

## Otolith chemistry as an indicator of movements of albacore (*Thunnus alalunga*) in the North Atlantic Ocean

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**Abstract.** Albacore (*Thunnus alalunga*) in the North Atlantic Ocean is currently managed as a single well-mixed stock, although this assumption remains contentious. We measured stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) and trace elements (Mg, Mn, Sr, Ba) in otoliths of albacore collected from two feeding grounds, namely the Bay of Biscay and Atlantic offshore waters, and compared them among sampling locations and life history stages. Measurements in otolith core, post-core and edge were used to determine whether albacore from these two regions have the same nursery origin and migratory patterns. We found no clear evidence of distinct nursery grounds based on otolith core chemistry, but Sr : Ca and Mg : Ca were different in the post-core portions of albacore from the two locations, suggesting residency in different regions during the early juvenile stage. Otolith edge chemistry, particularly stable isotopes and Sr : Ca, proved to be a valuable tool for classifying individuals to their capture locations. Annual cycles of Sr : Ca ratios were visible along life history transects, likely reflecting migratory patterns between water masses of differing salinity, but the timing of Sr : Ca cycles differed between the two groups. Differentiation in trace element concentrations in the otolith post-core and the timing of Sr : Ca cycles suggest the occurrence of two migratory contingents of albacore in the north-east Atlantic Ocean.

**Additional keywords:** migratory contingents, population structure.

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### Introduction

Albacore (*Thunnus alalunga*) is a highly migratory species that is widely distributed throughout the Atlantic, Pacific and Indian oceans. In the North Atlantic Ocean, the occurrence of three genetically distinct stocks is assumed: northern and southern Atlantic stocks (separated at 5°N) and the Mediterranean stock (Arrizabalaga *et al.* 2004; ICCAT 2012; Montes *et al.* 2012). Albacore is a temperate to subtropical tuna species and the distribution of the North Atlantic stock ranges between 0 and 50°N (Lehodey *et al.* 2014). Albacore are primarily exploited by surface fisheries operating in the Bay of Biscay and adjacent waters during the summer, and around the Azores Islands during summer and autumn (ICCAT 2011, 2012). In addition, Chinese Taipei longliners target albacore in the central and western North Atlantic Ocean throughout the year. Past decades have seen significant decline in albacore fisheries in the Bay of Biscay region, partly because fleets targeting albacore have diminished (Santiago 2004) but also because the spawning stock size has declined significantly (ICCAT 2014). Although the declining trend of North Atlantic albacore catches can be

explained by different biotic and abiotic factors, studies suggest environmental variability may influence the distribution and migratory patterns of albacore (Ortiz de Zárate *et al.* 1998; Bard 2001; Goñi and Arrizabalaga 2005; Dufour *et al.* 2010), which may explain recently observed changes in fisheries, such as the lack of albacore within the Bay of Biscay in some years. These episodes have raised important population questions about stock structure and movements, which are critical for stock assessment and management of the fishery. It is essential to include population structuring during stock assessments, otherwise overexploitation and eventual collapse of less-productive sub-populations may occur, with underexploitation of more productive populations (Hilborn *et al.* 2004). For North Atlantic albacore, it is unknown whether declines within the Bay of Biscay are linked to environmental changes or whether a subpopulation is being depleted. Unfortunately, the life cycle and population structure of albacore in the North Atlantic Ocean is poorly understood (Lehodey *et al.* 2014).

The albacore fishery in the North Atlantic Ocean is currently managed as a single well-mixed stock, although the population

structure of this species remains contentious. Studies carried out in the late 1960s based on catches of mature albacore and the presence of larvae indicated that spawning of albacore occurs in the Sargasso Sea and northern Venezuela (Koto 1969; Ueyanagi 1971). More recent investigations have reported spawning activity in the eastern tropical Atlantic indicated by the presence of several mature female albacore at the spawning stage (Ortiz de Zárate *et al.* 2004). Nevertheless, the sample size of that study was limited, and the reproductive biology of North Atlantic albacore remains largely unknown. A recent study of South Pacific albacore has found synchronised reproductive activity over a broad tropical area, with spawning peaking during the austral spring and summer months (Farley *et al.* 2013). In the North Atlantic, the spawning area and time remains uncertain, and the occurrence of possible subgroups spawning in different regions or with different behavioural traits is still not resolved. Several approaches have been used to address the population structure of albacore in the North Atlantic Ocean, including molecular genetics, blood group analyses and electronic and conventional tagging (Arrizabalaga *et al.* 2004; Cosgrove *et al.* 2010). However, there has been no agreement about whether North Atlantic albacore exist as a single well-mixed stock or whether this group comprises several subpopulations or contingents. Population genetic structure investigations using microsatellite markers have revealed some insights into genetic heterogeneity with the potential presence of three subpopulations within the North Atlantic Ocean stock (Davies *et al.* 2011), whereas single nucleotide polymorphisms did not detect significant heterogeneity (Albaina *et al.* 2013; Montes *et al.* 2012). Furthermore, there is some evidence to suggest that different groups with a different migration pattern may also exist within the North Atlantic Ocean (Bard 1981; Fonteneau 2010).

During the summer, juvenile, subadult (ages 1–4) and some larger adult specimens (age 5+) migrate towards the eastern North Atlantic Ocean, where recreational and bait boat fisheries operate along the coastal shelf of the Bay of Biscay, and a trolling fishery catches albacore in the open ocean zone. It is still unknown whether the albacore found in these two regions represent discrete subpopulations, migratory contingents or have age-specific distribution patterns influenced by environmental conditions. Consequently, a more refined understanding of the movement and stock structure is needed to effectively manage the species.

Recent studies using otolith chemical signatures as natural markers have shown to be a valuable tool for fishery ecologists in understanding the spatial ecology of tuna species (e.g. Wang *et al.* 2009; Wells *et al.* 2012, 2015; Rooker *et al.* 2014; Fraile *et al.* 2015). Otoliths are calcified structures that grow continuously throughout the life of the fish, and their chemical composition can provide continuous chronological records of the environment, allowing discrimination between groups of fish with different origins as well as reconstruction of life history and dispersal trajectories between habitats (Campana *et al.* 1994). Recent work by Macdonald *et al.* (2013) applying otolith trace element chemistry to albacore in the South Pacific Ocean provided some insights into connectivity between larval sources and adult populations of albacore across this region.

In the present study we use both stable isotopes and trace elements in otoliths of albacore to improve the current

understanding of the North Atlantic Ocean population structure and movement of individuals within this region. Our aim was to assess the feasibility of otolith chemistry as a tool to study movement patterns of albacore and to provide insights into its population substructure. Specifically, we combined otolith stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) and trace element:Ca ratios (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca) in the otolith edge to determine whether fish captured within the Bay of Biscay and those caught in offshore waters of the North Atlantic Ocean have inhabited different water masses before their capture. Then, the nursery origin of these two groups was explored by analysing chemical composition of otolith core and near-core areas. Finally, trace element chemistry was analysed along the otolith growth axis to retrospectively detect movements along the life history of the fish.

