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UNIVERSITY OF SOUTHAMPTON

FACULTY OF NATURAL AND ENVIRONMENTAL SCIENCES

School of Ocean and Earth Science

**THE RETROSPECTIVE GEOLOCATION OF FREE-LIVING MARINE
ORGANISMS USING STABLE ISOTOPES**

by

Katie St. John Glew

Thesis for the degree of Doctor of Philosophy

April 2018

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

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THE RETROSPECTIVE GEOLOCATION OF FREE-LIVING MARINE ORGANISMS USING STABLE ISOTOPES

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Isoscapes are spatially explicit models describing isotopic variability due to spatial differences in physical, chemical and biological processes across natural environments. Marine isoscapes are being increasingly developed to address a range of ecological questions, from better understanding space use and foraging behaviours to determining individual trophic feeding positions and assigning animals or animal products to their origin. However, many marine isoscapes lack suitable data coverage and resolution or explicit measures of variance, necessary for assignment. This research aims to advance isoscape prediction methodologies and develop isoscape assignment techniques to benefit marine conservation and management.

I have demonstrated two different methods of isoscape prediction. The first, ordinary kriging of *in situ* $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements of lion's mane jellyfish (*Cyanea capillata*) across the North Sea, producing highly accurate isoscapes. The second, a Bayesian hierarchical modelling approach incorporating multiple species of *in situ* jellyfish samples and additional environmental data to produce highly precise $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ UK shelf sea isoscapes. Both techniques provided greater than 80% assignment accuracy to areas representing 40% of each isoscape. North Sea assignments were comparable to light based data loggers and UK shelf sea assignment accuracy was approximately 80% when assigning to ICES subareas. I also demonstrated marine isoscape use in seabird foraging behaviour research, by refining over winter feeding positions during the vulnerable moult period of UK breeding guillemots (*Uria aalge*), razorbills (*Alca torda*) and Atlantic puffins (*Fratercula arctica*). The three sympatric species frequented slightly different areas and fed over different trophic positions, with high individual variability. Foraging responses also differed between winters with contrasting environmental conditions, with razorbill and puffin populations displaying different adaptation strategies.

This study addresses current limitations of sample collection constraints in marine isoscape predictions and highlights the potential of animal assignments to isoscapes as a useful tool to aid conservation, fisheries management and traceability.

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Academic Thesis: Declaration Of Authorship

I, Katie St. John Glew declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

THE RETROSPECTIVE GEOLOCATION OF FREE-LIVING MARINE ORGANISMS USING STABLE ISOTOPES

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as:

Chapter 3: Trueman, CN, MacKenzie, KM & St John Glew, K (2017) Stable isotope-based location in a shelf sea setting: accuracy and precision are comparable to light-based location methods. *Methods Ecol. Evol.* 8:232–240

Signed:

Date:

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Chapter 1 Thesis Introduction

1.1 Thesis aims and objectives

The research presented in this thesis aims to advance the field of marine isotope ecology by improving spatial models of isotopic variability (isoscaples), and exploring the potential of these models to identify location and clarify trophic ecology. In particular, this project aims to quantify the benefits of isoscape-based methods for conservation and spatial based management decisions. I focus my research within the UK Shelf Sea region and on using isoscape-based geographic assignment techniques as a tool to investigate over winter foraging behaviours of UK seabird populations. In addition I highlight the potential of such techniques in future fisheries traceability research. The specific objectives are to:

- Gain a thorough understanding of the current status of marine isoscape research.
- Build carbon and nitrogen isoscape models across the North Sea using *in situ* Lion's mane jellyfish (*Cyanea capillata*) samples and simple spatial interpolation tools, and quantify the accuracy and precision of geographic assignment that can be achieved.
- Combine isoscape assignment and data logger information to refine estimates of feather moult locations and understand individual trophic variability of populations of guillemots (*Uria aalge*), razorbills (*Alca torda*) and Atlantic puffins (*Fratercula arctica*) breeding in the North Sea.
- Compare foraging behaviours during moult of seabird populations during two contrasting survival winters, using newly developed coupled North Sea isoscape assignment and data logger techniques.
- Explore the use of Bayesian hierarchical statistical methods in isoscape prediction to incorporate multi species jellyfish samples and environmental variables in carbon and nitrogen UK Shelf Sea isoscape predictions and assess assignment accuracy to these newly developed isoscaples.
- Create the first marine sulfur isoscape across the UK Shelf Seas using *in situ* jellyfish samples.

1.2 Thesis structure

The thesis begins with a thorough review of marine isoscapes (chapter 2). I then focus on North Sea isoscapes and example assignment applications, starting with initial isoscape prediction and assignment method development (chapter 3) followed by two seabird isoscape assignment studies (chapters 4 and 5). Secondly, the thesis explores the use of new isoscape development techniques to enable the prediction of carbon, nitrogen and sulfur isoscapes across the UK Shelf Sea range (chapter 6).

Chapter 2 takes the form of a review chapter, introducing isotope ecology and analysing the current status of marine isoscape research. All known published marine isoscapes are presented and critically assessed on sample data and modelling methods used. Current applications of marine isoscapes are also discussed. Sections of this chapter have been accepted for publication in:

Trueman, C.N & St. John Glew (2018) Isotope tracking of marine animals. In: Hobson, KA & Wassenaar, L.I. (eds.) Tracking Animal Migration with Stable Isotopes (2nd Edition).

Author contributions: KSG wrote the review chapter as it appears within this thesis. Sections were taken from this thesis and included within the above publication.

Chapter 3 is a method development chapter where $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes are produced for the North Sea using ordinary kriging of *in situ* sampled lion's mane jellyfish isotope measurements. Scallops from known locations are assigned to the North Sea isoscapes to assess accuracy and precision of assignments. Herring samples are also assigned to the North Sea isoscapes and assignment locations compared to fisheries survey data. This chapter is published in Methods in Ecology and Evolution as:

Trueman, CN, MacKenzie, KM & St John Glew, K (2017) Stable isotope-based location in a shelf sea setting: accuracy and precision are comparable to light-based location methods. Methods Ecol. Evol. 8:232–240

Author contributions: KSG collected and processed the data and carried out data analysis. CT, KM and KSG developed the method and CT wrote the code for isoscape modelling and assignments. CT wrote the manuscript with input from KSG and KM. As CT devised the methods used within this chapter, he is the appropriate first author of the publication. KSG applied these methods throughout the subsequent chapters within this thesis and this chapter was included to describe the applied methods in detail.

Chapter 4 assigns seabird feather isotope values to the carbon and nitrogen isoscapes modelled in chapter 3 to refine at sea winter moult locations of UK breeding guillemots, puffins and razorbills, equipped with light based geolocator tags. This chapter couples two independent geolocation methods to quantify species-specific foraging locations and individual trophic variability. This chapter is “In Press” at Marine Ecology Progress Series as:

St. John Glew, K, Wanless, S, Harris, M.P, Daunt, F, Erikstad, K.E, Strøm, H & Trueman, C.N. (2018) Moult location and diet of auks in the North Sea, inferred from coupled light-based and isotope-based geolocation.

Author Contributions: KSG, CNT, SW and MPH conceived the project. SW, MPH, FD, KEE and HS deployed the geolocators and SW and MPH collected the feather samples. KSG carried out the sample and data analysis and wrote the manuscript. All authors provided editorial advice.

Chapter 5 uses the coupled isoscape assignment and data logger geolocation methods developed in chapter 4 to compare foraging behaviours during winter moult of puffins and guillemots within the North Sea, during two contrasting survival and environmental condition winters. This chapter is in preparation for submission for publication as:

St. John Glew, K, Wanless, S, Harris, M.P, Daunt, F, Erikstad, K.E, Strøm, H, Speakman, J.R, Kürten, B, & Trueman, C.N. Sympatric auk species show contrasting foraging responses to adverse marine conditions during winter moult.

Author Contributions: KSG, CNT, SW and MPH conceived the project. SW, MPH, FD, KEE and HS deployed the geolocators and SW and MPH collected the feather samples. JRS was involved with seabird feather isotope analysis. BK provided pipefish stable isotope data. KSG carried out the sample and data analysis and wrote the manuscript. All authors provided editorial advice.

Chapter 6 uses an INLA approach to model carbon, nitrogen and sulfur isoscapes across the UK shelf seas using *in situ* samples consisting of seven different species of jellyfish and environmental data. North Sea isoscape isotopic patterns and assignment accuracy and precision are compared to those modelled in chapter 3. Assignment accuracy to the UK Shelf Sea carbon and nitrogen isoscapes, over different precision thresholds, are calculated and assignment accuracy to known ICES Subareas discussed in terms of potential use for spatial based management decisions. This chapter has been divided into two papers which are in preparation for submission as:

St. John Glew, K, Graham, L, McGill, R & Trueman, C.N. Spatial models of carbon, nitrogen, and sulfur stable isotope distributions (isoscapes) across a shelf sea: an INLA approach.

St. John Glew, K, Graham, L, McGill, R & Trueman, C.N. The use of isoscapes in marine spatial based management: A UK shelf sea case study.

Author Contributions: KSG and CT conceived the project. RM carried out stable isotope analysis and provided data interpretation expertise and advice. LG provided INLA and data analysis expertise and advice. KSG collected and carried out sample and data analysis and wrote the chapter and manuscripts. All authors provided editorial advice.

