Isotopic evidence of limited exchange between Mediterranean and eastern North Atlantic fin whales

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Rationale: The relationship between stocks of fin whales inhabiting the temperate eastern North Atlantic and the Mediterranean Sea is subject to controversy. The use of chemical markers facilitates an alternative insight into population structure and potential borders between stocks because the two areas present dissimilar isotopic baselines.

Methods: Baleen plates, composed of inert tissue that keeps a permanent chronological record of the isotopic value of body circulating fluids, were used to investigate connectivity and boundaries between the stocks. Values were determined by continuous flow isotope ratio mass spectrometry.

Results: Stable isotopes confirm that, while the two subpopulations generally forage in well-differentiated grounds, some individuals with characteristic Atlantic values do penetrate into the Mediterranean Sea up to the northernmost latitudes of the region. As a consequence, the border between the two putative subpopulations may be not as definite as previous acoustic investigations suggested. The discriminant function obtained in this study may assist researchers to use baleen plate isotopic data to assign the origin of fin whales of uncertain provenance.

Conclusions: This study strengthens the stock subdivision currently accepted for management and conservation while recognizes a low level of exchange between the Mediterranean and temperate eastern North Atlantic subdivisions.

Fin whales make wide-range movements and their migration typically spans thousands of kilometres, traits that would in principle hinder the occurrence of demographic subdivisions within a given ocean basin. However, in the North Atlantic Ocean fin whales have long been known to actually subdivide into subpopulation units, with little individual interchange, and which for management purposes are commonly denominated ‘stocks’. In 1966, Jonsgård[1] was the first to comprehensively investigate such structuring and, after considering the apparent differences in the trajectory of the catch per unit effort of various localities where the species had been exploited, the iodine values of the oil extracted from the captured individuals and their mean body size, he inferred the existence in the basin of at least six stocks. Later, evidence from further and more refined catch per unit effort series, differences in age at sexual maturation and the results of marking programs led the International Whaling Commission (1977)[2] to slightly modify Jonsgård’s proposal and to agree a structuring of the North Atlantic into seven management subdivisions.

One of these subdivisions embraced the temperate waters of the eastern North Atlantic comprised between the British Isles, France, the Iberian Peninsula and northern Africa up to the 19°N line. Although marking with internal tags was conducted off NW Spain in the past, all tags were recovered in the same whaling grounds and thus did not provide evidence for stock structure. As a consequence, the rationale for that subdivision relied on a parallel decline in catch per unit of effort observed by Jonsgård[1] between the British Isles and the Iberian Peninsula during the 1920s, a fact that was accepted as evidence of connectivity between these areas. At that time, and despite absence of any supporting evidence for connectivity, the fin whales inhabiting the Mediterranean Sea were straightforwardly incorporated into the ‘British Isles-France-Iberian Peninsula-northern Africa’ stock. However, this was modified when later research showed that adult fin whales and calves were present year round in the western Mediterranean[3] and that individuals from the Mediterranean Sea and the western North Atlantic differed both genetically[4,5] and in their organochlorine pollutant concentrations and profiles[6], intrinsic markers which are frequently used to differentiate populations. The Mediterranean Sea was thus considered a new subdivision, with the Gibraltar Straits as the border separating from the adjoining Atlantic stock. Despite the persistence of some uncertainties about the actual

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subdivisions, this demographic structure has been adopted as the basis for management and conservation by the International Whaling Commission, ACCOBAMS, and the International Union for the Conservation of Nature, among other organizations.

However, the genetic studies warned that the isolation between the Mediterranean Sea and Atlantic stocks was not complete and estimated the exchange between them at about two females per generation, without clarifying whether such exchange was unidirectional or bidirectional. This appeared to be confirmed by satellite tagging, because from eight whales successfully tagged in the north-western Mediterranean Sea one crossed the Straits of Gibraltar towards the Atlantic and moved up to central Portugal, and by acoustic recordings that showed that whales producing songs attributable to the northern North Atlantic subpopulation were detected in the south-western Mediterranean Sea. However, this last result does not demonstrate an actual exchange between Atlantic and Mediterranean stocks, but just that the border between them is not the Straits of Gibraltar. Actually, whales are observed every year crossing through the Straits of Gibraltar both directions and the prevalence of Atlantic immigrants into the south-western Mediterranean is also true for other groups of marine vertebrates.

Chemical markers may facilitate an alternative insight into population structure and potential borders between stocks. In particular, stable isotope values in tissues are known to reflect the environment in which individuals obtain their food. Here we use baleen plates, an inert tissue that keeps a permanent chronological record of the isotopic value of body circulating fluids, to investigate connectivity and boundaries between the stocks inhabiting the Mediterranean and the temperate waters of the eastern North Atlantic. The two areas present dissimilar isotopic baselines, the $\delta^{15}N$ and $\delta^{13}C$ values in the NW Iberian Peninsula being higher than in the Mediterranean Sea, thus providing the necessary differentiation. The results will not only be useful to gauge the degree of exchange between putative subpopulations the main focus of the present study, but could provide the basis for the assignment of origin to individual whales in the future.