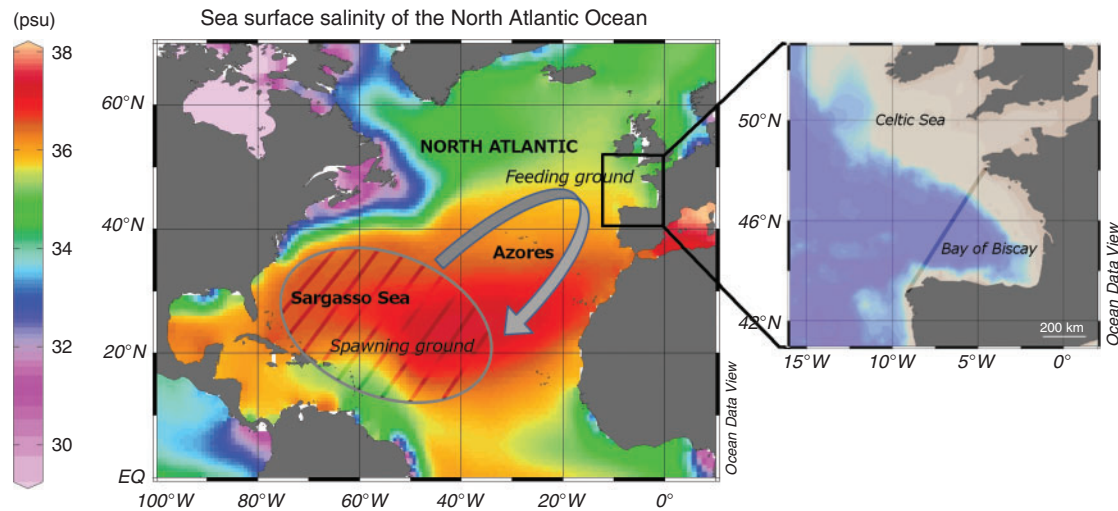
## Materials and methods

### Study location

The Bay of Biscay is located in the eastern North Atlantic Ocean, bounded by the western coast of France and the northern coast of the Iberian Peninsula, and is connected with the Celtic Sea in the north (Fig. 1). The Iberian margin is characterised by a narrow continental shelf, whereas the eastern margin of the Bay of Biscay has a wide shelf, with significant freshwater input. The oceanography of the Bay of Biscay is characterised by a variety of mesoscale features varying according to climatic and seasonal conditions. These characteristics have a strong effect on the biological production of the area and likely affect the distribution, abundance, and movement of albacore (Sagarminaga and Arrizabalaga 2014).

### Sample collection

Albacore ( $n = 167$ ) were collected in the eastern North Atlantic Ocean by commercial bait boat and trolling fleets over a 3-year period, between 2009 and 2011, during their summer migration (June–October) to the Bay of Biscay and surrounding waters. Samples were divided into two regions: (1) Bay of Biscay; and (2) offshore waters of the eastern North Atlantic Ocean, including the Celtic Sea, hereafter termed ‘offshore’ (Fig. 1). The division of these two regions was based on the lack of albacore within the Bay of Biscay in some recent years compared with a regular presence of albacore around the Azores Islands and southern Celtic Sea. This observation has raised the question of whether these two units comprise different subpopulations that differ in size or migratory patterns. Both Bay of Biscay samples ( $n = 69$ ) and offshore samples ( $n = 98$ ) were obtained in collaboration with on-board observers, distributors and fish processing manufacturers. Spatial and temporal distribution of catches depended on the activity of the commercial fleet over the study period, which varied among regions. Continuous sampling occurred from June to October in offshore waters, whereas albacore within the Bay of Biscay were only captured during August and October. The straight fork length (FL) of each fish was measured to the nearest centimetre (Table 1). The otolith collection used in the present study comprises albacore from different cohorts and collection dates in order to obtain a representative sample from each region.



**Fig. 1.** Study area in the eastern North Atlantic Ocean showing sea surface salinity (left) and bottom bathymetry (right). The spawning ground of the albacore (*Thunnus alalunga*) in the North Atlantic Ocean is located in tropical waters around the Sargasso Sea and the feeding grounds are located in the eastern North Atlantic Ocean between the Azores islands, Celtic Sea and the Bay of Biscay (based on Fonteneau 2010). Annual migration between the spawning ground and the feeding ground (grey arrow) remains uncertain. Samples used in the present study were collected in the Bay of Biscay, southern Celtic Sea and Azores Islands (right). Albacore tuna caught within the Bay of Biscay and those caught in offshore waters of the North Atlantic Ocean have been divided into two groups. Salinity data are from the World Ocean Atlas 2009 (Antonov *et al.* 2010) using Ocean Data View software (Brown 1998).

**Table 1.** Summary table of albacore (*Thunnus alalunga*) collected within the Bay of Biscay (inshore) and offshore waters (offshore) of the North Atlantic Ocean between 2009 and 2011

Age was estimated using the growth curve of Santiago and Arrizabalaga (2005). FL, fork length

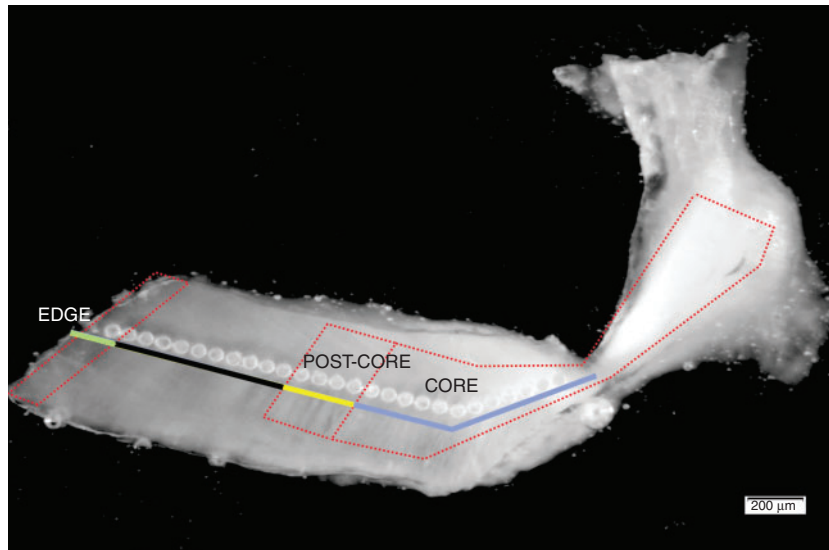
Analytical method	Area	Year	Number of individuals	FL range (cm)	Mean FL (cm)	Estimated age range (years)	Mean age (years)	Catch dates
Stable isotopes	Inshore	2009	13	75.0–89.0	82.51	3–5	4	2–10 August 2009
	Inshore	2010	34	58.0–98.0	86.26	1–6	4	1–21 October 2010
	Inshore	2011	11	55.0–93.0	70.73	1–5	2	17–26 October 2011
	Offshore	2009	24	48.0–82.0	68.28	1–4	2	13–22 July 2009
	Offshore	2010	48	51.5–99.5	74.62	1–6	3	18 June–12 October 2010
	Offshore	2011	14	56.0–87.0	76.29	1–4	3	14 July–23 August 2011
Trace elements	Inshore	2010	11	81.0–92.0	86.72	3–5	4	1–21 October 2010
	Offshore	2010	12	97.2–109	103.16	6–9	7	8–12 October 2010