Chapter 7 briefly summaries the findings within this thesis and highlights the benefits and limitations of the isoscape assignment approach. Future work and applications of UK Shelf Sea assignments are suggested and discussed in terms of conservation, fisheries management and traceability.

Chapters 3-6 have been published, submitted or prepared for journal submission, and as such may contain overlap of introductory comments and methodologies and are written in the style of the respective journals. All data and R code scripts are stored in the Git Hub repository found: <https://github.com/katiestjohnnglew/Thesis>

Chapter 2 Marine Isoscapes Review

2.1 Introduction

Stable isotopes of elements occur naturally within the environment with the abundance of heavy and light forms varying significantly within and between different compounds (Farquhar et al., 1989). The spatio-temporal variation in the isotopic composition of water, nutrients and animal and plant tissues provides information about physical, chemical and metabolic processes occurring within the natural ecosystem. Stable isotopes have long been recognised as useful indicators across both terrestrial and aquatic systems (Dansgaard, 1954, Farquhar et al., 1989, Bowen, 2010a). With increased data availability and modelling tools, research into the biogeochemical processes behind spatio-temporal variations in the distribution of stable isotope ratios, and what they can reveal about global ecosystems and the organisms that live within them, has greatly expanded (Bowen et al., 2009).

In low temperature systems operating far from chemical equilibrium, and particularly during biochemical reactions, the ratio of heavy to light isotopes varies due to reaction kinetics, with bond formation or phase transformations involving the heavier isotope generally requiring more energy (Ramos and Gonzalez-Solis, 2012). Consequently, reaction products are typically enriched in the light isotope and unconsumed reactants contain more of the heavier isotope (isotopic fractionation). Most biogeochemical and ecological applications involving stable isotopes are based on oxygen, hydrogen, carbon, nitrogen, and sulfur (Ramos and Gonzalez-Solis, 2012), while isotopes of calcium, zinc and mercury are generating increasing interest, particularly as analysis via multi-collector ICP-MS instrumentation becomes more reliable and affordable.

Isotope ratios are expressed as δ values in parts per thousand (‰), where $\delta = (R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}$, and where R is the ratio of the heavy to light isotope. All δ values are reported relative to an internationally recognised laboratory standard with a specific isotopic ratio, therefore the δ value is a measure of how much more of the heavier isotope is present within the sample, in comparison to the standard. Fractionation is a measure of the difference in isotopic ratio at the start and end of the chemical or biological process, and is expressed as a Δ value, where $\Delta_{A-B} = \delta_A - \delta_B$.

Isotope ratios vary spatially, at varying scales, across terrestrial, aquatic and marine landscapes due to differences in the hydrological cycle, fluid dynamics, nutrient cycling and biological processes exerting effects on isotopic abundance (Bowen, 2010a). For example increased temperatures likely lead to increased reaction timing and a reduction in the

preferential uptake of the lighter isotope, increasing the isotopic ratio in the products, and reducing abundance of heavier isotopes within the reactants (Hofmann et al., 2000, Bowen, 2010b, Trueman et al., 2012b). In addition age of substrates (Crowley et al., 2017), differences in biological pathways (Farquhar et al., 1989, Montoya et al., 2002) and physical processes (Tagliabue and Bopp, 2008) also add to isotope ratio differences across space. To utilise this spatial isotopic variation, or determine values in unmeasured regions, isotope maps (isoscapes) are produced.

An isoscape is a mechanistic or statistical model of the distribution of isotope ratios across space (Bowen, 2010a, West et al., 2010). Isoscapes have been used to determine origin of animals (Hobson et al., 1999b), plants (West et al., 2006) and humans (Ehleringer et al., 2008), track migration patterns (Hobson, 1999, Norris et al., 2006, Cherel and Hobson, 2007, Hobson and Wassenaar, 2008, Hobson et al., 2010), infer diet segregation and trophic ecology (Olson et al., 2010, Young et al., 2010, Jennings and van der Molen, 2015), determine traceability within forensic, food and consumer goods contexts (Kelly et al., 2005, Chesson et al., 2008, Chesson et al., 2010, Chiocchini et al., 2016) and understand complex ecological processes (LeGrande and Schmidt, 2006, Bowen et al., 2010).

2.1.1 Terrestrial isoscapes

2.1.1.1 Inorganic

Global inorganic oxygen and hydrogen isoscapes, based on kinetic fractionation occurring within the hydrological cycle, are the most explored examples of natural isotopic spatial variation (Bowen, 2010b, Bowen, 2010a). The degree of isotopic fractionation associated with evaporation of water at the ocean surface varies predictably with temperature. In regions of low evaporation (cooler, high latitudes) lighter isotopes are preferentially evaporated, whereas within regions of high evaporation (warmer, lower latitudes), less preferential loss of the lighter isotopes to the gas phase occurs (Fig. 2.1). As low latitude air masses are transported northwards, precipitation events then preferentially remove the heavier isotopes ^2H and ^{18}O from the atmosphere (Bowen, 2010b). As the air masses cool, each subsequent rain out event will have reduced relative abundance of heavy isotopes. The combined effect of increased preferential removal of light isotopes from surface oceans and reduced precipitation of heavy isotopes at higher latitudes, results in strong gradients of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values, with the highest isotopic ratios observed in the tropics (Bowen, 2010a, Bowen, 2010b)(Fig. 2.1). Isotopic gradients are also observed with elevation and distance inland, with heavier isotopes precipitated out at lower altitudes and closer to the sea, resulting in decreased isotopic ratio with increased elevation and coastal distance (Bowen, 2010a). Again, in high evaporation regions on land, lighter isotopes are

preferentially evaporated from surface waters, further increasing the terrestrial latitudinal isotope gradient. Surface waters incorporate into all biological, geological and manmade materials, resulting in mirrored isotopic gradients in plants, animals, ground water and soils (Bowen, 2010b).

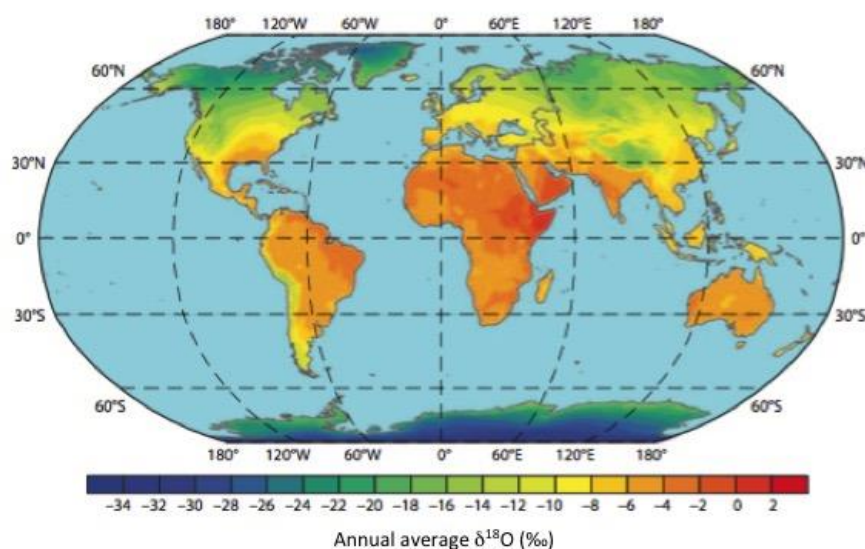


Figure 2.1 Long-term annual average precipitation $\delta^{18}\text{O}$ isoscape, estimated using data from the Global Network of Isotopes in Precipitation (Bowen, 2010a)

Strontium isotopes are also used for studying spatial aspects of modern, historical and fossil ecosystems (Crowley et al., 2017) such as distinguishing between different origins of freshwater fish (e.g. Atlantic salmon (Kennedy et al., 1997, Kennedy et al., 2000), and Chinook salmon (Brennan and Schindler, 2017)), migratory song birds (Chamberlain et al., 1997, Sellick et al., 2009) and mammals (Vogel et al., 1990, Radloff et al., 2010). Dissolved strontium isotopes are released into soils, surface waters and streams due to the weathering of rocks, and are incorporated into plants and animal tissues via diet and direct uptake by aquatic organisms (Kennedy et al., 1997, Kennedy et al., 2000, Crowley et al., 2017). ^{87}Sr is produced through the radioactive decay of ^{87}Rb over geological time, whereas the absolute quantity of ^{86}Sr does not deviate with time, therefore older rocks, and those with higher Rb/Sr ratios will develop higher $\delta^{87}\text{Sr}$ values (Crowley et al., 2017). The isotopic ratio of the watershed subsequently reflects the age and the composition of the underlying soils and rocks (Kennedy et al., 2000). Strontium isotopes are particularly useful for distinguishing between geographic origin as they do not fractionate during uptake and assimilation, so the $\delta^{87}\text{Sr}$ of the animal tissue will be the same as the surrounding environment (Kennedy et al., 1997, Crowley et al., 2017), however strontium analysis is expensive so the scale and resolution of samples is currently limited.

2.1.1.2 Organic

Carbon isoscapes have also been developed for vegetation, taking advantage of the metabolic differences in CO₂ fixation by C3 and C4 plants (Farquhar et al., 1989, Bowen, 2010a, Still and Powell, 2010). The C4 photosynthetic pathway results in greater fractionation, and therefore a lower isotopic ratio compared to C3 plants (Wassenaar and Hobson, 2000). Geographic distribution of C4 plants is predictably linked to climatic variables, and model-based estimates of C3:C4 abundance can be used to generate global models of spatial variation in $\delta^{13}\text{C}$ values in plants (Still and Powell, 2010). Plant-based isoscapes can then be used to study the origin of plant based material and animals which consume plants.