**Experimental**

**Sample collection and analysis**

The stable isotope data used for this research originated from two different sources: (i) the information provided by Bentaleb et al. from ten baleen plates (BP1–BP10), corresponding to nine fin whales stranded in the Mediterranean Sea during the period 1975–2002 (seven from the northern-western basin and two from the south-western basin), and (ii) the analysis of further baleen plates from five individuals caught off north-western Spain (Canelíñas whaling station, identified as A–E, Fig. 1), during the whaling season of 1985 (July to October). Because stable isotope values are known to fluctuate along the longitudinal axis of the baleen plate following seasonal changes in area of residence and/or feeding regimes, in both cases each plate was subsampled at regular intervals along the longitudinal axis to incorporate into the analysis the expected variation occurring throughout the biological cycle.

For storage and analytical procedures (that were almost identical to those of the present study) in the first set of baleen plates, see Bentaleb et al. In the case of the second set, the baleen plates were stored dry until analysis. Before sampling, they were cleaned of surface oils and adhered material using a steel palette knife, steel wool and a chloriform/methanol solution (2:1), and subsequently subsampled with a micromilling device at 1 cm intervals, including the unerupted section beneath the gum. Forty-one subsamples were obtained from each baleen plate. Approximately 0.3 mg of powdered baleen plate was weighed into tin capsules (3.5 × 5 mm) and combusted at 1000 °C in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA; Thermo Finnigan, Bremen, Germany). The analyses were performed at the Centres Científics i Tecnològics of the University of Barcelona, Barcelona, Spain.

Stable isotope abundances, expressed in delta notation ($\delta$), where the relative variations of stable isotope values are expressed in ppm (‰) deviations from the predefined international standards, were calculated as:

$$\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where X is $^{13}C$ or $^{15}N$, and $R_{\text{sample}}$ and $R_{\text{standard}}$ are the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios in the sample and standard, respectively. The standards were Vienna Pee Dee Belemnite (V-PDB) calcium carbonate for carbon and atmospheric nitrogen (air) for nitrogen. International isotope secondary standards of known $\delta^{13}C$ values from the International Atomic Energy Agency (IAEA, Vienna, Austria), namely polyethylene (IAEA CH$_2$, $\delta^{13}C = -31.8{‰}$), graphite (USGS24, $\delta^{13}C = -16.1{‰}$) and sucrose (IAEA CH$_{12}$, $\delta^{13}C = -10.4{‰}$), were used for calibration at a precision of 0.2‰. For nitrogen, international isotope secondary standards of known $\delta^{15}N$ values, namely (NH$_4$)$_2$SO$_4$ (IAEA N1, $\delta^{15}N = +0.4{‰}$) and IAEA N2, $\delta^{15}N = +20.3{‰}$) and KNO$_3$ (IAEA NO$_3$, $\delta^{15}N = +4.7{‰}$), were used for calibration at a precision of 0.3‰.

![Figure 1. Sampling locations and important areas in the Mediterranean Sea and Atlantic Ocean (triangle = this study; black dots = Bentaleb et al.).](image-url)
Statistical analysis

Initial statistical screening showed that variances of the $\delta^{13}C$ and $\delta^{15}N$ values between individual baleen plates were not homogenous according to the Levene test. Moreover, in the Mediterranean individuals, the number of subsamples analyzed was highly dissimilar between individuals. As a consequence, comparison using the full dataset of samples and including the ‘individual’ factor as a variable to control for pseudoreplication was not possible. Therefore, the comparison between whales from the different areas was based on the mean of all $\delta^{13}C$ and $\delta^{15}N$ values from each specimen, a procedure that was moreover expected to smooth the seasonal variability likely to exist within the plate. In doing so, the mean values from each group of whales were normally distributed according to the Lilliefors test and were homoscedastic according to the Levene test. A t test was used to compare the two datasets of baleen mean values.

