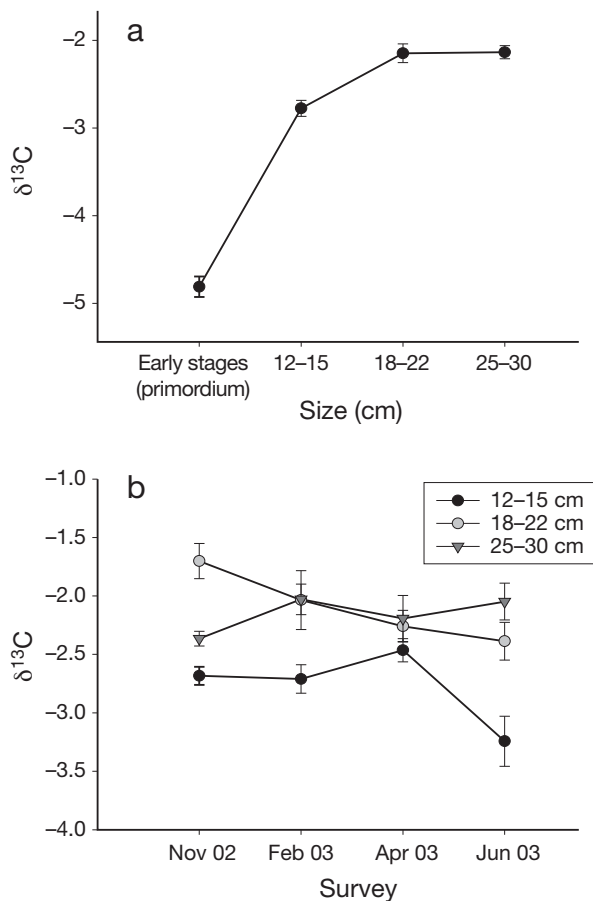


Fig. 7. *Merluccius merluccius*. (a)  $\delta^{13}\text{C}$  of the core area of all the individuals and edge samples of the 3 size ranges. (b)  $\delta^{13}\text{C}$  at the edge throughout the surveys for the 3 size ranges

$p > 0.05$ ). However, there were significant differences between seasons (ANCOVA,  $F_{3,47} = 2.83$ ,  $p < 0.05$ ; Fig. 7b), as June was lower compared to all the other seasons. Moreover, each size range showed different seasonal variations in carbon isotopes (size range–season interaction, ANCOVA,  $F_{6,47} = 2.89$ ,  $p < 0.05$ ; Fig. 7b). November and June showed different values between the 18 to 22 and 25 to 30 cm size ranges (Fig. 7b). The small size range (12 to 15 cm) varied seasonally in different ways to the larger size ranges and showed a drastic decrease in June (Fig. 7b).

## DISCUSSION

The present study provides new insights into the environmental and trophic conditions experienced by European hake *Merluccius merluccius* at 2 life-history phases that contribute to the recruitment success: the period of pre-settlement to the bottom (approximately first 2 mo of life) and of aggregation of recruits on the

shelf-break grounds. Ontogenetic changes in habitat and the consequent shift in the trophic level of food intake explain the isotopic differences between early life-history stages and recruits and core and otolith edge, respectively. The seasonal variation in isotopic composition at the edge of otolith of recruits would show a combination of: (1) the relative composition of trophic resources of hake in both the demersal system and the water column (i.e. related to the maxima of productivity on the thermocline) and (2) the possible consequence of variations in the vertical migration of hake up to the thermocline habitat at night (e.g. Bozzano et al. 2005 and references therein). Water oxygen isotopic values ( $\delta^{18}\text{O}_{\text{VSMOW}}$ ) were included in the present study and showed low variability, as has been shown before below the subsurface layers of the water column in the western Mediterranean (Pierre 1999). The variation in the correlation between  $\delta^{18}\text{O}_{\text{VSMOW}}$  and salinity between SO and CA is not surprising, as the  $\delta^{18}\text{O}_{\text{VSMOW}}$ /salinity relationship appears to be specific for each water mass as a result of distinct hydroclimatic conditions in the formation (e.g. excess of evaporation or freshwater input for instance) (Pierre 1999). Again, these results stress the need for sampling the water to measure  $\delta^{18}\text{O}_{\text{VSMOW}}$  in the water instead of using proxies based on  $\delta^{18}\text{O}_{\text{VSMOW}}$ /salinity relationships for an accurate estimation of water temperatures based on  $\delta^{18}\text{O}$  in the carbonate.