#### Analytical method

Sagittal otoliths of albacore were extracted for chemical analysis. After removing the adhering organic tissues, whole otoliths were briefly soaked in nitric acid (1%) to eliminate any remaining biological material and cleaned with deionised water (Rooper *et al.* 2008). One otolith per fish was embedded in epoxy resin and sectioned using a Buehler low-speed Isomet saw (Buehler Ltd, Lake Bluff, IL, USA) fitted with a diamond-edged blade to obtain a transverse section that included the core. Otolith sections were washed with nitric acid (1%) and deionised water to remove any post-mortem and handling contamination (Davies *et al.* 2010). Transverse sections were cut for stable isotope and trace element analyses (1.5 and 0.5 mm respectively). Transverse sections were first polished with 1200-grit paper moistened with distilled water, and were further

polished with a micro cloth and 0.3- $\mu$ m alumina powder to ensure a smooth surface. Sections were glued in a sample plate using Crystalbond thermoplastic glue (Crystalbond 509, Buehler Ltd). Otoliths of 144 individuals caught in 2009, 2010 and 2011 were used for stable isotope analysis, whereas those from 23 individuals caught in 2010 were used for trace element studies.

For stable isotope analysis, we followed a similar procedure to that used by Wells *et al.* (2015) for North Pacific albacore. The portion of the otolith corresponding to the first translucent zone (approximately first 6 months of life, hereafter called the 'core') and the most posterior margin of each otolith (which represents the fish's most recent growth, hereafter referred to as the 'edge') were milled using a high-resolution New Wave Research MicroMill System (New Wave Research, Fremont,



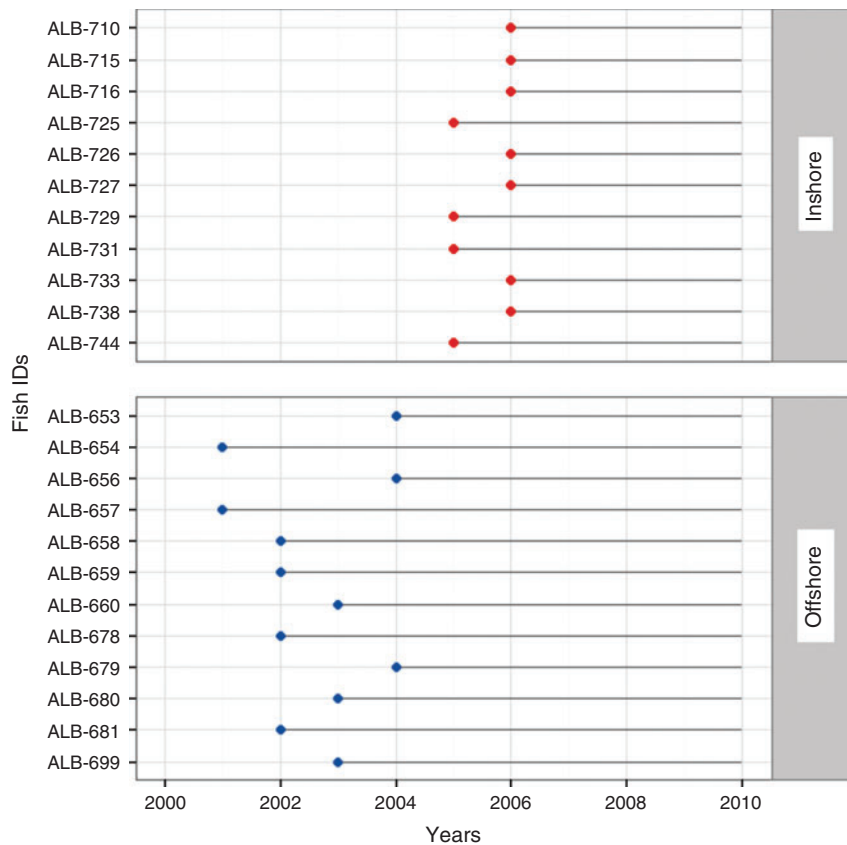
**Fig. 2.** Transverse section of an adult albacore (*Thunnus alalunga*) sagittal otolith showing laser ablation spots performed from the late larvae–early juvenile period (core), to age-0 recruit (post-core) and otolith edge, together with the portions milled for stable isotope analysis (core and edge). The diameter of ablation spots is 40  $\mu\text{m}$ .

CA, USA) consisting of a microscope and imaging system controlled by computer software. A series of 14 drill passes of 55- $\mu\text{m}$  depth were run over a programmed drill path using a 300- $\mu\text{m}$  diameter carbide bit. Carbon ( $\delta^{13}\text{C}$ ) and oxygen ( $\delta^{18}\text{O}$ ) stable isotopes of otolith powder were analysed using an automated carbonate preparation device (KIEL-III, Thermo Fisher Scientific, Waltham, MA, USA) coupled with a gas-ratio mass spectrometer (Finnigan MAT 252, Thermo Fisher Scientific) at the Environmental Isotope Laboratory of the University of Arizona. All isotope values are reported according to standards of the International Atomic Energy Agency in Vienna (Vienna Pee Dee Belemnite, VPDB).

