Stable-isotope based location in a shelf sea setting: accuracy and precision are comparable to light-based location methods.

Clive N. Trueman¹, Kirsteen M. MacKenzie¹,², Katie St John Glew¹

Running title: Stable isotope-based location in shelf seas

Word count: 6986

Ocean and Earth Science, University of Southampton Waterfront Campus, Southampton SO143ZH

Institute for Marine Research, Tromsø, Norway

CN Trueman: email Trueman@noc.soton.ac.uk
1. Retrospective determination of location for marine animals would facilitate investigations of migration, connectivity and food provenance. Predictable spatial variations in carbon and nitrogen isotopes in primary production across shelf seas provide a basis for stable isotope-based location.

2. Here we assess the accuracy and precision that can be obtained through dietary-isotope based location methods. We build isoscapes from jellyfish tissues and use these to assign scallops of fixed and known individual location, and herring with well-understood population-level distributions in the North Sea.

3. Accuracy and precision for retrospective isotope-based location in the North Sea was of a similar order to light-based location devices, with 75% of individual scallops assigned correctly to areas representing c.30% of the North Sea, with a mean linear error on the order of 10^2km. Applying assignment methods to an alternative migratory species (herring) resulted in ecologically realistic assignments consistent with fisheries survey data.

4. Location methods based on dietary isotopes such as carbon and nitrogen recover the spatial origin of nutrients assimilated into tissues and this may not correspond directly to the physical location if either the test animal or its prey is highly migratory. Stable isotope based location can be applied to any marine-feeding organism or derived food product, but the ecological meaning of any assigned area will be more difficult to interpret for large, high trophic level, migratory animals with relatively slow isotopic assimilation rates.
Key-words: Isoscape, assignment, provenance, connectivity, migration, spatial, marine, geolocation
Introduction

Understanding animal movements is fundamental to population dynamics, predator-prey relationships, nutrient and energy fluxes within food webs and management of human-animal interactions. In comparison to terrestrial animals, marine (and aerial) animals encounter relatively few static, physical barriers to movement and dispersal over areas large in comparison to body sizes is a common phenomenon. In the context of marine fisheries, mislabelling of fishery products has emerged as a major problem on global markets (Marko et al. 2004; Wong and Hanner, 2008; Nielsen et al. 2012, Cawthorn et al. 2012). Tracing marine food from origin to sale is a key aim of regulatory organisations worldwide. At present there are few effective retrospective analytical methods available to test claims of spatial origin of traded seafood.

Marine spatial ecology is undergoing a revolution with rapid developments in telemetry and electronic tagging technology with the deployment of large static acoustic arrays, satellite geo-location and the development of ever smaller less invasive data storage tags (Hunter et al. 2003; Righton et al. 2007; Block et al. 2011). Nonetheless, direct tagging of marine animals still requires capture and recovery of tags, and processing of data, and is relatively costly (Ramos & Gonzalez-Solis 2012). Furthermore, while tagging experiments reveal individual movements in high resolution, by definition, they cannot be applied retrospectively. Natural tags provide an attractive supplement to direct location tools. Natural location methods typically attempt to link the chemical (or parasite) composition of the test animal’s tissues to known spatial variations in the environment (Hobson 1999, Graham et al. 2010, Seminoff et al.
In recent years, stable isotope location has proven effective at reconstructing long-distance migrations in terrestrial, particularly avian, ecology (Rubenstein & Hobson 2004; Wunder and Norris 2008; Hobson et al. 2012). Statistical models of spatial variation in the isotopic composition of precipitation (Bowen, 2010), vegetation (West et al. 2007; Still & Powell 2010, ) and higher taxa tissue (Vander Zanden et al. 2015) have been developed in many environments and termed isoscapes. Such isoscapes can provide a base model to assign geographic origin to a tissue of interest, following calibration between the media used to construct the isoscape and the species and tissue to be assigned (Wunder & Norris 2008). A relatively mature literature has developed describing the construction of isoscapes, the statistical considerations surrounding geographic assignment based on isoscapes, and application of isoscapes to track animal movements (West et al. 2010).