## Environmental influences on hake recruits and juveniles

Due to the excessively narrow range of temperatures (independent variable) measured at locations where fish were caught (13.02 to 13.35°C), we cannot provide an accurate  $\delta_c - \delta_w$  versus temperature relationship for European hake. However, our estimated values of  $\delta_c - \delta_w$  are consistent with previous studies on different fish species carried out in laboratory conditions (Kalish 1991b, Thorrold et al. 1997, Gao et al. 2001, Høie et al. 2004b). All those investigations concluded that deposition occurs at or near equilibrium in fish otoliths. The temperature equation used in the present study was established and validated from another gadoid species, Atlantic cod, and has proved very similar to that obtained for other species (see references above) and also close to the inorganic precipitation aragonite (Kim et al. 2007).

Temperatures estimated from the core areas, corresponding to fish younger than 2 mo old were  $>15^\circ\text{C}$  for 49% of the core areas analysed, and for almost 90% of the fish they were  $>14^\circ\text{C}$ . These results highlight the use that early life stages of hake make of the warmer productive layers around the thermocline (ca. 40 to

75 m) before settlement to the bottom. The distribution of hake larvae is probably associated with the layers of maximum productivity (deep chlorophyll and zooplankton maxima) during the seasonal water column stratification (Olivar et al. 2003), whilst there are no vertical gradients during seasons with vertical mixing, when hake might make higher vertical diel displacements in search of their prey (Olivar et al. 2003, Sabatés 2004). During the night, large numbers of hake larvae gather at 60 to 80 m depth, while during the day vertical distribution can extend up to 10 m from the surface (Sabatés 2004).

The temperature estimates from the otolith edge for fish caught in SO were systematically higher than for fish caught in CA, and these significant differences stand irrespective of the error of measurement (between 1.1 and 1.3°C; Høie et al. 2004b). Values and seasonal variability of estimated temperatures in SO are consistent with temperature variation above the thermocline, whereas temperatures estimated for fish from CA seem more closely associated to the limited temperature variations that characterise the depths below the thermocline (Fig. 4). This suggests a close association of hake in SO with the depth of the thermocline (~50 m) during the season when the thermocline is fully developed, as opposed to fish caught in CA. The maximum temperature experienced by CA fish in April indicates a shallower and warmer feeding habitat in this season. The different behaviours in relation to the thermocline of fish inhabiting SO and CA may be caused by: (1) food availability and/or (2) juvenile fish vertical migrations. The different timing in the development of the thermocline (López-Jurado et al. 2008) and the stronger persistence of frontal thermohaline structures at the shelf-break along the northern side of Mallorca (in the Balearic basin off Sóller) may contribute to increase the biomass of the meso-zooplankton (Cartes et al. 2008), because frontal systems enhance zooplankton biomass (Sabatés et al. 1989). This different use of the water column indicates a high adaptation capacity of this species to the environmental and trophic conditions in nursery areas and on recruitment grounds (Sánchez & Gil 2000). The reported seasonal and geographic variability of European hake diel vertical migrations has been shown to be associated with the feeding behaviour of juveniles (Bozzano et al. 2005). European hake is an opportunistic predator (Velasco & Olaso 1998), and juveniles move into mid-water at night in response to similar movements of their prey (Bozzano et al. 2005). The significantly lower otolith  $\delta^{18}\text{O}$  values in the smallest recruits (12 to 15 cm) indicate that these often inhabited the warmer shallow waters close to the thermocline. These results reinforce the hypothesis suggesting that the youngest hake are more likely to carry out

vertical migrations in the water column because they have more specialist feeding habits (Orsi-Relini et al. 1989, Bozzano et al. 1997), in contrast to the generalist feeding habits of adult hake (Velasco & Olaso 1998, Carpentieri et al. 2005).

