



Stock discrimination of European conger eel (*Conger conger* L.) using otolith stable isotope ratios

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ABSTRACT

European conger eel (*Conger conger*) is a common and widely distributed fish in the NE Atlantic and Mediterranean. Although there is increasing evidence that stocks of European conger eel are in decline, there is little published material on the population structure or management of the species. Stable isotope ratios, namely $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, measured by standard mass spectrometric techniques in whole otolith samples of juvenile conger eels sampled in April/May 2006 from NE Atlantic (Azores, Madeira and North Portugal) and Mediterranean (Mallorca) exploited stocks provided location-specific signatures. Isotopic ratios for conger eel were similar to those reported for other marine species ($\delta^{18}\text{O}\text{‰}$, mean \pm SE: 1.64 ± 0.04 , 1.87 ± 0.02 , 1.26 ± 0.03 , 2.41 ± 0.02 and $\delta^{13}\text{C}\text{‰}$: -1.78 ± 0.08 , -2.09 ± 0.07 , -3.43 ± 0.15 , -2.73 ± 0.12 , respectively for North Portugal, Azores, Madeira and Mallorca). Relationships between isotopic ratios ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) and otolith mass were not significant. Seawater temperature could partially explain the differences in $\delta^{18}\text{O}$ of otoliths among the fishery locations. The $\delta^{18}\text{O}$ signatures of the Portuguese mainland coastal specimens were, however, lower than expected values, possibly as a result of the freshwater input of the Douro River located in the vicinity of this fishery area. The inter-site variation of carbon isotopic signatures was likely related to slight differences in diets or DIC of the water. The distinct isotopic signatures suggest low levels of connectivity between the fishing grounds and that conger eels are relatively sedentary during the juvenile phase. Isotopic signatures in the otoliths of *C. conger* clearly discriminated between the NE Atlantic and the Mediterranean fishery areas providing support for treatment of these fisheries as different management units.

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1. Introduction

The European conger eel (*Conger conger* L.) is an important commercial and recreational fishing species of the NE Atlantic and Mediterranean (Lythgoe and Lythgoe, 1971; Bauchot and Saldanha, 1986), captured primarily by bottom-trawl, hook and line gears (Figueiredo et al., 1996). The few fishery management studies on European conger eel indicate that it is currently not overexploited (O'Sullivan et al., 2003). The total world catch for 2008 was 16,594 t, with the largest catches being recorded by France (4412 t) and Spain (4029 t) (FAO-FIGIS, 2008). Despite being a geographically widespread and important fisheries resource, knowledge of the population structure and ecology of *C. conger* is scarce and mainly

concerns the early life history (Strehlow et al., 1998; Correia et al., 2002, 2003, 2006a), feeding ecology (Cau and Manconi, 1984), and reproductive biology (Cau and Manconi, 1983; Fannon et al., 1990; Sbaihi et al., 2001; O'Sullivan et al., 2003; Correia et al., 2009).

Naturally spawning individuals have not been observed and reports of the occurrence of maturing specimens are scarce (Fannon et al., 1990). The only well known conger eel spawning site is located in the Mediterranean, near the Island of Sardinia (Cau and Manconi, 1983). However, it is thought that the European conger may also spawn in the eastern North Atlantic, around the Azores (Correia et al., 2002, 2003). It is also believed that when the European conger eel reach sexual maturity at 5–15 years age, adults undergo a terminal spawning in deep waters in summer (Lythgoe and Lythgoe, 1971; Wheeler, 1985). The absence of males and ripe or spent females in the coastal inshore waters, suggests that sexual maturation occurs during the migration towards the deep-sea spawning areas and that inshore fisheries primarily target juveniles (Cau and Manconi, 1983, 1984; Sbaihi et al., 2001; O'Sullivan et al.,