2.1.2 Marine Isoscapes

In the marine environment, it is more difficult to generate isoscape models due to the difficulty in obtaining sufficient reference samples over appropriate spatial and temporal scales to account for the isotopic variability resultant from dynamic changes in climate, ocean circulation and biological community compositions (Lorrain et al., 2009, Hobson et al., 2010, Trueman et al., 2012a). Hydrogen and oxygen isotopes are less useful in a marine context, as spatial variations associated with surface evaporation, freshwater input and water mass mixing are buffered by the large water volume (MacKenzie et al., 2014). Therefore in marine systems spatial differences in organic carbon and nitrogen isotopes, as a result of strong biogeochemical gradients, are more ecologically informative.

Strong isotopic gradients and isotopically distinct regions within and between marine basins have been identified and exploited to yield ecological information (Schell et al., 1989, Hobson et al., 1997, Cherel and Hobson, 2007, Witteveen et al., 2009, Ramos and Gonzalez-Solis, 2012, Lorrain et al., 2014), but relatively few continuous surface marine isoscape models have been published, certainly in comparison with the terrestrial environment. In this review I will critically assess the known published marine isoscapes and discuss isoscape creation, limitations, and current applications in marine ecological research.

2.2 Marine isoscapes

2.2.1 Inorganic isoscapes

2.2.1.1 Oxygen

$\delta^{18}\text{O}$ values of seawater vary at regional and ocean basin scales (LeGrande and Schmidt, 2006, McMahon et al., 2013a). Seawater $\delta^{18}\text{O}$ values are linearly related to salinity due to the association with freshwater inputs and evaporation (Epstein and Mayeda, 1953, Trueman et al., 2012a). Lower $\delta^{18}\text{O}$ values occur in high latitudes, with large volumes of fresh water input (northern polar latitudes $\delta^{18}\text{O} = -3\text{--}0\text{‰}$), whereas higher $\delta^{18}\text{O}$ values are observed in highly evaporative regions, where lighter isotopes are preferentially removed, such as subtropical gyres (North Atlantic Subtropical gyre $\delta^{18}\text{O} = 0.5\text{--}1.5\text{‰}$) and semi enclosed basins (Mediterranean Sea $\delta^{18}\text{O} = 1\text{--}2\text{‰}$) (LeGrande and Schmidt, 2006, Trueman et al., 2012a, McMahon et al., 2013a). Although spatially variable, seawater $\delta^{18}\text{O}$ isotopic range ($-3\text{--}2\text{‰}$) (LeGrande and Schmidt, 2006) is relatively small in comparison to precipitation $\delta^{18}\text{O}$ range ($-36\text{--}0\text{‰}$) (Bowen and Revenaugh, 2003).

Oxygen isotope composition is measured directly from seawater (LeGrande and Schmidt, 2006, Torniainen et al., 2017), or from the mineral carbonate components of animals' bones, shells and teeth (Trueman et al., 2012a, Detjen et al., 2015, Matthews et al., 2016, Torniainen et al., 2017) which precipitate in isotopic equilibrium with the surrounding seawater (Trueman et al., 2012a, Detjen et al., 2015, Matthews et al., 2016). This relationship is influenced by temperature dependant fractionation, and $\delta^{18}\text{O}$ values of biominerals additionally reflect the temperature at mineral formation (Trueman et al., 2012a, Detjen et al., 2015, Torniainen et al., 2017). Oxygen isotopes have useful applications in marine ecological studies (Vighi et al., 2015, Torniainen et al., 2017), particularly $\delta^{18}\text{O}$ measured within fish otoliths which have been used to geolocate fish to their foraging grounds (Darnaude et al., 2014), determine migrations (Trueman et al., 2012a, Torniainen et al., 2017) and discriminate between different populations (Rooper et al., 2008a, Darnaude et al., 2014). Oxygen isotopes measured within otoliths also provide a useful tool for studying vertical migrations within deep-sea communities, as temperature and salinity gradients occur with depth (Longmore et al., 2011, Chung, 2015, Trueman et al., 2016). The isotopic compositions of otoliths have also been used to reconstruct past ocean temperatures and study temperature fluctuations and community response to localised climate change (Devereux, 1967, Jones and Campana, 2009). However due to the small isotopic range, the benefits are limited to regions with distinct temperature and/or salinity gradients, such as some semi enclosed or marginal seas or regions with relatively extreme evaporation rates (Trueman et

al., 2012a, McMahon et al., 2013a). In addition, Darnaude et al. (2014) found considerable variability in $\delta^{18}\text{O}$ temperature fractionation in wild plaice studied within the North Sea, suggesting climate reconstruction using $\delta^{18}\text{O}$ ratios in otoliths should be applied with caution.

2.2.1.2 Dissolved Inorganic Carbon (DIC)

Inorganic carbon in the oceans exists primarily in the carbonate (CO_3^{2-}) ion, but also as HCO_3^- with a dynamic equilibrium established between the proportions of carbon held in differing species. The isotopic composition of dissolved inorganic carbon (DIC) as CO_3 varies spatially due to differential CO_2 fractionation during air-sea gas exchange (Tagliabue and Bopp, 2008, McMahon et al., 2013a), and differing rates of uptake of CO_2 during primary production. In regions of CO_2 invasion, surface $\delta^{13}\text{C}_{\text{DIC}}$ values are relatively negative compared to ocean regions where outgassing occurs (McMahon et al., 2013a). Spatial distributions of $\delta^{13}\text{C}_{\text{DIC}}$ are also dependant on surface water residence time, ocean circulation and mixing (Tagliabue and Bopp, 2008). Fractionation during photosynthesis also influences oceanic $\delta^{13}\text{C}_{\text{DIC}}$ values, as phytoplankton preferentially uptake the lighter ^{12}C , causing higher $\delta^{13}\text{C}_{\text{DIC}}$ values in surface water in highly productive areas (McMahon et al., 2013a, Torniainen et al., 2017). Upwelling events, then reduce $\delta^{13}\text{C}_{\text{DIC}}$, as isotopically lighter DIC is brought to the surface (McMahon et al., 2013a). However in most regions, local spatial variability in $\delta^{13}\text{C}_{\text{DIC}}$ values due to productivity, are homogenised by chemical and physical processes (Tagliabue and Bopp, 2008, Schmittner et al., 2013).

Global DIC isoscapes and mechanistic isotope models have been used extensively to provide information on oceanic mixing processes and the global carbon pump (Schmittner and Somes, 2016) and on smaller scales to study movement across carbon gradients (Torniainen et al., 2017). However global $\delta^{13}\text{C}_{\text{DIC}}$ values vary by less than 3‰ (Lynch-Stieglitz et al., 1995), giving DIC isoscapes limited application in large scale ecological studies.

2.2.2 Organic Isoscapes

Whereas inorganic isotope measurements reflect the ambient water column, isotopic ratios of structural nutrient elements, such as carbon, nitrogen and sulfur, represent biological processes, primarily photosynthesis and isotopic change throughout the food web (Ramos and Gonzalez-Solis, 2012)(Fig. 2.2).

2.2.2.1 Sulfur

In open ocean environments, $\delta^{34}\text{S}$ values are assumed to be relatively uniform at approximately 21‰ with little spatial variability due to extensive mixing (Peterson and Fry, 1987). Marine sulfur isotopic composition is predominantly controlled by the large isotopic

fractionation that occurs during bacterial sulfate reduction where lighter ^{32}S isotopes are preferentially reduced to sulfide during marine sedimentation, resulting in distinct isotopic differences between pelagic and benthic environments with depleted $\delta^{34}\text{S}$ sulfides in marine sediments (-10‰) (Bottrell and Newton, 2006)(Fig. 2.2). Strong isotopic differences also occur between marine and terrestrial ecosystems, with freshwater $\delta^{34}\text{S}$ values ranging between -5 and 15‰ dependant on bedrock lithology, atmospheric deposition extent and anthropogenic inputs (Weber et al., 2002). These distinct isotopic differences result in clear $\delta^{34}\text{S}$ gradients between estuarine and coastal environments, with increased $\delta^{34}\text{S}$ ratios observed with distance from shore (Fry, 2002)(Fig. 2.2). In addition, $\delta^{34}\text{S}$ values also vary dependant on the source of primary production, with pelagic phytoplankton, macro algae and benthic primary producers varying in $\delta^{34}\text{S}$ ratio (Connolly et al., 2004, Niño-Torres et al., 2006).

As little fractionation of sulfur occurs between trophic levels ($0 - 3\text{‰}$ from marine phytoplankton to predators), isotopic gradients are also observed in coastal consumers foraging throughout and between salinity gradients, and foraging on different primary producers at the base of the food web, with $\delta^{34}\text{S}$ ratios often used to investigate coastal and estuarine resource use and connectivity between marine and freshwater ecosystems (MacAvoy et al., 1998, Weber et al., 2002, Lott et al., 2003).

