ICES Journal of Marine Science

ICES Journal of Marine Science (2015), 72(2), 625–634. doi:10.1093/icesjms/fsu156

Original Article

Origin of Atlantic bluefin tuna (*Thunnus thynnus*) in the Bay of Biscay

Igaratza Fraile[1]*, Haritz Arrizabalaga[1], and Jay R. Rooker[2]

[1]*Marine Research Division, AZTI Tecnalia, Herrera Kaia, Portualdea z/g, Pasaia E-20110, Spain*
[2]*Department of Marine Biology, Texas A&M University, 1001 Texas Clipper Road, Galveston, TX 77553, USA*

**Corresponding author: e-mail: ifraile@azti.es*

Fraile, I., Arrizabalaga, H., and Rooker, J. R. Origin of Atlantic bluefin tuna (*Thunnus thynnus*) in the Bay of Biscay. – ICES Journal of Marine Science, 72: 625–634.

Received 10 March 2014; revised 8 August 2014; accepted 11 August 2014; advance access publication 12 September 2014.

We used carbon and oxygen isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) in otoliths as a tool for identifying the nursery origin of Atlantic bluefin tuna (*Thunnus thynnus*) caught in the eastern North Atlantic Ocean (Bay of Biscay). Juvenile and adult bluefin tuna were collected over three consecutive years (2009—2011) using the regional bait boat fleet. Otolith $\delta^{13}C$ and $\delta^{18}O$ values of bluefin tuna were measured by mass spectrometry, and values were compared with a reference sample of yearling bluefin tuna from eastern (Mediterranean Sea) and western (Gulf of Mexico) nurseries to determine nursery origin. Maximum likelihood estimates based on otolith $\delta^{13}C$ and $\delta^{18}O$ values indicated that the overall contribution of western migrants to the Bay of Biscay fishery was <1% and varied over the years assessed. A small number of potential western migrants (2.7%) was detected in 2009, and most of these fish appear members of the abundant 2002 and 2003 year classes. In contrast, the Bay of Biscay fishery was composed exclusively (100%) of eastern origin bluefin tuna in 2010 and 2011, suggesting that this fishery is supported almost exclusively by the eastern spawning area but transatlantic western population may contribute to this fishery in a few years.

Keywords: mixing rate, otolith, stable isotope, *Thunnus thynnus*.

Introduction

The chemical composition of otoliths (ear stones) often reflects the ambient conditions of the water mass inhabited by marine fish, and thus these chemical tags represent natural markers that can be used to assess an individual place of origin or environmental history (Campana, 1999). Factors influencing the incorporation of stable carbon and oxygen isotopes in otoliths have been examined in both field (Pruell *et al.*, 2012) and laboratory (Thorrold *et al.*, 1997; Høie *et al.*, 2004) trials, confirming that oxygen isotopes are deposited at near equilibrium with that of the water, while carbon isotopes are influenced by kinetic and metabolic effects (Secor *et al.*, 1995; Schwarcz *et al.*, 1998; Rooker *et al.*, 2001). Variation in the seawater oxygen isotope composition is mainly controlled by salinity, although it is also influenced by temperature and sea ice formation (Craig and Gordon, 1965). At a global scale, different water masses have distinct isotopic signatures, and as such, the resulting chemical tags in otoliths from individuals occupying different regions can be used to examine movement at both small (10–100 km) and large (>1000 km) scales (Gao and Bean, 2008; Rooker *et al.*, 2008b; Shiao *et al.*, 2009; Kimirel *et al.*, 2013). Otoliths grow throughout the fish life providing a chemical chronology over the entire life of a fish, with material deposited during the first year of life serving as natural marker of the individual nursery origin (Campana, 2005).

Previous studies have demonstrated that $\delta^{13}C$ and $\delta^{18}O$ in otolith of tunas can be used to reliably predict their nursery origin (Rooker *et al.*, 2008a; Shiao *et al.*, 2010; Wells *et al.*, 2012). For Atlantic bluefin tuna, otolith $\delta^{13}C$ and $\delta^{18}O$ values are significantly more enriched in Mediterranean Sea than the Gulf of Mexico spawning/nursery areas (Rooker *et al.*, 2008a; Schloesser *et al.*, 2010), which is in accord with observed geographic variation in seawater $\delta^{13}C$ and $\delta^{18}O$ for eastern and western nurseries (LeGrande and Schmidt, 2006; McMahon *et al.*, 2013). These chemical markers have recently been used to document widespread mixing of eastern and western bluefin tuna populations, while showing high rates of natal homing by both populations (Rooker *et al.*, 2008a). Information from otolith chemistry studies using these markers as well as advances in electronic

tagging (Block *et al.*, 2001, 2005) and genetics (Carlsson *et al.*, 2006, 2007; Albaina *et al.*, 2013) have advanced our understanding of bluefin tuna population connectivity. However, our understanding of mixing rates of the two populations across the North Atlantic, although crucial in stock assessment models (Taylor *et al.*, 2011), is still unclear.

The movement of the Atlantic bluefin tuna into the Bay of Biscay occurs between the end of spring and the beginning of autumn, with juvenile and adolescent tuna feeding on the abundant prey (Cort, 1990; Dufour *et al.*, 2010; Goñi and Arrizabalaga, 2010; Logan *et al.*, 2011). Tagging experiments have shown connection between bluefin tuna in the Bay of Biscay, the western Atlantic Ocean, and the Mediterranean Sea (de la Serna *et al.*, 2001; Rodríguez-Marín *et al.*, 2005; Arregui *et al.*, 2006). According to some authors, the Bay of Biscay represents a foraging area for both eastern and western origin bluefin tuna (Fromentin and Powers, 2005). In the Bay of Biscay, Atlantic bluefin tuna are caught by the Spanish bait boat fleet and catch rates from this fishery used to obtain the only juvenile abundance index available for the eastern population (Rodríguez-Marín *et al.*, 2003; Santiago and Arrizabalaga, 2012). Despite its importance, little is known about the origin of bluefin tuna in the Bay of Biscay.

The aim of this study was to use otolith $\delta^{13}C$ and $\delta^{18}O$ to predict the origin of bluefin tuna in the Bay of Biscay. This region is believed to be frequented by large numbers of adolescent bluefin tuna of eastern origin, but similar to the US Atlantic Ocean, this region may represent an important mixing area of eastern and western populations. Isotopic values of adolescent bluefin tuna were compared with reference samples (yearlings from the eastern and western nurseries (Rooker *et al.*, 2014)) to assess the degree of mixing and transatlantic movement by bluefin tuna of western origin to the Bay of Biscay.

Material and methods

Otoliths were collected during summer, when bluefin tuna foraged in the Bay of Biscay (Figure 1). On-board observers, distributors, and fish processing manufacturers participated in the collection of bluefin tuna caught in the Bay of Biscay and surrounding areas over a 3-year period (2009–2011), with a total of 47 sampling days. Fork length (*FL*, cm) and total weight (*W*, kg) of bluefin tuna were measured to the nearest 0.1 cm and 0.1 kg. Yearling (up to 18 months), juvenile (ages 2, 3, and 4), and adult (age 5+) bluefin tuna were caught between June and October by the commercial bait boat fishery targeting albacore (*Thunnus alalunga*) and bluefin tuna (*Thunnus thynnus*). Individual age was estimated using the von Bertalanffy *FL*–age relationship for the eastern Atlantic bluefin tuna, and age-at-maturity was that of the eastern population (ICCAT, 2012; Cort *et al.*, 2014). Sizes of bluefin tuna ranged from 64 to 182 cm fork length, with age 2 and age 6 fish being the predominant age classes sampled in 2009, while most bluefin tuna caught in 2010 and 2011 were of age 2 (Figure 2). Otoliths of yearling tuna ($n = 24$) were combined with the reference sample set, while juvenile and adult bluefin tuna otoliths ($n = 281$) were selected for origin determination by chemical analysis. Otolith selection was based on fish size and fishing year to have a wide coverage in terms of time and age groups (Table 1).