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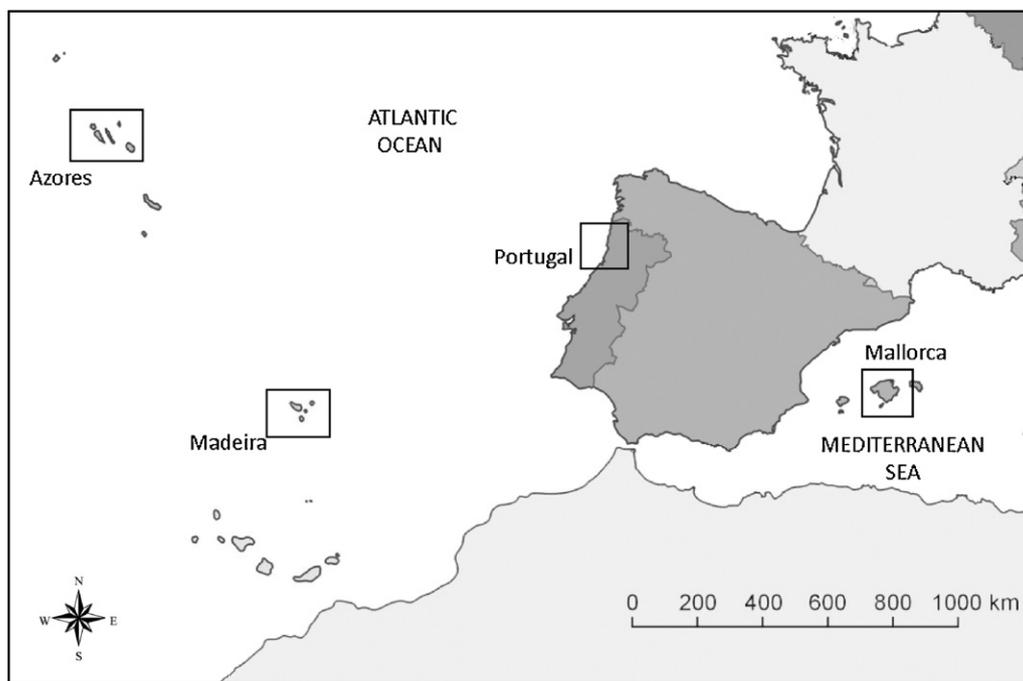


Fig. 1. Map of the NE Atlantic and Mediterranean showing the four conger eel sampling locations.

2003). There is a bathymetric partition of this species according to the sex-ratio: tending to zero (i.e. all females) from 0 to about 400 m and reaching near 0.5 between 400 and 800 m (Cau and Manconi, 1983). However, no information exists about the migration routes and ranges at the juvenile and the adult stages. A previous study (Correia et al., 2006b) on the conger eel population structure using mtDNA provided some evidence for the existence of genetic differentiation among local populations, suggesting that the conger eel does not comprise a single panmictic population. However, detail of conger eel stock structure and how it relates to key fishery areas is presently limited.

To implement management strategies for the sustainable exploitation of *C. conger* stocks in the NE Atlantic and the Mediterranean, there is a need for a better knowledge of the population structure and individual movements in *C. conger*. Appropriate spatial management and resource sharing arrangements depend on knowing whether fisheries in different areas should be regarded as a single stock or whether there are a number of smaller, essentially non-mixing, population units. Several studies have shown that stable oxygen ratios ($^{18}\text{O}/^{16}\text{O}$) in otoliths can be used as a proxy of the ambient sea temperature (Thorrold et al., 1997; Radtke et al., 1996; Høie et al., 2004). Otolith stable carbon isotope ratios ($^{13}\text{C}/^{12}\text{C}$) are also influenced by fish metabolism and diet (Schwarcz et al., 1998; Høie et al., 2003). In addition to these two factors, otolith stable carbon isotope ratios may be influenced by the isotope ratios of the dissolved inorganic carbon (DIC) in the water (Thorrold et al., 1997; Patterson, 1999; Solomon et al., 2006). The relative contribution of these various factors to otolith carbon isotope ratios is likely to vary both in space and in time, and among species. Both isotope ratios have been used successfully as natural tags for fish stock discrimination studies (Edmonds and Fletcher, 1997; Bastow et al., 2002; Gao et al., 2004; Huxham et al., 2007; Dufour et al., 2008; Hidalgo et al., 2008).

In this study we compared the carbon and oxygen isotopic ratios in the otoliths of *C. conger* juveniles captured from four main fishing grounds in the NE Atlantic (Azores, Madeira and North Portugal) and Mediterranean (Mallorca) to determine whether these can be used to assess the degree of separation between stocks, to investi-

gate population connectivity between these areas and to trace fish's origin.