A visual inspection of the raw baleen plate isotope data suggested that Mediterranean and Atlantic individuals separated into two independent clusters, with the only exception of individual BP1 from Bentaleb et al.\cite{125} occupying an intermediate position (Fig. 2). Linear discriminant analysis was used to investigate statistical assignment of this controversial individual to one origin group. The comparison between the isotopic niches of the two putative subpopulations was carried out by standard ellipses analysis (SEAc) performed using SIBER (Stable Isotope Bayesian Ellipses in R\cite{26}) as a measure of the mean core population isotopic niche. The standard ellipse of a set of bivariate data was calculated from the variance and covariance of the data and contained approximately 40% of the data. SEAc was considered to be appropriate when analyzing niche widths between groups because it is unbiased with respect to sample size, thus allowing robust comparison among datasets of different sample size.\cite{26} Differences in isotopic niche space between two different groups may indicate differences in the type of prey eaten or differences in the area where they forage. Convex Hull Area method\cite{26} (polygon around the most extreme data points on the isotope bi-plot) was also used to consider the entire variability in the stable isotope values. SEAc and Convex Hull Area method allowed us to assess the degree of overlap between datasets. In these analyses, the input data were the isotopic values of all baleen plate subsamples instead of the mean values from the baleen plates. SEAc and Convex Hull area were performed using the R statistical computing package. The statistical program SPSS 15.0 was used for the other tests.

RESULTS

The stable isotope values fluctuated along the longitudinal axis of baleen plates. However, the pattern of variation was highly variable among individuals, both for the $\delta^{13}C$ and $\delta^{15}N$ values. Comparison of mean values between the two datasets revealed that whales from the Atlantic were enriched in both $^{15}N$ and $^{13}C$ as compared with those from the Mediterranean ($t = 4.49$, $df = 13$, $p < 0.001$ for $\delta^{13}C$ and $t = 6.68$, $df = 13$, $p < 0.001$ for $\delta^{15}N$; $n_{\text{Mediterranean}} = 5$, $n_{\text{Atlantic}} = 10$ for both stable isotope ratios). Furthermore, the magnitude of the differences between the two datasets matched the magnitude of the differences between Atlantic\cite{24} and Mediterranean\cite{25} krill (Meganyctiphanes norvegica) (Fig. 3), thus confirming that they were not caused by dissimilarities in laboratory procedures.

However, a closer look of the data biplot (Fig. 2(a), Table 1) revealed a 7.77% overlap between the convex hull area of the two datasets, but not in the standard ellipses area. Interestingly, all the Mediterranean samples overlapping with the Atlantic dataset came from a single individual (BP1) that stranded at the core of the north-western Mediterranean foraging area (Fig. 1). The discriminant analysis successfully classified all the samples as Atlantic or Mediterranean, except those from whale BP1. The resulting discriminant function was $X = -3.74 + (1.846^{15}N + 0.78^{13}C)$ and the statistics that this function produced for the eastern North Atlantic
population: centroid (mean): 2.41; standard deviation: 0.99; ranges: -0.38 to 4.26, and for the Mediterranean population: centroid (mean): -2.92; standard deviation: 1.00; ranges: -5.03 to -0.66. Accordingly, when the analysis was repeated with whale BP1 excluded, the Atlantic and Mediterranean whales did not exhibit any overlap in their stable isotopic values either in the convex hull or in the standard ellipses area (Fig. 2(b)).

**DISCUSSION**

The present study clearly shows that isotopic values, both for $\delta^{15}$N and $\delta^{13}$C, were higher in the Atlantic individuals than in those from the Mediterranean Sea. In the case of $\delta^{15}$N this could be explained by the two subpopulations feeding at different trophic levels, because the nitrogen stable isotope increases at each trophic level.\[^{[19]}\] This effect should be discarded because the main prey of fin whales in both areas is almost exclusively *Meganyctiphanes norvegica* (for the Atlantic Ocean\[^{[1,24,27]}\]; for the Mediterranean Sea\[^{[3,23,28]}\]). Therefore, this difference can be more reasonably attributed to different isotopic baselines between the two areas. Graham et al.\[^{[29]}\] produced marine carbon and nitrogen isoscapes for the Atlantic Ocean based on a meta-analysis of published plankton $\delta^{13}$C and $\delta^{15}$N values and, according to their maps, both $\delta^{15}$N and $\delta^{13}$C are higher in the northeast Atlantic than in the Mediterranean Sea, thus matching the differences found here. This scenario is further supported by the isotopic values in other taxa such as squids\[^{[29]}\] or determined directly in samples of *M. norvegica* from the two areas (for the Atlantic Ocean\[^{[24]}\] and for the Mediterranean Sea\[^{[25]}\]) which, after having been corrected for the baleen plate enrichment factor determined by Borrell et al.\[^{[22]}\] also matched the results here obtained (Fig. 3). Nevertheless, differences between the Atlantic and the Mediterranean are larger and more consistent for $\delta^{15}$N than for $\delta^{13}$C values, both for *M. norvegica*\[^{[12,24,25]}\] and whales (this study).