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to determine the trace element chemistry of the otolith. The system consisted of a laser ablation system (Nd:Yag 213 nm; New Wave Research) coupled to a mass spectrometer (X Series 2 ICP-MS; Thermo Electron Corporation, Cheshire, UK.). Otolith sections were placed in a sealed chamber and viewed through a microscope connected to MicroMill-specific software. Ablations were performed using a 40- $\mu\text{m}$  spot size with a 10.00-Hz pulse rate at 70% energy ( $6.5 \text{ J cm}^{-2}$ ). Each otolith was analysed using individual spots along the ventral arm of the otolith, beginning in the region corresponding to the early life stage of the fish and moving outward to the outer margin of the otolith until the edge (Fig. 2). The time frame in the life of the tuna represented by each laser spot varies from a few weeks, when the spot is close to the core, to several months for spots at the edge (Wells *et al.* 2013; Renck *et al.* 2014). Age determination for each ablation point was done on the same otolith used for elemental analysis by examining polished otolith sections under a microscope with transmitted light, and counting the annual formation of narrow opaque and wide translucent bands along the ventral arm. The methodology outlined by Wells *et al.* (2013) was applied to sectioned otoliths for the reading and interpretation of the growth

increments. Otoliths from 23 individuals collected in 2010 and ranging in age from 4 to 10 years provided elemental signatures recorded between the hatch year and collection year (Fig. 3). To account for instrumental drift and sensitivity and to determine the precision of trace element measurements, a glass reference standard (National Institute of Standards and Technologies, NIST 612 SRM, US Department of Commerce) with known elemental concentrations was analysed prior and after otolith analysis. Mean values measured by the instrument have been compared with the recommended values (Jochum *et al.* 2011). In addition, the accuracy of the measurements was monitored with repeat measurements of the US Geological Survey (USGS) carbonate reference sample (MACS-3 pressed powder pellet). The measurements and the known values of Ca were used to correct for differences in ablation yields between standards and unknown samples. Calcium was used as an internal standard to account for variation in ablation efficiency, which is caused by variations in the mass of the material ablated (Campana *et al.* 1994). Four isotopes ( $^{25}\text{Mg}$ ,  $^{88}\text{Sr}$ ,  $^{55}\text{Mn}$  and  $^{138}\text{Ba}$ ) were measured from the core through all the layers to the outer edge of each otolith using the LA-ICP-MS system. Readings below detection limits were not used in analyses; in addition, ablation spots with a standard error  $>20\%$  were discarded from the analysis. The selection of elements was based on their occurrence in concentrations above the detection limit and their stability within a growth band. Elemental concentrations (ppm) were calculated based on relative abundance of the isotopes. Data reduction and processing were performed using the software package Iolite version 2.2 (School of Earth Sciences, The University of Melbourne, Melbourne, Vic., Australia; available from <http://www.iolite.org.au>, accessed 12 December 2012), an application that operates within IGOR Pro ver. 6.2 (WaveMetrics, Inc., Lake Oswego, OR, USA, see <http://www.wavemetrics.com>, accessed 5 December 2012; Paton *et al.* 2011; Paul *et al.* 2012). Finally,





**Fig. 3.** Years of otolith chemistry data for each albacore from the back-calculated harvested date until captured year based on direct age estimates. Horizontal lines represent individuals, grouped by sampling location, with line length indicative of lifetime and thus data availability.

calcium concentration was assumed from the stoichiometry of aragonitic calcium carbonate ( $400\,000\ \mu\text{g Ca g}^{-1}$  otolith), and the concentrations of other elements (ppm,  $\mu\text{g g}^{-1}$ ) were expressed as the element : Ca ratio (Ludsin *et al.* 2006). Ablation spots that resulted within the first translucent zone and first opaque zone (hereafter referred to as ‘core’ and ‘post-core’ respectively) were averaged to obtain a mean elemental concentration for each of the periods. The ‘edge’ was defined as the last ablation spot at the outer margin of the ventral arm of the otolith.

#### Otolith $\delta^{18}\text{O}$ prediction

Summer otolith  $\delta^{18}\text{O}$  values were predicted using sea surface temperature (SST) and salinities (SSS) of the Sargasso Sea between the 2001 and 2006 time frame that corresponds with the birth year of the sampled individuals. Summer SST and SSS of the Sargasso Sea were derived from GLORYS2V3 global reanalysis using Copernicus Marine Environment Monitoring Service (CMEMS, see <http://marine.copernicus.eu>, accessed December 2015) products, and spatially averaged for the entire Sargasso Sea, which is considered the nursery ground of the North Atlantic albacore. Seawater oxygen isotope ratio ( $\delta^{18}\text{O}_w$ ) was predicted from SSS using the equation provided by Harwood *et al.* (2008) and corrected to the VPDB scale using the relationship from Friedman and O’Neil (1977). The effect of temperature on

the fractionation of oxygen isotopes was calculated with the inorganic aragonite relationship from Kim *et al.* (2007).

#### Statistical analysis

Normality of data was tested in both otolith core and edge measurements using a Shapiro–Wilk test, and log transformation was applied to trace element data to approach normality and stabilise variance. Interannual and monthly variability was assessed by multivariate analysis of variance (MANOVA) and Kruskal–Wallis tests. Similarly, one-way analysis of variance (ANOVA) was used to determine whether interannual differences on predicted otolith  $\delta^{18}\text{O}$  values for the Sargasso Sea between 2001 and 2006 were significant. Ontogenetic variability on stable isotopic composition was analysed using the general linear model (GLM) with FL as an explanatory variable on otolith edge measurements for each of the predefined groups. Different statistical methods were applied stepwise to define the variation in the chemical composition of otoliths from each environment tested (Bay of Biscay and offshore waters). In a first exploratory analysis, comparisons of otolith chemical composition between the albacore caught within the Bay of Biscay and those caught in offshore waters were made with the null hypothesis of no difference, and the Mann–Whitney–Wilcoxon test was used as the test statistic. In a second step, habitat discrimination capacity between these two groups based

on otolith chemistry was evaluated by statistical classification methods. Recent work comparing the classification accuracy of different statistical methods has shown that machine learning methods often have greater classification efficiency with fewer assumptions than discriminant function analyses (Mercier *et al.* 2011). In the present study, two different statistical methodologies were selected: (1) a classical classification method, namely quadratic discriminant analysis (QDA); and (2) a data-mining technique capable of machine learning and pattern recognition, namely artificial neural network (ANN). Cross-validation was used to determine the accuracy of each classification method in order to select the best methodology and reduce subsequent misclassification errors when applying this classifier to predict fish movements. To get reliable results, we measured prediction efficiency over 1000 replicates and calculated a mean accuracy for each combination of elements. For each of the methods, all element combinations were tested and the optimal list(s) of elements for fish classification were selected. Prediction efficiency between all element combination and classification methods was measured by cross-validation. A detailed description of the statistical methodology is given in Mercier *et al.* (2011).

In order to compare migratory patterns between the two groups, core to edge transects were transformed into equally spaced observations using linear interpolation and generating a regular time series of elemental concentration from 0 to 4 years. Principal component analysis (PCA) was used to examine the leading patterns and temporal variations of the Sr:Ca ratio in albacore from the Bay of Biscay and offshore waters during the juvenile period (0–4 years). Data transformation and ordination analysis were performed using ‘zoo’ (Zeileis and Grothendieck 2005) and ‘vegan’ (J. Oksanen, F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O’Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, and H. Wagner, see <http://CRAN.R-project.org/package=vegan>, accessed September 2015) packages implemented in the R CRAN environment (R Foundation for Statistical Computing, Vienna, Austria, see <http://www.R-project.org/>, accessed September 2015).