2. Materials and methods

2.1. Fish collection and age estimation

In this study, 20 juvenile specimens of conger eels (TL: 95 ± 3 cm; size range: 75.0–115.0 cm) were sampled from each of four fish markets in the Azores (Faial), Madeira (Funchal), northern Portugal (Matosinhos) and Mallorca (NW Mediterranean) (Fig. 1). To ensure that the fish collected were all juveniles, *C. conger* specimens were captured through the local artisanal fishery mainly by hook and pots in shallow coastal waters (10–30 m depth) from late April to early May 2006, i.e. just prior to *C. conger*'s summer breeding season (Wheeler, 1985; Cau and Manconi, 1983). Because sex ratio depends on depth, with males normally absent shallower than 400 m (Sbaihi et al., 2001; O'Sullivan et al., 2003; Vallisher et al., 2007; Correia et al., 2009) and size at first maturity in females occurring at 200 cm (Whitehead et al., 1986), it was highly likely that all the fish sampled were immature females.

In the laboratory, total length (TL, cm) and whole mass (M , g) were measured for all fish sampled. Sagittal otoliths were removed with plastic forceps, cleaned with Milli-Q water, air-dried in a laminar-flow cabinet, weighed (0.001 g) and stored dry in Eppendorf tubes. Ageing conger eels by standard protocols for otoliths is difficult due to the existence of false rings (Correia et al., 2009). However, based on the average fish length and Von Bertalanffy growth curves for the species (Sbaihi et al., 2001; O'Sullivan et al., 2003; Correia et al., 2009) a mean age of 5 years was estimated for the specimens used in this study.

2.2. Otolith chemical analyses

Isotopic analyses of otoliths carbonate (whole left otoliths) were carried out at the Stable Isotope Laboratory (LABISE) of the Federal University of Pernambuco, Brazil. For carbon and oxygen isotopic determinations, CO_2 was extracted from powdered carbonates in

Table 1
Location, sampling date, number of specimens (*n*), fish length, otolith weight, isotopic values and sea surface temperatures (SSTs).

| Location | Date | <i>n</i> | Fish length (cm) Mean (\pm SE) | Otolith weight (mg) Mean (\pm SE) | $\delta^{13}\text{C}$ (‰VPDB) Mean (\pm SE) | $\delta^{18}\text{O}$ (‰VPDB) Mean (\pm SE) | SST ($^{\circ}\text{C}$) Mean (\pm SE) |
|----------|------------|----------|--------------------------------------|---|---|---|--|
| Portugal | April 2006 | 20 | 96.0 (\pm 1.9) | 73.5 (\pm 1.8) | -1.78 (\pm 0.08) | 1.64 (\pm 0.04) | 16.77 (\pm 0.03) |
| Azores | April 2006 | 20 | 98.9 (\pm 3.8) | 97.1 (\pm 4.1) | -2.09 (\pm 0.07) | 1.87 (\pm 0.02) | 19.20 (\pm 0.04) |
| Madeira | May 2006 | 20 | 90.5 (\pm 4.6) | 62.4 (\pm 5.9) | -3.43 (\pm 0.15) | 1.26 (\pm 0.03) | 20.45 (\pm 0.04) |
| Mallorca | April 2006 | 20 | 90.9 (\pm 2.5) | 85.3 (\pm 3.4) | -2.73 (\pm 0.12) | 2.41 (\pm 0.02) | 18.61 (\pm 0.04) |

SE, Standard error. Note: for more details about the SST values please see Section 2.

a high vacuum line after reaction with anhydrous orthophosphoric acid for 12 h at 25 $^{\circ}\text{C}$ (Craig, 1957). The released CO_2 was analyzed for carbon and oxygen isotopes in a double inlet, triple collector (SIRA III) mass spectrometer, using BSC (Borborema Skarn Calcite) as the reference gas. BSC was calibrated against NBS (National Bureau of Standards)-18, NBS-19 and NBS-20. The precision of analysis was better than 0.1‰ based on multiple analyses of this internal standard. The results are expressed in the notation $\delta\text{‰}$ (per mil) in relation to international VPDB (Vienna Pee-Dee Belemnite) scale (Epstein et al., 1953).

2.3. Sea surface temperatures

Sea surface temperatures (SSTs) can be useful for fish habitat mapping, even in the case of demersal and benthic fish species when *in situ* bottom temperatures are not available (e.g. Beentjes et al., 2002; Hunter et al., 2004; Leathwick et al., 2006). For broad-scale ecological studies, it may be acceptable to infer variation of bottom water temperature from SSTs, since satellite-derived datasets are often significantly correlated with *in situ* logger data for shallow water depths (Smale and Wernberg, 2009). SSTs of the four sampling locations were obtained from an open access historical database of the monthly mean sea surface temperatures (NOAA Extended Reconstructed SST V3 data) provided by the NOAA-ESRL Physical Sciences Division. Age-specific average SSTs were calculated for each location prior to fish capture over the entire fishing area (12 \times 12 Nautical Miles \sim 500 km^2).