Sagittal otoliths extracted from bluefin tuna were cleaned of tissues, soaked briefly in dilute nitric acid (1%), then rinsed with deionized water. After drying, otoliths were weighted to the nearest 0.01 mg using an electronic balance then stored dry until further processing. When the weight or size of the selected bluefin tuna was not available (e.g. frozen heads collected by distributors or fishers), it was predicted using the linear relationship between otolith weight and fish weight ($W_f = 0.932W_o - 15.392$; $R^2 = 0.94$. Figure 3).

One otolith per fish was randomly selected for carbon and oxygen isotope analysis. Samples were prepared for chemical analysis as described by Rooker *et al.* (2008b). Otoliths were embedded with Epofix resin (Struers), and transverse sections of ~1.5 mm thick were cut across the core using an IsoMet low-speed saw. Sections were glued to a sample plate with thermoplastic glue. The otolith portion corresponding to material deposited during the yearling period (hereafter called "otolith inner region") was

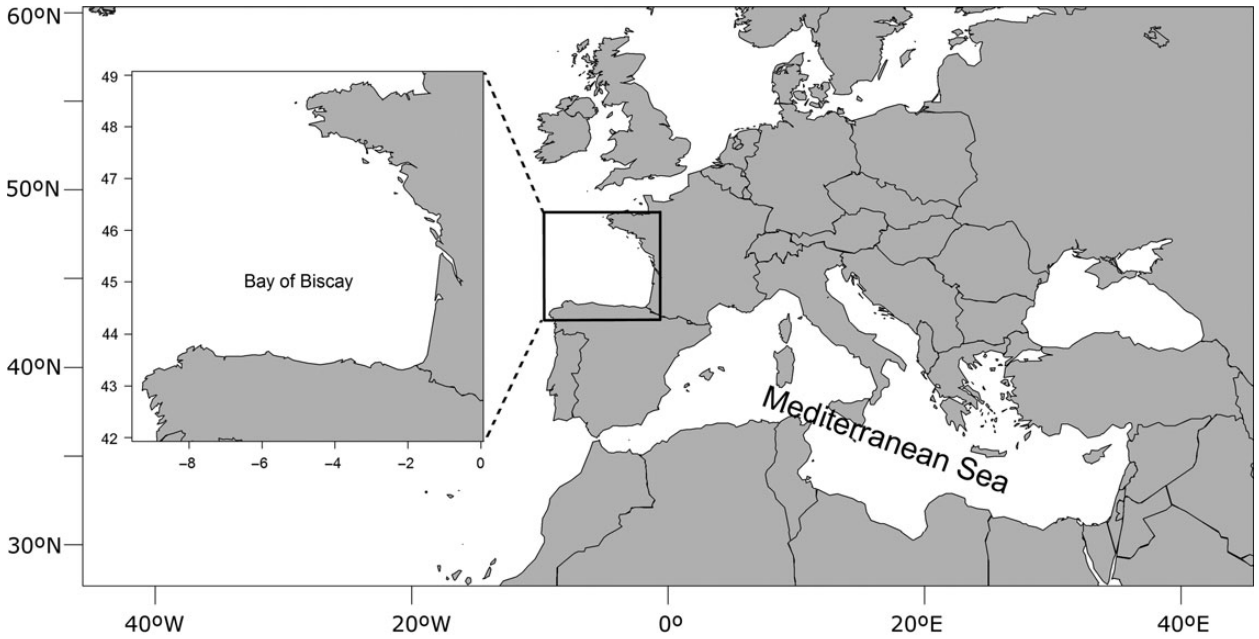

Figure 1. Study area, the Bay of Biscay, in the eastern North Atlantic Ocean.

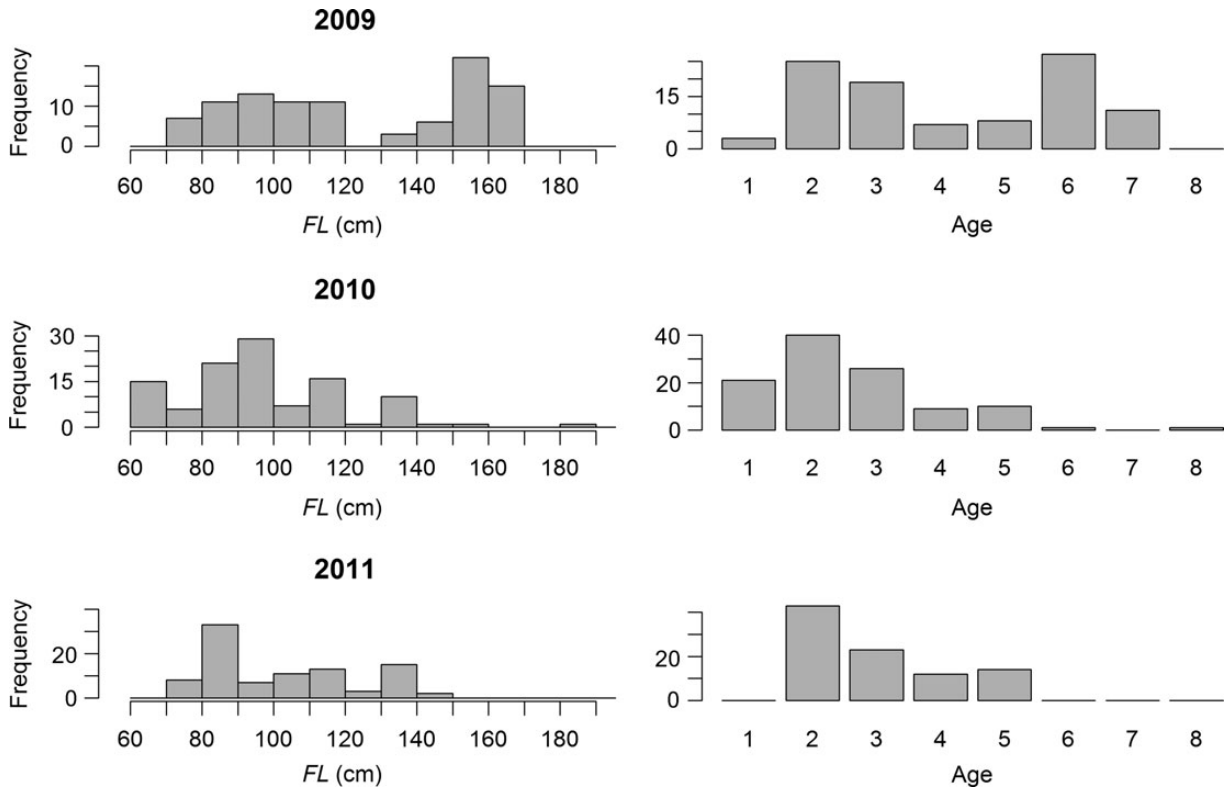

Figure 2. Fork length (*FL*) and age of Atlantic bluefin tuna (*T. thynnus*) collected between 2009 and 2011 from the Bay of Biscay. Size classes were divided into yearling (up to 18 months), juvenile (ages 2, 3, and 4), and adult (age 5^{+}) bluefin tuna.

Table 1. Sample size, mean length, weight, and estimated age of Atlantic bluefin tuna (*T. thynnus*) collected from the Bay of Biscay during the summers of 2009, 2010, and 2011 used for origin assignment.

