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Stable isotopes suggest fine-scale sexual segregation in an isolated, endangered sperm whale population

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Running page head: Fine-scale sexual segregation in sperm whales.

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Abstract

Sexual segregation is common among marine mammals, leading to intraspecific differences in diet, diving behaviour, home range size, and even latitudinal distribution and migratory patterns. Sperm whales (*Physeter macrocephalus*) present one of the most extreme examples of sexual dimorphism both in size and social structure, with males and females segregating at different latitudes across most of their range, but the underlying ecological drivers remain unclear. Studying fine-scale dietary and habitat differences where the sexes occur in sympatry could therefore provide insights into the mechanisms underpinning their large-scale segregation. In this study, we analysed the carbon and nitrogen stable isotope values in the skin of males and females from an isolated, endangered population inhabiting the Mediterranean Sea, sampled in a region where the sexes occur and feed regularly in the summer months but show subtle differences in habitat preference. We found marked differences in both carbon and nitrogen isotopic values between the sexes, indicating that they could be targeting prey items in different trophic levels and habitats. Combined with the evidence from habitat modelling studies, our results suggest that female and male sperm whales segregate even in the latitudinally restricted Mediterranean population, at a much smaller scale. This sympatric, fine-scale sexual segregation suggests that reduction of competition may have been a key factor in the evolution of the species' social structure and large-scale latitudinal segregation.

Keywords: dietary differences, sexual segregation, dimorphism, sperm whale, Mediterranean Sea.

1. INTRODUCTION

Sexual segregation is a widespread phenomenon in animal ecology (Ruckstuhl 2007, Main 2008), with important implications for both the theoretical understanding of population and ecosystem dynamics, and the design of effective conservation efforts (Wearmouth & Sims 2008). Among marine mammals, key top-down drivers of marine community structure (Bowen 1997, Williams et al. 2004, Baum & Worm 2009), social and habitat segregation can result in sex differences in diet, diving behaviour, home range size, and even latitudinal distribution and migratory patterns (Wearmouth & Sims 2008). Such variation has been described in many pinnipeds (e.g., Lesage et al. 2001, Breed et al. 2006, Tucker et al. 2007, Bailleul et al. 2010, Kernaléguen et al. 2016) and some cetaceans (e.g., Marcoux et al. 2012, Gavrilchuk et al. 2014), particularly in species that show a high degree of body size dimorphism. Reproductive status, energetic requirements and intersexual competition have been proposed as potential explanations for these differences (Wearmouth & Sims 2008), but the proximate or ultimate mechanisms driving the evolution of sexual segregation are still debated (Ruckstuhl 2007).

The sperm whale (*Physeter macrocephalus*) is highly sexually dimorphic, with males being substantially larger than females. These morphological differences are thought to be largely due to sexual selection (Cranford 1999, Whitehead 2003). In most studied populations, social structure and broad-scale habitat use is also strikingly different between the sexes: females and young form long-lasting social units at latitudes approximately $<40^{\circ}$, while males become increasingly solitary with age and simultaneously move to increasingly higher latitudes (Whitehead 2003). The species is characterised by a diet mainly composed of mesopelagic cephalopods (Kawakami 1980, Clarke et al. 1993, Santos et al. 1999, Roberts 2003, Foskolos et al. 2020).

Analyses of stomach contents from stranded individuals have suggested some differences in diet between the sexes, with males targeting larger and deeper prey items and consuming additional non-cephalopod prey at high latitudes (e.g., Clarke et al. 1993, Best 1999, Flinn et al. 2002, Evans & Hindell 2004, Teloni et al. 2008). In addition to the latitudinal separation when not breeding, males and females also appear to show subtly different habitat preference in regions and periods where they co-occur (e.g., Best 1999, Gregr & Trites 2011, Pirotta et al. 2011, 2020). Females tend to have higher feeding success in these areas, leading to the hypothesis that they outcompete males for the highest quality habitat (Clarke et al. 1988, Best 1999, Whitehead 2003). Together with reproductive requirements (e.g., the need to care for young individuals), these dietary and habitat differences could thus provide insights into the mechanisms underlying the evolution of sperm whale social structure, size dimorphism and ecology.

Stable isotope analysis is a powerful tool to investigate variation in diet among individuals or groups of individuals (Post 2002), but limited by a low taxonomic resolution (Nielsen et al. 2018). Using this technique on sperm whale skin samples, Ruiz-Cooley et al. (2004) detected dietary differences between males and females in the Gulf of California. However, given isotope turnover rates, the authors suggested these differences could reflect the diet of adult males in high-latitude feeding grounds or any period of fasting during their migration to lower latitudes, i.e. their large-scale latitudinal segregation, rather than sympatric differences in the area where they co-occurred with social units and were sampled. Such suggestions are consistent with the ontogenetic changes found in other studies (Mendes et al. 2007b). Similarly, Borrell et al. (2013) found sexual differences in isotopic values in sperm whale dentine samples from the Eastern North Atlantic, which they attributed to large-scale segregation and population structuring.