In our study, the relationship between $\delta^{18}\text{O}$ values of otolith carbonate and SSTs was explored with the expectation that if separate stocks exist the isotopic signature of their carbonate otoliths would correlate with the water temperature where they resided due to temperature dependent fractionation of $^{18}\text{O}/^{16}\text{O}$ during the precipitation of the otolith carbonate. It was also assumed that there was no significant variation in the isotopic composition of the ambient seawater across the oceanic areas of interest. Mean $\delta^{18}\text{O}$ values calculated for each region were plotted against corresponding mean monthly SSTs.

2.4. Statistical analyses

After testing for normality (Shapiro–Wilk Test, $P > 0.05$) and homogeneity of variances (Levene's Test $P > 0.05$) $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values were analyzed by an analysis of covariance (ANCOVA). Otolith weight is considered to be a proxy for age and also variation in growth, so otolith mass was used as the covariate in ANCOVA. Location was treated as a fixed factor. A post hoc Tukey HSD test was used to examine the existence of any significant differences in the individual isotopic ratios of carbon and oxygen among the four fishery areas.

Multivariate analysis of variance (MANOVA) and linear discriminant function analysis (LDFA) were used to explore the variation of multi-isotopic signatures among locations. For MANOVA we reported the approximate *F*-ratio statistic for the most robust test of multivariate statistics (Pillai's trace). Pairwise comparisons after MANOVA were done using the Hotelling's *T*-square test. LDFA was

used to classify individuals to sampling locations based on their otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values. Classification accuracies of the discriminant functions for each location were evaluated using the percentage of correctly classified individuals from jackknife (leave-one-out) cross-validation analyses.

The statistical analyses were performed using the software SYSTAT 12. Results are presented as means \pm standard errors (SE). A level of significance (α) of 0.05 was used for all statistical procedures.

3. Results

The mean otolith isotopic ratios obtained from the four sampling sites ranged from -3.43 to -1.78‰ for $\delta^{13}\text{C}$ and 1.26 to 2.41‰ for $\delta^{18}\text{O}$ (Table 1). For each location both isotopic ratios showed no significant relationships (ANCOVA; $P > 0.05$) with otolith mass (Fig. 2A and B). For $\delta^{13}\text{C}$, 54% of the sum of squares was explained by location (ANCOVA, $n = 80$, $P < 0.05$) and otolith mass was not significant (ANCOVA, $n = 80$, $P > 0.05$) (Table 2). For $\delta^{18}\text{O}$, 91% of the sum of squares was explained by location (ANCOVA, $n = 80$, $P < 0.05$) and otolith mass was not significant (ANCOVA, $n = 80$, $P > 0.05$) (Table 3).

