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Multi-decadal and ontogenetic trophic shifts inferred from stable isotope ratios of pinniped teeth

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Identifying and characterizing top predators' use of trophic resources provides important information about animal ecology and their response to changing conditions. Information from sources such as stable isotopes can be used to infer changes in resource use as direct observations in the wild are difficult to obtain, particularly in the marine environment. Stable carbon and nitrogen isotope values were recovered from the canine teeth of grey seals collected from haul outs in the central North Sea in the 1970/1980s (n = 44) and 2000s (n = 25), spanning a period of marked ecosystem changes in the region. Extracting material deposited during juvenile and adult life-stages, we reconstructed a multi-decadal record of $\delta^{15}N$ and $\delta^{13}C$ variation. Using established correlations between stable isotope ratios and sea bottom temperature we created a proxy for baseline isotopic variability to account for this source of temporal change. We found 1) a significant long-term decline in juvenile grey seal $\delta^{15}N$ values, suggesting trophic position has decreased over time; 2) a decline in adult $\delta^{15}N$ values and contraction in stable isotopic niche space after the North Sea regime shift, signifying both a decline in trophic position and change in foraging habits over the 20th century; and 3) evidence for dietary segregation between juvenile and adult animals, showing juvenile individuals feeding at a lower trophic position and in more nearshore areas than adults. Our results demonstrate the efficacy of mining archived biological samples to address ecological questions and imply important ontogenetic and long-term shifts in the feeding ecology of a top predator. Long-term changes in grey seal trophic dynamics may be partly in response to well documented ecosystem changes in the North Sea. Such indirect monitoring of marine predators may have utility when set in the context of ecosystem assessments where paucity of long-term monitoring data is prevalent.

Major changes in the eastern North Atlantic marine ecosystem have occurred over recent decades in association with global patterns of climate change (Beaugrand 2009). In the North Sea, multiple abrupt ecosystem shifts ('regime shifts') in the plankton community have been characterized, related to a change in temperature and dominant climatic oscillations in the region, the Atlantic multi-decadal oscillation (AMO) and the North Atlantic oscillation (NAO) (Beaugrand et al. 2014). Variance in the timing and productivity of the plankton community can exert bottom-up influence on the dynamics of upper trophic level organisms through, for example, affecting fish recruitment and growth (Beaugrand et al. 2003a, Frederiksen et al. 2006, Rijnsdorp et al. 2009). In addition, the past century has seen intense human exploitation of marine resources that can also drive top–down changes in ecosystem trophic dynamics (Engelhard et al. 2014, Speirs et al. 2016). Changing marine conditions combined with competitive interactions with fisheries can influence top predators such as seabirds and marine mammals through direct (e.g. sea ice, by-catch) and indirect mechanisms (e.g. food availability; Trites et al. 2006, Frederiksen et al. 2006). Consequently, marine apex predators can provide information about the overall health of the marine ecosystem (Boyd et al. 2006). Over time, variance in their diet and foraging habits may signal that ecosystem-level shifts have occurred. However, detecting and quantifying long-term change in marine systems is difficult without dedicated monitoring programs.

Grey seals *Halichoerus grypus* are important top predators across their range in the northeast Atlantic, foraging on a wide range of benthic and demersal species in the neritic environment (Prime and Hammond 1990, Hammond and Wilson 2016). Against a backdrop of potentially major changes to prey availability (Rijnsdorp et al. 2009, Engelhard et al. 2014), grey seal populations have increased in many parts of their range, recovering in part from human exploitation in the 19th and 20th centuries. Approximately 38% of the global grey seal population breeds along the coast of the United Kingdom (UK) and patterns of demographic change vary between UK coastal regions. Since comprehensive

monitoring began in 1984 the number of grey seal pups produced at North Sea haulouts around the UK has continued to increase exponentially, while the rate of increase has slowed or completely leveled off at haulouts in the north and northwest of the country (SCOS 2016). One possible consequence of a consistent 3% year^{-1} increase in the number of North Sea grey seals over the last half of the 20th century is an increase in intraspecific competition, albeit not demographically limiting. This is evidenced by the colonisation of new breeding areas and increases in the number of individuals at existing haulouts (SCOS 2016).

As central place foragers, grey seals can range widely at sea while foraging or transiting between haulout sites, but tend to return to the area from which they left. Trips at sea usually last 2–3 days (McConnell et al. 1999). Breeding occurs in autumn typically on remote islands or coasts. Grey seals are capital breeders: females give birth to a single pup which is suckled for ca 18 days before the female returns to sea (SCOS 2016). Juveniles are recruited into the breeding population at 5–6 years (Hewer 1964). Telemetry studies demonstrate that adult grey seals utilise areas ca 100 km offshore at-sea (Jones et al. 2015), spending much of their time at the sea floor (Photopoulou et al. 2014), but are physiologically capable of exploiting the marine environment available within UK shelf seas ($<$ 200 m). Little information is available regarding juvenile grey seal movement at sea. In general, sandeels *Ammodytes* spp. are the main contributor by weight to the diet of grey seals in the North Sea. Other important species include cod *Gadus morhua*, whiting *Merlangius merlangus*, plaice *Pleuronectes platessa*, lemon sole *Microstomus kitt*, dover sole *Solea solea* and bullrout *Myoxocephalus scorpius* (Hammond and Wilson 2016).

Faecal analysis provides a comprehensive and quantitative estimate of grey seal diet composition. However, data collection and processing constraints limit broad-scale analyses to decadal occurrences, providing snapshots of diet through time (Hammond and Wilson 2016). Knowledge of sex and age-related variation in grey seal diet is limited as data are primarily collected from mixed sex and age cohorts. In addition, prey composition from scats may be biased towards those individuals that return more frequently to haulout or prey items recently ingested. Seals that travel considerable distances to forage may be under-represented; prey without hardparts (e.g. cartilaginous fish) and large prey (e.g. salmon) may also be under-represented if the head is not consumed (Harris 2006).

For long-lived generalist and opportunistic predators such as seals, changes in trophic level or foraging distribution are likely to manifest over several years and the analysis of naturally occurring stable isotope ratios provides additional information regarding predator diet. The method quantifies the ratio of heavy to light stable isotopes in consumer tissues, and changes therein can be interpreted in relation to the stable isotope ratios of assimilated prey (DeNiro and Epstein 1978, 1981). The technique relies on an understanding of the pathways of stable isotopic fractionation associated with the assimilation of dietary nutrients. For example, preferential reaction of the lighter isotope of nitrogen, ^{14}N, during anabolism leads to the preferential excretion of ^{14}N and an increase in consumer tissue δ^{15}N values relative to diet (DeNiro and Epstein 1981, Post 2002, McCutchan et al. 2003). Often more important for δ^{13}C variation are large spatial gradients in δ^{13}C at the base of the marine food web influenced by productivity, variation in the growth and morphology of differing taxa of primary producers, and the concentration of aqueous CO_2. Well-characterised gradients in the natural abundance of carbon isotopes include gradients in δ^{13}C values from nearshore or benthic (less negative) to offshore or pelagic (more negative) habitats due to differences in productivity (France 1995); and global-scale latitudinal gradients driven by the concentration of aqueous CO_2. Broadly, pelagic systems in cold high-latitude waters have lower δ^{13}C values than mid-latitude pelagic systems (Tagliabue and Bopp 2008, Graham et al. 2010). These spatial patterns may be reflected in δ^{13}C values of consumer tissues and are used to infer likely foraging areas (Cherel and Hobson 2007, Trueman et al. 2016). Thus, δ^{15}N and δ^{13}C values can provide both process-based (e.g. trophic linkage) and source-based (e.g. foraging distribution) information about consumers (Peterson and Fry 1987, Newsome et al. 2007).