1 Therefore, it remains to be confirmed whether males and females vary in their diet when they co-
2 occur at lower latitudes.

3 A small population of sperm whales inhabits the Mediterranean Sea and is classified as
4 ‘Endangered’ in the IUCN Red List (Rendell & Frantzis 2016). Genetic and photo-identification
5 studies have shown that the population is isolated from the North-east Atlantic (Drouot et al.
6 2004a, Engelhaupt et al. 2009, Carpinelli et al. 2014, Alexander et al. 2016). Relative to sperm
7 whales in other oceans, males in this population are thought to be generally smaller (Drouot et al.
8 2004b, Frantzis et al. 2014), possibly implying smaller differences in energy requirements and
9 diving capabilities compared to females. Analysis of stomach contents of stranded Mediterranean
10 individuals suggested a diet dominated by cephalopods of the genera *Histioteuthis* and
11 *Octopoteuthis* (Roberts 2003, Foskolos et al. 2020).

12 Within this enclosed basin, the potential for the large-scale latitudinal segregation between sexes
13 observed in other oceans is much reduced due to geographical constraints. Information on
14 movements within the Mediterranean is limited, but photo-identification studies in the western
15 basin have demonstrated that individuals can move between the area of the Ligurian Sea and
16 Gulf of Lions, the Strait of Gibraltar, and the waters around the Balearic archipelago (Spain),
17 covering distances up to ~1,600 km (Drouot-Dulau & Gannier 2007, Carpinelli et al. 2014,
18 Rendell et al. 2014). The Balearic archipelago is an area where both female groups and singleton
19 males are regularly encountered during summer monitoring surveys (Pirota et al. 2011, 2020,
20 Rendell et al. 2014). Both sexes are observed consistently engaging in long dives while actively
21 searching for prey (as suggested by their echolocation activity), indicating they are feeding in
22 this area in the summer months. At least in this season, the sexes thus appear to be sympatric at
23 the regional scale, but there is evidence of varying habitat preferences on finer spatial scales of

1 approximately 10 km (Pirotta et al. 2011, 2020, Jones et al. 2016). Specifically, groups are
2 encountered in deeper, warmer waters characterised by lower sea level anomaly compared to
3 singletons, suggesting that the sexes may be segregating at these smaller scales (Pirotta et al.
4 2020). Therefore, the analysis of stable isotopes in this area could help our understanding of fine-
5 scale sexual segregation in the species.

6 While previous studies have measured stable isotope values in samples from Mediterranean
7 sperm whales (Mazzariol et al. 2011, Praca et al. 2011, Pinzone et al. 2015), none have explicitly
8 assessed isotopic differences between sexes. Here, we analysed the stable isotope values in the
9 skin of male and female sperm whales sampled around the Balearic Islands, with the aim of
10 exploring intraspecific differences in diet in a season where the sexes are encountered within the
11 same region. These differences could indicate the occurrence of sexual segregation in a low-
12 latitude area where the sexes are broadly sympatric. Dietary differences between males and
13 females could reflect a differential use of the water column or of portions of the distribution
14 range. In turn, this would affect their exposure to the human activities that threaten the
15 population's survival (Rendell & Frantzis 2016), thus highlighting the potential need for sex-
16 specific conservation strategies.

18 **2. MATERIALS & METHODS**

19 *2.1 Data collection*

20 Between May and September 2019, sperm whales were approached to collect biopsy samples
21 while at the surface between foraging dives using a 12-m auxiliary powered sailing yacht. We
22 distinguished between encounters with single individuals and encounters with groups, defined as

1 individuals (often including young animals) engaging in direct interaction or ‘moving together in
2 a coordinated fashion’ (Whitehead, 2003).

3 Biopsies were performed using a 150 lb pull crossbow (manufactured by Barnett International)
4 and arrows with stainless steel sampling heads of 0.5 cm diameter and 2.5 cm length (Ceta-Dart,
5 Denmark). Arrows were shot from a distance <15 m, aiming for the mid-lateral region of the
6 body, below the dorsal fin.

7 We successfully collected biopsy samples from 20 individual sperm whales (Fig. 1A). Most
8 individuals (90%) showed some short-term behavioural reaction to sampling, including startle,
9 defecation, shallow diving, and horizontal avoidance. All sampled individuals were subsequently
10 identified using photographs of their dorsal and caudal fin to avoid repeated sampling.

11 After retrieving the arrow, the sample was removed from the sampling head and divided in two
12 parts longitudinally. One half was directly frozen onboard in an electric freezer (for stable
13 isotope analysis), while the other half was placed in ethanol (for genetic sex determination). The
14 sampling heads were then sterilized using bleach and rinsed with 90% alcohol for subsequent
15 reuse.