			FL (cm)		*W* (kg)		Age (year)	
Sample type	**Sampling year**	***N***	**Mean**	***SD***	**Mean**	***SD***	**Mean**	***SD***
Eastern yearling	2009 – 10	24	69.1	3.2	6.7	1.1	1.1	0.2
Mixed sample	2009	97	125.6	31.8	43.1	29.2	4.5	1.8
Mixed sample	2010	87	104.7	18.7	22.9	13.1	3.5	1
Mixed sample	2011	97	104	19.8	22.7	13.2	3.3	1

FL, fork length (cm); *W*, total weight (kg); age estimation based on growth curve from Cort (1991).

powdered using a high-resolution New Wave Micromill System consisting of a microscope and imaging system, controlled by computer software. The drill path corresponding to the yearling period (Figure 4) was the same as the one used in previous studies (Rooker *et al.*, 2008a; Schloesser *et al.*, 2010). A 500 μm diameter carbide bit (Gebr. Brasseler, Germany) was used over the pre-programmed drill path repeating the path 14 times and milling ~55 μm depth per pass (770 μm in total). For each otolith ~1.0 mg of powdered material was collected in a glassine weighing paper.

Carbon and oxygen stable isotopes of otolith samples were analysed on an automated carbonate preparation device (KIEL-III) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252)

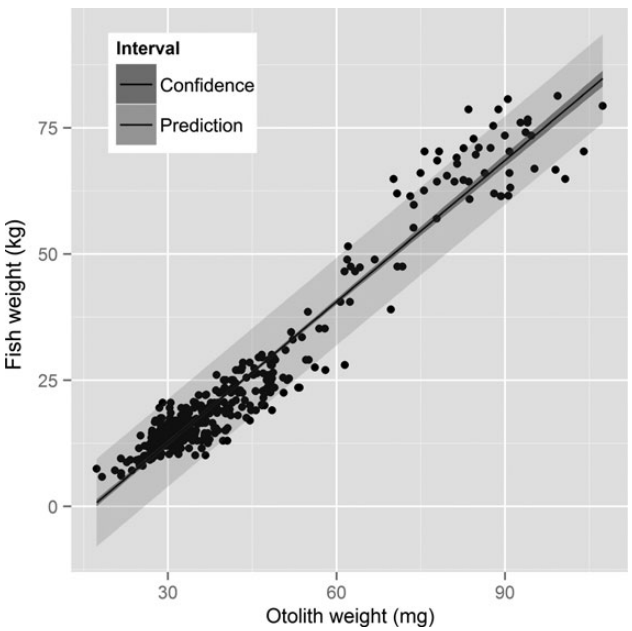

Figure 3. Linear regression between Atlantic bluefin tuna (*T. thynnus*) weight (W_f) and otolith weight (W_o) in the Bay of Biscay ($W_f = 0.932W_o - 15.392$; $R^2 = 0.94$). Prediction intervals (95%) and confidence intervals (95%) are represented by shaded bands. The dataset contains otoliths used for stable isotope analysis and additional otoliths collected in the Bay of Biscay region ($n = 434$).

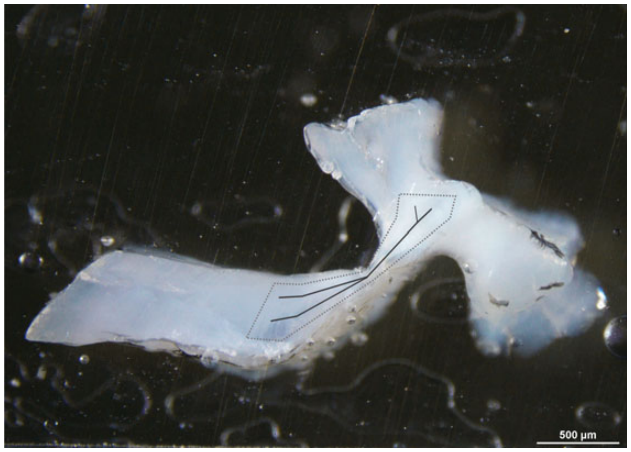

Figure 4. Transverse section of a juvenile (age 3) Atlantic bluefin tuna (*T. thynnus*) sagittal otolith showing the template for milling out the material from the inner region of the otolith (corresponding to the yearling period).

from the Environmental Isotope Laboratory of the University of Arizona. Powdered samples were reacted with dehydrated phosphoric acid under vacuum at 70°C. The isotope ratio measurement is calibrated based on repeated measurements of NBS-19 and NBS-18 and precision is ±0.1‰ for oxygen and ±0.08‰ for carbon isotopes (1 *SD*). The same mass spectrometer was used for the underlying baseline presented in Rooker *et al.* (2014), and thus inter-laboratory calibration was not needed. All isotope values are reported according to standards of the International Atomic Energy Agency in Vienna. $\delta^{13}C$ and $\delta^{18}O$ represent the ratio between the 13/12C and 18/16O in the sample, stated in per mil, relative to the Vienna Pee Dee Belemnite scale. Estimates of nursery origin of bluefin tuna were based on comparing otolith inner regions (otolith material deposited during the first year of life or yearling period) of juvenile and adult bluefin tuna to the baseline or reference samples of yearling bluefin tuna. Stable isotope composition in otoliths of yearling bluefin tuna was derived from a long-term baseline sample taken during the years 1998–2011 (Rooker *et al.*, 2014). The revised baseline includes only samples run at a single laboratory and all samples were processed using the same milling template for isolating the inner material. Cross-validated classification success (based on quadratic discriminant function analysis, QDFA) of the revised baseline was 90% for the east and 71% for the west. The age of juvenile and adult bluefin tuna in our study was between 2 and 7 years. Therefore, 100% of the samples were within the range of years covered by the baseline (1998–2011).

Generalized lineal models (GLMs) were used to determine whether otolith $\delta^{13}C$ and $\delta^{18}O$ values varied with sampling year and size of the fish. Separate GLMs were used to test for a year-class effect. Otolith $\delta^{13}C$ and $\delta^{18}O$ values for adolescent and adult bluefin tuna collected in the Bay of Biscay were compared with yearling baseline samples (Rooker *et al.*, 2014) and classified into eastern or western nurseries using maximum likelihood estimates (MLEs) from the mixed-stock analysis programme HISEA as described by Millar (1990). HISEA mixture model is based on a maximum likelihood estimation procedure based on finite-mixture distributions. Mixed-stock proportions and *SDs* were estimated by bootstrapping with 1000 replicates both the baseline dataset and the unknown (mixed) sample data. This procedure estimated the population composition of the Bay of Biscay fishery from our sample data and its associated error, assuming that there are no other nurseries not included in the baseline. Additionally, individual classification method was used to predict individual origin. This methodology calculates the probability that an individual fish belongs to a source population using the estimated discriminant model, rather than estimating an overall mixing rate. Carbon and oxygen isotope values of yearling baseline samples were used to build the model, and Bay of Biscay otoliths were then re-classified according to their isotopic values. As suggested by Mercier *et al.* (2011), different classification methods were tested with the baseline dataset: the classical QDFA, the Naive Bayes Bayesian classifier (NB), and a machine learning method namely the support vector machine (SVM). QDFA method is a common tool for classification analysis that models the likelihood of each class as a Gaussian distribution, and estimates the posterior probabilities for a given test point (Hastie *et al.*, 2001). NB is a probabilistic classifier based on Bayesian statistic. It tries to classify samples based on the probabilities of previously seen concentrations, assuming complete independence between the predictor variables (carbon and oxygen isotopes), although it performs well even when independence conditions are not met (Domingos and Pazzani, 1997). SVM method is a statistical learning system with associated learning algorithms that analyse data and recognize patterns to find the optimal separating hyperplane between the eastern and western samples (Vapnik, 1998). For the three classification methods, a subset of 75% of the data was randomly selected for model fitting and the remainder was used for model assessment. The method with the highest classification rate was used to predict the origin of our mixed sample. Given that the classification success of western origin baseline samples was ~70% (Rooker *et al.*, 2014), individuals were catalogued as western origin fish when the probability to be part of the western population was >70%. Consistency among the three classification methods was assessed by comparing the number of western migrants predicted with each method.