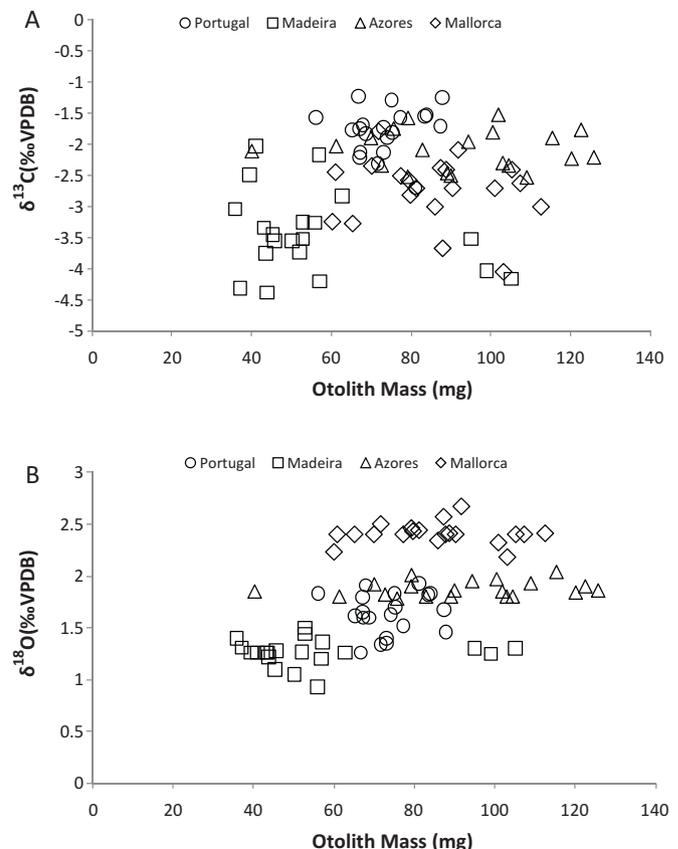


Fig. 2. $\delta^{13}\text{C}$ (A) and $\delta^{18}\text{O}$ (B) versus otolith mass for all data.

Table 2
ANCOVA for $\delta^{13}\text{C}$ values of otolith carbonate.

| Source | Df | SS | MS | F-ratio | P-value |
|----------------|----|--------|-------|---------|---------|
| Location | 3 | 21.272 | 7.091 | 29.162 | 0.000 |
| Otolith weight | 1 | 0.016 | 0.016 | 0.067 | 0.796 |
| Error | 75 | 18.236 | 0.243 | | |
| Total | 79 | 39.524 | | | |

Table 3
ANCOVA for $\delta^{18}\text{O}$ values of otolith carbonate.

| Source | Df | SS | MS | F-ratio | P-value |
|----------------|----|-------|-------|---------|---------|
| Location | 3 | 8.813 | 2.771 | 150.872 | 0.000 |
| Otolith weight | 1 | 0.001 | 0.001 | 0.046 | 0.830 |
| Error | 75 | 1.378 | 0.018 | | |
| Total | 79 | 9.692 | | | |

Tukey's HSD post hoc pairwise comparison tests showed significant differences among all locations for both isotopic signatures ($P < 0.05$), with the exception of the Azores and North Portugal ($\delta^{13}\text{C}$: Tukey Test, $n = 40$, $P > 0.05$; $\delta^{18}\text{O}$: Tukey Test, $n = 40$, $P > 0.05$) values. The relationship between oxygen isotope ratios and sea surface temperature indicated that water temperature could explain differences in $\delta^{18}\text{O}$ between the oceanic locations, with the exception of North Portugal (Matosinhos) location (Fig. 3). The $\delta^{18}\text{O}$ values from fish collected in North Portugal (Matosinhos) are lower than would be expected based on the temperature differences among the sampling areas (i.e. lowest temperature expected to show highest $\delta^{18}\text{O}$). Otolith $\delta^{18}\text{O}$ signatures were significantly correlated with SST for the Madeira, Mallorca and Azores areas ($P < 0.05$) (Fig. 3).

MANOVA indicated a significant difference in the multi-isotopic signatures of the whole otoliths (Pillai Trace; $F_{6152} = 1.534$; $P < 0.05$). All pairwise comparisons between sampling areas were significant (Hotelling's T -square, $P < 0.05$). The bi-plot using the two variables (oxygen and carbon) suggest that the isotopic signatures appear to be site specific (Fig. 4). LDFA based on whole otolith isotopic composition successfully discriminated the four groups, although some overlapping existed however between North Portugal and Azores (Fig. 5). Jackknife classification accuracy rates were 100%, 95%, 95% and 60% for Mallorca, Madeira, Azores and Portugal, respectively (Table 4). According to the between group F matrix ($df = 2, 75$) values from LDFA centroids for Portugal and Azores were closest (18.981); those for Madeira and Mallorca (358.699) are farthest apart.

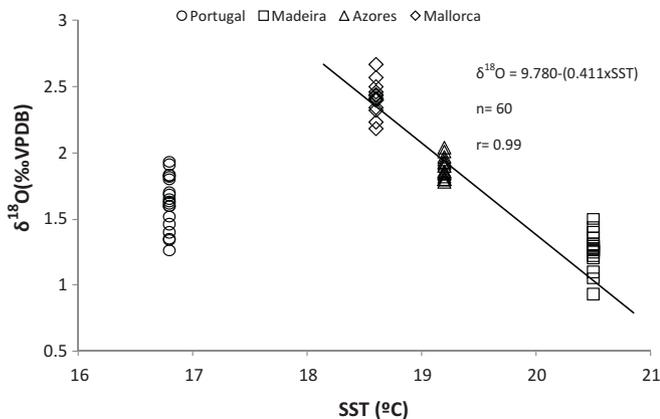


Fig. 3. $\delta^{18}\text{O}$ versus mean SST for all data.