2.2.2.2 Carbon

Phytoplankton $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}_{\text{plk}}$) are determined by the isotopic composition of the carbon source (typically, but not exclusively CO_3^{2-}), and the extent of isotopic fractionation during photosynthesis (Lee et al., 2005, Bowen, 2010a). Photosynthetic fractionation of carbon isotopes depends largely on the relative rates of diffusion of carbon into and out of the cell and is therefore influenced by cell size, growth rate and the concentration of aqueous CO_2 within the euphotic zone (Goericke and Fry, 1994, Laws et al., 1995, Popp et al., 1998, Graham et al., 2010, McMahon et al., 2013a). These factors are indirectly controlled by temperature (Hofmann et al., 2000, Graham et al., 2010, McMahon et al., 2013a)(Fig. 2.2). In cool, well mixed, high nutrient, high latitude regions, ambient CO_3 contents are high, and phytoplankton have slow growth rates and large cell sizes resulting in strong isotopic fractionation and therefore relatively negative $\delta^{13}\text{C}_{\text{plk}}$ values (Goericke and Fry, 1994, Graham et al., 2010). In warmer, low latitudes, dissolved CO_3 concentrations are reduced, cell size is smaller and growth rates faster, reducing isotopic fractionation leading to more positive $\delta^{13}\text{C}_{\text{plk}}$ values (Goericke and Fry, 1994, Graham et al., 2010).

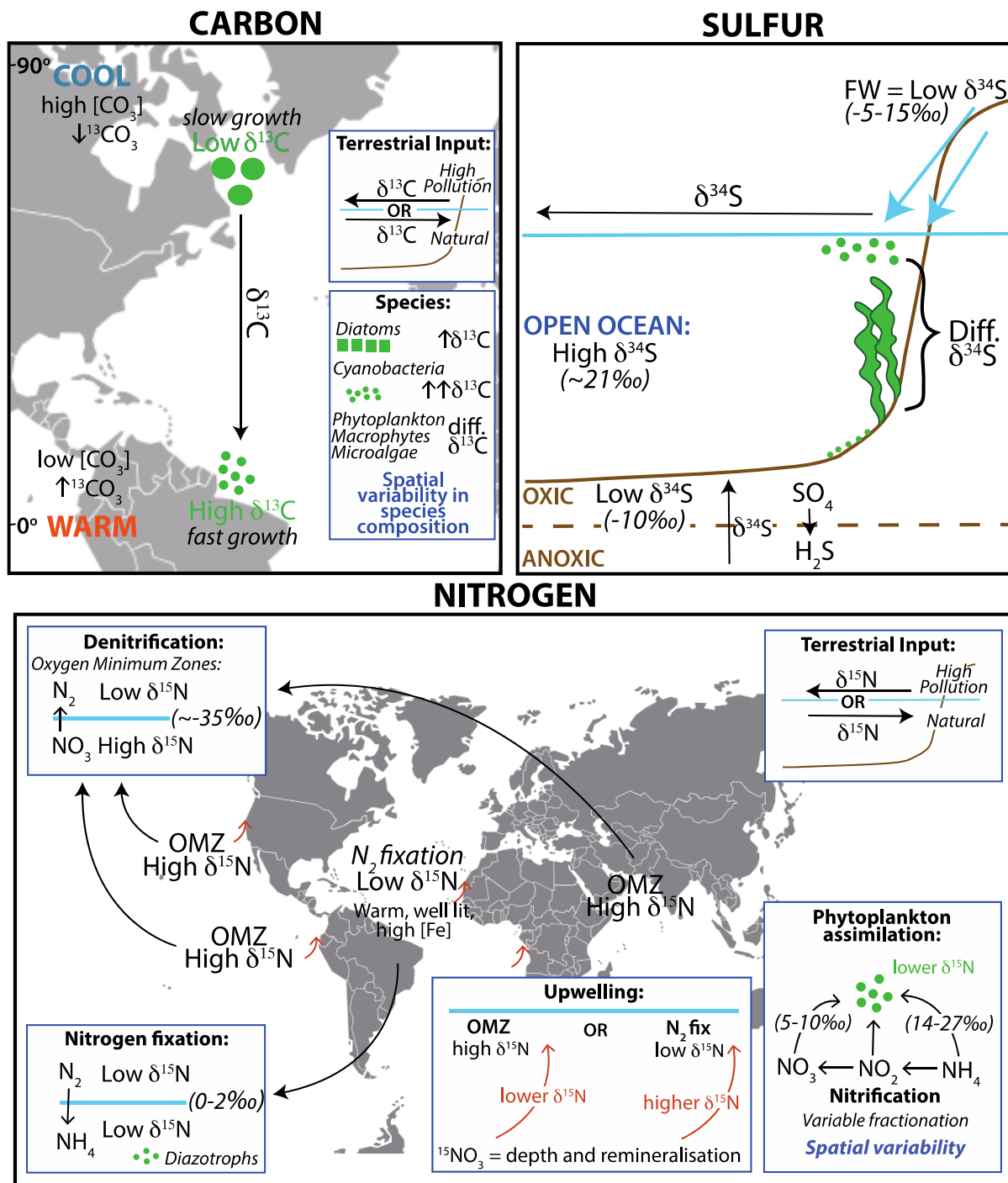


Figure 2.2 Schematic depicting the spatial variations observed in marine carbon, nitrogen and sulfur isotope ratios and the mechanisms driving these spatial differences.

The species composition of primary producers also affects the $\delta^{13}\text{C}_{\text{plk}}$ value within an area (Fig. 2.2). Diatoms are generally larger and faster growing compared to other phytoplankton types, with high nutrient uptake efficiency, resulting in reduced preferential uptake of the lighter carbon isotope and an increased $\delta^{13}\text{C}_{\text{plk}}$ ratio in high diatom abundance regions (Popp et al., 1998, Trueman et al., 2012a). In addition cyanobacteria are found to have the highest $\delta^{13}\text{C}$ values compared with all other marine phytoplankton (Carpenter et al.,

1997), due to reduced CO_2 uptake, small surface area to volume ratio and active transport of HCO_3^- into the cell (Levitan et al., 2007, Tchernov and Lipschultz, 2007). Throughout a bloom event uptake of CO_2 is even further reduced, increasing $\delta^{13}\text{C}$ value with time (Tchernov and Lipschultz, 2007).

2.2.2.3 Nitrogen

Nitrogen is present in numerous forms within the marine environment, and isotopic fractionation occurs as each form is converted to another (Fig. 2.2). Therefore, phytoplankton $\delta^{15}\text{N}$ values vary depending largely on the isotopic composition of their nitrogen source, which varies considerably depending on the biogeochemical nature of the ambient water column (Montoya, 2007, Graham et al., 2010, Trueman et al., 2012b, McMahon et al., 2013a). Fixation of atmospheric nitrogen by diazotrophs, such as the cyanobacteria *Trichodesmium*, favours incorporation of ^{14}N , but has a relatively small isotopic fractionation effect ($\Delta^{15}\text{N} = \sim -2$ to $+2\text{‰}$ (Ryabenko, 2013)). Organic nitrogen fixed by diazotrophs and other nitrogen fixers therefore has an isotopic composition close to or slightly lower than atmospheric N_2 (by definition 0‰) (Montoya et al., 2002, Montoya, 2007, McMahon et al., 2013a, Ryabenko, 2013). Nitrogen fixation is relatively energetically costly and marine diazotrophs are thought to be limited by light and potentially iron (Somes et al., 2010). Therefore the extent of nitrogen fixation is highest in warm, well-lit ocean regions with relatively low amounts of available nitrogen within the water column and within areas rich in atmospheric iron, such as the subtropical Atlantic Ocean.

Addition of nitrogen through fixation is balanced by loss of nitrogen through denitrification. In suboxic regions with excess dissolved nitrate, denitrification may occur whereby N_2 is lost to the atmosphere (Somes et al., 2010). This process strongly favours ^{14}N with a fractionation factor of $\sim 35\text{‰}$ (Ryabenko, 2013), leaving the dissolved nitrate pool enriched in ^{15}N . Denitrification primarily occurs in large oxygen minimum zones of the eastern tropical North Pacific, eastern tropical South Pacific and the Northern Arabian Sea.

Phytoplankton assimilate nitrogen as ammonium, nitrate or nitrite, favouring ^{14}N uptake (Graham et al., 2010), with varying fractionation effects, with isotopic fractionation greater in ammonium assimilation ($14\text{--}27\text{‰}$) in comparison to nitrate ($5\text{--}10\text{‰}$) (Sigman et al., 2009, Ryabenko, 2013). Oxidation of reduced ammonia into nitrite and nitrate occurs through nitrification carried out by ammonia and nitrite oxidising bacteria (Casciotti, 2009, Sigman et al., 2009). The extent of isotopic fractionation during nitrification is dependant on the external conditions and the microbial community, but with a global average isotopic effect of 5‰ (Somes et al., 2010). Upwelling of nitrate rich waters, following nitrification, may contribute to varying spatial patterns of $\delta^{15}\text{N}$ in surface waters, dependant on the

isotopic composition of the regions these water are upwelled into (low $\delta^{15}\text{N}$ regions with high nitrogen fixation, or high $\delta^{15}\text{N}$ regions characterised by denitrification) and the dominance of remineralised organic material from higher trophic level organisms (e.g. zooplankton faeces) within the upwelled water mass (Graham et al., 2010).

2.2.3 Shelf seas, coasts and estuaries

In shelf sea, coastal and estuarine environments further factors complicate spatial stable isotopic variation resulting in isotopic gradients at smaller scales than typically observed within open ocean settings.