Retrospective studies of stable isotope variation are possible for many body tissues that remain metabolically inert after formation, including teeth (Koch et al. 1994, Hanson et al. 2009), baleen (Schell 2000), bone (Christensen and Richardson 2008, Turner Tomaszewicz et al. 2017), calcified cartilage (Carlisle et al. 2015), and fish scales or otoliths (MacKenzie et al. 2011, Hanson et al. 2013). These structures have the advantage of recording both individual life history and long-term chemical signals. In contrast to faecal analysis, information contained in stable isotope ratios reflects a general dietary signal assimilated over the time the tissue grew (or accreted) and the isotopic signal was incorporated (isotopic turnover).

Comparing historical and modern stable isotopic data can detect shifts in predator diet in response to ecological change. However, interpretation of historical shifts must be set in the context of the dynamic biogeochemistry of the environment. Over decades, physical (e.g. temperature), chemical (e.g. nutrient supply), and biological (e.g. algal composition) processes governing isotopic fractionation at the base of the marine food web may change and become incorporated into δ^{13}C and δ^{15}N signals (Graham et al. 2010). Accounting for these sources of baseline variation in stable isotope studies is difficult as samples from primary producers or consumers are rarely available retrospectively. Recent developments to characterise spatial variation in marine stable isotope ratios, termed 'marine isoscapes', provide appropriate context in which to interpret observed shifts in predator values (Lorrain et al. 2015, Trueman et al. 2016). For example, two studies in the North Sea predicted spatial variation in δ^{13}C and δ^{15}N of a benthic filter feeder, the queen scallop *Aequipecten opercularis* using limited hydrodynamic variables such as depth, salinity and bottom temperature (Jennings and Warr 2003, Barnes et al. 2009). Sea bottom temperature (SBT) was a strong predictor of both queen scallop δ^{13}C and δ^{15}N values. Ten years later, MacKenzie et al. (2014) conducted a similar study with mesopelagic lion's mane jellyfish *Cyanea capillata* and re-analysed the queen scallop data. In particular, sea bottom temperature (SBT) explained 68% of variance in jellyfish δ^{15}N values and 35% of variance in δ^{13}C values (MacKenzie et al. 2014), and the broad spatial patterns described using

the explanatory SBT covariate were consistent with those found for queen scallops. The temporal separation of the studies suggests that the relationship between $\delta^{13}C$ and $\delta^{15}N$ values and sea bottom temperature may be relatively stable over decadal scales, opening the possibility to construct proxies of baseline $\delta^{13}C$ and $\delta^{15}N$ from long-term empirical measures of SBT.

Here, we assess long-term and age-dependent changes in the stable isotope values of grey seals in the central North Sea from tooth samples. We draw on developments of marine isoscapes for the North Sea to explore potential multi-decadal trophic shifts in grey seals, while accounting for a proxy measure of baseline variability. The use of canine teeth allows sampling from both early (juvenile) and end-of-life (adult) stages from the same individual to provide insight into ontogenetic changes in diet. Specifically, we address five key objectives: 1) account for proxy baseline $\delta^{13}C$ and $\delta^{15}N$ values over time; determine multi-decadal shifts in the 2) juvenile and 3) adult phases of grey seal spatial and trophic marine resource use inferred from $\delta^{13}C$ and $\delta^{15}N$ values; 4) quantify ontogenetic change in diet between juvenile and adult grey seals; 5) contextualise results of objectives 2) – 4) using stable isotope values of grey seal prey items.

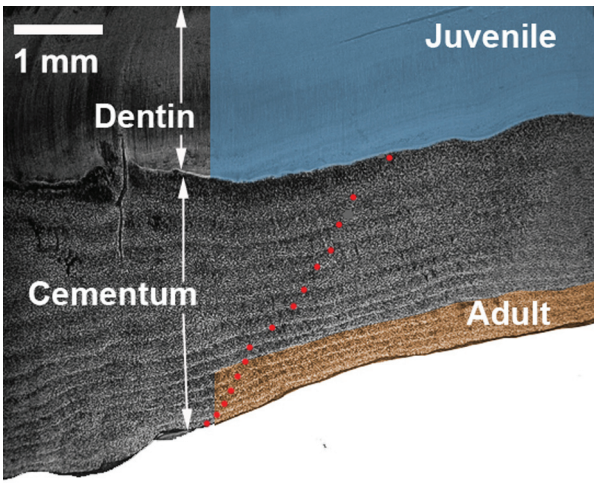

Figure 1. Polarised light micrograph of a female grey seal with an estimated age of 15 years. Red spots indicate growth layer groups (GLGs) counted in the cementum. This female died in 1981. Her estimated year of birth was 1966; pulp cavity occlusion occurs after ~ 4–5 years. 'Adult' collagen material was extracted from the outer surface of the tooth (orange); 'juvenile' collagen material was extracted across all layers present in the dentin (blue).

Material and methods

Sample collection and preparation

A sample of 44 archived grey seal canine teeth (held at the Sea Mammal Research Unit (SMRU), Univ. of St. Andrews) was randomly selected from adults legally culled in 1972 (n = 5) and 1981 (n = 39) at the Farne Islands (55°38′N, 1°37′W) under the provision of the Conservation of Seals Act (1970). The culls focused on removing adult females so the archive is heavily biased in this regard. Between 2005 and 2008, canine teeth were also collected from grey seal carcasses stranded (n = 16) or shot on the east coast of Scotland (n = 9) between 56°10′N, 2°32′W and 57°51′N, 3°58′W. Seals were shot to protect fisheries under the Conservation of Seals Act (1970) and licensed through the Moray Firth Seal Management Plan (Butler et al. 2011). Teeth were removed after a short boil (30 min) and cleaned manually which does not significantly alter stable isotope ratios of collagen (Deniro et al. 1985, Fernandes et al. 2014).

Grey seal teeth are formed from outer (non-vascularized) cementum layers and inner (vascularized) pulp cavity layers capped with a layer of enamel. The enamel layer is highly mineralized with only a small amount of protein; dentin and cementum, however, are composed of a carbonated hydroxyapatite–collagen matrix. The ratio of inorganic compounds to protein is similar in dentin and cementum and both tissues are considered to be relatively metabolically inert after deposition (Hobson and Sease 1998, Graber et al. 2016). Dentin is deposited within the pulp cavity of grey seal canine teeth until it occludes the cavity by age 4 or 5 (Fig. 1, area marked 'Dentin/Juvenile; Hewer, 1964). Cementum is deposited on the outer surface and deposition continues throughout life, with the outermost layers being the most recently deposited (see also figures in Hewer 1964, Frie et al. 2013).