16 *2.2 Laboratory analyses*

17 All stable isotope analyses were undertaken at the Centres Científics i Tecnològics of the
18 University of Barcelona (CCiT-UB). Subsamples of skin were defrosted, minced with scalpel and
19 scissors, and dried at 60 °C for 48 h. Dried samples were treated with a chloroform-methanol (2:1)
20 solution to extract lipids (Bligh & Dyer 1959). Lipid extraction can alter $\delta^{15}\text{N}$ values (Logan &
21 Lutcavage 2008, Smith et al. 2020), but was required to prevent the confounding effect of lipids
22 on isotopic values, in the absence of tissue- and species-specific lipid-normalization models

(Lesage et al. 2010). Given that the aim of the study was to assess differences between the sexes, this technique was deemed appropriate, since the consequences of lipid extraction are primarily relevant when comparing isotopic values with putative prey or samples from other laboratories. Subsequently, samples were dried at 60 °C for 48 h. The lipid content was determined as the difference in sample weight before and after the lipid-extraction treatment. Approximately 0.3 mg of each sample was weighed in tin capsules, automatically loaded, and combusted at 1,000 °C for analysis in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA Thermo Finnigan, Bremen, Germany). Standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were the Vienna Pee Dee Belemnite limestone (V-PDB) and the atmospheric nitrogen, respectively. International isotope secondary standards of known $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios in relation to V-PDB and atmospheric nitrogen, respectively, were used for calibration of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, namely: polyethylene (IAEA CH7; $\delta^{13}\text{C} = -31.8\text{‰}$), ammonium sulphate (IAEA N1; $\delta^{15}\text{N} = +0.4\text{‰}$ and IAEA N2; $\delta^{15}\text{N} = +20.3\text{‰}$), L-glutamic acid (USGS 40; $\delta^{13}\text{C} = -26.2\text{‰}$; $\delta^{15}\text{N} = -4.6\text{‰}$;) and caffeine (IAEA 600; $\delta^{15}\text{N} = 1,0\text{‰}$; $\delta^{13}\text{C} = -27,7\text{‰}$). The reference materials used are distributed by the International Atomic Energy Agency (IAEA) and were selected based on previous calibration experiments to ensure an optimum range of reference values. Calibration precision based on replicate measurements of the internal laboratory standards was $0.23 \pm 0.15 \text{‰}$ for $\delta^{13}\text{C}$ and $0.19 \pm 0.11 \text{‰}$ for $\delta^{15}\text{N}$. Results were expressed as per mil (‰) following the delta (δ) notation, according to the equation: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} (\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \cdot 10^3$, where R is the heavy-to-light isotope ratio ($^{15}\text{N}/^{14}\text{N}$; $^{13}\text{C}/^{12}\text{C}$).

The determination of the sex of sampled individuals was conducted at the DNA/Molecular Genetics laboratory in the School of Biological, Earth and Environmental Sciences of University College Cork. Genomic DNA was extracted from each ethanol-preserved skin subsample using

the Qiagen DNeasy® Blood and Tissue kit. Sex determination was carried out following Rosel (2003).

2.3 Statistical analysis

Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between males and females were tested using Gaussian linear models; assumptions of residual normality and homogeneity of variance across sexes were assessed using Shapiro-Wilks and Levene's tests, respectively. Some individuals were sampled as part of the same group, which could imply that their dietary choices were not independent. Therefore, we also refitted the models using a working independence correlation structure in a Generalised Estimating Equations (GEE) framework (Hardin & Hilbe 2003), where group identity was used as the blocking factor. A sandwich variance estimator provided robust estimates of precision accounting for the observed degree of autocorrelation within each block (Hardin & Hilbe 2003), which were used in a Wald's test to assess whether differences between the sexes were significant.

Frequentist standard ellipses (containing approximately 40% of the data) corrected for small sample sizes (SEA_C) were generated using package SIBER (Jackson et al. 2011) for R v3.6.0 (R Core Team 2019). The robust Bayesian framework implemented in SIBER was also used to estimate Bayesian standard ellipses (SEA_B) and compare isotopic niche widths for the two sexes.

An uninformative inverse Wishart prior with 2 degrees of freedom and scale matrix $\begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$ was used for the covariance matrix, while uninformative Gaussian priors with mean = 0 and precision = 0.001 were used for the means (package defaults). Markov chain Monte Carlo algorithms were run for 10^6 iterations (discarding the first 1,000 iterations as burn-in).

3. RESULTS

Genetic sexing indicated that seven sampled individuals were females, while the remaining thirteen were males. Among sampled males, seven were recorded as singletons. Additionally, three males were sampled from a group of seven, even though they were not visibly smaller than other sampled males. The remaining three males were sampled while grouped together (without other individuals), and included a smaller, probably younger individual observed closely interacting with the boat and engaging in aerial displays. All the sampled females were part of four separate groups.

Male $\delta^{15}\text{N}$ values ranged between 11.4 and 13.5 ‰ (mean $\pm$ SD: 12.4 ± 0.6), while female $\delta^{15}\text{N}$ values ranged between 10.8 and 11.6 ‰ (mean $\pm$ SD: 11.2 ± 0.3); $\delta^{13}\text{C}$ values ranged between -18.4 and -17.6 ‰ for males (mean $\pm$ SD: -18.0 ± 0.2), and between -18.6 and -18.2 ‰ for females (mean $\pm$ SD: -18.4 ± 0.1). The C:N ratio in the lipid-extracted skin samples fell between 3.1 and 3.6%, confirming that the lipid extraction process was effective and did not affect $\delta^{13}\text{C}$ values (Ryan et al. 2012, Giménez et al. 2017).