The observed seasonal variation in  $\delta^{13}\text{C}$  values obtained at the edge of the otoliths depends on length/age. Several studies on cod have shown no clear pattern of otolith  $\delta^{13}\text{C}$  associated with seasons (Weidman & Millner 2000, Jamieson et al. 2004). Although rearing experiments are the best way to control for metabolic and trophic patterns, 2 significant issues must be brought forward from our results. First, otolith  $\delta^{13}\text{C}$  showed a significant decrease during summer in the 12 to 15 size range individuals (Fig. 7b). Recruits at this size in summer are at the end of the recruitment season and showed lower physiological condition than recruits of February and April (Hidalgo et al. 2008). It suggests that fish recruited outside the main recruitment season were exposed to a different environmental scenario and poorer trophic conditions. Secondly, significant differences were observed in November in fish of the larger size ranges (Fig. 7b). The end of autumn and early winter was a period with low prey abundance for hake (Moranta et al. 2008) and also poor physiological condition of fish (Hidalgo et al. 2008), resulting in a lower selectivity of prey for both size ranges and thus in significantly lower otolith  $\delta^{13}\text{C}$ .

#### Ontogenetic changes of habitat and diet detected in the otolith composition

The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  isotopic composition of the core area and edge were different, reflecting the ontogenetic change of habitat typical of hake (Lombarte & Popper 2004), which results in settlement at the bottom at around 150 m depth (approximately 3.5 cm fish length; Morales-Nin & Moranta 2004). This change of habitat (from the pelagic to demersal system) encompasses a shift in feeding habit itself, reflected in an increase of the  $\delta^{13}\text{C}$  signal. These changes of habitat and feeding regime explain the different  $\delta^{13}\text{C}$  versus  $\delta^{18}\text{O}$  relationships between the core area and edge. Lower dispersion in  $\delta^{13}\text{C}$  from core areas compared to that from the edge determines the differences in the  $\delta^{13}\text{C}$  versus  $\delta^{18}\text{O}$  relationship.  $\delta^{13}\text{C}$  versus  $\delta^{18}\text{O}$  relationships of different groups would indicate the different trophic levels associated with different habitats (Gao et al. 2005) and/or different metabolic rates (Kalish 1991a, Gao et al. 2001) often driven by temperature variability (Høie et al. 2003, 2004a). Nothing is known about the fractionation of carbon isotopes during larval development and metamorphosis in hake. However, our results showed that the seasonal varia-

tion of  $\delta^{13}\text{C}$  in the core area is consistent with seasonal variation of estimated temperature. Moreover, the diet does not change during the early life stages of fish (zooplankton feeders, mainly copepods at different stages) at which the core area is deposited (Olivar et al. 2003). Therefore, the main source of carbon isotope variation in the core area is most probably due to variable metabolic rates (e.g. driven by temperature and activity), though possible maternal influence should not be disregarded (Thorrold et al. 2006).

$\delta^{13}\text{C}$  values in the otoliths showed a clear pattern of ontogenetic change, as has been observed in other species (Schwarcz et al. 1998, Gao & Beamish 2003, Gao et al. 2004). Together with the aforementioned increase associated with settlement, significant differences in carbon isotopes in the 12 to 15 cm fish size range compared to the larger size groups further indicate changes in feeding behaviour. Young hake feed mainly on small crustaceans such as euphausiids and mysids (Bozzano et al. 1997), whereas the mixed diet phase dominates the feeding regime of 18 to 30 cm fish (Carpentieri et al. 2005). This is consistent with the transition to a  $\delta^{13}\text{C}$  plateau at 18 to 22 and 22 to 30 cm size ranges (Fig. 7a). This pattern of ontogenetic variation in otolith  $\delta^{13}\text{C}$  is common to other species and results from predation at higher trophic levels being more enriched in  $^{13}\text{C}$  (see references above). We might expect an enrichment in  $^{13}\text{C}$  in the otoliths of larger hakes (>30 cm) when their diet is totally composed of fish as observed in other demersal species (Gao et al. 2001, 2004, Jamieson et al. 2004).