A longitudinal thin section (100–120 µm) was removed from the tooth root using a precision low speed diamond saw. Age was determined by counting the number of complete growth layer groups (GLGs) in the cementum from photographs of thin sections. A completed GLG consists of an opaque and a translucent layer (Fig. 1; Hewer 1964). Each tooth was read three times by one researcher (NNH) and once by another experienced researcher (S. Murphy, Galway-Mayo Inst. of Technology). Between 10–30 mg of powdered dentin or cementum was extracted from the dentin, representing juvenile material, and outermost portion of the cementum, representing adult material, using a hand-held rotary tool fitted with a diamond burr (600 µm tip). In both cases the outermost surface was first abraded to remove contaminating material. Within the dentin, material was milled across all layers present in the pulp cavity. For cementum samples, the outer < 1 mm was milled. GLGs are difficult to distinguish in the dentin but Hewer (1964) suggests width is fairly similar, declining somewhat with age. Because the pulp cavity is encased in dentin by age 4 or 5, collagen samples obtained from the dentin represented years 0 to ca 5 with a slight bias towards earlier years. Stable isotope values from this region of the tooth are hereafter referred to as 'juvenile' samples. Collagen samples obtained from the outermost margin of cementum represent the last several years of life (Fig. 1 area marked 'Adult'). Combining of several GLGs to produce a single sample for stable isotope analysis is not uncommon in species with small teeth/GLGs (Knoff et al. 2008), and we adopted this approach. Powdered dentin and cementum samples were demineralised with 0.5 M HCl for 24 h at room temperature to extract collagen, rinsed with deionised water until neutrality and lyophilized. Collagen samples were stored in plastic vials until stable isotope analysis.

A total of 37 prey (nine species) were collected as part of the international bottom trawl survey (IBTS) of the North Sea in August 2009 (Cruise Ref 1109S) to provide prey stable isotope dietary variation coinciding with recent seal tooth collections. A subsample of white muscle was excised, lyophilized and ground to a fine homogenous powder with mortar and pestle. Lipids were extracted from tissue using accelerated solvent extraction as per method A from Bodin et al. (2009): samples were processed with dichloromethane at 100°C and 1900 psi with a cell heat up time of 10 min followed by 10 min of static time before flushing (60% of total volume) and purging for 60 s. This combination of temperature, pressure and timing has least influence on $\delta^{15}N$ values (Bodin et al. 2009).

Stable isotope analysis

Between 1–1.6 mg of tooth collagen and 1 mg of fish white muscle were weighed into tin capsules for analysis. All samples were analysed by continuous-flow isotope ratio mass spectrometry (CF-IRMS) at the Univ. of St Andrews Facility for Earth and Environmental Analysis. The instrument was an elemental analyzer fitted with a zero-blank auto-sampler coupled to a mass spectrometer. Stable isotope results are reported in δ notation as parts per thousand (‰) deviation according to Eq. 1 (McKinney et al. 1950):

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

where X is ^{13}C or ^{15}N and R_{sample} is the corresponding ratio of heavy to light isotopes. $R_{standard}$ is Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2 for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Standard deviation of internal and external standards was within ± 0.20‰ and ± 0.25‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively.

Data analysis

Supplementary material Appendix 1 Fig. A1 shows a schematic flowchart of the analytical process, which comprises of five sections.

Baseline estimation

Using the relationship derived in Mackenzie et al. (2014) from lion's mane jellyfish, we predicted a proxy for spatial variation in baseline $\delta^{13}C$ and $\delta^{15}N$ ($\delta^{13}C_{base}$ and $\delta^{15}N_{base}$) in the North Sea from sea bottom temperature (SBT) using Eq. 2 and 3. Monthly mean SBT data between 1945 and 2008 was provided by ICES (available to download from <www.ices.dk>) for 0.5° × 1.0° ICES statistical rectangles in the North Sea.

$$\delta^{13}C_{base} = 0.336(\pm 0.08) \times SBT - 21.53(\pm 0.89) \quad (2)$$

$$\delta^{15}N_{base} = 0.48(\pm 0.06) \times SBT - 6.04(\pm 0.67) \quad (3)$$

Our aim was to extract an annual summary of variation in $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ over the timescale of the seal tooth archive within the context of North Sea grey seal life history. Isoscapes were limited to an appropriate spatial extent using grey seal at-sea usage maps (Jones et al. 2015). Jones et al. (2015) produced maps by combining movement data from 259 telemetry tags deployed on grey seals between 1991 and 2013 with terrestrial counts to map the distribution of the grey seal population around the UK. Grey seal numbers have been increasing in recent years and so the map, which was scaled to the 2013 population estimate, shows the largest extent of space use. Areas with low grey seal usage could potentially have different $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ values; therefore a spatial buffer was overlaid onto the baseline isoscapes to select the area where 90% of grey seal at-sea usage was located (Supplementary material Appendix 1 Fig. A2). Grey seals are wide-ranging predators and from the telemetry data available, seals from the east coast of Scotland (more recently collected samples) share at-sea foraging ranges with seals from the Farne Islands (historical samples; Jones et al. 2015). A single annual mean proxy $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ value was calculated by aggregating, with equal weighting, all grid cells within the 90% grey seal usage buffer. Because juvenile seal collagen represented an integration of up to five early years, baseline proxy values were further smoothed using a five-year equally weighted moving average.

In addition to variability in $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ due to SBT are global trends in $\delta^{13}C$ values caused by the decline in ^{14}C in atmospheric CO_2 as a result of increased combustion of fossil fuels, termed the Suess effect (Suess 1955, Keeling 1979). Change in relative proportions of carbon isotopes in the atmosphere has the effect of reducing $\delta^{13}CO_2$ over time, coupled with increased flux of CO_2 from oceans, to create an 'ocean ^{13}C Suess effect'. For the latter half of the 20th century, this has been estimated as –0.026‰ year^{-1} in the North Atlantic (Körtzinger et al. 2003). A scaling factor was applied to all grey seal tooth $\delta^{13}C$ records (both juvenile and adult) to account for the ocean Suess effect using Eq. 4 (Körtzinger et al. 2003):

$$\delta^{13}C_{adj} = \delta^{13}C_{obs} - 0.026 \times (2008 - t_i) \quad (4)$$

Where t_i is the year assigned to each $\delta^{13}C_{obs}$ value and $\delta^{13}C_{adj}$ are values adjusted for the Suess effect. Through trophic enrichment in ^{13}C between seals and their prey (Hobson et al. 1996), there may be attenuation of the ocean Suess effect with trophic level, and the adjustment applied here is likely to be conservative.