Results

A GLM explained 22% of the null deviance and showed a statistically significant (P-value < 0.05) year-to-year depletion in $\delta^{13}C$ values over the 3 years sampled (Table 2) and a significant increase in $\delta^{13}C$ values with fish size (slope = 0.0033, P-value < 0.05, see Figure 5). A separate GLM also showed a significant linear decrease of $\delta^{13}C$ values with year class (slope = −0.1062, $P < 0.05$) for the range of year classes covered (2002–2009), with 15% of the deviance explained. In contrast, $\delta^{18}O$ values did not differ significantly among the years sampled (mean ± 1.96*SD* = −0.75 ± 0.63‰, −0.75 ± 0.33‰ and −0.77 ± 0.39‰ for 2009, 2010 and 2011, respectively; P-value > 0.05), but a significant negative effect of fish size in $\delta^{18}O$ signatures was detected (slope = −0.0015, P-value < 0.05, 3% of null deviance explained).

Yearling bluefin tuna otoliths collected in the Bay of Biscay during 2009 and 2010 showed similar isotopic values to those of the eastern reference sample set, with mean $\delta^{13}C$ of −8.60‰ coinciding with the mean eastern signature of the existing baseline (Rooker *et al.*, 2014), and mean $\delta^{18}O$ of −0.76‰ in otoliths collected for this study compared with mean $\delta^{18}O$ −0.81‰ of the baseline. Values for $\delta^{13}C$ and $\delta^{18}O$ measured in otolith inner region of juvenile and adult bluefin tuna were similar to the values of the eastern reference sample of yearlings (Figure 6). MLEs showed that bluefin tuna from the Bay of Biscay are almost entirely composed of individuals from the Mediterranean Sea nursery

ground. For bluefin tuna collected in 2010 and 2011, HISEA indicated that our entire samples (100%) were of Mediterranean origin. *SD* of estimated proportions was 0.0%, indicating a high degree of confidence in this prediction. However, bluefin tuna of western origin were detected for both juveniles and adults collected in 2009, indicating that transatlantic movement (west to east) may have occurred for the western population of bluefin tuna. Estimated contribution of juvenile and adult bluefin tuna of western origin was 2.4 ($\pm$4.6) and 6.1 ($\pm$10.1%), respectively (Table 3). According to MLE, the presence of western migrants in the Bay of Biscay was not significant (95% of confidence interval), as the estimated proportion of western fish is within the statistical error ($\pm$1.96*SD*).

To further characterize the nature of potential western contributions to the Bay of Biscay, an individual classification approach was also conducted. The comparison of three classification methods to estimate individual nursery origin showed consistent results, with overall western contribution $<$5% always. Among the three,

Table 2. Summary of otolith $\delta^{13}C$ and $\delta^{18}O$ values of Atlantic bluefin tuna (*T. thynnus*) from the Bay of Biscay.

			$\delta^{13}C$ (‰)			$\delta^{18}O$ (‰)		
Sample type	**Sampling year**	**Size category**	**Range**	**Mean**	***SD***	**Range**	**Mean**	***SD***
Baseline	2009–2010	Yearling	−9.52 to −7.92	−8.60	0.44	−1.05 to −0.33	−0.76	0.16
Mixed	2009	Juv	−10.07 to −7.61	−8.44	0.51	−2.21 to 0	−0.66	0.34
		Adu	−9.21 to −6.96	−8.02	0.53	−1.7 to −1.4	−0.83	0.28
Mixed	2010	Juv	−9.51 to −7.64	−8.68	0.39	−1.1 to −0.28	−0.74	0.17
		Adu	−9.78 to −7.94	−8.66	0.58	−1.1 to −0.49	−0.80	0.18
Mixed	2011	Juv	−10.07 to −7.51	−8.93	0.51	−1.18 to −0.36	−0.77	0.19
		Adu	−9.74 to −8.02	−8.80	0.45	−1.11 to −0.34	−0.77	0.22

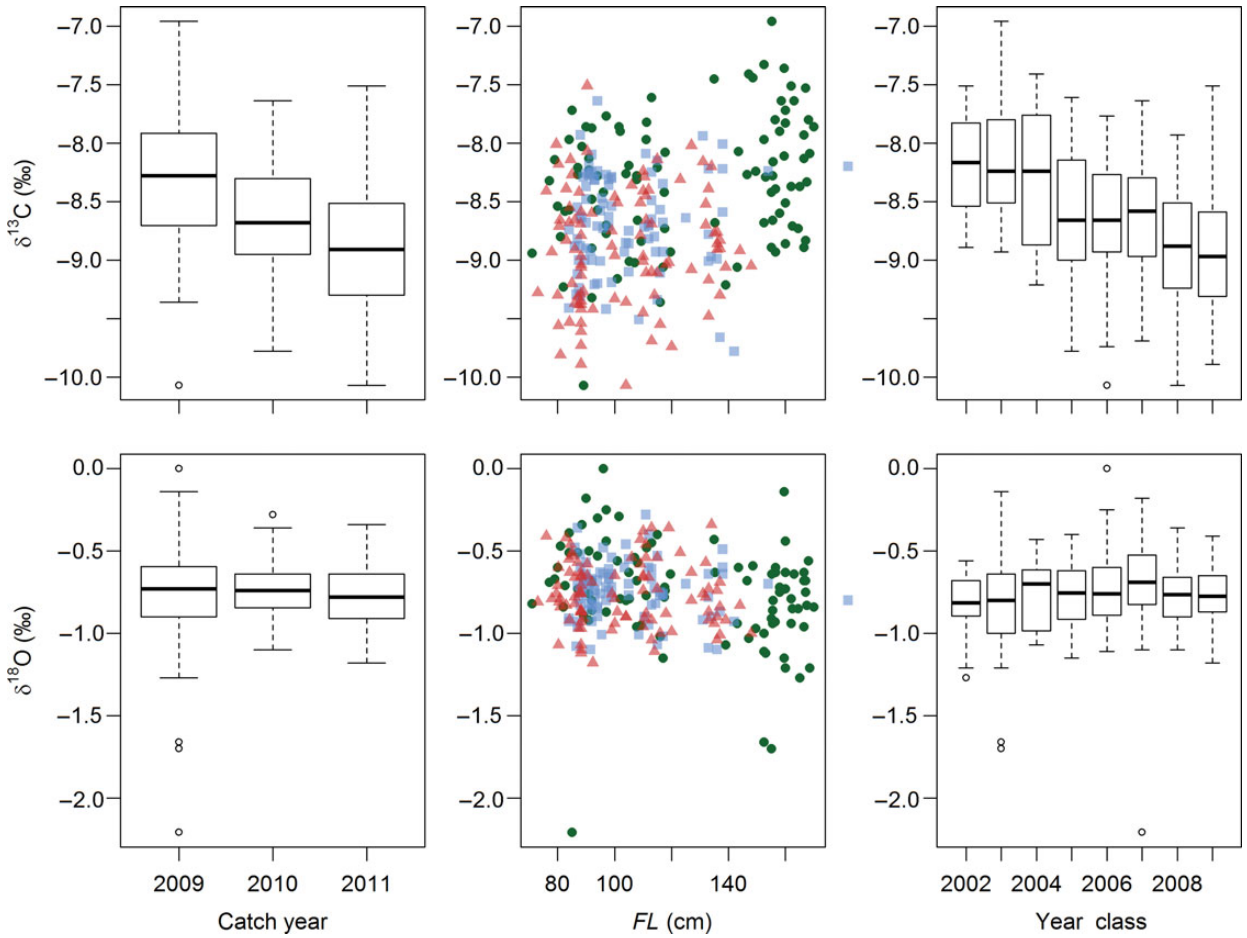

Figure 5. Otolith stable isotope composition ($\delta^{13}C$ and $\delta^{18}O$ in upper and lower panels, respectively) as a function of year caught (left panels), fork length (*FL*, central panels), and year class (right panels) for Atlantic bluefin tuna (*T. thynnus*) caught in the Bay of Biscay. Circles, squares, and triangles in central panels represent fish captured in 2009, 2010, and 2011 respectively.