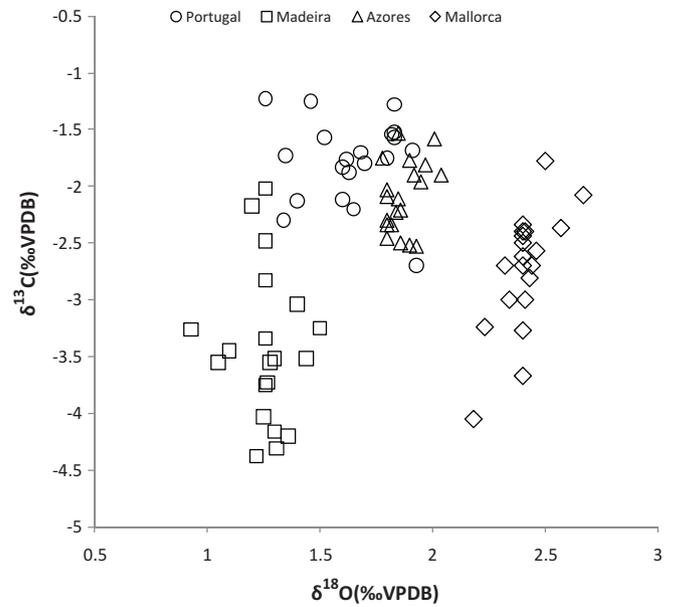


Fig. 4. $\delta^{13}\text{C}$ versus $\delta^{18}\text{O}$ for sagittal otolith carbonate from congers from all locations.

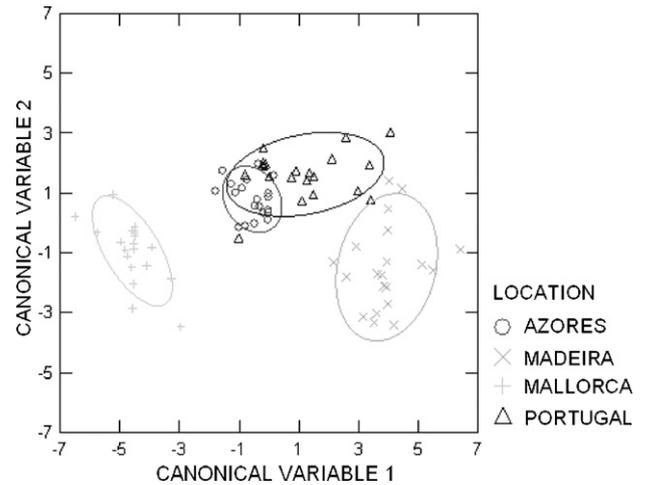


Fig. 5. Canonical variate plots displaying spatial differences in multi-isotopic signatures in whole otoliths from the four sampling locations along the NE Atlantic and Mediterranean. Ellipses represent 95% confidence intervals around the data, and data points represent individual fish.

Table 4
Jackknife classification matrix of age-5 *Conger conger* specimens based on whole otolith isotopic signatures used in LDFA.

| Real location | Predicted location | | | | % Correct |
|---------------|--------------------|---------|----------|----------|-----------|
| | Azores | Madeira | Mallorca | Portugal | |
| Azores | 19 | 0 | 0 | 1 | 95 |
| Madeira | 0 | 19 | 0 | 1 | 95 |
| Mallorca | 0 | 0 | 20 | 0 | 100 |
| Portugal | 7 | 1 | 0 | 12 | 60 |
| Total | 26 | 20 | 20 | 14 | 88 |

4. Discussion

Recent improvements in analytical techniques and technological advancements have led to a growing interest in the use of otolith geochemistry, especially $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values, to determine fish population structure, detect anadromy, reconstruct thermal and salinity history, and infer migration routes (Campana, 1999;

Campana et al., 2000). Variation in the isotopic composition of conger eel otoliths was evident for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ across four important European fishery areas: Azores, north Portugal, Madeira and Mallorca. Despite this variation, values of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ for conger eel were within the general ranges for marine species (-2 to $+4\text{‰}$ and -9 to $+1\text{‰}$, respectively for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) (Kalish, 1991; Iacumin et al., 1992).