Within coastal environments, a greater range of isotopically distinct primary producers are present than found within the open ocean. Terrestrial plants, macrophytes (seagrass and seaweed) and benthic algae all contribute to marine food webs, with varying extents, in addition to phytoplankton production (France, 1995, Fry, 2002, Niño-Torres et al., 2006). Macrophytes have naturally higher $\delta^{13}\text{C}$ ratios compared to phytoplankton, and exhibit large isotopic variability with differences of 6-8‰ observed within a single leaf of eelgrass *Zostera marina* and kelp *Laminaria longiruis* (Stephenson et al., 1984). Although macrophytes are considerable contributors of nutrients to the local food web and must be taken into account whilst studying consumers reliant on their production, their role in carbon flow to the wider marine ecosystem is limited (France, 1995). Benthic microalgae are also enriched in ^{13}C in comparison to pelagic phytoplankton by approximately 5‰ (France, 1995) likely due to reduced water turbulence and an increased boundary layer, decreasing the diffusion and uptake of isotopically light carbon isotopes (France, 1995). In regions with strong bottom currents, these isotopic differences between pelagic and benthic production may be dampened.

In general, marine derived nutrients are typically enriched in heavier isotopes, compared with freshwater derived material (Peterson and Fry, 1987), often resulting in strong, yet variable, isotopic gradients with distance from shore (Fry, 2002). However terrestrial run off and pollution can also influence isotopic composition, with sewage, agricultural and fisheries farm waste inputs relatively enriched in ^{15}N and ^{13}C (Vizzini and Mazzola, 2006, Montoya, 2007, McMahon et al., 2013a). In addition, water column depth can have a significant influence on isotopic composition, due to varying degrees of mixing and re-suspension of sedimented organic matter. Within the North Sea, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values co-vary with bottom temperature, likely due to the extent of water column mixing, with largest isotopic differences observed across depth gradients (Jennings and Warr, 2003, Barnes et al., 2009b, MacKenzie et al., 2014).

2.2.4 Trophic effects on carbon and nitrogen isotopes

The isotopic composition of both carbon and nitrogen changes throughout the food web due to isotopic fractionation (trophic discrimination), occurring at each stage along the biological pathway (Fig. 2.3). The lighter isotope is preferentially assimilated into newly synthesised metabolic waste products and therefore excreted, resulting in an accumulation of heavier isotopes within an individual's tissues (Hobson et al., 2010, Ramos and Gonzalez-Solis, 2012). During nitrogen fractionation ^{14}N amine groups are preferentially removed during amino acid deamination and transamination processes, resulting in an enriched ^{15}N nitrogen pool (Vander Zanden and Rasmussen, 2001, McCutchan et al., 2003). Heavier ^{13}C accumulates due to excretion of lighter ^{12}C during respiration, but fractionation values are relatively low (Peterson and Fry, 1987, McCutchan et al., 2003). Consumers are typically reported to be enriched by 3-3.4‰ in $\delta^{15}\text{N}$ (Minagawa and Wada, 1984), and ~ 1 ‰ in $\delta^{13}\text{C}$ (Peterson and Fry, 1987) in comparison to their prey (Fig. 2.3).

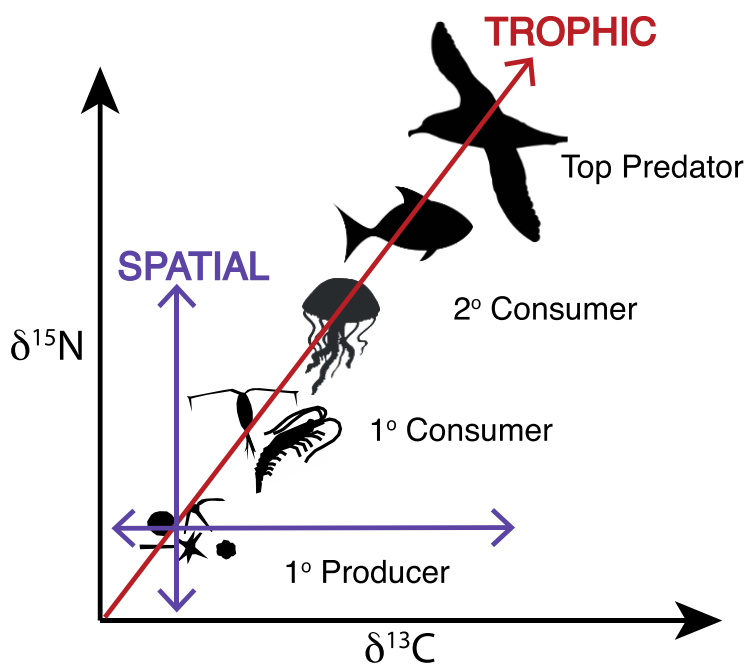


Figure 2.3 Schematic demonstrating trophic enrichment of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in a marine food web

However evidence suggests more variability in fractionation factors between consumer and diet than first thought, with variation observed between organisms with different diet types such as carnivores and herbivores (Mill et al., 2007), different protein and lipid contents and positioned at different levels within a food web (Deniro and Epstein, 1978, McCutchan et al., 2003, Vanderklift and Ponsard, 2003). Different physiologies such as ammonia, uric acid or urea excretion pathways and body condition such as starvation or

stress (Vander Zanden and Rasmussen, 2001, McCutchan et al., 2003, Vanderklift and Ponsard, 2003) also contribute to fractionation variability. In addition sample preparation, such as using muscle tissue or whole organisms has also been found to alter trophic fractionation values (McCutchan et al., 2003). This trophic discrimination and variability between values need to be considered when producing and utilising organic marine isoscapes. Carbon and nitrogen isoscapes have been produced across the marine environment, using a variety of animal tissues, over numerous spatial and temporal scales (Table 2.1, Fig. 2.4), and for use across many different ecological applications.

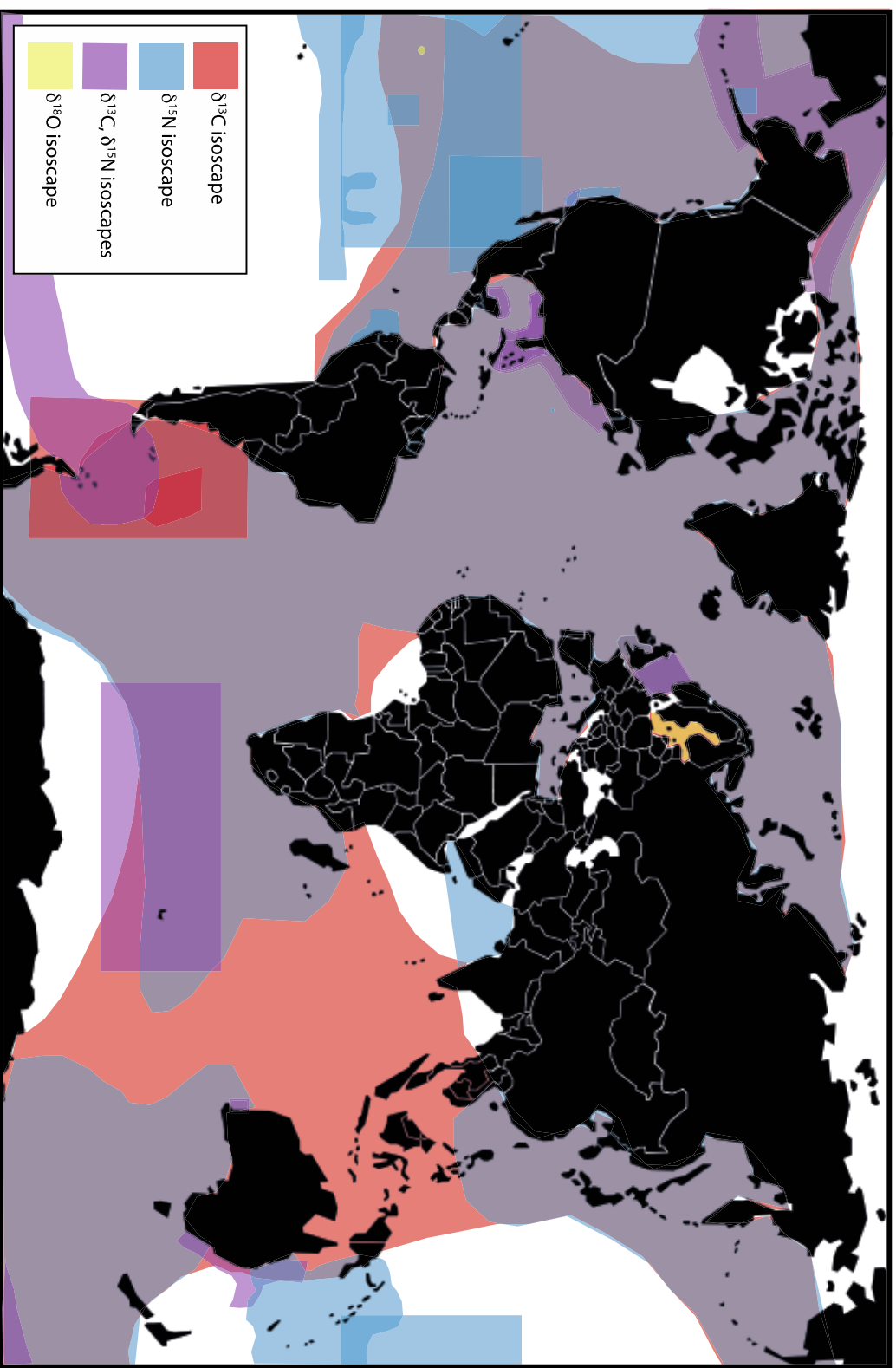


Figure 2.4 Locations of all known published marine $\delta^{13}\text{C}$ (organic), $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ isoscapes. Global isoscapes were not included in this figure.