## Results

Interannual variability of  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values measured at the otolith edge were not significantly different ( $P > 0.05$ , MANOVA;  $P > 0.05$ , Kruskal–Wallis). Moreover, otolith  $\delta^{18}\text{O}$  values predicted using SST and SSS fields from the Sargasso Sea did not differ from 2001 to 2006, and were therefore not considered in further analysis. GLM for otolith edge  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values with FL as the explanatory variable indicated that ontogenetic influence was not significant for either isotope. Otolith  $\delta^{13}\text{C}$  values for albacore from the North Atlantic Ocean ranged from  $-9.17$  to  $-6.35\text{‰}$ , whereas  $\delta^{18}\text{O}$  of the same otoliths ranged from  $-1.67$  to  $0.11\text{‰}$ .

### Capture locations

Collection locations in the Bay of Biscay and offshore waters of the North Atlantic Ocean were clearly separated based on otolith edge chemistry when all year-classes were pooled. Separation between the two locations was based primarily on stable isotope composition and Sr:Ca ratio ( $P < 0.05$ , Mann–Whitney–Wilcoxon test). Otolith edge  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  were more enriched

for albacore captured in offshore waters compared with those captured within the Bay of Biscay (Table 2; Figs 4, 5). A significant month effect was detected for otolith edge  $\delta^{18}\text{O}$ , indicating that  $\delta^{18}\text{O}$  values across capture months (from June to October) were not consistent. In contrast, no significant differences in otolith edge  $\delta^{13}\text{C}$  were observed by month ( $P > 0.05$ , Kruskal–Wallis). However, given that the discriminatory power of  $\delta^{18}\text{O}$  was not significant (Table 3), discrimination of the two capture locations was not affected by month-to-month variability of  $\delta^{18}\text{O}$ . Among the trace element analyses at the otolith edge, significant spatial differences were present only for Sr:Ca, with albacore captured in offshore waters having higher mean values than those captured in the Bay of Biscay (mean  $\pm$  s.d.  $5.63 \pm 0.36$  v.  $4.82 \pm 0.23$  mmol mol<sup>-1</sup> respectively). Differences for the remaining elements measured at the edge were not significant (Table 2). Classification accuracy of albacore to their capture locations based on otolith edge chemistry varied between 66 and 90% depending on the statistical classification method and chemical elements included (Table 3). Capture locations were discriminated with a moderate accuracy (66 and 69% using ANN and QDA approaches respectively) based on  $\delta^{13}\text{C}$  measurements, whereas trace element chemistry was more efficient in discriminating between the Bay of Biscay and Atlantic offshore waters. QDA was identified as the best classification method for trace element signatures, because fish caught from the two different geographic regions could be accurately classified to their capture locations (90% of fish correctly classified). The ANN method was also successful in classifying otolith edge signature, with 85% accuracy. In both cases, element combination displaying the greatest accuracy (selected by a stepwise variable selection procedure) included the Sr:Ca ratio.

### Otolith core and post-core chemistry

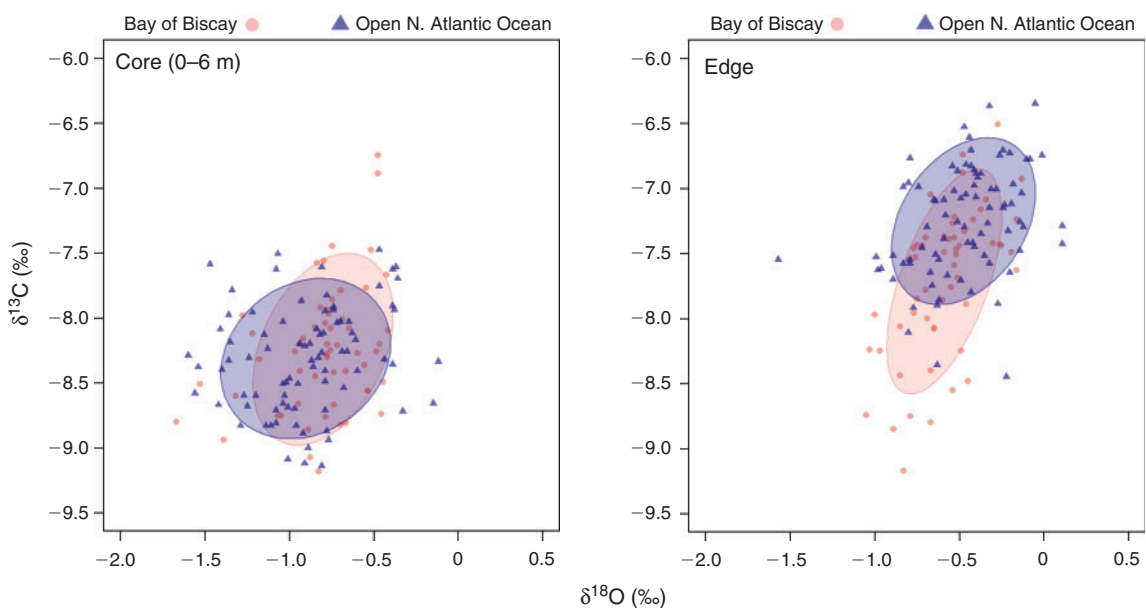
Chemical signatures in otolith cores were analysed to identify nursery origin of albacore. Otolith core  $\delta^{18}\text{O}$  values were significantly higher in otoliths of albacore caught in the Bay of Biscay ( $P < 0.05$ , Mann–Whitney–Wilcoxon test), whereas no significant difference in otolith core  $\delta^{13}\text{C}$  was found between the two groups (Table 2; Fig. 4). Among the elements that occurred above instrument detection limits within the core, Mn:Ca ratios were significantly different between the two groups, with higher ratios of this element found in albacore from the Bay of Biscay ( $P < 0.05$ , Mann–Whitney–Wilcoxon test; Table 2; Fig. 5). The remaining elements were undifferentiated across the two capture locations.