SEA_C for males and females did not overlap (Fig. 1B). Using 10,000 posterior draws of SEA_B, a very small overlap was confirmed (mean = 3.6%; median = 0%). Stable isotope values for one sampled male differed markedly from other male $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, falling within the female convex hull; this was the smaller individual sampled in a group of three. The Gaussian models highlighted that males had significantly higher $\delta^{15}\text{N}$ (coefficient estimate = 1.22, standard error = 0.23, t -value = 5.23, $p < 0.001$) and $\delta^{13}\text{C}$ (coefficient estimate = 0.45, standard error = 0.09, t -value = 5.00, $p < 0.001$); the tests on model residuals suggested that the assumptions of normality and homogeneity of variance between groups were met. The GEE analysis suggested

1 that these differences remained significant even after accounting for any correlation within social
2 groups ($\delta^{15}\text{N}$: $\chi^2 = 49.3$, $p < 0.001$; $\delta^{13}\text{C}$: $\chi^2 = 27.5$, $p < 0.001$).

3 Males' isotopic niche width had a 98% probability of being larger than females' (ratio of the
4 posterior modes = 3.1; Fig. 2). However, the probability of a sex difference in niche width
5 dropped substantially when excluding the smaller male with female-like isotope values (mode
6 ratio = 1.6; 79% probability of males' niche width being larger than females'; Fig. 2).

8 **4. DISCUSSION**

9 We detected clear differences in the $\delta^{15}\text{N}$ and, to a smaller extent, $\delta^{13}\text{C}$ values of male and
10 female sperm whales sampled around the Balearic archipelago (Spain), where both sexes are
11 observed to feed regularly in the summer months. Isotopic differences between the sexes in other
12 ocean basins have been attributed to large-scale latitudinal segregation (Ruiz-Cooley et al. 2004,
13 Borrell et al. 2013). Males in the Mediterranean cannot undergo such an extended latitudinal
14 migration, but photo-identification evidence suggests they do move between the Balearics, the
15 north-western basin, and the Strait of Gibraltar (Drouot-Dulau & Gannier 2007, Carpinelli et al.
16 2014). Females could also move (Rendell et al. 2014), although social groups are believed to be
17 more philopatric (Whitehead 2003). If individuals experience different isotopic baselines during
18 movements within the western basin, this could be reflected in their isotopic values (Graham et
19 al. 2010). However, given the short isotope turnover rates in odontocete skin (less than 1-1.5
20 month; e.g., Browning et al. 2014, Giménez et al. 2016), the fact that samples were collected
21 over a period of four months, and the within-sex consistency in our sample, stable isotope values
22 are likely indicative of differences in the prey targeted by males and females when they are

1 occurring around the Balearic archipelago. Despite the preliminary nature of our study, these
2 results could therefore support the existence of marked, intraspecific dietary differences in sperm
3 whales belonging to this isolated, endangered population.

4 Higher $\delta^{15}\text{N}$ in males could indicate that they were feeding either on prey species at a higher
5 trophic level, or on larger individuals of the same species (Hooker et al. 2001, Ruiz-Cooley et al.
6 2004, Newsome et al. 2010), in line with the limited available evidence from stomach content
7 analysis in the Mediterranean (I. Foskolos, personal communication) and other areas (Best 1999,
8 Evans & Hindell 2004), and from stable isotopes in teeth (Mendes et al. 2007a, Borrell et al.
9 2013). The difference in $\delta^{13}\text{C}$ was small compared to the corresponding analytical precision
10 ($0.23 \pm 0.15 \text{ ‰}$). However, as supported by the results of the statistical analysis and the separate
11 clustering of male and female samples along this axis (Fig. 1B), the variability within each group
12 was smaller than that between groups, suggesting that differences in $\delta^{13}\text{C}$ could be ecologically
13 relevant. If confirmed, this could indicate that the sexes were occupying habitats that differed at
14 a fine scale, in accordance with visual and acoustic surveys in this and other low-latitude areas
15 indicating that females are generally observed further offshore or in waters with distinct
16 oceanographic characteristics (Best 1999, Gregr & Trites 2011, Pirotta et al. 2020).