Table 2.1 Summary table of all known published marine isoscapes. Isotope, baseline organism, interpolation method and temporal scale are listed.

Location	Isotopes	Author	Continuous /Discrete?	Bulk / CSIA -AA	Baseline organism	Model/ in situ samples	Method	Variance?	Time scale	Even spatial coverage of in situ data?
Global										
Global	$\delta^{15}\text{N}$	(Somes et al., 2010, Somes et al., 2013)	Continuous	Bulk	POM	Model	Climate model + marine ecosystem model	No	Simulate over different time scales	NA
Global	$\delta^{13}\text{C}$, $\delta^{13}\text{C}$ -DIC	(Hofmann et al., 2000)	Continuous	Bulk	DIC,POC	Model	3D model of phytoplankton and zooplankton community dynamics	No	Simulate over different time scales	NA
Global	$\delta^{13}\text{C}$, $\delta^{13}\text{C}$ -DIC	(Tagliabue and Bopp, 2008)	Continuous	Bulk	DIC,POC	Model	Physical model + PISCES ocean-ecosystem model (Aumont and Bopp 2006)	No	Simulate over different time scales	NA
Global	$\delta^{13}\text{C}$ -DIC	(Schmittner et al., 2013)	Continuous	Bulk	DIC	Model	Physical + marine ecosystem model (Schmittner et al 2008)	No	1990-2005	NA
Global	$\delta^{13}\text{C}$	(Magozzi et al., 2017)	Continuous and discrete	Bulk	Plankton	Model	Coupled physics-biogeochemistry model (NEMO-MEDUSA), used to estimate carbon isotopic fractionation.	Variance reported within discrete provinces	2001-2010, annually averaged	NA
Global	$\delta^{18}\text{O}$	(LeGrande and Schmidt, 2006)	Continuous	Bulk	Seawater	In situ (Schmidt et al 1999)	Nearest neighbour interpolation of isotopically distinct regions	No	50 years	No

Global and Atlantic Ocean	$\delta^{18}\text{O}$	(Trueman et al., 2012a)	Continuous	Bulk	Seawater and fish otoliths	In situ seawater samples + temperature data	Fish otolith isotope values estimated from measured seawater samples (Schmidt et al 1999), and SST data	No	30+ years	No
Atlantic										
Atlantic Ocean	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{13}\text{C}$ -DIC, $\delta^{18}\text{O}$, $\delta^2\text{H}$	(McMahon et al., 2013b, McMahon et al., 2013a)	Continuous	Bulk	Seawater and zooplankton	In situ (published data)	Ocean Data View: Data Interpolating Variation Analysis	Misfits calculated	Data compiled across numerous years	No
Gulf of Mexico	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Vander Zanden et al., 2015)	Continuous	Bulk	Loggerhead turtles (scute tissue)	In situ	GIS - ordinary kriging	Variance isoscape	2011/2012	Yes
Gulf of Mexico	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Radabaugh et al., 2013)	Continuous	Bulk	3 fish species (muscle tissue), benthic algae and POM	In situ	Ordinary kriging	No	2009/2010	Yes
Gulf of Mexico	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Radabaugh and Peebles, 2014)	Continuous	Bulk	7 fish species (muscle tissue), benthic algae and POM	In situ + environmental data	Multiple regression models between fish isotope data and environmental variables. Ordinary kriging of data.	Variation plots from mean isotopic value of each species	2009/2010	Yes
Bermuda Platform	$\delta^{15}\text{N}$	(Fourqurean et al., 2015)	Continuous	Bulk	Seagrass	In situ	Ordinary kriging	No	2006-2008	No
North Sea, Irish Sea and English Channel	$\delta^{15}\text{N}$	(Jennings and Warr, 2003)	Continuous	Bulk	Queen scallops	In situ + environmental data	Linear model used to predict $\delta^{15}\text{N}$ values at environmental data points.	Model variance assessed	2001	No

North Sea, Irish Sea and English Channel	$\delta^{13}\text{C}$	(Barnes et al., 2009)	Continuous	Bulk	Queen scallops	In situ + environmental data	Linear model used to predict $\delta^{13}\text{C}$ values at environmental data points.	Model variance assessed	2001	No
North Sea	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(MacKenzie et al., 2014)	Continuous	Bulk	Lion's mane jellyfish	In situ + environmental data	Ordinary kriging, linear model used to predict isotope values at environmental data points. Ordinary kriging of modelled data.	Variance isoscape	2011	No
North sea	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Trueman et al., 2017)	Continuous	Bulk	Lion's mane jellyfish	In situ	Ordinary kriging	Variance isoscape	2015	Yes
English Channel	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Kopp et al., 2015)	Continuous	Bulk	Queen Scallops (Data from Jennings and Warr, 2003, Barnes et al., 2009))	In situ	Ordinary kriging	No	2001	No
Baltic Sea	$\delta^{18}\text{O}$, $\delta^{13}\text{C}$ -DIC	(Tornaiainen et al., 2017)	Continuous	Bulk	Seawater + Atlantic salmon otoliths + salinity data	In situ	Otolith isotope values estimated from predictive model using seawater $\delta^{18}\text{O}$ and salinity data. Ocean Data View: Data Interpolating Variation Analysis .	Simple sensitivity analysis	2010/2011	Yes
NW Atlantic	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Ceriani et al., 2014, Ceriani et al., 2017)	Continuous	Bulk	Loggerhead turtles (skin tissue)	In situ	ARC GIS - Bayesian kriging	Cross validation statistics	2009-2013	No

Pacific										
Bering, Chukchi and Beaufort Seas	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Schell et al., 1998)	Discrete	Bulk	Zooplankton	In situ	Grouped samples into regions separated by different water masses - average value in each area	No	1985-1994	Yes
Bering Sea	$\delta^{15}\text{N}$	(Jones et al., 2014)	Continuous	Bulk	Krill, pollock, thick-billed murre and black-legged kittiwake muscle	In situ	Arc GIS - inverse distance weighting interpolations. Cross shelf pattern observed, and modelled using all species.	No	2008/2009	Yes
Equatorial Pacific	$\delta^{15}\text{N}$	(Graham et al., 2010)	Continuous	Bulk	Bigeye and yellowfin tuna muscle samples	In situ	Isotope values normalized against average value for the species in that region, differences plotted across space.	No	Unknown	No
Eastern Tropical Pacific	$\delta^{15}\text{N}$	(Olson et al., 2010)	Continuous	Bulk	Copepods and yellowfin tuna	In situ	GAM models to predict copepod $\delta^{15}\text{N}$ values across space. Tuna isotope values - ordinary kriging.	No	Copepods = 2003, tuna 2003-2005.	Yes (copepods)
Pacific atoll	$\delta^{18}\text{O}$	(Dejeu et al., 2015)	Continuous	Bulk	Barnacles attached to green sea turtles + seawater isotope values (LeGrande and Schmidt, 2006)	In situ + model	Arc GIS - Barnacle oxygen isotopes predicted from water isotope values and SST. Contour maps.	No	2011	Yes (Seawater data)

California Coast	$\delta^{15}\text{N}$ - CSIA-AA	(Volkshoori and McCarthy, 2014)	Continuous	CSIA-AA	Mussels - but measuring primary productivity	In situ + model	Model isotope values and latitude. Arc GIS - simple interpolation.	No	2009/2010	Yes
Southern Californian Bight	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Kurle and McWhorter, 2017)	Continuous	Bulk	POM	In situ	Arc GIS - Inverse distance weighting. Mapped distance from mean isotopic values.	No	2012-2013 (seasonal)	Yes
Temperate Australasia	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Mackey et al., 2015)	Continuous	Bulk	Red algae	In situ + environmental data	Linear model used to predict $\delta^{13}\text{C}$ values at environmental data points. Ocean Data View - Data Interpolating Variation Analysis.	No	2013 (summer and winter)	No
South West Pacific	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Pethybridge et al., 2015)	Continuous	Bulk	Albacore tuna	In situ + environmental data	GAM model created using SST, Chl and lat/long to predict isotope values. Contour plots created.	No	2009/2010	Yes
South East Pacific	$\delta^{15}\text{N}$	(Young et al., 2015)	Continuous	Bulk	Yellowfin tuna	In situ + model	GAM model of yellowfin tuna and other predatory fish isotope values, adjusted with modelled baseline (Somes et al 2013)	No	Integrated over many years	No
Eastern Tropical pacific	$\delta^{15}\text{N}$	(Zupcic-Moore et al., 2017) – data from (Tesdal et al., 2013)	Continuous	Bulk	Surface sediment samples	In situ	Contour interpolation plot	No	Integrated over many years	No

Western and Central Pacific	$\delta^{15}\text{N}$	(Houssard et al, 2017)	Continuous	Bulk	POM and yellowfin and bigeye tuna	In situ	GAM models of POM and tuna, using a two dimension thin plate regression spline fitted to the samples by location.	No	1992-2015	No
Upper Gulf of California	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Rodríguez-Pérez et al., 2018)	Continuous	Bulk	Sediment and zooplankton	In situ	Ordinary kriging with linear variogram model	No	2010/2011	Yes
Southern Ocean										
Southern Ocean	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Jaeger et al., 2010)	Continuous	Bulk	Albatross blood plasma	In situ	Interpolation of blood plasma isotopes	No	2008	No
Southern Ocean	$\delta^{13}\text{C}$	(Quillfeldt et al., 2010)	Continuous	Bulk	Phytoplankton and POM	In situ	Arc GIS - nearest neighbour interpolation	No	Integrated over many years	No
South Georgia	$\delta^{13}\text{C}$	(Ceia et al., 2015)	Continuous	Bulk	Albatross blood and plasma	In situ	ARC GIS - natural neighbour interpolation	No	May-Oct-2009	No
West Antarctic	$\delta^{15}\text{N}$, $\delta^{13}\text{C}$	(Brault et al., 2018)	Continuous	Bulk	Zooplankton	In situ	Ocean Data View: Data Interpolating Variation Analysis	No	2007-2011	No

2.3 Isoscape prediction

Isoscapes are essentially models of spatial variation in the stable isotope composition of a defined substrate, ideally expressed for a specific time period. Isoscape models are produced by spatial interpolation of values measured at known locations (Schell et al., 1998, Hobson et al., 1999b, Cherel and Hobson, 2007, Jaeger et al., 2010, McMahon et al., 2013a), from statistical inference where isotope values are predicted from another measurable variable (Wunder, 2012, MacKenzie et al., 2014) or through empirical models (Table 2.1).