Otolith post-core values of Mg:Ca and Sr:Ca ratios were significantly different between the two groups ( $P < 0.05$ , Mann–Whitney–Wilcoxon test; Table 2; Fig. 5). Mean Sr:Ca was lower and Mg:Ca higher for fish captured within the Bay of Biscay. Statistical classification techniques using  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values measured at the otolith core showed low evidence of differentiation between the two groups (58–60%). Based on trace element chemistry, optimal discrimination was attained from Mn:Ca, with classification accuracy ranging from 78 to 86% using QDA and ANN techniques respectively. Classification analyses, based on otolith post-core element:Ca ratios produced noticeable separation between albacore caught in the two regions (cross-validated classification success was between

**Table 2.** Mean ( $\pm$ s.d.) element : Ca ratios in otoliths of albacore (*Thunnus alalunga*) caught in two regions, namely the Bay of Biscay (Inshore) and offshore waters (offshore) of the North Atlantic Ocean

Otolith zones are divided into the core, post-core and edge. Significant differences among the two regions ( $\alpha=0.05$ ) are shown in bold. nm, element not measured or below detection limit

Otolith portion	Capture location	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	Mg : Ca ( $10^{-4}$ mol mol $^{-1}$ )	Ba : Ca ( $10^{-6}$ mol mol $^{-1}$ )	Sr : Ca ( $10^{-3}$ mol mol $^{-1}$ )	Mn : Ca ( $10^{-7}$ mol mol $^{-1}$ )
Core	Inshore	$-8.23 \pm 0.25$	<b><math>0.80 \pm 0.14</math></b>	$4.56 \pm 1.24$	$2.21 \pm 0.52$	$3.71 \pm 0.24$	<b><math>9.88 \pm 0.87</math></b>
	Offshore	$-8.30 \pm 0.21$	<b><math>0.90 \pm 0.17</math></b>	$3.43 \pm 0.87$	$2.70 \pm 0.55$	$4.08 \pm 1.05$	<b><math>5.37 \pm 0.47</math></b>
Post-core	Inshore	nm	nm	<b><math>3.34 \pm 0.82</math></b>	$2.57 \pm 0.57$	<b><math>4.26 \pm 0.31</math></b>	nm
	Offshore	nm	nm	<b><math>1.93 \pm 0.30</math></b>	$3.12 \pm 0.82$	<b><math>5.80 \pm 1.87</math></b>	nm
Edge	Inshore	<b><math>-7.72 \pm 0.29</math></b>	<b><math>-0.59 \pm 0.11</math></b>	$2.55 \pm 1.01$	$2.65 \pm 0.53$	<b><math>4.76 \pm 0.18</math></b>	nm
	Offshore	<b><math>-7.25 \pm 0.21</math></b>	<b><math>-0.47 \pm 0.14</math></b>	$3.31 \pm 2.16$	$3.05 \pm 0.98$	<b><math>5.62 \pm 0.36</math></b>	nm



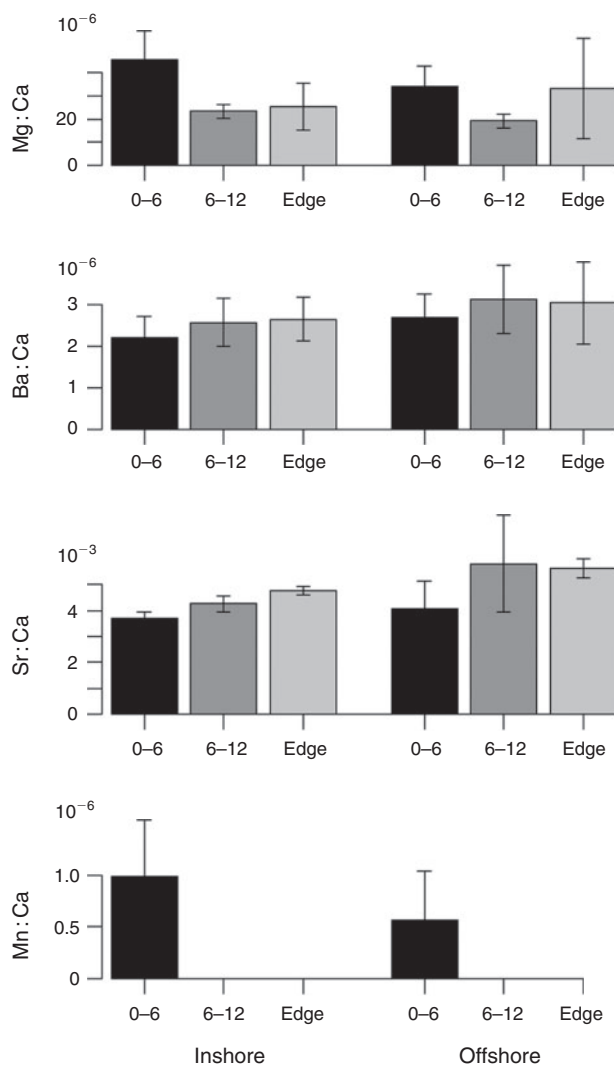
**Fig. 4.** Confidence ellipses (1 s.d.) and individual data points for otolith  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  composition measured at the core and edge of the otolith in albacore (*Thunnus alalunga*) captured within the Bay of Biscay compared with those captured in offshore waters of the North Atlantic Ocean.

80 and 81% depending on the statistical method selected). Regardless of the method used for prediction, habitat discrimination was maximised when the combination of Sr : Ca and Mg : Ca was used to separate the two regions. Details about the optimal combination of elements and the classification success of each classification method are given in Table 3.

#### Trace element transects

Three of the four trace elements (Mg, Sr and Ba) were well above the detection limits along the entire transect. Based on back-calculated harvested year, the time frame covered by the ablation transects ranged from 2001 to 2010 (Fig. 2). Maximum Mg : Ca was observed close to the otolith core and decreased rapidly during the age-0 period, showing little fluctuation along the rest of the otolith transect (Fig. 6). Otolith Sr : Ca was relatively low and stable during the first few months of life, and then fluctuated through the entire life history. Although the timing of the first Sr : Ca cycling varied among individuals,

marked Sr : Ca cycling was visible during the juvenile stage (ages-1–4), with a high Sr : Ca ratio often corresponding with the opaque bands. In most of the otoliths, annual periodicity in Sr : Ca persisted through the adult stage (age-4+), although in some cases concentrations remained relatively stable during the adult stage (e.g. ALB-658 in Fig. 6). The timing of the first peak in otolith Sr : Ca varied between groups: All albacore caught in the Bay of Biscay showed Sr : Ca peaks at age-1, whereas in the majority of the offshore samples (55%) first peaking in Sr : Ca occurred after the first year or it was less pronounced. PCA applied to the time series of otolith Sr : Ca during the juvenile stage (0–4 years) showed that individuals captured in the Bay of Biscay and offshore waters were grouped into two distinct clusters (Fig. 7). The first two axes of the PCA explained 78% of the total variation, and the scores of these two axes were significantly different between albacore caught in the Bay of Biscay and offshore waters ( $P < 0.05$ , MANOVA). Otolith Ba : Ca fluctuated considerably along the transects and the



**Fig. 5.** Mean ( $\pm$ s.d.) element : Ca ratios in otoliths of albacore (*Thunnus alalunga*) captured in the Bay of Biscay (inshore) and offshore waters of the North Atlantic Ocean. Element : Ca values are shown for the otolith core (late larvae–early juvenile period), post-core (age-0) and edge (recent habitat use). Otolith Mn : Ca ratios in the edge and post-core portions were below the detection limit.

patterns were not consistent among individuals. A significant positive correlation between Sr : Ca and Ba : Ca was found in 43% of otoliths (e.g. ALB-681), but in most otoliths no clear relationship was found between cycles of these two elements. Otolith Mg : Ca was inversely correlated with both Sr : Ca and Ba : Ca concentrations (e.g. ALB-715) for 76 and 57% of samples respectively.