Isotope-based location comprises a geo-statistical spatial model, a calibration between the model and the species and tissue to be assigned, and a probabilistic comparison between model and measured data. Isoscapes derived from the same species and tissues as those that will be assigned in theory provide the most robust method of assignment. However, the practical and financial limitations associated with sampling and analysing tissues of each migratory species across the full potential foraging range are considerable. Therefore the potential for isotope-based geo-location is greatly increased if multiple taxa can be referred to a single isoscape model. The accuracy and precision available for isotope-based location therefore depends on the variance associated with the underlying geostatistical isoscape model, in situ variability in the isotopic compositions of both the organism used to construct the model and
in the tissues to be assigned, and the uncertainty inherent in linking the isotopic compositions of the tissues to be assigned to the baseline isoscape (i.e. calibration of the isoscape to the tissue of interest, Wunder & Norris 2008). Considerable debate remains around the most effective way to incorporate error and uncertainty into stable isotope-based geographic assignment methods (Wunder & Norris, 2008; Van Wilgenburg et al. 2011; Wunder 2012; Bowen et al. 2014, Vander Zanden et al. 2015). Wunder (2008) provides a thorough review of the assumptions inherent in isotope-based location, focussing on hydrogen and oxygen isotope based geo-assignment specifically in migratory birds.

Isotope-based location is not as well developed in marine settings and very few robust assessments of the accuracy and precision obtained using isotope based location have been developed in marine settings (Vander Zanden et al. 2015). In marine systems oxygen and hydrogen isotopes are relatively spatially constant, so alternative isotope systems are needed to provide spatial information (Trueman et al. 2012). The isotopic composition of carbon and nitrogen in marine primary production is predictably heterogeneous over spatial scales ranging from tens to thousands of kilometres (Jennings and Warr, 2003; Somes et al. 2010; McMahon et al. 2013; Radabaugh et al. 2013, Jennings & van der Molen 2015), and is passed through the food chain. Assigning location based on carbon and nitrogen isotopic compositions therefore effectively tracks the spatial origin of primary production fuelling higher trophic level production rather than the direct spatial location of the animal tested. Nonetheless carbon and nitrogen isotopes have been used extensively to track animal movements

Marine carbon and nitrogen isoscape models are generated by interpolation from spatially explicit samples (Schell et al. 1998; McMahon et al. 2013). Sessile invertebrates such as filter feeding bivalves have often been used to produce spatial isotope models (e.g. Jennings & Warr, 2003). However, the distribution of sessile invertebrates is limited by water depth and substrate type resulting in systematic variance in spatial coverage of reference samples across the study region. Environmental correlates such as water temperature, depth and salinity have been used to predict isotopic compositions in areas with no reference samples (Jennings & Warr 2003; Barnes et al. 2009; MacKenzie et al. 2014), but the resulting isoscape models are strongly dependent on the location of the reference samples and the assumption that regression relationships between environmental drivers and isotope values derived in the sampled region are constant throughout the wider study area. The uncertainty associated with any predicted isotope value increases with (a) the error associated with the regression model, (b) the spatial distance from the reference sites and (c) isotopic or environmental differences between conditions at the predicted site and the mean of the combined reference sites. Estimating the spatially varying uncertainty associated with regression-based isoscape models is not trivial (Bowen & Ravenaugh 2003), and has not been attempted for marine isoscapes. An alternative approach lies in selecting pelagic reference organisms that are widely distributed, but may have larger between-individual variance associated with movement or diet ecology. Scyphomedusan jellyfish provide an attractive potential target due to their ubiquitous distributions, rapid growth and short
lifespans (MacKenzie et al. 2014). While scyphomedusan jellyfish are mobile, movement is relatively passive and isotopic assimilation rates are fast. The isotopic half-life for the moon jellyfish Aurelia aurita, for example, is estimated at c.10 days (D’Ambra et al. 2014). The distance travelled by jellyfish during the window of isotopic assimilation is therefore likely to be short compared to the spatial scales of isotopic variance in open waters. Jellyfish may be a poor choice for spatial isotope modelling in coastal areas where isotopic variability occurs at smaller spatial scales.

Here we assess the precision and accuracy associated with using spatial gradients in carbon and nitrogen isotopes to assign origin to animal tissues across a relatively large shelf sea area. We derive carbon and nitrogen isoscape from lion’s mane jellyfish Cyanea capillata expanding on the dataset and methods outlined in (MacKenzie et al. 2014, Fig. 1). The North Sea is a shallow semi-restricted shelf sea in the North Atlantic ocean with a total area of around 650,000km², sustaining one of the productive fisheries in the world. The North Sea comprises a seasonally-stratified northern basin with a mean depth >50m, and a shallower southern basin that is not stratified. In this study we quantify the accuracy and precision associated with isotope-based geo-location in the North Sea using two independently-determined datasets of stable isotope compositions of the sessile queen scallop Aequipecten opercularis (Jennings et al. 2002, Jennings & van der Molen, 2015). We then identify feeding locations of 351 herring Clupea harengus caught at known locations throughout the North Sea.