2.3.1 Variance

The production of isoscapes is based on models which reduce complex natural processes into mechanisms that can be generalized across time and space (Bowen et al., 2010). Isoscape models are based on numerous assumptions and uncertainties including baseline organism physiological differences, temporal and spatial variability (Graham et al., 2010), spatial interpolation errors and the spatial resolution of the data (Wunder and Norris, 2008b). Variability in isotope values may also result from analytical error during stable isotope analysis, because output values are averages dependant on run time calibrations; repeated analyses of the same sample rarely produces the same result (Jardine and Cunjak, 2005, Wunder and Norris, 2008b). The reliability of an isoscape model and its utility across multiple applications, is dependant on the ability to quantify these variance-generating processes across the geographic region of interest (Wunder and Norris, 2008b, Wunder and Norris, 2008a). To date, marine isoscapes often lack associated measures of uncertainty, with very few published isoscapes quantifying and reporting variability or measures of model predictability across isoscape space (MacKenzie et al., 2014, Vander Zanden et al., 2015, Magozzi et al., 2017, Trueman et al., 2017). In order to produce an isoscape that can be used effectively for geographic assignment, measures of variance must be considered at each stage of the isoscape model design process.

2.3.2 Continuous vs. discrete, scale and resolution

Isoscapes can be continuous gridded prediction surfaces, with a specific isotope value per cell, or discrete, with an average isotope value applied across defined regions (Schell et al., 1998, Magozzi et al., 2017). Discrete isoscapes are dependent on predetermined boundaries, segregating the study area by different water masses (Schell et al., 1998) or predefined biogeographic provinces (Magozzi et al., 2017), requiring prior regional knowledge to define these potentially arbitrary boundaries (Wunder, 2010). Continuous

isoscapes may allow a more detailed approach, enabling the study of isotopic gradients across space, but their accuracy and practical value depends on the quality and distribution of spatial reference samples relative to the spatio-temporal scale of isotopic gradients. However, ultimately all isoscapes are discrete; grid cells in continuous surfaces are defined boundaries with a specific isotope value per cell. Therefore, the distinction between discrete and continuous isoscapes is related to scale and data resolution (Wunder, 2010).

Existing marine isoscapes range across different spatial scales from single shelf seas (Jennings and Warr, 2003, Barnes et al., 2009b, Radabaugh and Peebles, 2014, Vander Zanden et al., 2015, Trueman et al., 2017) or coastal regions (Vokhshoori and McCarthy, 2014, Fourqurean et al., 2015, Rodríguez-Pérez et al., 2018), to ocean basins (Jaeger et al., 2010, Quillfeldt et al., 2010, McMahon et al., 2013a) and across global oceans (LeGrande and Schmidt, 2006, Somes et al., 2010, Trueman et al., 2012a, Magozzi et al., 2017) to address different ecological questions.

In order to produce a statistically useful isoscape, data points must be evenly distributed across the entire spatial extent of the region of interest. If measurement locations are patchily distributed, some regions of the isoscape will have higher or lower variance and skew the isoscape, potentially amplifying isotopic differences in some regions, and smoothing variability in others. In addition, for geolocation purposes, uniform spatial coverage of data and therefore variance, is critical in reducing assignment bias to higher data coverage regions. Therefore ultimately, the method used to construct the isoscape model is dependent on the spatial resolution of data available, the size of the region of interest and the aimed use of the isoscape on completion (Wunder, 2010). Small-scale isoscapes produced to study complex biogeochemical processes, track individual movement patterns within specific regions, or geolocate an animal to its origin will benefit from a continuous approach, if enough evenly distributed data are available. Alternatively, large scale isoscapes designed to study differences in isotope ratios across broad geographical, management or political boundaries, with limited data availability or reliant on extensive simplifications, will benefit from a coarser resolution discrete approach (Wunder, 2010, Magozzi et al., 2017, Bird et al., 2018).

2.3.3 Baseline Organism

For inorganic isoscapes, isotopes can be measured directly from the water column ($\delta^{13}\text{C}_{\text{DIC}}$ and $\delta^{18}\text{O}$), or by correcting for mineralisation effects (e.g. in otoliths), using sea water $\delta^{18}\text{O}$ and surface temperature measurements. For organic isoscapes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$), isotope samples need to be taken from tissues of organisms at even spatial resolutions across the region of interest; the chosen animal is termed the baseline organism.

Spatially referenced isotope ratios can be measured within the target organism sampled at known locations (Graham et al., 2010, Jaeger et al., 2010, Radabaugh et al., 2013, Ceia et al., 2015, Vander Zanden et al., 2015), reducing variance associated with trophic discrimination adjustment uncertainty. However sufficient sample collection across the region of interest is rarely feasible due to limited distribution or prevalence of the target species and time and financial constraints, increasing probability of uneven spatial variance. For large-scale isoscapes, isotopes are commonly measured in easily-sampled low trophic level organisms, increasing the ability to collect a uniform distribution of samples and expanding the range of applications for a single isoscape model (McMahon et al., 2013a, Fourqurean et al., 2015, Mackey et al., 2015, Kurle and McWhorter, 2017, Trueman et al., 2017). Consumers are effective baseline organisms, as they may integrate short-term isotopic variability (Schell et al., 1998, Jennings and Warr, 2003, Radabaugh et al., 2013, Jones et al., 2014, Lorrain et al., 2014, MacKenzie et al., 2014, Trueman et al., 2017) and are less influenced by dynamic environmental fluctuations compared to primary producers especially in marine systems (Post, 2002). Ideally organisms used as reference samples to construct isoscape models will be sessile or with reduced movements and will be well distributed across space expressing no preference for specific patchily distributed habitats (Jennings and Warr, 2003, Barnes et al., 2009b, Lorrain et al., 2014, MacKenzie et al., 2014, Trueman et al., 2017). If benthic organisms are used as reference taxa, feeding method and isotopic depth gradients must also be considered. When using baseline organisms, the isotopic spacing between the reference organism and the subsequent study species must be estimated. Large-scale isoscapes may extend over the ranges of several reference taxa, adding additional complexity to isoscape generation. To date, while isoscapes have been generated by compiling data from multiple species, none have explicitly quantified the additional variance effects introduced by compiling across species.

Sample tissue type also requires consideration (Trueman et al., 2012a). Tissues with high metabolic rates, such as blood and liver, represent recent diet consumption and snapshots in isotopic time (Hobson, 2005, Wolf et al., 2009, Ramos and Gonzalez-Solis, 2012), whereas tissues with lower metabolic rates, such as bone collagen integrate diet over longer time periods (Hobson, 2005, Wolf et al., 2009, Ramos and Gonzalez-Solis, 2012). Tissue choice will therefore result in representation of different time periods (Tucker et al., 2014). If studying short term processes over varying temporal scales, faster metabolic tissues will be useful, whereas researching animal movement patterns over seasonal or yearly time scales will require longer turn over time tissues.

To reduce trophic enrichment factor uncertainty and primary producer variability, compound specific isotope analysis of amino acids (CSIA-AA) can be used to produce

isoscapes (Vokhshoori et al., 2014, Vokhshoori and McCarthy, 2014). Temporally integrated baseline primary production isotopic ratios can be determined from measuring consumer source amino acids (Popp et al., 2007, Lorrain et al., 2014), beneficial in dynamic coastal environments (Vokhshoori and McCarthy, 2014). However CSIA-AA is expensive and currently impractical for most applications of large scale spatial isoscape modelling.

2.3.4 Method of Interpolation

Sample-based isoscapes rely on the prediction of values at unknown sites from measured values at known locations using interpolation, based on Tobler's first law (Tobler, 1970) stating that variables at nearby locations are more closely related and have more similar values compared with variables at sites more widely separated in space (Bowen, 2010a, Wunder, 2010). Marine isoscapes have been produced by interpolating between *in situ* data, predicting isotopic ratios using alternative environmental variables or combining both *in situ* and predictive approaches.

