Population-specific sex and size variation in long-term foraging ecology of belugas and narwhals

Supplementary text

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Material and methods

We present additional details on the samples and the laboratory procedures below.

Laboratory analyses

We removed lipids from the samples with 10 ml 2:1 chloroform/methanol (v/v) under sonication for 1 h. After removing the solvent, we dried the samples under normal atmospheric pressure for 24 h. We demineralized the samples in 10 ml of 0.5 M HCl for 4 h while agitating them by an orbital shaker. After demineralization, we rinsed the samples to neutrality with Type I water, and heated them at 75 °C for 36 h in 10^{-3} M HCl to solubilize the collagen. We freeze dried the water soluble collagen. We treated the collagen with 10 ml 10:5:4 chloroform/methanol/water (v/v/v) under sonication for 1 h to remove any residual lipids. After centrifugation, the chloroform/methanol layer was removed and the methanol was evaporated out of the water layer at 60 °C for 24 h. We freeze dried and weighed the samples before adding 0.5 mg into tin capsules for elemental and isotopic analysis. We determined carbon and nitrogen stable isotopic and elemental compositions using a Nu Horizon (Nu Instruments, UK) continuous flow isotope ratio mass spectrometer at Trent University, Canada. We calibrated stable carbon and nitrogen isotopic compositions relative to the VPDB and AIR scales using USGS40 and USGS41a (glutamic acid) [1,2]. Analytical uncertainty was monitored using four internal reference materials in addition to USGS40 and USGS41a: SRM-1 (caribou bone collagen, long-term average $\delta^{13}C$ = −19.40±0.08 ‰, $\delta^{15}N$ = +1.82±0.11 ‰), SRM-2 (walrus bone collagen, long-term average $\delta^{13}C$ = −14.82±0.06 ‰, $\delta^{15}N$ = +15.59±0.14 ‰), SRM-14 (polar bear bone collagen, long-term average $\delta^{13}C$ = −13.68±0.08 ‰, $\delta^{15}N$ = +21.61±0.16 ‰), and SRM-17 (phenylalanine, long-term average $\delta^{13}C$ = −12.45±0.04 ‰, $\delta^{15}N$ = +3.17±0.15 ‰). The observed isotopic compositions for these standards are presented in table S2. For samples analyzed in duplicate, the mean difference between pairs was 0.06 ‰ for $\delta^{13}C$ and 0.11 ‰ for $\delta^{15}N$. Standard uncertainty was determined to be ±0.11 ‰ for $\delta^{13}C$ and ±0.21 ‰ for $\delta^{15}N$. We also adjusted the $\delta^{13}C$ values

to correct for the change in atmospheric and oceanic dissolved inorganic carbon that has occurred since the late 19th century due to industrialization (the “Suess Effect”; [3,4]), following [5], although we used 0.014 for the annual rate at which $\delta^{13}C$ has declined for a particular water body [6].

Statistical analyses

Data on the condylobasal length of the narwhal skulls were from [53]. Many beluga skulls were broken, thus to increase sample size we used another length measure on the skull (figure S1), which was highly correlated with condylobasal (called total here) skull length (R^2=0.95,P<0.01, figure S2). Overall, we could get skull measurements from 81 out of the 106 belugas and narwhals.

Results

Detailed results, which are presented in short in the discussion of the main manuscript

Bone collagen $\delta^{13}C$ and $\delta^{15}N$ differ significantly among populations (n=106, ANOVA, F(2,103)=554.8, P<0.01 for $\delta^{13}C$; ANOVA, F(2,103)=62.3, P<0.01 for $\delta^{15}N$, table S3). $\delta^{13}C$ and $\delta^{15}N$ values are significantly higher in WG belugas than in both WG and EG narwhals, and significantly higher in WG narwhals than in EG narwhals (Tukey-HSD, P<0.01, table S3, figure S3).

Bone collagen $\delta^{13}C$ does not differ significantly between males and females in any population (WG belugas: t=-0.31,df=20, P=0.76; WG narwhals t=-0.62, df=37, P=0.54; EG narwhals: t=-1.1, df=36, P=0.27, table S3, figure 1b). Bone collagen $\delta^{15}N$ is significantly higher in males than in females in both WG belugas (t=-2.4, df=20, P=0.03) and EG narwhals (t=-2.7, df=36, P=0.01). We do not find any significant differences in $\delta^{15}N$ between males and females in WG narwhals (t=1.43, df=34, P=0.16, table S3,figure 1b).

$\delta^{13}C$ and skull length are marginally positively correlated in WG belugas (R^2=0.16, P=0.05), WG and EG narwhals (R^2=0.24, P=0.01, R^2=0.22, P=0.01, respectively, figure S5). The correlation between $\delta^{13}C$ and skull length is significantly positive for female WG narwhals and female EG narwhals (R^2=0.41, P=0.01, R^2=0.25, P=0.05, respectively, figure 3).

We find a positive correlation between $\delta^{15}N$ and skull length in WG belugas (R^2=0.41, P<0.01) and EG narwhals (R^2=0.40, P<0.01), but not in WG narwhals (R^2=0.01, P=0.79,

figure S5). When dividing the dataset by sex, correlation is significantly positive for male WG belugas and male EG narwhals (R^2=0.73, P<0.01, R^2=0.48, P<0.01, respectively, figure 3).

The isotopic niche areas of WG belugas (SEA_B=0.56 ‰^{2}), WG narwhals (SEA_B=0.46 ‰^{2}) and EG narwhals (SEA_B=0.51 ‰^{2}) do not differ significantly in size (all proportions p<0.95 and >0.05, table S3, figures S3 and S4). There is no overlap between the niches of WG and EG narwhals, and little overlap between belugas and WG narwhals (ellipse overlap was 9%).