### Effects of temperature on 2 consecutive annual recruitments

Since annual recruitment in 2003 was much lower than in 2004 and was related to changes in environmental conditions (Massutí et al. 2008), it follows that the early life stages were subjected to different environmental conditions. Hake recruits were detected from February to April 2004 in the surveys conducted off the Balearic Islands (Hidalgo et al. 2008). Those fish would have hatched from approximately May to September 2003. The core areas of fish with hatching dates related to the same season in summer 2002 presented lower temperatures. This is consistent with the mean temperature values measured in the summer surveys between 15 and 80 m depth (data from historical surveys Medar/Medatlas database, IFREMER), being  $15.41^\circ\text{C}$  in 2002 and  $15.95^\circ\text{C}$  in 2003. Thus, different environmental conditions could alter the bottom-up forcing of the system and the ocean productivity, which would produce matches and mismatches between zooplankton production and fish larvae (Cush-

ing 1990). However, more extensive and inter-annual studies must be performed to test whether the warmer temperatures in 2003 compared to 2002 might have resulted in a more successful recruitment in 2004 than in 2003. Estimated temperatures in SO for the same period were significantly higher than those estimated in CA, suggesting that individuals recruiting in SO could have experienced different environmental conditions (higher temperatures) than those in CA. This is consistent with recent evidence, which suggests a probable input of early life stages hatched in Spanish mainland areas and having experienced different environmental conditions (Hidalgo 2007). These pelagic stages would be transported by currents to the Balearic Islands and recruited at the shelf-break of northern Balearic locations, connecting mainland and island populations.

To sum up, the results of our study indicate that stable oxygen and carbon isotope compositions in hake otoliths respond to ontogenetic changes in habitat and feeding behaviour. High mobility, especially for recruits of this species, is reflected in the stable oxygen isotope values. Results from the core area of the otoliths might be used as a tool for identifying differences in environmental conditions experienced by early life stages, which ultimately affect recruitment success in different years. Carbon isotopes clearly reflect ontogenetic changes in diet. Nevertheless, seasonal variability is still different throughout the hake's life, depending on the seasonal variability of its food source. In spite of the stable regime below the thermocline in the Mediterranean Sea, otolith isotopic compositions are useful for studying recruitment processes, ontogenetic changes in habitat related to feeding behaviour and for providing information on early life stages.