Finally, the annual proxy for baseline stable isotope variation was then subtracted from observed predator $\delta^{15}N$ and Suess-effect adjusted $\delta^{13}C$ values to calculate baseline-corrected $\delta^{13}C_{corr}$ and $\delta^{15}N_{corr}$. These 'corrected' values were used in the remainder of data analyses. They represent a measure of top predator stable isotopic deviation from a proxy baseline.

Long-term trends in juvenile signal

Temporal changes in juvenile $\delta^{13}C_{corr}$ and $\delta^{15}N_{corr}$ values were assessed using a linear model with Gaussian error distribution. Year was included as a continuous covariate. To centre the data values within the 4–5 years represented by the juvenile signal contained in the tooth collagen, year assigned to juvenile $\delta^{13}C_{corr}$ and $\delta^{15}N_{corr}$ data was lagged by two years after estimated year of birth. Sex was not included as a covariate because few males were present in the historical samples (1970–1980s), and within the more recent samples some carcasses could not be accurately assigned to either sex. A comparison between male (M) and female (F) seals from

the 2000s data where sex could be reliably assigned (M = 6, F = 11), showed highly overlapping 95% confidence intervals on the mean of both adult and juvenile $\delta^{13}C_{corr}$ and $\delta^{15}N_{corr}$ but these are small sample sizes (Supplementary material Appendix 1 Fig. A3). Cause of death (culled, stranded, shot) was not included in the models because we reasoned the stable isotope values (representing diet and behavior) deposited while animals were young were unlikely to be affected by the ultimate cause of morbidity as adults. However, we tested for significant differences between model residuals with respect to cause of death (Supplementary material Appendix 1). Model residuals were inspected for normality and Wald–Wolfowitz runs test of randomness was used to detect temporal autocorrelation. Residuals from each fitted model were checked for homogeneity of variance assumptions. We tested for a temporal trend using second order Akaike information criterium for small sample sizes (AICc). Akaike weights were calculated for each model:

$$w_i = \frac{\exp(-0.5\Delta_i)}{\sum_{r=1}^{N}\exp(-0.5\Delta_r)} \quad (5)$$

where $\Delta_i = AICc_i - AICc_{min}$, N is the number of possible models, $AICc_i$ is the AIC value of model i, and $AICc_{min}$ is the minimum AIC value (Burnham and Anderson 2002, Burnham et al. 2011). We considered models with $\Delta_i > 3$ to indicate the best fit.

Long-term shifts in adult stable isotope values

Adult age-at-death ranged from 2 to 32 years old (Table 1). The dentin cavity occludes at 4–5 years, so young animals have the same early years of life reflected in adult and juvenile portion of the canine. For the remainder of the analyses, individuals younger than 5 years old were removed (four observations from teeth collected in 2000s).

Animal deaths were clustered in time (Table 1), and two adult groups were defined: animals culled in the 1970s/1980s, and dead stranded in the 2000s. Through assimilated diet, animals occupy a unique isotopic niche described by variation in $\delta^{13}C$ and $\delta^{15}N$. The area of a standard ellipse estimated from these data describes isotopic niche space (Newsome et al. 2007, Jackson et al. 2011). Bayesian standard ellipse areas (SEA) were fitted using the R package SIBER, using vague normal priors for mean adult $\delta^{13}C_{corr}$ and $\delta^{15}N_{corr}$ values ($\mu = 0$, $\sigma^2 = 10^3$), and a vague Inverse-Wishart prior for the covariance matrix (details in Jackson et al. 2011). We also assessed differences in mean adult $\delta^{13}C_{corr}$ and $\delta^{15}N_{corr}$ values between the 1970s/1980s and 2000s groupings using linear regression, assuming a Gaussian error distribution and visually inspecting residuals for homogeneity of variance. The adult sample included animals from 5 to 32 years, and we included age-at-death in these models to test for significant differences between individuals due to age.

Our adult sample represented material laid down over the last several years of life, and the stable isotopic value of this sample could be affected by the cause of death if the ultimate cause of morbidity had manifested over the same time period. For seals that were shot, long-term illness was not the ultimate cause of death but the primary cause of death of necropsied stranded seals in the UK is physical trauma or bacterial infection (e.g. pneumonia; <www.strandings.org>). Therefore, we tested for differences in adult $\delta^{13}C_{corr}$ and $\delta^{15}N_{corr}$ related to the manner of death (dead stranded versus shot) within the group of samples collected in the 2000s.

Table 1. Summary of grey seal *Halichoerus grypus* tooth samples included in the present analysis. See Supplementary material Appendix 1 Table A1 for the full dataset.

Year of death	Grouping	Location	Age range	Birth year range	n
1972	1970s/1980s	Farne Islands	16–29	1943–1956	5
1981	1970s/1980s	Farne Islands	6–32	1949–1975	39
2005	2000s	East coast Scotland	7	1998	1
2006	2000s	East coast Scotland	2–11	1995–2004	4
2007	2000s	East coast Scotland	12	1995	1
2008	2000s	East coast Scotland	3–25	1983–2005	19

Ontogenetic change

We used life-stage as a categorical predictor in mixed effects linear regression models to test for significant differences between mean $\delta^{15}N$ and $\delta^{13}C$ values of juvenile and adult life-stages within individual grey seal teeth. Individual was specified as a random effect. Any association between the magnitude of the shift (e.g. adult $\delta^{15}N_{corr}$ – juvenile $\delta^{15}N_{corr}$) within an individual and their age-at-death was tested using ordinary least squares linear regression. In both cases we assumed a Gaussian error distribution and visually inspected residuals for homogeneity of variance. We also tested for overall changes in isotopic niche space using the methods described above.

Potential prey

Potential prey items were selected based on the results of previous faecal sample analyses (Hammond and Prime 1990, Hammond and Grellier 2006, Hammond and Wilson 2016) The 37 prey items collected in 2009 as part of the present study were supplemented with freely available data (Jennings and Cogan 2015), which included white muscle samples collected between 2002 and 2009 in the central North Sea within the 90% grey seal usage buffer. To allow comparison between the two prey datasets, $\delta^{13}C$ values from Jennings and Cogan (2015) were lipid corrected using C:N ratios for fish muscle tissue (Logan et al. 2008) (Eq. 6):

$$\delta^{13}C_{corr} = \delta^{13}C_{bulk} + 4.401 \times \mathrm{In}(C:N) - 4.7632 \quad (6)$$

We did not correct consumer values for tissue-specific fractionation factors (i.e. the difference between dietary protein and pinniped tooth collagen stable isotope values) to compare directly with prey data because none are available. As an appproximation, $\Delta^{13}C_{collagen\text{-}diet}$ is usually assumed to be ca + 3–5‰ (Jim et al. 2004, Koch 2007) and $\Delta^{15}N_{collagen\text{-}diet}$ is ca + 3‰ (Newsome et al. 2010) for animals subsisting on a relatively high protein diet (such as pinnipeds).