$\delta^{18}\text{O}$ in aragonitic otoliths appear to be deposited at, or close to, equilibrium with ambient water (Devereux, 1967; Kalish, 1991; Iacumin et al., 1992). However, due to the temperature dependent fractionation of ^{18}O and ^{16}O during precipitation of CaCO_3 the variation in $\delta^{18}\text{O}$ of otoliths is primarily a function of the seawater temperature that fish experience under constant salinity and ambient $\delta^{18}\text{O}$ (Thorrold et al., 1997; Gao et al., 2001; Høie et al., 2004). Assuming that seawater $\delta^{18}\text{O}$ is constant, lower $\delta^{18}\text{O}$ values indicate higher temperatures (Ashford and Jones, 2007). Differences in the average seawater temperature between the locations where fishing occurs are mainly due to latitude, regional circulation patterns or residence depth. Therefore, seawater temperatures should provide the basis for explaining and predicting differences in oxygen isotopic compositions among separated populations or stocks (Edmonds and Fletcher, 1997; Edmonds et al., 1999; Bastow et al., 2002). In general SSTs are largely governed by latitude and can be inferred from otolith oxygen isotopic signatures (Thorrold et al., 1997; Gao et al., 2001; Høie et al., 2004).

Although the $\delta^{18}\text{O}$ values observed across the sampling area followed the expected trend in relation to water temperature (i.e. higher oxygen isotopic signatures for the areas with lower SST values), the Portugal mainland location (Matosinhos) did not fit the expected pattern and should have displayed higher $\delta^{18}\text{O}$ value based on the SST when compared with the variation across the other locations. The Northeast Portugal fishing area is the only area in this study near a major input of freshwater, the Douro River. This river located in the Northwest Portugal is responsible for a major input of freshwater to the Portuguese inner shelf, with an average freshwater discharge of $488\text{ m}^3\text{ s}^{-1}$ (Vieira and Bordalo, 2000). The Douro River drains into the Atlantic Ocean at $41^\circ 08' \text{ N}$ and $08^\circ 42' \text{ W}$, adjacent to the Matosinhos sampling area. This area is known to have a low-salinity lens (Western Iberian Buoyant Plume, WIBP) formed by river discharge and continental run-off extending along the shelf off Northwest Iberia (Otero et al., 2008). $\delta^{18}\text{O}$ of water is known to vary strongly in relation to salinity (Elsdon and Gillanders, 2002; Kerr et al., 2007) and often with depth, latitude and potentially other environmental factors (Tan et al., 1983; Bastow et al., 2002). The input of freshwater from the Douro River, was likely an important factor in explaining the relatively low otolith $\delta^{18}\text{O}$ values in Matosinhos otoliths.

Although the differences in oxygen isotope ratios between the three marine salinity sites (excluding Portugal) appeared to be influenced by seawater temperature, it would be useful to compare the slope of this relationship to those found by other studies that have experimentally validated $\delta^{18}\text{O}$ otolith–temperature relationships (e.g. Høie et al., 2004). However, ambient $\delta^{18}\text{O}$ estimates from the NOAA Global Seawater Oxygen-18 Database (Schmidt et al., 1999) are not available for Azores and Madeira areas. Furthermore, as in previous studies, the assumption has been made, or is implicit, that the isotopic composition of seawater across the oceanic area of interest is constant enough for temperature to be the overriding factor determining the $\delta^{18}\text{O}$ composition of the otolith carbonate (Edmonds and Fletcher, 1997; Newman et al., 2000; Stephenson et al., 2001).

In contrast to the well-known temperature dependent fraction of otolith $\delta^{18}\text{O}$, the $\delta^{13}\text{C}$ of otolith carbonate is dependent of a variety of exogenous and endogenous factors. Metabolic rate is considered an important endogenous factor, with higher metabolic rates resulting in greater depletion on ^{13}C in otoliths (Kalish, 1991).

This explains the general trend of increased $\delta^{13}\text{C}$ otolith as fish age due to decreased metabolic rate (Kalish, 1991; Gillooly et al., 2001; Bastow et al., 2002). $\delta^{13}\text{C}$ values measured in fish otoliths can also be influenced by ontogenetic changes in trophic levels that comprise the fishes diet (Schwarcz et al., 1998; Gao and Beamish, 2003; Gao et al., 2004), and may also reflect geographic variation in the $\delta^{13}\text{C}$ of the DIC of the ambient water (Thorrold et al., 1997; Patterson, 1999; Solomon et al., 2006).