17 A qualitative comparison with other published sperm whale isotopic values in the Mediterranean
18 (Mazzariol et al. 2011, Praca et al. 2011, Pinzone et al. 2015) suggests some variation among
19 studies (Fig. 3). Female sperm whales in our study also appear to have lower $\delta^{13}\text{C}$ than other
20 Mediterranean deep-diving odontocetes, but comparable $\delta^{15}\text{N}$, whereas males are characterised
21 by higher $\delta^{15}\text{N}$ than long-finned pilot whales *Globicephala melas* and lower $\delta^{13}\text{C}$ than Risso's
22 dolphins *Grampus griseus* (Fig. 3) (Praca et al. 2011, Pinzone et al. 2015). Given the expected
23 diet to skin discrimination factor in odontocetes (estimated to be approximately 1.5 ‰ for $\delta^{15}\text{N}$,

1 and 1 ‰ for $\delta^{13}\text{C}$ in bottlenose dolphins *Tursiops truncatus*; Browning et al. 2014, Giménez et
2 al. 2016) and reported values in putative prey samples (Praca et al. 2011, Fanelli et al. 2013,
3 Navarro et al. 2013, Valls et al. 2017), our stable isotope results are broadly compatible with a
4 diet of Histioteuthidae (Fig. 3), as indicated by previous stable isotope (Praca et al. 2011) and
5 stomach content analysis (Roberts 2003, Garibaldi & Podestà 2014, Foskolos et al. 2020). The
6 comparison of a small number of stomachs from stranded males and females in the Eastern basin
7 suggested that both sexes were mainly targeting the cephalopod *Histioteuthis bonnellii*, but
8 female diet was characterised by a higher proportion of the smaller congener *H. reversa*, which
9 was also more prevalent in the diet of calves (I. Foskolos, personal communication). These
10 observations are in agreement with the isotopic differences between the sexes we detected.
11 However, uncertainty remains on the species or sizes targeted by male and female sperm whales
12 in the Mediterranean and on the ecology of their prey. Further stomach content data and putative
13 prey sampling are required to elucidate the diet of the two sexes in this region.

14 A series of mechanisms could act, either alone or in concert, to produce the observed sex
15 differences in diet and the resulting fine-scale habitat segregation (Ruckstuhl 2007, Wearmouth
16 & Sims 2008). Males, being larger, could have different energy requirements and diving
17 abilities, which would allow them to target larger, deeper prey. Social affinities (that is, the
18 tendency to associate with members of the same sex) are also possible in this highly social
19 species (Whitehead 2003, Wearmouth & Sims 2008). Alternatively, social units might be
20 responding to reproductive requirements, such as the need to care for young individuals with
21 limited diving abilities, or to feed more intensely to cover the costs of gestation and lactation. As
22 a result, females might also be more efficient at extracting resources, outcompeting males by
23 scramble competition and forcing them to target different prey (Whitehead 2003, Breed et al.

2006). Sexual segregation would thus reduce intersexual competition when sexes occur in the same region, while allowing males to access the energy they need to sustain their size (Beck et al. 2007).

Determining whether observed differences are ancestral in the species or the result of adaptation in this isolated population would require further investigating dietary patterns outside the Mediterranean and a better understanding of the population's phylogenetic history (Engelhaupt et al. 2009). It has been long recognised that male and female sperm whales from populations inhabiting other ocean basins are sexually segregated at a large, latitudinal scale (Whitehead 2003). The evidence from our study, combined with existing habitat modelling results (Pirota et al. 2011, 2020), suggests that dietary differences occur even in this latitudinally restricted population and that the sexes could also segregate at a much smaller scale. Segregation between the sexes could act as a mechanism underpinning intraspecific resource partitioning in sperm whales, which is increasingly recognised as an important ecological and evolutionary process (e.g., Field et al. 2005, Svanbäck & Bolnick 2007).

Our results contribute to shed further light on the poorly understood ecology of the Mediterranean sperm whale population. Dietary differences may suggest that the broad sympatry between the sexes in the basin, possibly resulting from the latitudinal restrictions to male migration, could increase intraspecific competition for resources (Rendell & Frantzis 2016). Moreover, male and female feeding ecology might influence their movements at multiple scales (e.g. between and within portions of the Mediterranean, and over the seasons), which could help clarify why social units are mostly encountered in specific areas, and specific habitats within those areas (Frantzis et al. 2014, Pace et al. 2018, Pirota et al. 2020). Finally, understanding the species' ecology will help explore some of the differences in social behaviour observed in the

basin (e.g. the smaller size of social units compared to oceanic populations; Rendell & Frantzis 2016).

Dietary analysis should be repeated to expand the small sample size and investigate whether detected differences are consistent between seasons and years (Guerra et al. 2020). Comparison of male and female diving behaviour (particularly in terms of targeted depth layer) and feeding success would also contribute to further clarify the mechanisms underlying these sexual differences, although this analysis would have to account for any diurnal vertical migration of the prey. The GEE analysis suggested that any lack of independence among samples from the same group did not affect the significance of the effect of sex, but more samples could be used to assess group-specific dietary preferences (Marcoux et al. 2007). The sampling of several males in clusters, which were considered group encounters in the field, suggests that the social structure of this population, and the resulting isotopic differences, might vary significantly from those in other oceans, where considerable variation has already been documented (Whitehead et al. 2012). Further confirmatory sampling would also help quantify any geographic variation in diet within the sexes (for example, see potential differences among females sampled at different latitudes in Fig. 1) or within individuals (Guerra et al. 2020), as well as sex differences in niche width, possibly related to differences in size and metabolism, termed the forage selection hypothesis (Ruckstuhl 2007, Main 2008). Finally, we observed that one small (possibly younger) male showed similar isotopic values to females: this is consistent with the notion of ontogenetic shifts in sperm whale diet suggested by analysis of stable isotopes in teeth (Mendes et al. 2007b, Borrell et al. 2013), and with previous findings of similarities in the diet of females and immatures (e.g., Ruiz-Cooley et al. 2004), but again more independent samples are needed for confirmation.