Table 3. Maximum likelihood classification (percentage and *SD*) of Atlantic bluefin tuna (*T. thynnus*) collected in the Bay of Biscay.

		2009			2010			2011			All		
		N	East	West	*N*	East	West	*N*	East	West	*N*	East	West
Juveniles	Clas. (%)	51	97.7	2.4	79	100	0	87	100	0	217	99.3	0.7
	SD	51	2.3	2.3	79	0	0	87	0	0	213	0.6	0.6
Adults	Clas. (%)	46	93.9	6.1	8	100	0	10	100	0	64	97.7	2.4
	SD	46	5.1	5.1	8	0	0	10	0	0	64	2.6	2.6
All	Clas. (%)	97	97.4	2.7	87	100	0	97	100	0	281	99.6	0.4
	SD	97	2.5	2.5	87	0	0	97	0	0	281	0.4	0.4

Table 4. Summary data for western Atlantic bluefin tuna (*T. thynnus*) migrants captured in the Bay of Biscay (based on QDFA).

Fish code	$\delta^{13}C$	$\delta^{18}O$	*FL* (cm)	Probability western (%)	Capture date (dd/mm/yyyy)	Estimated age (year)	Estimated year class
HL09-BFT-45	−8.42	−1.70	155.00	99	22/07/2009	6.5	2003
HL09-BFT-51	−7.36	−1.15	159.50	92	22/07/2009	6.8	2002
HL09-BFT-53	−8.86	−1.21	160.00	77	22/07/2009	6.8	2002
HL09-BFT-56	−7.33	−1.11	152.50	86	22/07/2009	6.3	2003
HL09-BFT-59	−7.97	−1.66	152.50	99	22/07/2009	6.3	2003
HL09-BFT-109	−7.72	−2.21	85.00	100	22/07/2009	2.4	2007
HL09-BFT-201	−8.09	−1.21	168.50	89	30/07/2009	7.5	2002
HL09-BFT-208	−8.37	−1.27	165.00	93	30/07/2009	7.2	2002

Age is estimated using the age–length relationship based on von Bertalanffy growth model for the western population (Restrepo *et al.*, 2010).

QDFA performed best and attained the highest classification accuracy with the baseline dataset, with an overall classification success of 86.4% compared with 74.6% for NB and SVM. Thus, QDFA was further used to classify the mixed sample of the Bay of Biscay. Based on the QFDA results and the adopted classification approach, eight individuals were classified as western fish in the 2009 sample (Table 4 and Figure 6). Although otolith sampling extended throughout the entire summer (from June to October) for three consecutive years, with a total of 47 sampling days, all 8 western bluefin tuna that visited the Bay of Biscay were caught from 22 July 2009 to 30 July 2009. These individuals show depleted $\delta^{18}O$ values (mean $\pm$ *SD* = −1.44 $\pm$0.38‰, Table 4). Except one juvenile, these tunas ranged between 159.5 and 168.5 cm *FL* (Figure 7) with most retrospectively assigned to the 2002 or 2003 year class.

Discussion

Otolith $\delta^{18}O$ values observed for bluefin tuna from the Bay of Biscay showed little temporal variation, whereas a depletion of $\delta^{13}C$ values over time was observed. Overall, younger bluefin tuna showed depleted $\delta^{13}C$ values compared with older individuals (Figure. 5), generating scattering of data points in Figure 6 for otoliths collected in 2009, when most of the adult bluefin tuna were sampled. This variability could partially be due to the re-mineralization of small amount of calcium carbonate deposited originally at the outer edge of the otolith during subadult or adult stage by the otolith portion surrounding the core area (Shiao *et al.*, 2009). Consequently, when milling the otolith powder throughout the yearling portion of the otolith, a small amount of material deposited at subadult and adult stages may have mixed up with the "real" early life stage material, generating a higher dispersion in the data points. Depletion of $\delta^{13}C$ in bluefin tuna otoliths, although at smaller rates, has also been reported in otoliths of bluefin tuna from the western Atlantic (Schloesser *et al.*, 2009). Such depletion of $\delta^{13}C$ in bluefin otoliths was attributed to the Suess effect, which is caused by an increase of atmospheric CO_2 derived from the combustion of fossil-fuels and deforestation, causing a decrease in atmospheric $\delta^{13}C$ and, in turn, a decrease of $\delta^{13}C$ in biogenic carbonates (Verburg, 2007). The year-to-year decrease of $\delta^{13}C$ values observed in otoliths of bluefin tuna from the Bay of Biscay could be induced, at least partly, by the cited Suess effect. Other physical and biological processes, such as seasonal upwelling events and associated shifts in oceanic production and vertical transport may also generate interannual variability in carbon isotopes (McMahon *et al.*, 2013). Still, because the differentiation of the two stocks is mostly determined by oxygen isotope values, these variations in $\delta^{13}C$ do not influence mixed-stock estimates. For otolith $\delta^{18}O$ values, interannual variation accounted for <0.1% of the total variance, demonstrating the temporal stability of this proxy to differentiate eastern and western populations. $\delta^{18}O$ in aragonite is positively related to seawater salinity and inversely related with temperature (Campana, 1999; Rohling and Cooke, 1999; Bastow *et al.*, 2002). Salinification of the western Mediterranean Sea during the last decades has been already reported by several authors (Vargas-Yáñez *et al.*, 2005). Given that it includes the Balearic archipelago, one of the main spawning areas for eastern bluefin tuna and connected with the Bay of Biscay as demonstrated by tagging studies (de la Serna *et al.*, 2001; Rodríguez-Marín *et al.*, 2005; Aranda *et al.*, 2013), this might partly explain the depletion of $\delta^{18}O$ in older bluefin individuals compared with younger ones as described in this study.

The presence of western migrants in the Bay of Biscay was limited with no indication of western origin bluefin tuna in 2 of the 3 years sampled. Still, we did detect a small number of potential western migrants in 2009, mostly adults, indicating that transatlantic movement may occur for this population. However, given that MLEs tend to be biased when some of the stocks in the mixed fishery are low contributors (Millar, 1990; Rooker *et al.*, 2014), the western contribution might be eclipsed by the dominant eastern group, and the eastern proportion underestimated. Data were re-analysed using QDFA, and the high probabilities predicted by QDFA raise the