Results

All stable isotope values obtained from grey seal canines are given in Supplementary material Appendix 1 Table A1. A summary of mean stable isotope variation and corrected values between age and collection periods is given in Table 3.

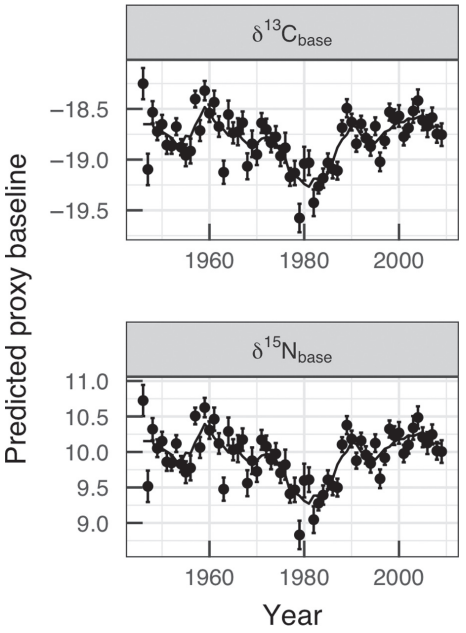

Figure 2. Mean (and 95% confidence interval) annual proxy stable isotope baseline values predicted for the area of the North Sea isoscape limited to 90% of grey seal *Halichoerus grypus* at-sea usage. The isoscape was calculated from relationships specified in Mackenzie et al. (2014) between stable isotope values of a mesopelagic jellyfish *Cyanea capillata* and sea bottom temperature. A five-year running mean was used to smooth the time series (black line).

The biochemical quality of the organic material extracted from demineralized tooth samples identifies it as collagen. Mean carbon to nitrogen ratio (C:N) was 3.48 (95% CI: 3.39, 3.56) and was not significantly different between dentin and cementum collagen extractions (ANOVA $F_{1,135} = 1.281$, $p = 0.26$).

Proxy baseline estimation

Mean annual proxy $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ for the North Sea isoscape constrained by grey seal at-sea usage is shown in Fig. 2. Temporal patterns follow variation in SBT as specified in Eq. 2 and 3. Perturbations in SBT (and hence proxy baseline isotope values) associated with the North Sea regime shift in the 1980s (Beaugrand 2004) are easily discernible in the annual mean isotope values as a shift towards more negative values (lower SBT).

Long-term trends in juvenile signal

A model describing juvenile $\delta^{15}N_{corr}$ as a function of year was much more likely given the data than an intercept-only model (Akaike weight = 0.99; Table 2). Accounting for baseline variation, juvenile $\delta^{15}N_{corr}$ declined by an average of 0.025 (95% CI: –0.04, –0.013)) ‰ $year^{-1}$ between the 1950s and early 2000s (Fig. 3a). Accounting for baseline variation and the Suess effect, there was no discernable trend in juvenile $\delta^{13}C_{corr}$ values although there was considerable heterogeneity between individuals (Fig. 3b). A model of juvenile $\delta^{13}C_{corr}$ with an annual trend had the lowest AICc value but it was not considerably lower than the intercept-only model (Δ AIC < 3; Table 2).

Long-term shift in adult stable isotope values

Adult stable isotopic niche area was considerably contracted in seals collected in the 2000s compared to those culled in the 1970s/1980s (Fig. 4) and this could not be explained by different proxy baseline variances over those periods (Supplementary material Appendix 1). Mean standard ellipse area in the more recent samples was 1.85‰^{2} (95% credible intervals: 1.16, 2.65) compared with an area of 4.52‰^{2} (95% credible intervals: 3.29, 6.01) for the older samples. Niether adult $\delta^{13}C_{corr}$ nor $\delta^{15}N_{corr}$ were related significantly to animals' age-at-death ($p > 0.10$ in both cases), but the contraction in isotope niche space indicated by the above analysis was accompanied by a significant reduction in $\delta^{15}N_{corr}$ values (–1.78 between 1970s/1980s grouping and 2000s grouping, 95% CI: –2.25, –1.22). $\delta^{13}C_{corr}$ values were not significantly different between the two time periods. Within the recently collected group, cause of death (shot versus dead stranded) had no significant effect on mean $\delta^{13}C_{corr}$ nor $\delta^{15}N_{corr}$ values (OLS regression, $p = 0.08$ and $p = 0.86$ respectively).

Ontogenetic change

Within individuals, clear stable isotopic shifts were apparent between juvenile and adult collagen lifestages (Fig. 5). $\delta^{15}N$ values were on average 1.96‰ (95% CI: 1.68, 2.24) higher in adult material than juvenile material and 97% of individuals showed a positive shift. $\delta^{13}C$ values were on average 0.87‰ (95% CI: 0.57, 1.17) lower in adult material than juvenile material and 77% of individuals showed a negative shift (after correction for proxy baseline and the Suess effect). There was no association between the magnitude of the shift in $\delta^{15}N$ or $\delta^{13}C$ and animal age at death (OLS regression, $p = 0.15$ and $p = 0.91$ respectively), but the magnitude of ontogenetic shift in $\delta^{15}N$ values was significantly lower in the 2000s group compared to the 1970s/1980s group (OLS regression, $p < 0.001$). In general, stable isotope space occupied by juveniles appeared to be smaller than that of adults (Fig. 5). Mean standard ellipse area of adult samples

Table 2. Akaike information criteria metrics for candidate models describing variation in juvenile grey seal stable nitrogen and carbon isotope values recorded between the late 1940s and early 2000s. $\delta^{15}N_{corr}$ and $\delta^{13}C_{corr}$ refers to stable values corrected for a proxy of baseline variation by subtracting estimated annual $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ from measured $\delta^{15}N$ and $\delta^{13}C$ (corrected for the Suess effect).

Stable isotope	Covariates	AIC differences	AIC weight	β
$\delta^{15}N_{corr}$	Year	0	0.998	–0.025 (–0.04, –0.013)
	Intercept only	13	0.002	
$\delta^{13}C_{corr}$	Year	0	0.72	0.008 (0.0002, 0.015)
	Intercept only	1.89	0.28	

Table 3. Summary of mean (95% confidence intervals) measured stable carbon and nitrogen values, and corrected values. $\delta^{15}N_{corr}$ and $\delta^{13}C_{corr}$ refers to stable values corrected for a proxy of baseline variation by subtracting estimated annual $\delta^{15}N_{base}$ and $\delta^{13}C_{base}$ from measured $\delta^{15}N$ and $\delta^{13}C$ (corrected for the Suess effect, Körtzinger et al. 2003).