**Materials and methods**
STABLE ISOTOPE SAMPLES

Following methods described in MacKenzie et al. (2014), 66 individuals of *C. capillata* were sampled from 52 stations in the North Sea in August 2015 during the International Bottom Trawl Survey on board the RV Cefas Endeavor. Jellyfish were collected, weighed and measured, and a section of bell tissue (mesoglea) removed and immediately frozen. Jellyfish ranged in size from 80 to 240mm in diameter (mean = 107mm, $\sigma = 3.25$mm). In the laboratory, tissues were washed 3 times with water to remove any soluble nitrogenous materials, re-frozen prior to freeze-drying, sub-sampling and submission for isotopic analyses. Capture locations, body sizes and isotope data for jellyfish tissues are reported in Table S1 and locations are illustrated in Fig. 1.

351 individual herring were captured at 41 known locations within the North Sea during September 2011 as part of the International Bottom Trawling Survey. Fishing was conducted from the R.V. “Cefas Endeavor”. After capture, herring were weighed, dorsal muscle was excised and frozen prior to analysis. Herring under 200mm standard length were grouped as ‘small’ fish, likely to represent juveniles, whereas fish greater than 200mm standard length are likely to be mature (ICES, 2012). Muscle samples were freeze dried, ground to a powder and analysed for carbon and nitrogen isotopic composition. Capture locations, body sizes and isotope data for herring muscle are reported in Table S2 and locations are illustrated in Fig. 2.
Analyses were performed by either OEA laboratories or Elemtex laboratories, Cornwall, UK. Accuracy and precision were monitored through laboratory internal standards (USGS 40 and USGS 41 and a bovine liver standard) and repeat blind analyses of an in-house comparison standard (ARCOS glutamic acid) nested within samples. Accuracy in both laboratories for δ\(^{13}\)C and δ\(^{15}\)N values was within 0.1‰ of long-term average values for this standard, and precision was 0.2‰ for δ\(^{13}\)C and 0.17‰ δ\(^{15}\)N values.

Jellyfish bell tissue δ\(^{13}\)C values showed a significant negative linear relationship with C:N ratios (\(p = 4.54 \times 10^{-5}\), slope = -0.047, Adjusted R\(^2\) = 0.2), implying a variance component related to the concentration of isotopically light lipids within the sample. To correct for potential lipid-related variance in δ\(^{13}\)C values, measured δ\(^{13}\)C values were adjusted to those predicted for a lipid-free protein (atomic C:N ratio of 3.4) using linear regression between δ\(^{13}\)C values and C:N ratios. We did not apply alternative arithmetic lipid correction terms as the measured C:N ratios are close to those expected from pure protein with a small range (mean = 3.6, \(\sigma = 0.15\)) implying that linear corrections are equally effective (Kiljunen et al. 2006), and we therefore prefer to use correction terms derived from the species and individuals studied. Lipid-corrected δ\(^{13}\)C values of jellyfish show a positive correlation with bell diameter, accordingly they were normalised to the median diameter (107mm):

\[
\delta^{13}C_{s, \text{cor}} = \delta^{13}C_{\text{cor}} + (\text{Diameter} - 10.73) \times 0.19
\]
eqn 1
Herring muscle contained varying C:N ratios, and δ\textsuperscript{13}C values were corrected for lipid content arithmetically (Kiljunen et al. 2006).

Isotopic data from queen scallops was recovered from Jennings & Warr (2003) and Barnes et al. (2009) for scallops sampled between 25 July and 29 September 2001, and from S. Jennings (pers. comm. 2016) for scallops sampled in similar locations in the summer of 2010 (Jennings & van der Molen, 2015). Up to seven individual scallops were sampled in each area. Locations of capture sites are shown in Fig. 2, Details of sampling, preparation and analytical methodologies are provided in Jennings & Warr (2003), Barnes et al. (2009) and Jennings & van der Molen, 2015.

STATISTICAL ASSIGNMENT METHODS

Statistical models of spatial variation in the isotopic composition of carbon and nitrogen in jellyfish tissues sampled in 2015 were drawn from the lipid and size-corrected isotope data using Linear Kriging. Isoscapes are presented in Fig. 1 together with the associated spatial variances, and locations of jellyfish sampled to create the isoscapes. Raster files of the isoscape values are provided as supplementary data.

In isotope-based Geo-assignment, the likelihood or probability of the sample originating from a given location or cell in the isoscape depends on the isotopic difference between the sample and cell value relative to the total variance in the isoscape. As described above, much of the difficulty associated with isotope-
based location lies in quantifying sources of variance, a problem that is particularly acute when using environmental correlates to extend predictions into regions with no reference samples.