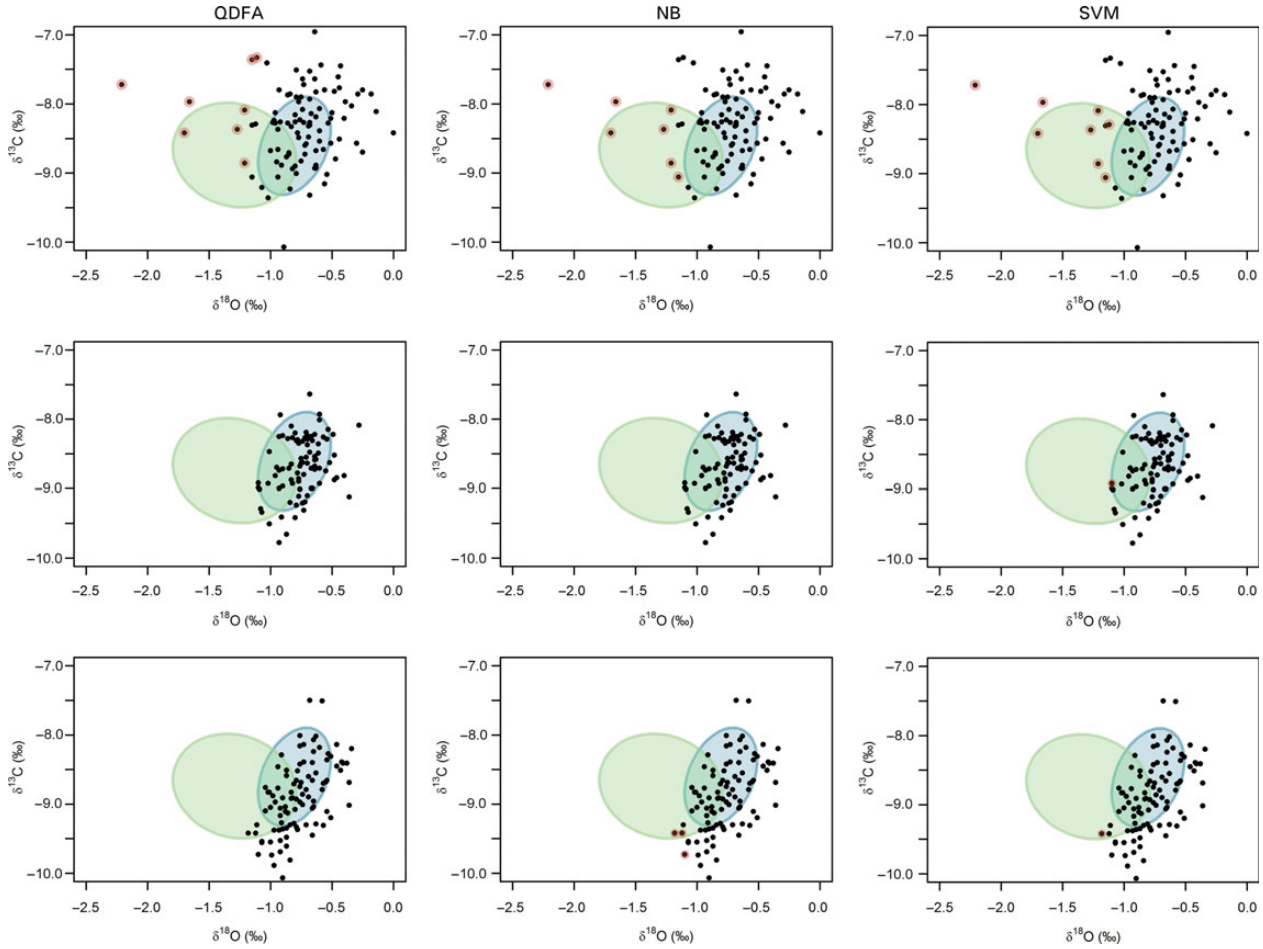

Figure 6. $\delta^{13}C$ and $\delta^{18}O$ confidence ellipses constructed with the baseline samples from western (green) and eastern (blue) nurseries (1 *SD* or $P = 68\%$; Rooker *et al.*, 2014). Points represent otolith $\delta^{13}C$ and $\delta^{18}O$ values of Atlantic bluefin tuna (*T. thynnus*) from the Bay of Biscay caught in 2009 (upper panels), 2010 (middle panels) and 2011 (lower panels). Red circles denote individuals with a probability >70% of being born in the western Atlantic Ocean predicted by QDFA (left), NB (middle), and Support Vector Msachine (right).

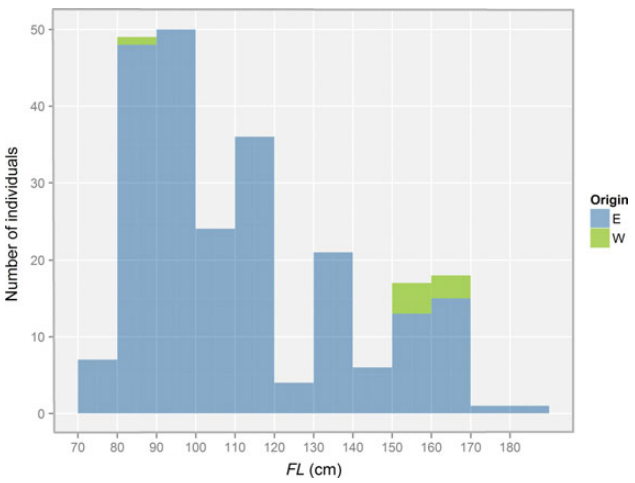

Figure 7. Fork length of Atlantic bluefin tuna (*T. thynnus*) from the Bay of Biscay classified as eastern (blue) and western (green) by individual origin assignment based on quadratic discriminant analysis. Individuals with a probability >70% (based on QDFA) are included in the classification.

confidence in their western origin. The small proportion of juvenile western bluefin tuna detected in the Bay of Biscay in 2009 could have joined an adult bluefin tuna school in the western Atlantic Ocean and migrate to the east as a mixed school of small and large bluefin tuna. During 2010 and 2011, perhaps the lack of western migrants in these samples could be due to the limited number of adults examined (especially ages 6 and older). Based on our results, it appears that the Bay of Biscay is utilized primarily as a juvenile feeding ground of the eastern bluefin tuna population, and thus the fishery in this area is supported by the production of bluefin tuna in the Mediterranean Sea. Results from our analysis also suggest that the observed west to east transatlantic movement of western origin bluefin tuna to the Bay of Biscay is negligible, supporting the current practice of using catch rates from the Bay of Biscay fishery as an indicator of the eastern population abundance in stock assessments (ICCAT, 2012). However, given that the eastern population is ~10 times larger than that of the western population (ICCAT, 2012), the presence of western fish in the Bay of Biscay during 2009 is very relevant for the Western stock assessment, and suggests that a considerable fraction of the western population may move across the Atlantic Ocean to feed in the Bay of Biscay and/or surrounding waters of the Northeast Atlantic.

Previous authors have reported transatlantic movement of bluefin tuna using electronic and conventional tags, with high variability among seasons, age classes, locations, years, and decades (Lutcavage *et al.*, 1999; Block *et al.*, 2001*et al.*, 2005; Arregui *et al.*, 2006). However, the origin of bluefin tuna displaying transatlantic movement was unclear until recently. Using chemical markers in otoliths (Rooker *et al.*, 2008b) demonstrated that transatlantic movements of bluefin tuna were significant with a large fraction of adolescent bluefin tuna on foraging areas in the US Atlantic Ocean originating from the eastern spawning area. Our results indicate adolescent bluefin tuna of western origin do not show the same level of transatlantic movement to the Bay of Biscay. Still, contribution rates in this region will be heavily influenced by disparity in the sizes of eastern and western populations because a large proportion of western origin fish would need to migrate to the east before it could be detected. Putative western migrants detected in this study were mostly adults of 67 years old. Given that eastern population reaches maturity earlier than the western population, the western bluefin tuna classified as adults would in fact correspond to western adolescents. Thus, our findings of transatlantic movement of age 6 and age 7 individuals of western origin (i.e. adolescents) to the Bay of Biscay appears in accord with transatlantic movement patterns described by Rooker *et al.* (2008b) for adolescents of eastern origin.

Although sampling was carried out at 2–5 days intervals during three consecutive summers, with 47 sampling days, all western origin fish were captured in two days and within a 10-day period, suggesting that western origin bluefin may enter the Bay of Biscay occasionally. West to east transatlantic movements therefore may fluctuate greatly over years, with migration events occurring in sporadic pulses. We hypothesize that such migration pulses could be linked to demographic aspects (e.g. relative population sizes and density-dependent range contraction/expansion events in both populations), environmental aspects and/or age-dependent behaviour of bluefin tuna (Mather, 1980; Sibert *et al.*, 2006).