The only exception to this general rule was one whale analyzed by Bentaleb et al.\[^{[12]}\] that stranded in the Mediterranean (BP1) and whose isotopic values matched better the Atlantic than the Mediterranean baselines. Because this whale had been sampled about 15 years before the other Mediterranean sampled individuals, Bentaleb et al.\[^{[12]}\]...
proposed that these higher δ¹⁵N values might reflect a
temporal change in the Mediterranean δ¹⁵N baseline.
However, in our statistical analyses the discriminant function
assigned this controversial individual with reliability to the
Atlantic subpopulation. Moreover, the convex hull and the
standard ellipses analyses performed to define trophic niche
similarly matched it to the Atlantic subpopulation (Fig. 2).
Further, if this particular individual is examined in more
detail it can be seen that the most recently formed part of the
baleen plate had the most depleted value of ¹⁵N in the
whole plate and, indeed, that value fell within the typical
values found in the Mediterranean individuals. This
suggested that the newly formed layers in the baleen plate
were in the process of acquiring the Mediterranean signature,
indicating a recent transit into Mediterranean waters. As a
consequence, all evidence indicates that BP1 was indeed an
Atlantic individual that migrated into the Mediterranean
Sea and stranded soon after on the coast of France.

Two individuals (BP8 and BP10) which stranded on the
coast of Málaga, i.e. in the south-western Mediterranean,
matched the isotopic values of Mediterranean whales
although the values of one of them (BP10) were close to the
Atlantic dataset. It has been proposed that fin whales visiting
this area would indeed be part of the Atlantic subpopulation
because the acoustic parameters detected by archival bottom-
mounted audio recorders matched those attributable to the
north-eastern North Atlantic subpopulation,[13] so if these
two individuals actually originated from the Atlantic stock
they would have had to be foraging in the Mediterranean
Sea for at least 2 years, the approximate period recorded in
the baleen plates analysed.

Thus, the information previously available and the results
here presented all concur to confirm the existence of two
differentiated subpopulations occurring in the temperate
eastern North Atlantic Ocean and the Mediterranean Sea, although
the border between them may be not as definite as the
acoustic investigations suggested.[13] The collective evidence of at least some individuals with stable isotopic
values characteristic of the Atlantic found in the
northernmost western Mediterranean Sea, the results from
genetic studies pointing to the existence of some limited
exchange,[6] and the movement of one individual tagged with
a satellite mark in the Mediterranean that crossed the Straits
of Gibraltar and reached the Atlantic coast of central
Portugal,[11,12] all confirm the occurrence of recurrent
exchange between the subpopulations inhabiting these two
water masses.

The actual distribution ranges are in both cases difficult to
determine. The Mediterranean subpopulation is known to
mainly feed in the Ligurian Sea and in the northern zone of
the Balearic Islands during the summer but afterwards it is
thought that it would disperse around the southern
Mediterranean with individuals conducting during the
winter sporadic incursions to de Gulf of Cadiz, the central
coast of Portugal or even further north. The ranges of the
temperate eastern North Atlantic subpopulation are more
uncertain, particularly because the very own identity and
composition of the subpopulation is unclear. The region
witnessed a severe whaling period during 1921–1927 that
resulted during the few years of exploitation in the catch of
over 6000 individuals.[30] The Gulf of Cadiz, where
observational records from nineteenth century open-boat
whalers show that the species was originally very
abundant,[31] sustained the bulk of the catch. Afterwards, fin
whales almost vanished from the area; subsequent whaling operations there focused on the sperm whale, with only
marginal catches of fin whales and, even today, after seven
decades have passed from the last removals, the density
appears to be extremely low.[14,15] However, further north,
on the coast of Galicia (NW Spain), whaling was conducted
on a clearly more robust subpopulation until 1985, when a
global moratorium on commercial whaling was established
by the International Whaling Commission.[30] The distinct
trajectory of the subpopulations occupying the two temperate
eastern North Atlantic areas has been taken as suggestive of
them being independent stocks, with the one occupying the
Gulf of Cadiz having been wiped out by the massive
removals made in the 1920s.[32] The few individuals that are
nowadays seen in the area or crossing the Gibraltar Straits
are thought to be either the negligible remains of the original
local subpopulation of the Gulf of Cadiz or stragglers from
other neighbouring subpopulations.

Results from this study have proved the previously
accepted separation between Atlantic and Mediterranean fin
whale subpopulations, but also confirmed that some Atlantic
individuals wander into the Mediterranean Sea and that
therefore the subpopulation borders are not as strict as
previously thought.[13] This is of relevance to the conservation
and management of the species. The discriminant function
obtained in this study may assist researchers to use baleen
plate isotopic data to assign the origin of fin whales of
uncertain provenance. Further research is needed to more
precisely determine the degree of connectivity between
the subpopulations and the actual location of their boundaries.
Stable isotope studies conducted in combination with
genetics, particularly on fin whales sampled at the Gibraltar
Straits and neighbouring areas from where potential
stragglers may stem from, may assist in establishing
demographic structure, as has been the case with other
balaenopterid species.[33]

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