As our isoscape model does not contain values predicted from regression models, variance associated with the isoscape is composed of a spatially varying term related only to the physical distance between sample points estimated from the kriging process, and a fixed term reflecting measurement error and between-individual variance (Bowen et al. 2014). Measurement error associated with jellyfish analyses determined as the standard deviation from 13 replicate analyses of the glutamic acid standard was 0.2‰ for δ^{15}N and 0.1‰ for δ^{13}C analyses. Between-individual variances in jellyfish isotope compositions were estimated from jellyfish sampled both in 2011 (MacKenzie et al. 2014) and in 2015 as 1.69‰ and 1.04‰ for δ^{13}C_{cor} and δ^{15}N values respectively. These between-individual variance estimates are similar to those provided for gelatinous zooplankton by Nagata et al., (2015) and Fleming et al., (2015), particularly when accounting for the marked effect of size on isotopic variance in the Fleming et al. (2015) data. Total uncertainty in the assignment isoscape was given by:

\[ \sigma^2_{iso \ (x,y)} = \sigma^2_{k.iso \ (x,y)} + \sigma^2_{m.iso \ (x,y)} + \sigma^2_{bi.iso \ (x,y)} \quad \text{eqn 2} \]

where \( \sigma^2_{iso \ (x,y)} \) is the pooled variance associated with the isoscape prediction, \( \sigma^2_{k.iso \ (x,y)} \) is the variance associated with the spatial interpolation model, \( \sigma^2_{m.iso} \)
$(xy)$ is the variance associated with measurement error and $\sigma^2_{bi.iso \ (x,y)}$ is the variance associated with in situ between-individual variation.

Measurement error associated with $\delta^{15}$N analyses of scallop tissues was $<0.2\%$, and the mean standard deviation between individual scallops was 0.8%, similar to between-individual variance in $C.\ capillata\ \delta^{15}$N values

(Jennings & Warr 2003, Jennings & van der Molen 2015). We estimate associated measurement precision associated with $\delta^{13}$C values in scallop tissues as 0.2%, similar to measurement errors associated with $\delta^{13}$C analyses of $C\ capillata$.

Between-individual variance in lipid-corrected $\delta^{13}$C values of scallops across 22 stations sampled in 2010 was 0.21% (Jennings pers. comm. 2016).

Pooled error associated with the measurement of scallop stable isotope compositions is therefore given by:

$$\sigma^2_{assign \ (x,y)} = \sigma^2_{m.assign \ (x,y)} + \sigma^2_{bi.assign \ (x,y)}$$  \hspace{1cm} eqn 3

where $\sigma^2_{assign \ (x,y)}$ is the pooled variance associated with the isoscape prediction,

$\sigma^2_{m.assign \ (x,y)}$ is the variance associated with measurement error and $\sigma^2_{bi.assign \ (x,y)}$ is the variance associated with in situ between individual variation.

Uncertainties associated with calibration between the isoscape model and the tissue to be assigned were estimated from the combined uncertainty associated with trophic separation and trophic fractionation between jellyfish and scallops (e.g Wunder & Norris 2008). Trophic separation between jellyfish and scallops was constrained from known diet preferences. Scallops are filter-feeding molluscs sustained primarily on detrital phytoplankton and
microzooplankton. Lion’s mane jellyfish are opportunistic pelagic predators consuming a range of macro-zooplankton and larval/juvenile fish. The jellyfish sampled in 2015 encompassed a relatively narrow size range from 80 to 240mm bell diameter equivalent to a wet mass of c. 100-500g, and no systematic size-related difference in trophic level between sampled individuals is expected (Fleming et al., 2015). Mass balance (Ecopath) modelling of the North Sea community (Mackinson & Daskalov, 2007) estimates scallop and gelatinous zooplankton trophic levels as 2.8 and 3.6 respectively. We therefore estimate the trophic distance between *C. capillata* and *A. opercularis*, as a single trophic level and assign uncertainty to that estimate with standard deviation of 0.25, ensuring that 95% of the estimates of trophic distance between scallops and *C. capillata* fall between 0.5 and 1.5 trophic levels.

Isotopic fractionation between tissue and diet (trophic fractionation) is estimated as 3.4‰ for nitrogen and 1‰ for carbon (Vander Zanden & Rasmussen, 2001) with a standard deviation of 0.5‰ ensuring that 95% of the estimates of isotopic trophic fractionation fall between 2.4 and 4.4‰ for nitrogen and between 0 and 2‰ for carbon. We then created 10,000 trophic fractionation and trophic distance values drawn from the distributions described above and estimated the distribution of isotopic separation values between jellyfish and scallops.