Metabolic and trophic effects are probably responsible for the reported increase in $\delta^{13}\text{C}$ of fish otoliths until the age of maturity, since both effects would result in an increase in otolith carbon signatures with age (Mulcahy et al., 1979; Kalish, 1991; Gaultie, 1996; Schwarcz et al., 1998). The different carbon isotopic signatures observed among locations may be the result of slight differences in the $\delta^{13}\text{C}$ values of metabolically derived carbon (fish diet). Additionally, since conger eel have a long larval leptocephalus stage that could last about two years (Correia et al., 2006a,b), dissolved-inorganic carbon (DIC) incorporated in otolith during the early life history stage could also play an important role. However, additional information on the DIC of the ambient water among locations and the fish diet for each region will be necessary to better interpret these differences.

In summary, the variation in $\delta^{18}\text{O}$ of otoliths observed in this study appeared to relate to water temperature and salinity, and we suggest that diet and DIC water content may have been responsible for the differences observed in otolith $\delta^{13}\text{C}$. As in previous studies of population structure (Edmonds and Fletcher, 1997; Edmonds et al., 1999; Bastow et al., 2002), knowledge of the underlying causes of the stable isotopic composition of otolith carbonate is not necessary for the use of measured differences as an aid in delineating stocks. This is valid for stable oxygen isotope measurements as well as for carbon. If fish were mixing over the whole area occupied by a species on a time scale that averaged out their isotope signatures, i.e. with each fish spending equal lengths of time in the whole range of temperatures that the species can be exposed to, they would be expected have similar isotopic signatures. If, however, they remained completely separated for most of their lives, with the bulk of otolith carbonate laid down within a single environment with its own characteristic temperature and ambient chemistry regime, then the isotopic signatures would reflect that environment and would be different for the different regions.

ANCOVA results of oxygen and carbon isotope values indicated that location is the most important source of variation. However, the post hoc univariate tests showed no significant differences in isotopic values between Azores and North Portugal and the F -ratio value was the lowest of the pairwise comparisons. This suggests some similarity between these two water masses, possibly a result of the NE Atlantic circulation pattern, and the fact that the Azores and Portuguese currents are both branches of Gulf Stream System (Käse and Krauss, 1996). Another possibility, already suggested, relates to the Azores spawning ground potentially being a source of recruits to the northern European conger populations (Lythgoe and Lythgoe, 1971). The MANOVA and LDFA allowed us to clearly identify four groups corresponding to each sampling location. This suggested low levels of individual movement of the conger eels during their juvenile phase. Feeding grounds are apparently discrete and if the individuals do originate from the same spawning grounds during the long leptocephalus larval stage, it is not long enough for otolith deposition to dominate the isotopic signal accumulated during the five year juvenile stage prior to sample collection.

Our results suggest that despite not knowing their spawning locations, after the larval dispersion to the European and North Africa coasts (Strehlow et al., 1998), the juveniles became relatively sedentary and show high fidelity to their growing and feeding areas. Since it is accepted that at the onset of the breeding season conger eel probably undergo a migration to deeper waters and stop feed-

ing (Sbaihi et al., 2001; O'Sullivan et al., 2003; Correia et al., 2009), analysis of the otoliths of *C. conger* adults caught on the spawning ground(s) should allow us to answer the question of their origin and infer about their migration pathway. Juvenile conger eel from these locations can be regarded as discrete populations, although further work is required to determine if they are sourced from separate spawning sources. This study suggests that the populations of juvenile fish in the Azores, north Portugal, Madeira and Mallorca inshore fisheries are non-mixing and could be assumed to be separated units for management purposes. The isotopic differences found here indicate that the European conger eels from at least three distinct areas (Madeira, Majorca and Azores-Portugal) experience different environmental conditions for most of their lives and that migrations between these areas are rare or inexistent despite the large size of these fish (>80 cm). However, otolith isotopic signatures can be used in the future to retrospectively identify conger eels' origin, if applied to adults caught on the spawning ground(s). The observed isotopic site-specific differences in conger eel otoliths suggests a high level of site-fidelity until the age of 5 years and indicates that Mediterranean and Atlantic stocks could be managed separately, provided that their spawning grounds are confirmed later to be distinct.

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