## Discussion

Improving our understanding of population structure of commercially exploited fish stocks is needed to define appropriate management units for stock assessments. Otolith chemistry can provide valuable information on the life history, dispersal and stock characteristics of fish, and the present study demonstrates the potential of otolith chemical analyses to provide important

**Table 3.** Chemical composition of albacore (*Thunnus alalunga*) otoliths collected in the Bay of Biscay and offshore waters of the North Atlantic Ocean and comparison of classification methods

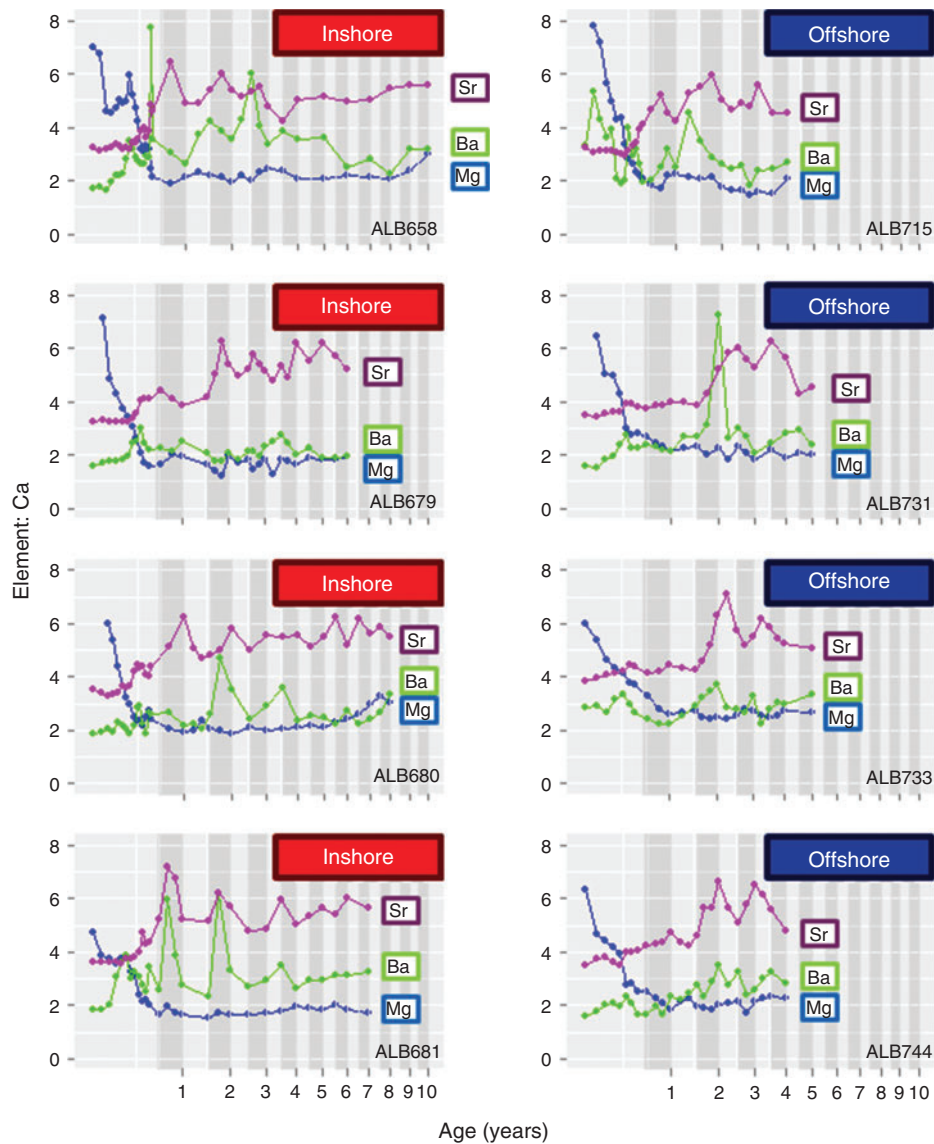
The combination of elements that resulted in the highest classification rate is shown together with the corresponding accuracy level. Accuracy is given as the percentage of fish classified correctly to the capture location. QDA, quadratic discriminant analysis; ANN, artificial neural network

Otolith region	Chemical analysis	Classification method	Element combination	Accuracy (%)
Core	Trace elements	QDA	Mn : Ca	78
		ANN	Mn : Ca	86
	Stable isotopes	QDA	$\delta^{13}\text{C}$	58
		ANN	$\delta^{18}\text{O}$	60
Post-core	Trace elements	QDA	Mg : Ca, Sr : Ca	80
	ANN	Mg : Ca, Sr : Ca	81	
Edge	Trace elements	QDA	Sr : Ca; Mg : Ca	91
		ANN	Sr : Ca; Ba : Ca	85
	Stable isotopes	QDA	$\delta^{13}\text{C}$	70
		ANN	$\delta^{13}\text{C}$	66

information about the stock structure and connectivity of albacore in the eastern North Atlantic Ocean. The otolith edge chemistry indicated that albacore captured within the Bay of Biscay and offshore waters of the North Atlantic Ocean have inhabited different water masses before capture. Among the trace elements that routinely occurred above instrument detection limits, otolith Sr : Ca was considered the most reliable marker as a geographic proxy for recent regional habitat use within the eastern North Atlantic Ocean, most likely generated by the salinity gradient of the study area (Fig. 1). QDA performed slightly better than ANN in discriminating the different water masses of the eastern North Atlantic Ocean. We found a lack of evidence of discrete nursery grounds for albacore from the North Atlantic Ocean (revealed by relatively invariant  $\delta^{13}\text{C}$ , Mg : Ca, Sr : Ca and Ba : Ca in the core across the two capture locations), although otolith  $\delta^{18}\text{O}$  and Mn : Ca were significantly different between the two groups. The low classification accuracy attained using both QDA and ANN methods (expected classification is 50% based on random assignment) indicates similarity in the core chemical signature of the two groups. Differences in Mn : Ca found in otolith core could be explained by physiological or structural factors rather than environmental drivers. Mn is introduced into the water column through different sources, such as terrestrial input, upwelling of Mn-rich waters or microbial oxidation, among others (Landing and Bruland 1980; Johnson *et al.* 1992; Klinkhammer and McManus 2001), but previous studies found variable and no conclusive effects of temperature and salinity on otolith Mn : Ca (Elsdon and Gillanders 2002). Elevated Mn concentration is often found in otolith primordia because maternal transfer appears to be the principal mechanism of incorporation (Brophy *et al.* 2004; Martin and Thorrold 2005; DiMaria *et al.* 2010). Thus, differences in otolith Mn : Ca, although statistically significant, would likely be due to proximity of the ablation spot to the primordium rather than environmentally driven.