Seven of the eight western migrants detected in the Bay of Biscay in 2009 were of age 67 based on age–length (weight) keys described by Restrepo *et al.* (2010). Thus, these individuals likely belonged to the 2002 or 2003 year class. The last stock assessment estimated strong 2002 and 2003 year classes (ICCAT, 2012). The 2003 cohort is known to be a strong year class of bluefin tuna that has made a dominant contribution to the recreational fishery in the western Atlantic Ocean. Recent work carried on in the US Atlantic coast by Secor *et al.* (2012) also found that western contribution was higher for the 2003 year-class compared with average mixing rates. Our results potentially provide further evidence that the 2002–2003 year classes were exceptionally strong in the western Atlantic Ocean, leading to an expansion to foraging grounds into the eastern Atlantic Ocean (i.e. Bay of Biscay). Under such demographic hypothesis, however, one would expect that western origin fish would be detected in the Bay of Biscay during a few consecutive years. Unfortunately, the lack of 2003 year-class otolith samples in 2010 and 2011 hinders the possibility to follow the temporal evolution of this cohort in the Bay of Biscay and thus confirm the hypothesis. But, regardless of the underlying mechanism(s) involved, our results suggest that considerable interannual variations in movement and mixing might occur across the Atlantic as suggested by Sibert *et al.* 2006). This complex dynamic, yet to be fully understood and characterized, undermines assessment and management of Atlantic bluefin tuna populations.

In conclusion, this study demonstrates the value of otolith stable isotope analysis for studying the connectivity between the eastern and western populations of Atlantic bluefin tuna. Results suggest that the contribution of individuals from the western nursery to the Bay of Biscay was limited, and this movement may have little influence in the eastern stock assessment, but could be potentially important for the Western stock assessment because a substantial fraction of the western population may move into the eastern management area.

Acknowledgements

The authors thank L. Naval and the staff from La Bacaladera for providing Atlantic bluefin tuna samples for this study. They are indebted to the crew from Attalaya Berria, Tukutuku, and Berriz Gure Naia vessels for their contribution on behalf of the bluefin tuna sampling programme. They thank the comments of two anonymous reviewers that improved the manuscript. This work was carried out under the provision of the ICCAT Atlantic Wide Research Program for Bluefin Tuna (GBYP), funded by the European Union, by several ICCAT CPCs, the ICCAT Secretariat and by other entities (see http://www.iccat.int/GBYP/en/Budget.htm). The contents of this paper do not necessarily reflect the point of view of ICCAT or of the other funders. This work was also supported by grant CTM2011-27505 by the Spanish Ministry of Economy and Competitiveness (MINECO), National Geographic Society (Grant W132-10), and the Basque Government (Grant 351BI20080022). This paper's contribution number is 666 from AZTI-Tecnalia (Marine Research).

References

Albaina, A., Iriondo, M., Velado, I., Laconcha, U., Zarraonaindia, I., Arrizabalaga, H., Pardo, M. A., *et al.* 2013. Single nucleotide polymorphism discovery in albacore and Atlantic bluefin tuna provides insights into worldwide population structure. Animal Genetics, 44: 678–692.

Aranda, G., Abascal, F. J., Varela, J. L., and Medina, A. 2013. Spawning behaviour and post-spawning migration patterns of Atlantic bluefin tuna (*Thunnus thynnus*) ascertained from satellite archival tags. PLoS ONE, 8: e76445.

Arregui, I., Arrizabalaga, H., and de la Serna, J. M. 2006. Preliminary approach to the experimental design of tagging campaings for movement rate estimations of east Atlantic bluefin tuna. Collective Volume of Scientific Papers ICCAT, 59: 769–788.

Bastow, T. P., Jackson, G., and Edmonds, J. S. 2002. Elevated salinity and isotopic composition of fish otolith carbonate: stock delineation of pink snapper, *Pagrus auratus*, in Shark Bay, Western Australia. Marine Biology, 141: 801–806.

Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A., *et al.* 2001. Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. Science, 293: 1310–1314.

Block, B. A., Teo, S. L. H., Walli, A., Boustany, A., Stokesbury, M. J. W., Farwell, C. J., Weng, K. C., *et al.* 2005. Electronic tagging and population structure of Atlantic bluefin tuna. Nature, 434: 1121–1127.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series, 188: 263–297.

Campana, S. E. 2005. Otolith science entering the 21st century. Marine and Freshwater Research, 56: 485–495.

Carlsson, J., McDowell, J. R., Carlsson, J. E. L., and Graves, J. E. 2007. Genetic identity of YOY bluefin tuna from the eastern and western Atlantic spawning areas. Journal of Heredity, 98: 23–28.

Carlsson, J., McDowell, J. R., Carlsson, J. E. L., Olafsdottir, D., and Graves, J. E. 2006. Genetic heterogeneity of Atlantic bluefin tuna

caught in the eastern North Atlantic Ocean south of Iceland. ICES Journal of Marine Science, 63: 1111–1117.

Cort, J. L. 1990. Biología y pesca del atún rojo, *Thunnus thynnus*, del mar Cantábrico. Publicaciones Especiales IEO, 4: 272.

Cort, J. L. 1991. Age and growth of bluefin tuna (*Thunnus thynnus* L.) of the Northwest Atlantic. Collective Volume of Scientific Papers ICCAT, 35: 213–230.

Cort, J. L., Arregui, I., Estruch, V. D., and Deguara, S. 2014. Validation of the growth equation applicable to the eastern Atlantic bluefin tuna, *Thunnus thynnus* (L.), using Lmax, tag-recapture and first dorsal spine analysis. Reviews in Fisheries Science & Aquaculture, 22: 239–255.

Craig, H., and Gordon, L. I. 1965. Deuterium and oxygen 18 variations in the ocean and marine atmosphere. *In Stable Isotopes in Oceanographic Studies and Paleotemperatures*, pp. 9–130. Ed. by E. Tongiogi, V. Lishi, and e F. Pisa, Spoleto, Italy.

de la Serna, J. M., Ortiz de Zárate, V., and Gómez, M. J. 2001. Actualización de los datos de marcado – recaptura de atÃ°n rojo (*Thunnus thynnus* L.) en el Atlántico Este y Mediterráneo. Collective Volume of Scientific Papers ICCAT, 52: 784–792.

Domingos, P., and Pazzani, M. 1997. On the optimality of the simple Bayesian classifier under zero-one loss. Machine Learning, 29: 103–130.

Dufour, F., Arrizabalaga, H., Irigoien, X., and Santiago, J. 2010. Climate impacts on albacore and bluefin tunas migrations phenology and spatial distribution. Progress in Oceanography, 86: 283–290.

Fromentin, J. M., and Powers, J. E. 2005. Atlantic bluefin tuna: population dynamics, ecology, fisheries and management. Fish and Fisheries, 6: 281–306.

Gao, Y., and Bean, D. 2008. Stable isotope analyses of otoliths in identification of hatchery origin of Atlantic salmon (*Salmo salar*) in Maine. Environmental Biology of Fishes, 83: 429–437.

Goñi, N., and Arrizabalaga, H. 2010. Seasonal and interannual variability of fat content of juvenile albacore (*Thunnus alalunga*) and bluefin (*Thunnus thynnus*) tunas during their feeding migration to the Bay of Biscay. Progress in Oceanography, 86: 115–123.

Hastie, T., Tibshirani, R., and Friedman, J. 2001. The Elements of Statistical Learning. Springer-Verlag, NewYork.

Høie, H., Otterlei, E., and Folkvord, A. 2004. Temperature dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua* L.). ICES Journal of Marine Science, 61: 243–251.

ICCAT (International Commission for the Conservation of Atlantic Tuna). 2012. Report of the 2012 Atlantic Bluefin Tuna Stock Assessment Session. Madrid, Spain, September 4–11, 124 pp.

Kimirel, I. A., Nagelkerken, I., Mgaya, Y. D., and Huijbers, C. M. 2013. The mangrove nursery paradigm revisited: otolith stable isotopes support nursery-to-reef movements by Indo-Pacific fishes. PLoS ONE, 8: e66320.

LeGrande, A. N., and Schmidt, G. A. 2006. Global gridded data set of the oxygen isotopic composition in seawater. Geophysical Research Letters, 33: L12604.