1 Aside from fundamental insights into the ecology of this isolated population and the evolutionary
2 forces that underpin the species' complex social structure, understanding patterns of sexual
3 segregation will be important for quantifying sex-specific rates of interactions with human
4 activities (Wearmouth & Sims 2008). Collisions with vessels, entanglement in fishing gear, and
5 exposure to pollutants (including noise) are some of the threats sperm whales face in the
6 Mediterranean Sea (Rendell & Frantzis 2016). The differences in feeding ecology between males
7 and females we have highlighted here could reflect contrasting habitat use or diving behaviour,
8 which, in turn, would imply a different susceptibility of the sexes to these threats. For example,
9 different diving depths or frequency may affect the time spent near the surface, where
10 individuals are vulnerable to vessel strikes (Laist et al. 2001). Moreover, the preferential use of
11 certain habitats may modulate the amount of traffic, noise, fishing gear or plastic debris an
12 individual is exposed to (e.g., De Stephanis et al. 2013, Erbe et al. 2014, Cózar et al. 2015, Breen
13 et al. 2016, Abrahms et al. 2019). Depending on a species' life history, the demographic rates of
14 one or both sexes may primarily influence population dynamics (Caswell 2001, Gerber & White
15 2014). Therefore, if sexual segregation were confirmed in Mediterranean sperm whales, the
16 successful conservation of this 'Endangered' population would require differential strategies
17 targeting specific threats to either females or males in distinct areas.

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Ethics statement

Data collection was carried out in compliance with Spanish regulations and under a permit to
JMB from the Directorate-General for Sustainability of the Coast and the Sea of the Ministry for
the Ecological Transition.

This study was approved by the Animal Welfare and Ethics Committee (AWEC) of the
University of St Andrews (UK).

Data availability

Data will be made publicly available on the Open Science Framework repository upon
acceptance.

Authors' contributions

EP and LR conceived the study, with contributions from all other co-authors; JMB, MC, EP and
LR collected the data; MV led the stable isotope analysis and interpretation of the results; ED
carried out the genetic sex determination; EP carried out the statistical analyses and drafted the
manuscript. All authors critically revised the manuscript, gave final approval for publication and
agree to be held accountable for the work performed therein.

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5. LITERATURE CITED

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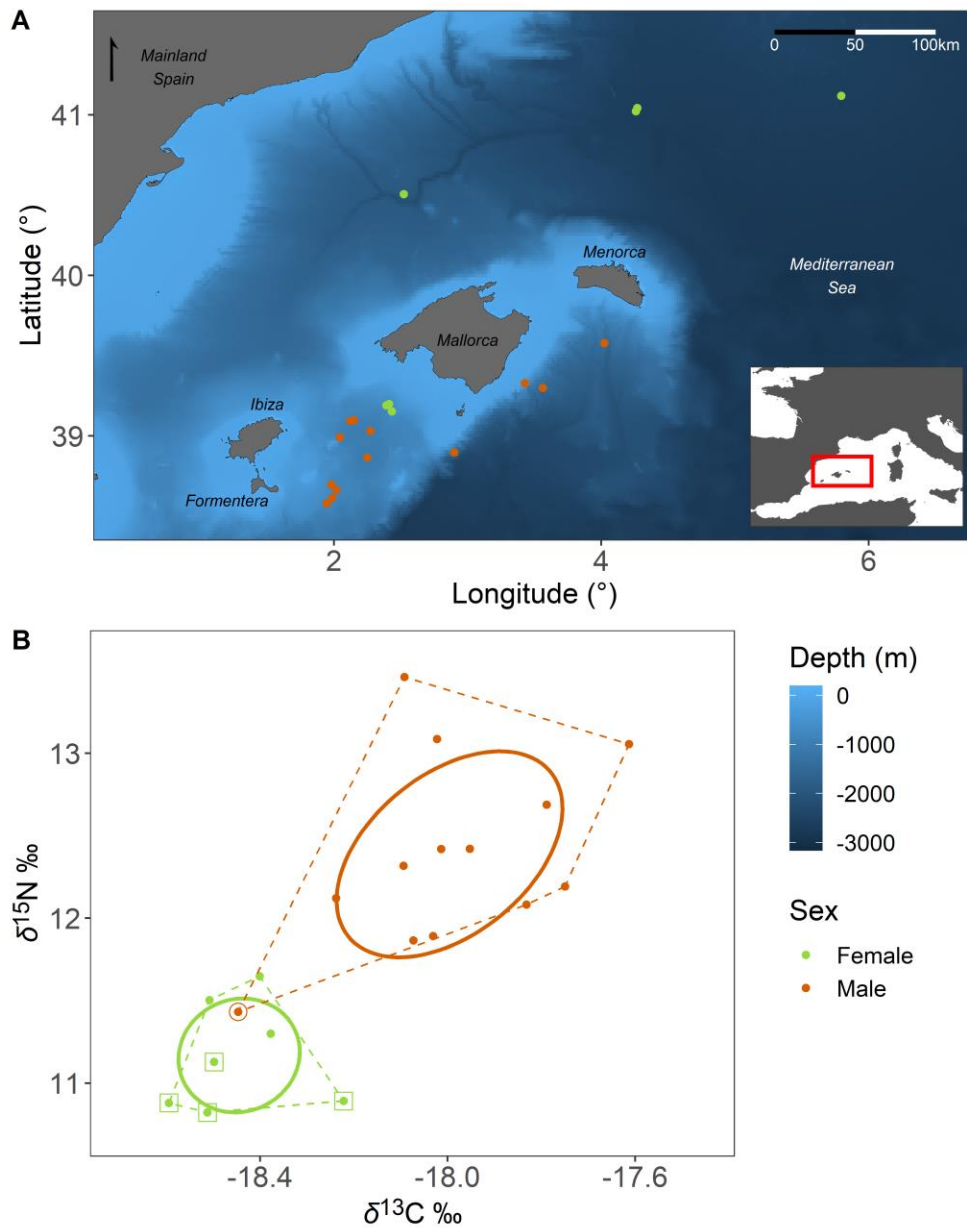
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9