We found no evidence that tuna captured in offshore waters and those found in the Bay of Biscay originated in different geographic regions, but differences in Sr : Ca and Ba : Ca in the



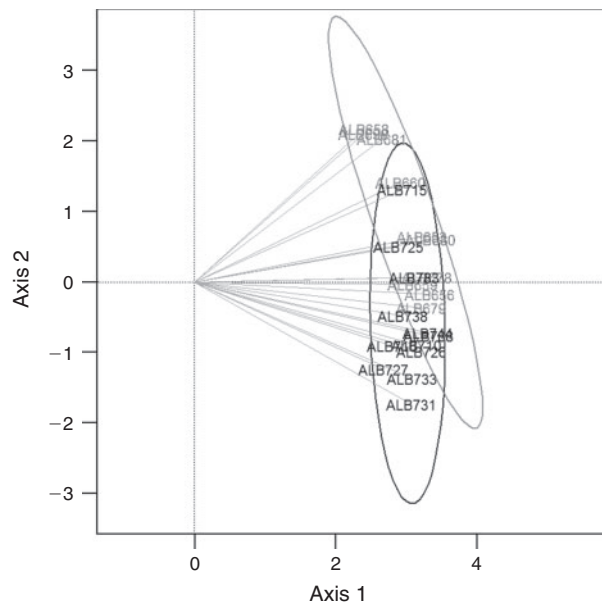


**Fig. 6.** Chronological changes in otolith Sr:Ca, Ba:Ca and Mg:Ca ratios measured along transects from the otolith core (representing nursery habitat) to the edge of the ventral arm (representing recent regional habitat use) for four representative samples from the Bay of Biscay (inshore) and offshore waters. Vertical bands represent consecutive otolith translucent (fast growth) and opaque (slow growth) zones. Otolith Ba:Ca is shown at  $10^{-6}$  mol mol $^{-1}$ , Mg:Ca at  $10^{-5}$  mol mol $^{-1}$  and Sr:Ca at  $10^{-3}$  mol mol $^{-1}$ .

post-core portion of the otolith suggest that the two groups resided in habitats with distinct physicochemical properties during the early juvenile stage (coinciding with the first winter). Alternatively, differences in the post-core could be explained by interannual variability in environmental conditions, because albacore captured in the two geographic areas were sampled from different cohorts (Fig. 3). However, if differences in otolith Sr:Ca and Ba:Ca were linked to interannual variability, differences would also be reflected in the core signature. Given that the core signature of albacore from the two regions were statistically similar, we conclude that differences in the post-core portion are linked to migrations to different habitats. Similarly, Wells *et al.* (2015) found little evidence to support

separate production zones of albacore for the eastern North Pacific, but found different recent environmental history of two subgroups of juvenile albacore inhabiting the California Current.

Differences in Sr:Ca along the otolith transects were observed between the two groups. Sr is incorporated into the otolith in proportion to that of ambient seawater composition (Secor and Rooker 2000) and, given its positive relationship with water salinity (Secor and Rooker 2000; Martin *et al.* 2004), otolith Sr:Ca is often used to reconstruct the environmental salinity history (Limburg *et al.* 2001; Jessop *et al.* 2002; Wang *et al.* 2009). Otolith Sr:Ca fluctuations in our samples were interpreted as annual migratory movements between water



**Fig. 7.** Principal components analysis (PCA) correlation biplot obtained for the time series of Sr:Ca ratio and 95% confidence ellipses on otoliths of albacore (*Thunnus alalunga*) captured in the Bay of Biscay (grey) and offshore waters (black) of the North Atlantic Ocean throughout the juvenile period (0–4 years). Axis-1 and axis-2 explain 78% of the total variation.

masses of differing salinities. Generally, peaks of Sr concentration coincided with narrow opaque bands (slow growth zone generally associated with winter), suggesting that during winter the albacore migrate to high-salinity regions, such as tropical and subtropical regions of the North Atlantic Ocean. The central Atlantic Ocean is considered the main wintering area for albacore (Bard 1981), although there are major uncertainties given the scarcity of fisheries data on juvenile albacore during the winter months. Migration to high-salinity waters is visible during the first winter for the individuals captured in the Bay of Biscay (100%). In contrast, most fish captured in offshore waters (55%) do not reflect a migration signal until age-2, or the amplitude of the otolith Sr:Ca cycle at age-1 is smaller, suggesting that the magnitude of movements that may take place at age-1 is more limited. PCA applied to otolith Sr:Ca transects from 0 to 4 years reinforces the partitioning of albacore migratory patterns during the juvenile stage into two units.

Based on the differences in otolith post-core Sr:Ca and Ba:Ca and the timing of Sr:Ca cycles, albacore in the North Atlantic Ocean could be classified into two different migratory contingents: (1) a group captured within the Bay of Biscay, with early life movements between different water masses (starting at age-1+); and (2) a group formed by individuals captured in offshore waters, with limited movements during the early life or annual migration starting at later stages.

Davies *et al.* (2011) described the potential presence of three different subpopulations within the eastern North Atlantic Ocean, which fits with our findings based on movement patterns. Furthermore, Bard (1981) reported that albacore captured in the Bay of Biscay (Cantabrian Sea) and offshore waters (Azores Islands) present different morphometric characteristics and hypothesised that different subpopulations may follow a

different migration route in the eastern North Atlantic Ocean. Despite the fact that we found no evidence of different nursery areas, our results support the occurrence of two migratory contingents of albacore in the eastern North Atlantic Ocean. Further research is needed to determine whether the two contingents follow different migration routes or differences in elemental chemistry are generated by the timing of the movements.

Observed differences in otolith chemistry between the two locations suggest that both classes of natural markers are useful, and further development and application of the approach for understanding the population structure of albacore in the North Atlantic Ocean is warranted. The combination of stable isotopes and trace elements in otoliths of albacore shows great potential for studying the variability in migration patterns, physiology and life histories among groups. Assessing interannual variations in stable isotopes and trace elements, as well as extending this work to the broader North Atlantic Ocean, will help clarify the highly complex population structure of albacore, which is crucial for sustainable management and exploitation of the fishery.

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