Scallop muscle and jellyfish bell tissue have contrasting biochemical compositions and therefore have potential for additional isotopic offsets. We do not know of any studies reporting isotopic discrimination between jellyfish bell tissue and coexisting muscle while accounting for trophic level. As all scallops are known to derive from the isoscape area, trophic-level corrected values
should lie within the total range of isotopic values within the isoscape. We therefore compare trophic level-corrected scallop data to the full range of $\delta^{13}C$ and $\delta^{15}N$ values contained in the isoscape, and apply the smallest offset term required to ensure that all measured scallop values lie within the range described by the isoscape. For scallops we therefore apply an additional tissue-specific adjustment of $+1\%$ ($\sigma = 0.5$) for $\delta^{15}N$ and $+0\%$ ($\sigma = 0.5$) for $\delta^{13}C$ values. The final correction also accounts for any systematic under or over-estimation of trophic differences or isotopic fractionation. The estimated variance associated with calibration between scallop and jellyfish tissues $\sigma^2_{calib}$ is therefore composed of the variance in estimated isotopic spacing across the 10,000 draws and the estimated variance around the remaining tissue calibration offset

$$\sigma^2_{calib(x,y)} = \sigma^2(TD \ast TF_{(x,y)}) + \sigma^2_{off (x,y)} \quad \text{eqn 4}$$

where $x$ and $y$ refer to $\delta^{13}C$ and $\delta^{15}N$ values respectively, TD is the distribution of trophic difference values, TF is the distribution of isotopic fractionation values and $\sigma^2_{off}$ is the estimated variance associated with the tissue offset.

We apply the method outlined above to quantify variance terms associated with assigning herring to the same $C.\ capillata$-defined isoscape. Herring are gape-limited zooplankton feeders with a similar diet and trophic level to predatory jellyfish. Ecopath modelling assigns herring a trophic level of 3.4 and gelatinous zooplankton a trophic level of 3.6 (Mackinson & Daskalov 2007). We therefore assign a trophic difference between herring and $C.\ capillata$ of -0.5 with a standard deviation of 0.5. Multiple individuals were sampled in all
41 locations, and mean between-individual standard deviations were 0.44‰ for δ15N and 0.39‰ for lipid-corrected δ13C values. Minimum tissue offset values between herring and jellyfish were estimated as described above as +2‰ (σ=0.5) for δ13C and +0.5‰ (σ 0.5) for δ15N. A summary of assignment conditions is provided in Table 1.

We follow the assignment approach outlined in Vander Zanden et al. 2015:

\[
f(x, y | \mu_i, \Sigma) = \frac{1}{(2\pi \sigma_x \sigma_y \sqrt{1 - \rho^2})} \times \exp \left( -\frac{1}{2(1 - \rho^2)} \left[ \frac{(x - \mu_x)^2}{\sigma_x^2} + \frac{(y - \mu_y)^2}{\sigma_y^2} + \frac{2\rho(x - \mu_x)(y - \mu_y)}{\sigma_x \sigma_y} \right] \right)
\]

where \( f(x, y | \mu_i, \Sigma) \) represents the probability that an individual with adjusted isotopic composition (δ13C=x and δ15N=y) originates from a given cell (i) within the isoscape with mean isotopic composition equal to the components of vector \( \mu_i \), and variance co-variance matrix \( \Sigma \). \( \rho \) is the correlation between δ13C and δ15N values throughout the isoscape, \( \sigma_x \) and \( \sigma_y \) are the pooled standard deviations in δ13C and δ15N values respectively given by the sum of the variances:

\[
\sigma_{(x,y)} = \sqrt{ (\sigma_{iso}^2 (x,y) + \sigma_{assign}^2 (x,y) + \sigma_{off calib}^2 (x,y)) }
\]
The range in pooled error terms across the isoscape for scallop assignment was 3.5-12.4‰ for δ\(^{13}\)C values and 5.5-11.6‰ for δ\(^{15}\)N values, approximately three times higher than the pooled error estimates provided by Vander Zanden et al. (2015) where no calibration was needed between isoscape and assignment tissue.

DISPLAYING ASSIGNMENT OUTCOMES

The outcome of stable-isotope based location can be displayed as continuous surfaces, but it is easier to describe accuracy and precision based on discrete assignments to a probable area defined by a probability threshold (i.e. an area containing all sites with an assignment probability higher than an arbitrarily fixed value). We use odds ratios to set threshold values (Van Wilgenburg et al. 2012, Vander Zanden et al. 2015). The odds of an event occurring is given by the probability of the event occurring relative to the probability of that event not occurring (or P/1-P). Thus a likely event has high odds. Here we define the odds ratio as the ratio of odds of the outcome occurring compared to the odds of the most likely outcome possible given the available data:

\[
\text{Odds Ratio} = \frac{(P/1-P)}{(P/1-P_{\text{max}})}
\]

eqn 7

By setting an odds ratio threshold, all cells with probability values greater than the threshold are defined as cells of likely origin. The reciprocal of the odds ratio gives the total proportion of data (and thus the total proportional area) expected within the threshold limit according to the normal probability density function.
For instance, an odds ratio threshold of 2:1 includes all cells representing the most likely $2^{\frac{1}{2}} = 50\%$ of all data outcomes and defines a region of likely origin that is 50% of the total isoscape area. The precision of isotope-based assignment is thus defined by the odds ratio threshold, and the accuracy is given by the proportion of assigned individuals where the true location is contained within the assigned area (Vander Zanden et al. 2015).