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## LITERATURE CITED

- Alemaný F, Oliver P (1995) Methods of hake in the Balearic Sea: a proposal of new growth model. *Cah Options Mediterran* 10:51–52
- Bozzano A, Recasens L, Sartor P (1997) Diet of the European hake *Merluccius merluccius* (Pisces: Merlucciidae) in the western Mediterranean (Gulf of Lions). *Sci Mar* 61:1–8
- Bozzano A, Sarda F, Rios J (2005) Vertical distribution and feeding patterns of the juvenile European hake *Merluccius merluccius* in the NW Mediterranean. *Fish Res* 73: 29–36
- Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanism and applications. *Mar Ecol Prog Ser* 188:263–297
- Carpentieri P, Colloca F, Cardinalle M, Belluscio A, Ardizzone GD (2005) Feeding habits of European hake (*Merluccius merluccius*) in the central Mediterranean Sea. *Fish-B NOAA* 103:411–416
- Cartes JE, Madurell T, Fanelli E, López-Jurado J (2008) Dynamics of suprabenthos–zooplankton communities around the Balearic Islands (NW Mediterranean): influence of environmental variables and effects on higher trophic levels. *J Mar Syst* 71:316–335
- Cushing DH (1990) Plankton production and year-class in fish populations—an update of the match mismatch hypothesis. *Adv Mar Biol* 26:249–293
- FAO (Food and Agricultural Organisation) (2006) FAO General Fisheries Commission for the Mediterranean. Report of the 9th session of the Scientific Advisory Committee. FAO Fish Rep 814. FAO, Rome
- Friedman I, O'Neill JR (1977) Compilation of stable isotope fractionation factors of geochemical interest. In: Fleisher M (eds) Data of geochemistry, 6th edn. US Geological Survey Professional Paper 440, Chap KK. US Geological Survey, p 1–12
- Gao Y, Beamish RJ (2003) Stable isotope variations in otoliths of Pacific halibut (*Hippoglossus stenolepis*) and indications of the possible 1990 regime shift. *Fish Res* 60: 393–404
- Gao Y, Schwarcz HP, Brand U, Moksness E (2001) Seasonal stable isotope records of otoliths from ocean-pen reared and wild cod, *Gadus morhua*. *Environ Biol Fishes* 61: 445–453
- Gao Y, Jøner SH, Svec RA, Weinberg KL (2004) Stable isotopic comparison in otoliths of juvenile sablefish (*Anoplopoma fimbria*) from waters off the Washington and Oregon coast. *Fish Res* 68:351–360
- Gao Y, Bargmann G, Brand U, Noakes D (2005) Stable isotopic and trace elemental compositions of otoliths and the stock structure of Pacific cod (*Gadus macrocephalus*). *Environ Biol Fishes* 74:335–348
- Goñi R, Adlerstein S, Álvarez F, Garcia M and others (2004) Recruitment indices of European hake, *Merluccius merluccius* (Linnaeus, 1758), in the Northwest Mediterranean based on landings from bottom-trawl multispecies fisheries. *ICES J Mar Sci* 61:760–773
- Hidalgo M (2007) Recruitment process and population dynamics of European hake (*Merluccius merluccius* L.) off the Balearic Islands: seasonal and interannual approach. PhD dissertation, University of Vigo, Vigo
- Hidalgo M, Massutí E, Moranta J, Cartes JE, Lloret J, Oliver P, Morales-Nin B (2008) Seasonal and short spatial patterns in European hake (*Merluccius merluccius*, L.) recruitment process at the Balearic Islands (NW Mediterranean): the role of the environment on distribution and condition. *J Mar Syst* 71:367–384
- Hoff GR, Fuiman LA (1993) Morphometry and composition of red drum otoliths: changes associated with temperature, somatic growth rate, and age. *Comp Biochem Physiol A* 106:209–219
- Høie H, Folkvord A, Otterlei E (2003) Effect of somatic and otolith growth rate on stable isotopic composition of early juvenile cod (*Gadus morhua*, L) otoliths. *J Exp Mar Biol Ecol* 289:41–58
- Høie H, Anderson C, Folkvord A, Karlsen Ø (2004a) Precision and accuracy of stable isotope signals in otoliths of pen-reared cod (*Gadus morhua*) when sampled with a high-resolution micromill. *Mar Biol* 144:1039–1049
- Høie H, Otterlei E, Folkvord A (2004b) Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua* L.). *ICES J Mar Sci* 61:243–251
- Jamieson RE, Schwarcz HP, Bratley J (2004) Carbon isotopic records from the otoliths of Atlantic cod (*Gadus morhua*) from eastern Newfoundland, Canada. *Fish Res* 68:83–97
- Kalish JM (1991a) <sup>13</sup>C and <sup>18</sup>O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. *Mar Ecol Prog Ser* 75:191–203
- Kalish JM (1991b) Oxygen and carbon stable isotopes in the otoliths of wild and laboratory reared Australian salmon (*Arripis trutta*). *Mar Biol* 110:37–47
- Kim ST, O'Neil JR, Hillaire-Marcel C, Mucci A (2007) Oxygen isotope fractionation between synthetic aragonite and water: influence of temperature and Mg<sup>2+</sup> concentration. *Geochim Cosmochim Acta* 71:4704–4715
- Lombarte A, Popper AN (2004) Quantitative changes in the otolithic organs of the inner ear during the settlement period in European hake *Merluccius merluccius*. *Mar Ecol Prog Ser* 267:233–240
- López-Jurado J, Monserrat S, Marcos M (2008) Hydrodynamic conditions at the Balearic Islands during 2003–2004. *J Mar Syst* 71:303–315
- Massutí E, Monserrat S, Oliver P, Moranta J and others (2008) The influence of oceanographic scenarios on the population dynamics of demersal resources in the western Mediterranean: hypotheses for hake and red shrimp off the Balearic Islands. *J Mar Syst* 71:421–438
- Maynou F, Leonart J, Cartes JE (2003) Seasonal and spatial variability of hake (*Merluccius merluccius* L.) recruitment in the NW Mediterranean. *Fish Res* 60:65–78
- Maynou F, Olivar MP, Emelianov M (2006) Patchiness of eggs, larvae and juveniles of European hake *Merluccius merluccius* from the NW Mediterranean. *Fish Oceanogr* 15: 390–401
- Morales-Nin B, Moranta J (2004) Recruitment and post-settlement growth of juvenile *Merluccius merluccius* on the western Mediterranean shelf. *Sci Mar* 68:399–409
- Moranta J, Quetglas A, Massutí E, Guijarro B, Hidalgo M, Diaz P (2008) Spatio-temporal variations in deep-sea communities off the western Mediterranean associated to different fishing exploitation rates. *J Mar Syst* 71: 346–366
- Olivar MP, Quilez G, Emelianov M (2003) Spatial and temporal distribution and abundance of European hake, *Merluccius merluccius*, eggs and larvae in the Catalan coast (NW Mediterranean). *Fish Res* 60:321–331
- Orsi-Rellini L, Capparena M, Fiorentino F (1989) Spatial-temporal distribution and growth of *Merluccius merluccius* recruits in the Ligurian Sea. *Observations on the 0 group*. *Cybiurn* 13:263–270
- Palomera I, Olivar MP, Morales-Nin B (2005) Larval development and growth of European hake *Merluccius merluccius* in the north-western Mediterranean. *Sci Mar* 69: 251–258