1 6. FIGURES



2

3 Figure 1. (A) Locations of sperm whale biopsy sample collection, coloured by sex, and (B) $\delta^{13}\text{C}$
 4 and $\delta^{15}\text{N}$ values in skin samples, with solid lines representing standard ellipses corrected for
 5 sample size (SEAC) and dashed lines representing convex hull areas, coloured by sex. Sample
 6 comprises 7 females and 13 males. The observation corresponding to the small male is circled,
 7 while females sampled at latitudes $>40^\circ$ are identified by squares.

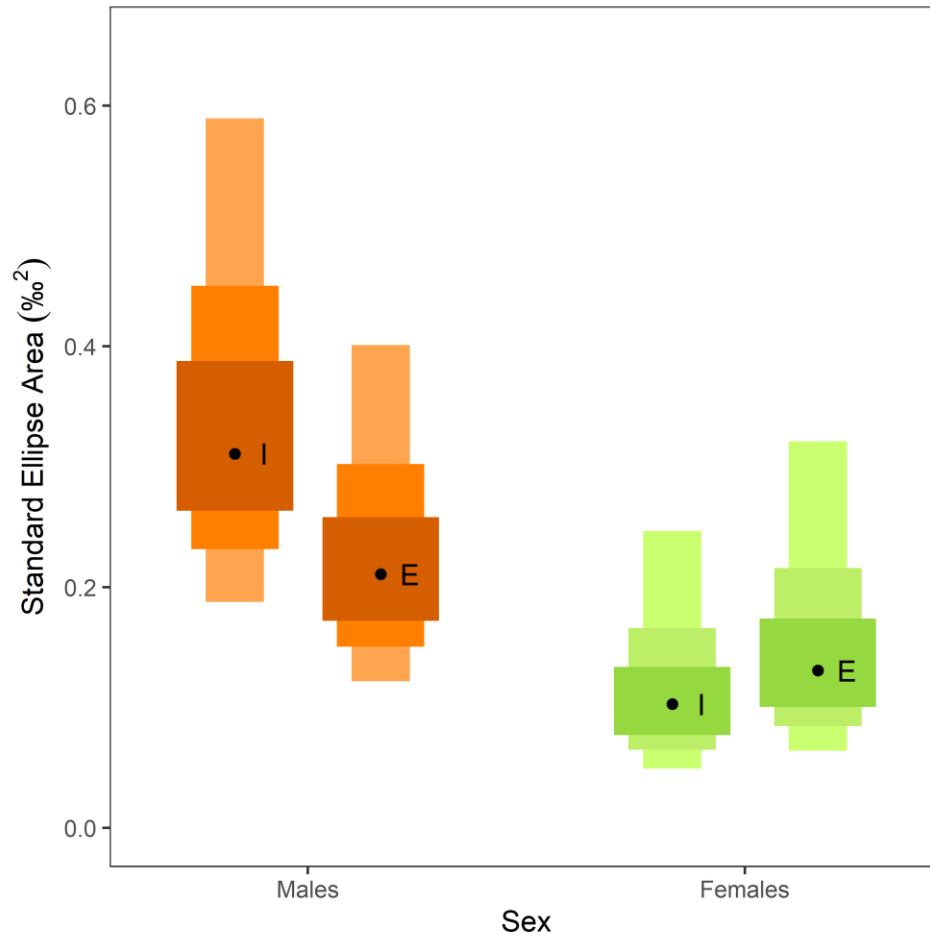


Figure 2. Posterior distribution of standard ellipse areas ($\% ^2$, representing estimated niche widths) for males and females from the Bayesian model in SIBER, including (I) or excluding (E) the small male with female-like isotope values. Therefore, males in our sample show larger niche width, but this is somewhat dependent on the inclusion of the smaller sub-adult male. Black dots represent the posterior modes, while the shaded boxes indicate the 50, 75 and 95% credible intervals (darker to lighter colours).