### Results

#### ISOSCAPES

The spatial isotope models (isoscapes) derived from *C. capillata* are shown in Fig. 1 A,B. Broad spatial patterns are similar to those shown in Jennings et al. 2003; Barnes et al. 2009; MacKenzie et al. 2014 and Jennings & van der Molen 2015, indicating consistent and temporally stable spatial isotopic gradients, and isotopic ranges that are conserved between pelagic and benthic feeding organisms. The newly derived isoscapes are drawn from samples with relatively regular spacing across the modelled area, and the variance associated with the new isoscope models is relatively low and constant across the region Fig 1 C,D.

#### ASSIGNMENT ACCURACY AND PRECISION

The accuracy associated with assigning a geographic origin to the two temporally distinct scallop tissue datasets considering uncertainties in calibration terms and between-individual variance is shown in Fig. 3. The assignment method provides better than random accuracy at all odds ratio
thresholds (Fig. 3). Assignments are >90% accurate when assigning to areas that on average represent >40% of the total area of the North Sea. Precision is enhanced at the expense of accuracy: Doubling the assignment precision to areas encompassing 20% of the total North Sea reduces accuracy to 50%. The mean linear error between the cell of maximum likelihood and the known location was 226 (σ = 137) km for the 2001 scallop data and 318 (σ = 114) km for the 2010 scallop data.

HERRING ASSIGNMENT

Herring were assigned to likely feeding areas using the assignment parameters outlined in Table 1. To report pooled results, individual herring areas were grouped according to body size. Following Van Wilgenburg et al. (2011), for each fish, cells designated as likely feeding areas were assigned a value of 1 and all other calls assigned a value of 0. Values were then summed for each cell across the total number of individual fish and divided by the total number of fish, providing an index of the most frequently assigned cells ranging between 0 and 1 (Fig. 4). Irrespective of capture location, larger fish are assigned to feeding areas in the central northern North Sea (Fig. 4A), consistent with summer fishery catches (ICES 2012, Fig. 4C). Smaller (juvenile) herring are assigned to feeding areas in the southern North Sea particularly around the German Bight (Fig. 4B), again consistent with locations of juvenile herring inferred from acoustic surveys (ICES 2012, Fig. 4D).

Discussion
Despite combined uncertainties associated with measurement, between individual variance, and calibration between an isoscape and measured tissues, isotope-based location was 75% accurate to 30% of the North Sea, equivalent to a spatial precision on the order of $10^5$ km$^2$. The mean linear error between the single cell of highest probability and the known location was between 200 and 300 km. Light-based location is widely used in animal ecology, but relatively few studies have tested accuracy of light based location. Where direct tests have been reported, mean errors of location by light range between around 200-400km (Phillips et al. 2004; Lisovski et al. 2012), approximately equivalent to linear errors reported here for isotope-based location methods.

The isoscape used here is derived from a mobile pelagic organism, but used to assign origin to a sessile benthic organism collected either 4 or 14 years prior to the samples used to derive the isoscape. This mismatch in sample collection time and organism functional group is deliberate, testing the degree to which isoscapes derived from a single reference organism can be used to assign a wide range of taxa over unspecified periods of time.

Short and long-term temporal variation in isotopic baselines could confound the use of isotopes for geolocation. Scallops have been sampled in 2001 and 2010, and jellyfish in 2011 (MacKenzie et al., 2014) and 2015. The regional distribution of isotope values was consistent across these four independent sampling dates,
although the exact location of boundaries between isotopically distinct regions varies slightly between sample suites. Consequently assignment accuracy is relatively consistent between the two test datasets (Fig. 3). This is consistent with broad hydrological control over spatial distribution of isotope values, modified by relatively minor intra-year variability (MacKenzie et al., 2014; Jennings & van der Molen 2015). While jellyfish mesoglea sample spring and summer production, scallops have a longer isotopic turnover times and likely integrate annual average production (Jennings & van der Molen 2015). The similarity between jellyfish and scallop isosymes further supports the argument that spring and summer primary production dominates biomass-weighted consumer tissue production in this strongly seasonal sea. At higher spatial resolution, or in coastal settings, isotopic compositions of primary production and dissolved organic matter are expected to vary more widely in both time and space (Kürten et al. 2013), and the spatial isotope models presented here are unlikely to perform well.