Logan, J. M., Rodríguez-Marín, E., Goñi, N., Barreiro, S., Arrizabalaga, H., Golet, W., and Lutcavage, M. 2011. Diet of young Atlantic bluefin tuna (*Thunnus thynnus*) in eastern and western Atlantic foraging grounds. Marine Biology, 158: 73–85.

Lutcavage, M., Brill, R. W., Skomal, G. B., Chase, B. C., and Howey, P. W. 1999. Results of pop-up satellite tagging of spawning size class fish in the Gulf of Maine: do North Atlantic bluefin tuna spawn in the mid-Atlantic? Canadian Journal of Fisheries and Aquatic Sciences, 56: 173–177.

Mather, F. J. 1980. A preliminary note on migratory tendencies and distributional patterns of Atlantic bluefin tuna based on recently acquired and cumulative tagging results. Collective Volume of Scientific Papers ICCAT, 9: 478–491.

McMahon, K. W., Hamady, L. L., and Thorrold, S. R. 2013. A review of ecogeochemistry approaches to estimating movements of marine animals. Limnology and Oceanography, 58: 697–714.

Mercier, L., Darnaude, A. M., Bruguier, O., Vasconcelos, R. P., Cabral, H. N., Costa, M. J., Lara, M., *et al.* 2011. Selecting statistical models and variable combinations for optimal classification using otolith microchemistry. Ecological Applications, 21: 1352–1364.

Millar, R. B. 1990. Comparison of methods for estimating mixed stock fishery composition. Canadian Journal of Fisheries and Aquatic Sciences, 47: 2235–2241.

Pruell, J. R., Taplin, B. K., and Karr, J. D. 2012. Spatial and temporal trends in stable carbon and oxygen isotope ratios of juvenile winter flounder otoliths. Environmental Biology of Fishes, 93: 61–71.

Restrepo, V. R., Díaz, G. A., Walter, J. F., Neilson, J. D., Campana, S. E., Secor, D., and Wingate, R. L. 2010. An updated estimate of the growth curve of western bluefin tuna. Aquatic Living Resources, 23: 335–342.

Rodríguez-Marín, E., Arrizabalaga, H., Ortiz, M., Rodríguez-Cabello, C., Moreno, G., and Kell, L. T. 2003. Standardization of bluefin tuna, *Thunnus thynnus*, catch per unit effort in the baitboat fishery of the Bay of Biscay (Eastern Atlantic). ICES Journal of Marine Science, 60: 1216–1231.

Rodríguez-Marín, E., Rodríguez-Cabello, C., de la Serna, J. M., Cort, J. L., Alot, E., Rey, J. C., Ortiz de Zárate, V., *et al.* 2005. A review of bluefin tuna juveniles tagging information and mortality estimation in waters around the Iberian Peninsula. Collective Volume of Scientific Papers ICCAT, 58: 1388–1402.

Rohling, E. J., and Cooke, S. 1999. Stable oxygen and carbon isotope ratios in foraminiferal carbonate. *In Modern Foraminifera*, pp. 239–258. Ed. by B. K. Sen Gupta. Kluwer Academic, Dordrecht, The Netherlands.

Rooker, J. R., Arrizabalaga, H., Fraile, I., Secor, D. H., Dettman, D. L., Abid, N., Addis, P., *et al.* 2014. Crossing the line: migratory and homing behaviours of Atlantic bluefin tuna. Marine Ecology Progress Series, 504: 265–276.

Rooker, J. R., Secor, D. H., De Metrio, G., Kaufman, A. J., Rios, A. B., and Ticina, V. 2008b. Evidence of trans-Atlantic movement and natal homing of bluefin tuna from stable isotopes in otoliths. Marine Ecology Progress Series, 368: 231–239.

Rooker, J. R., Secor, D. H., De Metrio, G., Schloesser, R., Block, B. A., and Neilson, J. D. 2008a. Natal homing and connectivity in Atlantic bluefin tuna populations. Science, 322: 742–744.

Rooker, J. R., Zdanowicz, V. S., and Secor, D. H. 2001. Chemistry of tuna otoliths: assessment of base composition and post-mortem handling effects. Marine Biology, 139: 35–43.

Santiago, J., and Arrizabalaga, H. 2012. Standardized bluefin tuna cpue indices of the Bay of Biscay baitboat fishery (1952–2011). ICCAT Standing Committee on Research and Statistics, SCRS/2012/100.

Schloesser, R. W., Neilson, J. D., Secor, D. H., and Rooker, J. R. 2010. Natal origin of Atlantic bluefin tuna (*Thunnus thynnus*) from the Gulf of St. Lawrence based on $\delta^{13}C$ and $\delta^{18}O$ in otoliths. Canadian Journal of Fisheries and Aquatic Sciences, 67: 563–569.

Schloesser, R. W., Rooker, J. R., Louchuoarn, P., Neilson, J. D., and Secor, D. H. 2009. Interdecadal variation in seawater $\delta^{13}C$ and $\delta^{18}O$ recorded in fish otoliths. Limnology and Oceanography, 54: 1665–1668.

Schwarcz, H. P., Gao, Y., Campana, S., Browne, D., Knyf, M., and Brand, U. 1998. Stable carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences, 55: 1798–1806.

Secor, D. H., Gahagan, B., and Rooker, J. R. 2012. Atlantic bluefin tuna stock mixing within the U.S. North Carolina recreational fishery, 2011–2012. Collective Volume of Scientific Papers ICCAT, 69: 947–954.

Secor, D. H., Henderson-Arzapalo, A., and Piccoli, P. M. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? Journal of Experimental Marine Biology and Ecology, 192: 15–33.

Shiao, J. C., Wang, S. W., Yokawa, K., Ichinokawa, M., Takeuchi, Y., Chen, Y. G., and Shen, C. C. 2010. Natal origin of Pacific bluefin tuna Thunnus orientalis inferred from otolith oxygen isotope composition. Marine Ecology Progress Series, 42: 207–219.

Shiao, J. C., Yui, T. F., Høie, H., Ninnemann, U., and Chang, S. K. 2009. Otolith O and C stable isotope compositions of southern bluefin tuna, *Thunnus maccoyii* (Pisces: Scombridae) as possible environmental and physiological indicatorss. Zoological Studies, 48: 71–82.

Sibert, J. R., Lutcavage, M. E., Nielsen, A., Brill, R. W., and Wilson, S. G. 2006. Interannual variation in large-scale movement of Atlantic bluefin tuna (*Thunnus thynnus*) determined from pop-up satellite archival tags. Canadian Journal of Fisheries and Aquatic Sciences, 63: 2154–2166.

Taylor, N. G., McAllister, M. K., Lawson, G. L., Carruthers, T., and Block, B. A. 2011. Atlantic bluefin tuna: a novel multistock spatial model for assessing population biomass. PLoS ONE, 6: e27693.

Thorrold, S. R., Campana, S. E., Jones, C. M., and Swart, P. K. 1997. Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. Geochimica et Cosmochimica Acta, 61: 2909–2919.

Vapnik, V. 1998. Statistical Learning Theory. WileyInterscience, New York.

Vargas-Yáñez, M., Salat, J., Fernández de Puelles, M. L., López-Jurado, J. L., Pascual, J., Ramírez, T., Cortés, D., *et al.* 2005. Trends and time variability in the northern continental shelf of the western Mediterranean. Journal of Geophysical Research, 110: C10019.

Verburg, P. 2007. The need to correct for the Suess effect in the application of $\delta^{13}C$ in sediment of autotrophic Lake Tanganyika, as a productivity proxy in the Anthropocene. Journal of Paleolimnology, 37: 591–602.

Wells, R. J. D., Rooker, J. R., and Itano, D. 2012. Nursery origin of yellowfin tuna in the Hawaiian Islands. Marine Ecology Progress Series, 461: 187–196.

Handling editor: Francis Juanes