The isotopic niche area of male WG belugas (SEA_B=0.85 ‰^{2}) and male EG narwhals (SEA_B=0.63 ‰^{2}) are larger than those of females (WG belugas: SEA_B=0.27 ‰^{2}, p>0.99; EG narwhals: SEA_B=0.30 ‰^{2}, p=0.99; figures 1b, 2, table S3). The SEA_B size is not significantly larger in male WG narwhals (SEA_B=0.37 ‰^{2}) than females (SEA_B=0.55 ‰^{2}, p=0.11). The niche overlap between sexes is 25% in WG belugas, 51% in WG narwhals, and 27% in EG narwhals.

Influence of locality and year of sampling on stable isotope values

Locality

All samples from East Greenland narwhals were taken from the same locality (Scoresbysund, figure 1a). For belugas, most individuals were taken from one site (Nuusuaaq), in addition to three individuals from a nearby site (Kullorsuaq, table S3). The two sites are recognized as being used by the same population [7].

The West Greenland narwhal population is composed of different stocks, which is the term used for summer aggregations that constitute management units based on distribution, telemetry, ecological and, in some cases, genetic data [7]. The stocks migrate between different fjords and sounds in the summer and offshore Baffin Bay in the winter. Different stocks can show different stable isotope signatures in soft tissues [7]. However, narwhals winter offshore in Baffin Bay, where most of the foraging occurs [8]. Therefore, we expect limited variation in stable isotopes in bone tissue along West Greenland, which reflects diets spanning several years. In our West Greenland narwhal dataset, we have sampled from from four different localities; two summer localities to the north: Melville Bay (n=7) and Qaanaaq (n=19) corresponding to the Melville Bay and Inglefield Bredning stocks, and two migratory areas further south: Qeqertarsuaq (n=7) and Uummannaq (n=7) (figure 1a, table S1). We do not know to which stocks individuals sampled in Qeqertarsuaq and Uummannaq come from. Narwhals from Melville Bay migrate through both Qeqertarsuaq and Uummannaq. But several other stocks migrate through these localities as well. Narwhals

from Qaanaaq (Inglefield Bredning stock) likely also winter offshore in Baffin Bay, but their migration is less understood [7].

We compared mean isotopic values among the four localities, using the statistical analyses detailed in the main manuscript, and we find no significant differences in $\delta^{13}C$ (ANOVA, $F(3,36)=1.8$, $P=0.16$, figure S6). We do find significant differences in $\delta^{15}N$ among localities (ANOVA, $F(3,36)=8.3$, $P<0.01$, figure S6); individuals from Melville Bay and Uummannaq have higher $\delta^{15}N$ than individuals from Qaanaaq and Qeqertarsuaq (P-values between <0.01 and 0.03).

Sample sizes for three of the localities were too small (n≤5) to test for any effect of sex. However, we ran the analyses for Qaanaaq, which comprised 12 males and 7 females, and find no significant difference in stable isotopes values between males and females (Student t-test, for $\delta^{13}C$ $t=0.47$, $df=17$, $P=0.64$ and for $\delta^{15}N$ $t=-0.45$, $df=7$, $P=0.67$, figure S7). Our findings indicate that locality differences potentially do not mask differences between sexes.

Variation in $\delta^{15}N$ within WG narwhals may result from resource partitioning among different stocks, which we analysed here as belonging to one population (figure S6). In West Greenland, different narwhal stocks migrate between various summer localities, and offshore in Baffin Bay during winter, where their ranges overlap, although they may utilize different parts of the Baffin Bay [9]. This may explain why we observe differences in $\delta^{15}N$ among narwhals sampled from different localities as they can represent different stocks. Those differences do not impact our inferences on species, areas, sex and size differences, but shows that geographical resource partitioning may be another mechanisms to decrease intra-specific competition. In summary, our results suggest the isotopic niche of WG narwhals may reflect geographical subdivision.

Year of sampling

All beluga samples were taken in the same time period, 1990-1994 (n=27), while narwhal samples were taken during two time periods: 1993-1995 (WG n=10, EG n=31) and 2002-2007 (WG n=30, EG n=8). There are no available data on the turn-over rate of bone collagen for any marine mammals. In humans aged 18-25 years, it is 2-3% per year [10,11]. If the turn-over rate in belugas and narwhals is comparable, our data should reflect ~20 years of the diet for mature adults. Even for subadults, the isotopic composition of bone collagen will represent the average diet over multiple years. Therefore, temporal changes may be buffered, as integration time will at least partially overlap between our two time periods.

$\delta^{13}C$ was marginally lower in 1993-1995 than 2002-2007 in WG narwhals (Student t test, t=-2.6, df=11, P=0.03), while $\delta^{15}N$ was marginally higher (Student t test, t=2.6, df=11.9, P=0.02, figure S8). However, sample sizes were uneven (1993-1995 n=10; 2002-2007 n=30), and the localities Qaanaaq and Qeqertarsuaq, which have lower $\delta^{15}N$ were only represented in 2002-2007, potentially introducing biases in our comparison. In EG narwhals, $\delta^{13}C$ and $\delta^{15}N$ did not differ significantly between time periods (Student t test, t=-1.0, df=12, P=0.34, and t =-0.9, df=17.3, P=0.39, respectively, figure S8). We therefore conclude that the sampling period has a limited effect on our results.

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