GEOGRAPHIC ASSIGNMENT OF MIGRATORY FISHES

Herring present a particular challenge for fishery management, as they exhibit complex migratory behaviour and variation in spawning strategies which change in response to environmental conditions, population sizes, age structures and harvesting (Dickey-Collas et al. 2010). North Sea herring feed in open waters in the northern North Sea in summer months, before migrating south and east to spawn in discrete locations dictated by the need for well-oxygenated coarse substrates. Larval herring drift eastwards within the southern North Sea...
towards the German Bight before recruiting to the adult population. Isotope-based geo-assignment captures this ontogenetic migration (Fig. 4), implying that isotope based location offers a promising additional tool for marine spatial ecology and management.

IMPLICATIONS FOR ECOLOGY, MANAGEMENT AND FOOD TRACEABILITY

Stable isotope-based retrospective location is well-established in terrestrial ecology, particularly for birds, but extension into marine environments has been slow due to the difficulty of obtaining baseline spatial isotope data. Here we show that isotopic baselines derived from carbon and nitrogen isotopic compositions of pelagic gelatinous zooplankton provide sufficient spatial resolution to rival light-based location in terms of accuracy and precision.

Determining location based on carbon and nitrogen isotope compositions records a fundamentally different ecological variable to other location methods. While data storage tag, satellite and water chemistry-based locations record the physical position of the animal, dietary isotope based locations record the likely spatial origin of nutrients assimilated during feeding. In sessile animals, or animals with a limited foraging range, feeding location and physical location will be effectively the same within the error of the assignment methods. In mobile animals (or animals feeding on mobile prey), however, assigned feeding location reflects the origin of primary production assimilated during feeding. Potentially, the location associated with assimilation of food may not necessarily correspond to the location where an animal spends the majority of its time.
Dietary isotope-based location provides additional ecological information beyond location at a fixed point in time, but interpreting the ecological meaning of dietary isotope ‘location’ in migratory animals requires some understanding of the timescale of isotopic assimilation relative to the rate and scale of movements across isotopic gradients. Herring are relatively small, metabolically-active, low trophic level (Mackinson & Daskalov 2007) fish, and isotopic equilibration is likely to occur with a half life on the order of c.50 days (Miller 2000; vander Zanden et al 2015). Consequently, isotopic-assignment areas for herring closely correspond to feeding areas. Dietary isotope-based identification of feeding grounds will be more problematic in animals where isotopic assimilation rates are slow with respect to movements across isotopic gradients. While static physical location tags (e.g. light or tidal-stream based location) can provide an answer to the question of where animals go (Hammerschlag et al. 2011), combinations of physical tags and isotopic location may go some way towards addressing questions of why animals spend time in particular regions.

The accuracy and precision of location methods based on carbon and nitrogen stable isotopes is highly dependent on the isotopic calibration between the baseline organism and the species and tissue to be assigned. Estimates of uncertainty associated with all steps in isotopic measurement, spatial modelling and calibration can be quantified and incorporated into assignment algorithms. Calibration methods and uncertainties must be reported with any stable isotope assignment. Nevertheless we suggest that stable isotope based geoassignment can be used in marine systems retrospectively to infer the location where the majority of nutrients were assimilated prior to capture. The method can in theory be applied to any marine feeding organism, but the ecological meaning of
any assigned area will be more difficult to interpret for high trophic level and migratory animals with relatively slow isotopic assimilation rates.
Acknowledgements

Simon Jennings (Cefas) is gratefully thanked for access to scallop isotope data.

Cefas staff and crew of the RV Cefas Endeavor kindly allowed KSG to participate
in the 2015 IBTS survey. Mike Wunder, Hannah Vander Zanden, Steve Van
Wilgenburg and staff of the ITCE Spatial course in Utah in 2015 are all thanked
for informative and constructive discussions that shaped the analyses performed
here. KSG is funded through the SPITFIRE NERC DTP partnership. The authors
would also like to thank the editors and reviewers whose constructive comments
improved the manuscript considerably.

Data accessibility

Jellyfish and herring isotope data and isoscape raster files are provided in the
supplementary information. Stable isotope data from jellyfish, herring and
previously published stable isotope data from Queen scallops sampled in 2001
are also available from (doi:10.5061/dryad.609hp). Stable isotope data from
queen scallops sampled in 2010 are available from Cefas. The owner of the data,
Simon Jennings, will be archiving it in Dryad shortly. The final version of this
manuscript will include a direct link to this data. If you are viewing the ‘Accepted
Articles’ version after September 2016, please search ‘North Sea stable isotope’
with Simon Jennings as the author in Dryad to find the relevant data.