Group	Tooth portion	Mean $\delta^{13}C$ (95% CI)	Mean $\delta^{15}N$ (95% CI)	Mean $\delta^{13}C_{corr}$ (95% CI)	Mean $\delta^{15}N_{corr}$ (95% CI)	n
1970s/1980s	juvenile	–12.94 (–13.11, –12.77)	16.17 (15.87, 16.46)	4.72 (4.56, 4.87)	6.21 (5.93, 6.49)	44
1970s/1980s	adult	–14.57 (–14.98, –14.17)	17.89 (17.58, 18.21)	3.91 (3.51, 4.31)	8.54 (8.23, 8.86	44
2000s	juvenile	–13.45 (–13.67, –13.22)	15.52 (15.21, 15.82)	5.00 (4.77, 5.23)	5.49 (5.16, 5.81)	25
2000s	adult	–14.59 (–14.91, –14.27)	16.75 (16.47, 17.03)	4.06 (3.73, 4.38)	6.61 (6.33, 6.89)	25

was 5.04‰² (95% credible intervals: 3.87, 6.21) compared with an area of 1.73‰² (95% credible intervals: 1.33, 2.16) for juvenile samples.

Potential prey

To provide some dietary context for the interpretation of ontogenetic shifts, we present stable carbon and nitrogen bi-plots for major seal prey groups in the central North Sea collected as part of the present study and from a larger published dataset (Jennings and Cogan 2015) (Fig. 6, collected in 2009 and between 2002 – 2009 respectively). $\delta^{15}N$ and $\delta^{13}C$ values of potential grey seal prey in the North Sea fell along a positive axis with carnivorous organisms such as cod, whiting and saithe) generally having higher $\delta^{15}N$ values than those feeding predominantly on benthic invertebrates (plaice, lemon sole) and zooplanktivorous fish (haddock, sandeel, herring, poor cod) (see also dataset in Supplementary material Appendix 1 Table A2). Sandeel mean $\delta^{15}N$ was higher than expected given that these fish feed primarily on primary consumers, however sample size was low (n = 7, not sampled in the North Sea in Jennings and Cogan 2015).

Discussion

Three patterns emerged from the analysis: long-term decline in $\delta^{15}N$ values of the juvenile portion of grey seal teeth, apparent contraction in adult isotopic niche space since the North Sea regime shift, and ontogenetic shift in diet between juvenile and adult animals. We assumed that animals used in the analysis were a representative sample of the population in the central North Sea.

Partitioning observable stable isotope variation between behavioural (e.g. shifting diet or geographic location) and environmental (e.g. shifting baseline) mechanisms is a challenge for bulk tissue analyses with a temporal element. Here, we assume that statistical relationships between physical properties of the North Sea marine environment, such as temperature and depth, and baseline $\delta^{13}C$ and $\delta^{15}N$, are stable over time. However, hind-casting $\delta^{13}C_{base}$ and $\delta^{15}N_{base}$ based on temperature alone is an oversimplification of the complex hydrological and biological processes that drive the

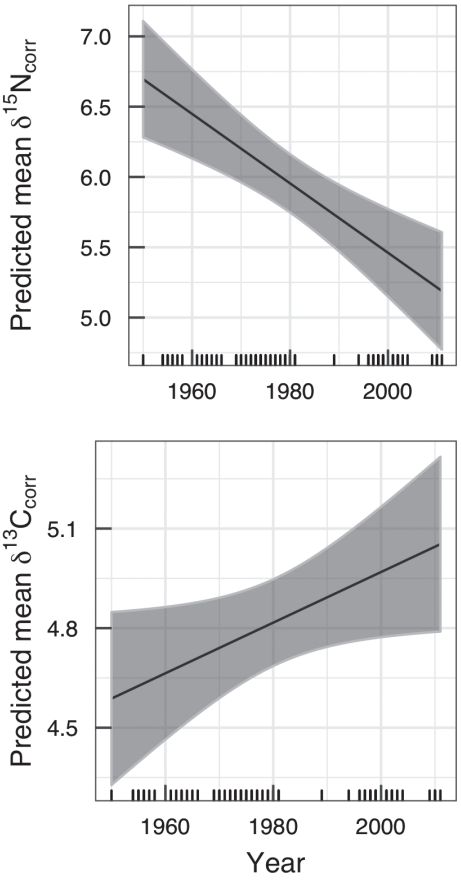

Figure 3. Linear trends in mean predicted $\delta^{15}N_{corr}$ (a) and $\delta^{13}C_{corr}$ (b) values retrieved from juvenile collagen in grey seal *Halichoerus grypus* canine teeth. The grey areas indicate 95% confidence intervals around the trend. $\delta^{15}N_{corr}$ and $\delta^{13}C_{corr}$ are observed values corrected for a proxy measure of baseline variability. Rug plots on the x-axis indicate individual data points.

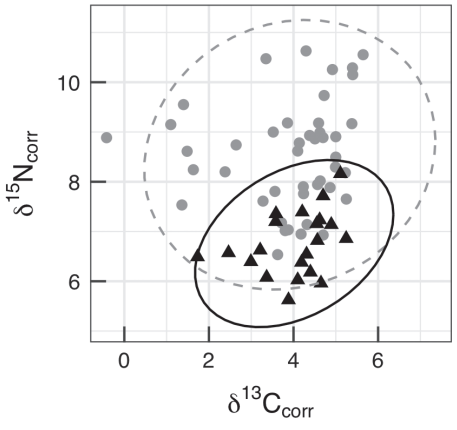

Figure 4. Standard 95% confidence ellipses of $\delta^{15}N_{corr}$ and $\delta^{13}C_{corr}$ values retrieved from adult collagen in grey seal *Halichoerus grypus* canine teeth collected in the 1970s/1980s (n = 44 from 1972 and 1981; grey points) and 2000s (n = 25 from 2005–2008, black triangles). $\delta^{15}N_{corr}$ and $\delta^{13}C_{corr}$ are observed values corrected for a proxy measure of baseline variability.

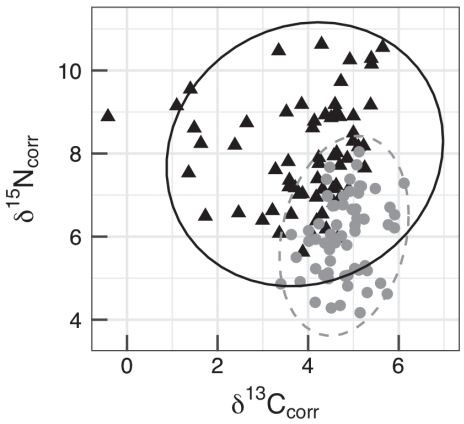

Figure 5. Standard 95% confidence ellipses of $\delta^{15}N_{corr}$ and $\delta^{13}C_{corr}$ values retrieved from juvenile (grey circles) and adult (black triangles) portions of individual grey seal *Halichoerus grypus* canine teeth (n = 64; 5 samples from animals < 5 years old were removed). $\delta^{15}N_{corr}$ and $\delta^{13}C_{corr}$ are observed values corrected for a proxy measure of baseline variability.

dynamics of stable isotope fractionation (Barnes et al. 2008, 2009, MacKenzie et al. 2014). Nevertheless, by incorporating a coarse proxy for baseline variability in $\delta^{13}C$ and $\delta^{15}N$ in our models, we aimed to provide a more robust historical record of grey seal stable isotope variation than would otherwise be available. This was especially important considering that the timeframe of stable isotope data obtained from archives and modern tooth samples spanned the 1980s,