- Pierre C (1999) The oxygen and carbon isotope distribution in the Mediterranean water masses. *Mar Geol* 153:41–45
- Recasens L, Lombarte A, Morales-Nin B, Torres GJ (1998) Spatio-temporal variation in the population structure of the European hake in the NW Mediterranean. *J Fish Biol* 53: 387–401
- Sabatés A (2004) Diel vertical distribution of fish larvae during the winter-mixing period in the north-western Mediterranean. *ICES J Mar Sci* 61:1243–1252
- Sabatés A, Gili JM, Pages F (1989) Relationship between zooplankton distribution, geographic characteristics and hydrographic patterns off the Catalan coast (western Mediterranean). *Mar Biol* 103:153–159
- Sánchez F, Gil J (2000) Hydrographic mesoscale structures and Poleward Current as a determinant of hake (*Merluccius merluccius*) recruitment in southern Bay of Biscay. *ICES J Mar Sci* 57:152–170
- Schwarcz HP, Gao Y, Campana SE, Browne D, Knyf M, Brand U (1998) Stable carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*). *Can J Fish Aquat Sci* 55: 1798–1806
- Stige LC, Ottersen G, Brander K, Chan KS, Stenseth NS (2006) Cod and climate: effect of the North Atlantic Oscillation on recruitment in the North Atlantic. *Mar Ecol Prog Ser* 325:227–241
- Thorrold SR, Campana SE, Jones CM, Swart PK (1997) Factors determining  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  fractionation in aragonitic otoliths of marine fish. *Geochim Cosmochim Acta* 61: 2909–2919
- Thorrold SR, Jones GP, Planes S, Hare JA (2006) Transgenerational marking of embryonic otoliths in marine fishes using barium stable isotopes. *Can J Fish Aquat Sci* 63: 1193–1197
- Velasco F, Olaso I (1998) European hake *Merluccius merluccius* (L., 1758) feeding in the Cantabrian Sea: seasonal, bathymetric and length variations. *Fish Res* 38:33–44
- Weidman CR, Millner R (2000) High-resolution stable isotope records from North Atlantic cod. *Fish Res* 46:327–342

*Editorial responsibility: Matthias Seaman, Oldendorf/Luhe, Germany*

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