References

Barnes, C., Jennings, S. & Barry, J.T. (2009) Environmental correlates of large-
scale spatial variation in the $\delta^{13}$C of marine animals. *Estuarine, Coastal and Shelf
Science*, 81, 368-374.


Supporting Information

Table S1. Locations of capture, bell diameter, bell weight and weight and stable isotope composition (δ^{13}C and δ^{15}N) for *C. capillata* recovered across the North Sea

Table S2. Locations of capture and stable isotope composition (δ^{13}C and δ^{15}N) for *C. harengus* recovered across the North Sea

C_raster.gri, C_raster.grd R-compatible raster files of the carbon isoscape model

N_raster.gri, N_raster.grd R-compatible raster files of the nitrogen isoscape model

CVar_raster.gri, CVar_raster.grd R-compatible raster files of spatial variance in the carbon isoscape model

NVar_raster.gri, NVar_raster.grd R-compatible raster files of spatial variance in the nitrogen isoscape model

Table 1: Assignment conditions adopted for stable isotope based location of scallops and herring against isoscapes derived from jellyfish tissues.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Isoscape jellyfish</th>
<th>Scallop calibration</th>
<th>Herring calibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement error</td>
<td>δ^{13}C: 0.1, δ^{15}N: 0.2</td>
<td>δ^{13}C: 0.2, δ^{15}N: 0.2</td>
<td>δ^{13}C: 0.2, δ^{15}N: 0.2</td>
</tr>
<tr>
<td>(σ)</td>
<td>measured</td>
<td>estimated / measured</td>
<td>measured</td>
</tr>
<tr>
<td>-----</td>
<td>----------</td>
<td>----------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Between-individual variance</td>
<td>$\delta^{13}C: 1.69, \delta^{15}N: 1.04$: measured</td>
<td>$\delta^{13}C: 0.2, \delta^{15}N: 0.7$: estimated / measured</td>
<td>$\delta^{13}C: 0.2, \delta^{15}N: 0.2$: measured</td>
</tr>
<tr>
<td>Trophic distance</td>
<td>NA</td>
<td>1 ($\sigma = 0.25$): literature estimate</td>
<td>-0.2 ($\sigma = 0.25$): literature estimate</td>
</tr>
<tr>
<td>Isotopic trophic fractionation</td>
<td>NA</td>
<td>$\delta^{13}C: 1(\sigma = 0.5), \delta^{15}N: 3.4(\sigma = 0.5)$: literature compilation</td>
<td>$\delta^{13}C: 1(\sigma = 0.5), \delta^{15}N: 3.4(\sigma = 0.5)$: literature compilation</td>
</tr>
<tr>
<td>Tissue specific fractionation</td>
<td>NA</td>
<td>$\delta^{13}C: +0(\sigma = 0.25), \delta^{15}N: +1(\sigma = 0.25)$: graphical estimate</td>
<td>$\delta^{13}C: +2(\sigma = 0.25), \delta^{15}N: +0.5(\sigma = 0.25)$: graphical estimate</td>
</tr>
<tr>
<td>Threshold odds ratio</td>
<td>NA</td>
<td>1.33</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Figure captions
Fig. 1. Isoscape models (A, B) and associated variances (C,D) for $\delta^{13}$C (A, C) and $\delta^{15}$N (B, D) values based on *C. capillata* sampled in September 2011. Sample stations indicated with filled circles.
Fig. 2 Locations of herring (open triangles) and scallop (2001 data: filled circles, 2010 data: open circles) samples within the North Sea.
Fig. 3 Accuracy and precision of assignment for the combined, 2001 and 2010 scallop datasets. Precision is defined by the probability threshold and expressed as the proportion of data (i.e. cells) considered as likely. Accuracy is assessed as the proportion of individual scallops where the threshold area contained the known sample location.
Fig. 4. Comparison of isotope-based feeding area assignments and fisheries survey data. A, B Most likely feeding areas for 351 North Sea herring sampled in September 2011 as derived from stable isotope-based location. Colours
represent the number of individual herring assigned to each grid square. A) herring >200mm standard length, B herring <200mm standard length. Open circles indicate capture locations. C. Spatial distribution of reported landings of adult herring (log_{10} tonnes) in quarters 2 and 3 of 2011, data from (ICES 2012). D. Estimated biomass of immature herring in June-July 2011 from combined acoustic surveys (ICES 2012).