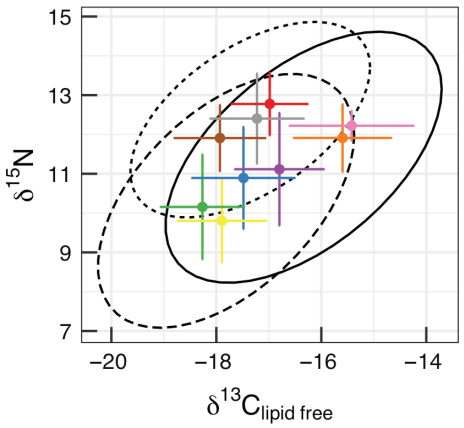

Figure 6. Stable carbon and nitrogen isotope bi-plot of grey seal *Halichoerus grypus* prey items collected in the North Sea. Data are from lipid extracted white muscle collected in 2009 as part of the present study (available in Supplementary material) and from lipid corrected white muscle collected between 2002 and 2009 (Jennings and Cogan 2015). 95% confidence ellipses are for three trophic groups based on fish diet: carnivorous fish (CF, dotted line), zooplankton-feeding fish (ZF, dashed line), and fish feeding on benthic invertebrates (BIF, solid line). Groupings were as in Das et al. (2003). Mean and 95% confidence intervals are also given for cod (red, *Gadus morhua*, n = 155, CF), haddock (blue, *Melanogrammus aeglefinus*, n = 169, ZF), herring (green, *Clupea harengus*, n = 130, ZF), lemon sole (purple, *Microstomus kitt*, n = 132, BIF), plaice (orange, *Pleuronectes platessa*, n = 82, BIF), poor cod (yellow, *Trisopterus esmarkii*, n = 181, ZF), saithe (brown, *Pollachius virens*, n = 144, CF), sandeel (pink, *Ammodytes tobianus*, n = 7, ZF), and whiting (grey, *Merlangius merlangus*, n = 196, CF).

a critical period of regime shift in the North Sea characterized by a switch from a cold-dominated regime to a warmer one (Beaugrand 2004). However, without empirical stable isotope variation in primary producers, we cannot exclude the possibility that the decline in consumer $\delta^{15}N$ reflects a shift in ^{15}N abundance in marine primary producers over time rather than a trophic shift.

Long-term trends in juvenile signal

Accounting for baseline changes in nitrogen stable isotope values, juvenile values declined by an average of 0.25‰ decade^{-1}, or 1.51 ‰ over the years represented by the tooth archive. As $\delta^{15}N_{base}$ could not be measured directly over time, a quantitative estimate of shift in trophic position cannot be reported, but the decline can be broadly interpreted as a relative decrease in trophic level over the study period. Assuming an average trophic shift of ca 3‰ (Newsome et al. 2010) the decline amounts to about half of a trophic level. The prey data presented in Fig. 5 are of limited value in interpreting temporal changes in juvenile $\delta^{15}N_{corr}$ because they do not overlap in time. However, while the absolute values cannot be extrapolated to historic seal data, the trophic relationships between, for example, sandeels (which feed on zooplankton) and species such as cod and whiting (which are more carnivorous), are unlikely to change over time. Broadly speaking, the decline in juvenile $\delta^{15}N_{corr}$ values can be interpreted as a shift from upper trophic level carnivorous species towards relatively lower trophic level planktivorous species.

Interpretation of declining juvenile grey seal $\delta^{15}N_{corr}$ values as a relative decline in trophic level is supported by results of traditional diet analysis and long-term assessments of the North Sea fish community. Sandeels are the predominant prey item for grey seals, and their relative importance in the diet of grey seals in the central North Sea has increased (up to 78.5% of the diet by weight in 2010/2011 compared to 63.9% and 62.2% in two previous assessments in 1985 and 2006 respectively; Hammond and Wilson 2016). Accounting for fish size, gadids tend to occupy higher trophic levels than sandeels or flatfish (Jennings et al. 2002). The observed decline in grey seal tooth $\delta^{15}N_{corr}$ values is consistent with the reduction in the relative importance of gadids in the diet of grey seals (down to 8.4% in 2010/11 from 29.5% in 1985) determined by faecal analysis (Hammond and Wilson 2016). Furthermore, broad-scale assessments of long-term research trawl data have demonstrated an unequivocal decrease in the size and stock biomass of large predatory fish in the North Sea (Speirs et al. 2016). A decline in the availability of large upper trophic level gadids (cod and saithe) may prompt grey seals to switch to other more available prey, such as sandeels (Smout et al. 2014).

Declining nitrogen stable isotope values also are consistent with an overall reduction in prey size available to grey seals. In addition to evidence for reduction in the size of large predatory fish due to fishing (Speirs et al. 2016), the length-at-age of lesser sandeels *Ammodytes marinus* retrieved from puffins *Fratercula artica* in the central North Sea declined by over 11 mm between 1973 and 2002 (Wanless et al. 2004). Trophic level, fish size, and tissue $\delta^{15}N$ values are explicitly linked (Jennings et al. 2008). Drivers for the decline in grey

seal tooth $\delta^{15}N$ values could be a combination of reduction in the size of prey available, and a shift away from foraging on larger prey species. This trend has been replicated in other marine predators sympatric with grey seals in the North Sea; both demersal fish community and harbour porpoise *Phocoena phocoena* $\delta^{15}N$ values declined over the 20th century (Jennings et al. 2002, Christensen and Richardson 2008), consistent with the present findings.

Long-term shifts in adult stable isotopic niche

As with juveniles, adult $\delta^{15}N_{corr}$ values declined between samples collected in the 1970s/1980s and the 2000s, perhaps driven by the same mechanisms of potentially declining availability of larger prey and a shift over time to foraging of prey species at lower trophic levels. While the more recent sample values were within the range of older samples, they were less diverse, and when combined with the changes in adult $\delta^{13}C_{corr}$, showed a contraction in stable isotopic niche area. This suggests that adult grey seals experienced a decline in trophic position and change in foraging habits over the study period. Mean adult grey seal $\delta^{13}C_{corr}$ values did not show a significant change over time (Fig. 4). However, carbon stable isotope values in animals collected in the 2000s were less variable compared to the 1970s/1980s. $\delta^{13}C$ values in the marine environment can be broadly related to foraging locations due to variation along distance (e.g. nearshore versus offshore), latitudinal and depth (e.g. benthic versus pelagic) gradients.