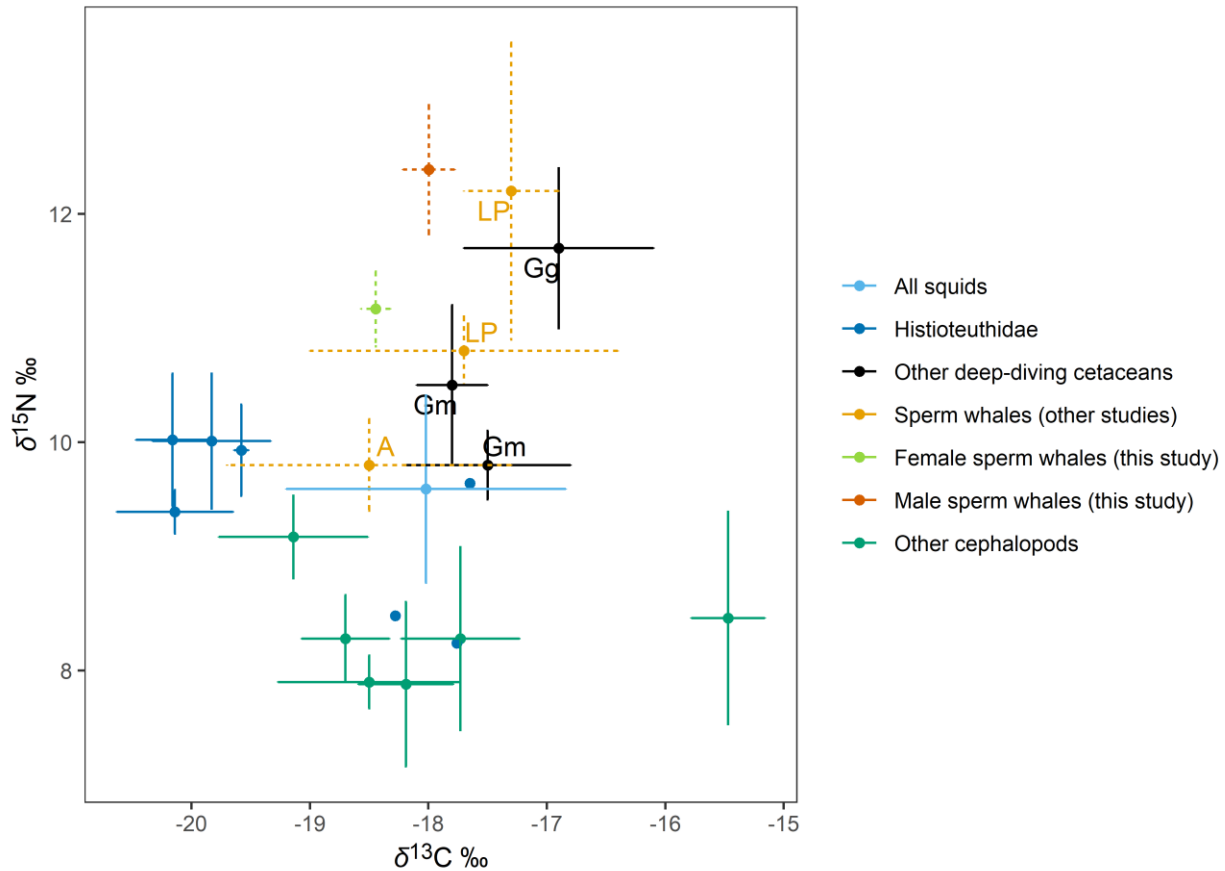


Figure 3. Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean and standard deviations) in samples of Mediterranean sperm whales (dashed lines), other deep-diving odontocetes, and putative prey (solid lines). Sperm whale samples include males and females from this study, individuals stranded along the Adriatic coast (A; Mazzariol et al. 2011), and individuals sampled in the Liguro-Provençal basin (LP; Praca et al. 2011, Pinzone et al. 2015). Other deep-diving odontocetes include long-finned pilot whales *Globicephala melas* (Gm) and Risso's dolphins *Grampus griseus* (Gg) (Praca et al. 2011, Pinzone et al. 2015). Putative prey include Histioteuthidae (*Histioteuthis reversa* and *Histioteuthis bonnellii*; Praca et al. 2011, Fanelli et al. 2013, Valls et al. 2017), other cephalopods (other *Teuthoidea*, *Octopoda*, *Sepioidea*; Praca et al. 2011, Fanelli et al. 2013, Valls et al. 2017) and all squids (Navarro et al. 2013). Isotopic values in prey were not corrected by the discrimination factors.