Whilst adult mean $\delta^{13}C_{corr}$ values remained consistent over time, the contraction in isotopic niche space is indicative of a less diverse diet, both in terms of composition and foraging location. Changes in available prey composition are unknown but one interpretation of a contraction in foraging range could be a greater reliance on sandeels, which occur in patchy aggregations at the seafloor (Wright et al. 2000). Trophic changes found in this study corroborate those in Hammond and Wilson (2016), and occur against a striking demographic backdrop; grey seal pup production in the North Sea is growing exponentially. There were less than 2000 pups estimated in the early 1980s, whereas in 2012 the estimate was greater than 10 000 pups (SCOS 2015). In theory, an increase in intraspecific competition with higher population densities may result in contracted foraging ranges for individuals departing from particular haulouts (Adams 2001, Morales et al. 2010). However, changes in individual foraging behavior, and the relationship to diet and intraspecific competition are still not well understood for this species.

It is difficult to assess the impact of the 1980s North Sea regime shift on upper trophic level predators such as seals. As long-lived, generalist and highly mobile predators, seals should be relatively buffered from short-term ecosystem perturbations. Rising marine temperatures have caused major biogeographic shifts in the distribution of several plankton and nekton species and are associated with poor survival conditions for larval cod (Beaugrand et al. 2003b, Beaugrand 2004), a species that was abundant in the diet of grey seals during the 1980s (Hammond et al. 1994). However, overfishing of species that are historically important in grey seal diet may have had synergistic effects on these predators (Kirby et al. 2009), contributing to the observed decline in average trophic level.

Ontogenetic change

The final pattern to emerge from the study is a clear increase in $\delta^{15}N$ and decrease in $\delta^{13}C$ values between juvenile and adult life-stages, indicative of an ontogenetic shift in diet. The magnitude of the shift in stable isotope values did not vary with age-at-death, suggesting little further change in foraging habits as animals continue to age past sexual maturity. However, animals collected in the later part of the time series (2000s) were more likely to have a smaller shift in $\delta^{15}N$ values than earlier samples (1970s/1980s). This could be related to stable isotopic variability that was in general lower for adults sampled in the 2000s (Fig. 4). Some individuals did not follow the general pattern of higher $\delta^{15}N$ values and lower $\delta^{13}C$ values into adulthood; we could find no common factor linking these individuals but in the case of $\delta^{13}C$ it is possible that our correction for the Suess effect, which raises $\delta^{13}C$ values obtained later in the time series, was in effect an over-correction causing adult $\delta^{13}C$ values to be more positive than juvenile values.

In general, the positive shift in $\delta^{15}N$ values between juvenile and adult lifestages is indicative of foraging at higher trophic levels. There is some trophic fractionation of $\delta^{13}C$ (ca 1‰ per trophic level; Newsome et al. 2010) but if trophic position were driving $\delta^{13}C$ values, we would expect adult $\delta^{13}C$ values to be higher than those of juveniles. We found the opposite (ca 0.87‰ decrease in $\delta^{13}C$ values), which suggests a degree of spatial segregation in foraging areas between younger and older (sexually mature) animals. While not explicitly tested here, the more positive $\delta^{13}C$ values retrieved from the juvenile portion of grey seal teeth may signify a greater reliance on carbon sources derived from areas relatively closer to shore and/or more benthic prey.

Due to the inability to discriminate age classes in faecal collection, and limited information on juvenile grey seal diet and movement, little is known about the diet and distribution of grey seals that have not yet recruited into the breeding population (Jones et al. 2015, Gallon et al. 2017). Tucker et al. (2007) found evidence for diet segregation between adult and juvenile (mostly yearlings) grey seals on Sable Island, Nova Scotia, Canada from carbon and nitrogen stable isotope values of skin samples. However, at the same location Beck et al. (2007) sampled six-month old juvenile fatty acid signatures and found a high degree of overlap with adult diet, although juvenile fatty acid signatures were significantly more varied than for adults. The authors speculate that naïve juveniles would be more unlikely than adults to show preference for particular prey groups as they learn to forage and develop dive capabilities. Our stable isotope samples represented more years of juvenile life (up to five years) and the results indicated more isotopic variability amongst adults compared with juvenile, albeit with considerable overlap (Fig. 5). This suggests that adults feed on a more varied diet, both in terms of trophic level and space, than juveniles. At Sable Island, young grey seals (< 1 year) had lower mass-specific oxygen stores and aerobic dive limits than adults, suggesting that juvenile diving capabilities may be limited (Noren et al. 2005), and telemetry data from

similarly aged seals indicated they can be excluded from foraging areas near breeding colonies (Breed et al. 2013). However, telemetry data from sexually immature (based on age–length relationships) juvenile grey seals suggested the proportion of time seals spent diving and the probability of travelling throughout the year was similar between adults and juveniles (Russell et al. 2015). The differences we found in stable isotope values between juveniles and adults may therefore reflect differences in prey acquisition capabilities or preference rather than a physiological disparity in diving capability.

Stable isotope data obtained from a single tooth explicitly links stable isotope variation over time for the same individual. Female grey seals reach sexual maturity aged 5–6 years (Hewer 1964), and so the juvenile life-stage in the present study represented pre-breeding females, and the adult life-stage represented breeding females. If female grey seals were to show ontogenetic changes in diet, it would most likely be detectable at this transition point where there is an increased demand on females to obtain sufficient energetic stores for gestation and lactation (Fedak and Anderson 1982). Larger fish tend to occupy higher trophic levels (Jennings et al. 2008) and to have higher energy densities (Pedersen and Hislop 2001). It is possible that females help meet the energetic costs of reproduction by including relatively more of these prey items in their diet.

Conclusions

Our stable isotope results suggest dietary segregation exists between juvenile (sexually immature) and adult grey seals. The observed trajectory of stable isotope change between life-stages would suggest that pre-breeding individuals feed at lower trophic levels (lower $\delta^{15}N$) and from carbon sources derived from more nearshore or benthic areas (higher $\delta^{13}C$). As central-placed foragers, nearshore areas close to grey seal haulouts are well utilized by adults (Jones et al. 2015). However, our results suggest nearshore areas may contribute more in terms of macronutrients to juveniles than to adults in the North Sea. This information, if confirmed through studies of habitat use and additional diet information (Flaherty and Ben-David 2010), could be pertinent to marine spatial planning, for example, when considering the placement of coastal anthropogenic developments.

Comparing stable isotope values over several decades showed a general decline in the trophic position grey seals coincident with a reduction in the breadth of prey available to them, or with a shift towards greater reliance on fewer prey types. We find concordance of the present stable isotope results with faecal analyses, changes in the North Sea fish community, and other stable isotope studies to be compelling evidence to suggest a real shift in grey seal diet over the 20th century. However, in the future, developments in compound-specific stable isotope analyses may go some way towards alleviating the problem of shifting baselines in long-term stable isotopic studies (Ohkouchi et al. 2015).

Grey seals generalist diet is likely an advantage in a dynamic marine ecosystem where prey populations can exhibit rapid and large fluctuations; their diet broadly reflects what is abundant and geographically available to them (Engelhard et al. 2014, Smout et al. 2014). Despite the long-term changes to their diet, grey seals ability to successfully respond to changing trophic conditions is evidenced by their continued population growth.

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Supplementary material (available online as Appendix oik-04441 at <www.oikosjournal.org/appendix/oik-04441>). Appendix 1.