Nearest neighbour interpolation is the simplest form of interpolation, where unknown grid cells adopt the value of the nearest neighbouring pixels. This method is quick and simple and has been used by Quillfeldt et al. (2010) and LeGrande and Schmidt (2006) to produce large scale marine isoscapes, however there is a loss of detail and neighbouring data points are ignored. Natural neighbour interpolation proportionately applies weights to a subset of close input data points to calculate the unknown cell. This method is simple and does not infer trends over the entire surface area, but enables a smoother prediction surface as shown by Ceia et al. (2015). Inverse distance weighting is a deterministic approach, estimating unknown cell values by weighted averaging of values in the nearest neighbouring cells. The predicted value is directly based on surrounding measured values and has been used by Jones et al. (2014) and Kurle and McWhorter (2017) to produce Bering Sea and Californian coastal isoscapes. Kriging is a more sophisticated geostatistical method, based on statistical modelling incorporating autocorrelation of the data. The kriging approach initially performs exploratory analysis of the data, fits a variogram model and then uses this model to predict missing values. Kriging has been used to produce numerous small-scale detailed marine isoscapes (Radabaugh et al., 2013, Ceriani et al., 2014, Radabaugh and Peebles, 2014, Vander Zanden et al., 2015, Trueman et al., 2017). Kriging not only produces a prediction surface, but also a measure of uncertainty surrounding these predictions, which is particularly important for geolocation applications.

Ocean Data View (Schlitzer, 2002) provides the Data Interpolating Variational Analysis (DIVA) gridding software (Barth et al., 2010) which automatically interpolates inputted data using a finite element method, which subdivides the area into smaller models and then

reassembles these finite elements, to produce the entire continuous surface. It is increasingly being used in marine isoscape creation (McMahon et al., 2013a, Mackey et al., 2015, Torniainen et al., 2017, Brault et al., 2018) due to its ability to automatically take into account coastlines and boundaries between water masses and optimally interpolate sparse and heterogeneously distributed data over a regular grid. DIVA is also able to compute error maps, objectively identifying areas with poor sample coverage and misfit plots, representing the difference between observational and modelled data (Barth et al., 2010). As with any “black box” modelling method, care needs to be taken to ensure the data input is appropriately interpolated and the methods are fully understood before use; although DIVA is able to “optimally interpolate” unevenly distributed data, the resultant isoscape will only be as good as the quality and resolution of the data supplied.

If sufficient, regularly-spaced sample data are unavailable, simple interpolation methods are inappropriate as prediction surfaces will be skewed by single data points widely separated in space (Magozzi et al., 2017). In these cases, predictive modelling approaches have been adopted, making use of determined statistical relationships between isotope ratios and evenly distributed environmental data (Bowen and Revenaugh, 2003). For example, static variables such as depth, latitude and longitude and more dynamic variables such as sea surface temperature and salinity have all been shown to co-vary with plankton isotope ratios (Jennings and Warr, 2003, Barnes et al., 2009b, MacKenzie et al., 2014). Statistical relationships between potential environmental predictors and measured isotope values are initially modelled from the spatially referenced isotope dataset, then used to predict isotope values in regions covered by environmental variable data but with no associated reference isotope values. General linear models (GLMs), where linear relationships are determined, have been used in various regions to predict marine isoscape surfaces (Jennings and Warr, 2003, Barnes et al., 2009b, Trueman et al., 2012a, MacKenzie et al., 2014, Radabaugh and Peebles, 2014, Mackey et al., 2015, Torniainen et al., 2017). Alternatively general additive models (GAMs) are also used (Olson et al., 2010, Pethybridge et al., 2015, Young et al., 2015). GAMs enable a curve to be fitted to the data and do not assume linear relationships, potentially increasing isotope prediction accuracy. However such relationships are difficult to interpret and may not be appropriate when ecological interactions aim to be inferred from the isoscape. Environmental data modelling approaches are strongly dependant on *in situ* sample location and assume the derived relationships are constant throughout study space (Trueman et al., 2017). Regression model error, spatial differences between *in situ* samples and environmental differences between prediction location and data averages all contribute to isoscape uncertainty (Trueman et al., 2017). Ultimately, isoscapes are most robust and appropriate for a wide range of applications, when produced using geostatistical interpolation of high spatial resolution *in situ* data, evenly distributed across the study area.

2.3.5 Global ocean scale models

To produce global scale isoscapes, coupled ocean physics-biogeochemistry models can provide global phytoplankton isotopic composition predictions (Hofmann et al., 2000, Tagliabue and Bopp, 2008, Somes et al., 2010, Schmittner et al., 2013, Somes et al., 2013, Magozzi et al., 2017). The models generate global scale representations of environmental variables, such as sea surface temperature, concentration of dissolved CO₂, growth rates and concentrations of different phytoplankton taxa, combined with physical oceanography and are used in combination to predict isotope ratios (Magozzi et al., 2017). Various model combinations have predicted global carbon and nitrogen isotope ratios, based on different assumptions and combinations of variables.

Models are inexpensive and practical alternatives to extensive sample collection and allow the segregation of isotopically distinct regions and the study of broad scale oceanic and ecological processes and long distance migratory routes of wide ranging animals (Young et al., 2015, Magozzi et al., 2017). Predictive models also enable the exploration of isotopic change over temporal scales, by repeatedly simulating isoscapes over specific time periods to investigate dynamic processes, or integrated and consistent isotopic patterns with time (Somes et al., 2013, Magozzi et al., 2017). However, modelled isoscapes produce coarse predictions and lack local scale detail. Model estimates are based on assumptions and offer a simplified representation of isotopic space (Magozzi et al., 2017). Progression of model based isoscapes are currently limited by the lack of ability to quantify uncertainty and validate predictions (Young et al., 2015, Magozzi et al., 2017), making global isoscape models generally unsuitable for geolocation applications.

2.3.6 Temporal variability

Marine nutrient availability and photosynthesis rate fluctuates over seasonal, yearly and longer time scales, influencing $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios (Hannides et al., 2009, Quillfeldt et al., 2015, Kurle and McWhorter, 2017), with temporal variability outweighing spatial variability in some highly dynamic regions (Quillfeldt et al., 2015). Isotope temporal variability is particularly prevalent within primary producers, whereas variations are dampened in higher trophic level organisms due to slower tissue turnover rates (Montoya, 2007, Graham et al., 2010, Radabaugh et al., 2013, Ceia et al., 2014, Ceia et al., 2015), although significant seasonal and yearly fluctuations have also been observed in predators (Kurle et al., 2011, Quillfeldt et al., 2015). In some marine environments isotope ratios remain relatively stable (Jones et al., 2014, Ceia et al., 2015), for example North Sea broad scale isotopic patterns have remained relatively consistent over decadal to centennial time periods (Jennings and Warr, 2003, Barnes et al., 2009b, Barrett et al., 2011, MacKenzie et al., 2014, Trueman et al., 2017), likely

due to the dominant influence of static environmental variables on isotopic composition within this area (MacKenzie et al., 2014).

In dynamic coastal regions, or areas with complex circulation and upwelling patterns, (Kurle and McWhorter, 2017), previously developed isoscapes may not be appropriate for current applications (Kurle et al., 2011, Kurle and McWhorter, 2017). Marine isoscapes have been developed representing various time scales dependant on the sample organism, tissue and collection time; from seasons (Radabaugh et al., 2013, MacKenzie et al., 2014, Ceia et al., 2015, Trueman et al., 2017), to one to two years (Jennings and Warr, 2003, Vander Zanden et al., 2015) to integration of data over multiple years (LeGrande and Schmidt, 2006, Quillfeldt et al., 2010, McMahon et al., 2013a). Understanding the time frame represented by the isoscape and local baseline temporal variability, are essential for appropriate isoscape applications, determining isoscape resampling requirements and deciding whether isotope models should be extrapolated temporally (Graham et al., 2010, Ramos and Gonzalez-Solis, 2012, Quillfeldt et al., 2015). Mechanistic models are able to provide a potential solution, by estimating likely seasonal and interannual range in baseline isotopic variability, by monitoring the variation of the main driving mechanisms within the specific region.

2.4 Isoscape applications

Understanding baseline isotope values within the marine environment has useful applications for animal ecology such as tracking animal movements across space, determining natal origin and understanding migratory connectivity and energy flow throughout the marine food web (Schell et al., 1998, Bowen et al., 2009, Graham et al., 2010, McMahon et al., 2013a, Garza, 2016). In addition, stable isotope analysis for studies of trophic ecology require an understanding of baseline isotope variation for the accurate interpretation of results (MacKenzie et al., 2014, Kopp et al., 2015). Studying spatial isotope gradients also provides useful information for research into ecosystem health, nutrient limitation and biogeochemical processes (Bowen, 2010a, Fourqurean et al., 2015, Pethybridge et al., 2015).

2.4.1 Trophic Ecology

Estimates of trophic positions and resource acquisition are important for food web modelling and understanding resource use, trophic transfer efficiency, predator to prey body-size ratios, competition and niche segregations, diet flexibility and adaptability to changing prey availability due to anthropogenic pressures and species range shifts and accumulation of contaminants (Olson et al., 2010, Karnovsky et al., 2012, Wiley et al., 2012, Watt et al., 2013, Jennings and van der Molen, 2015, Kopp et al., 2015, Young et al., 2015). Consumer carbon and nitrogen stable isotope ratios reflect prey isotope values and are used to infer food web

energy sources and predator trophic level (Deniro and Epstein, 1978, Post, 2002, Lorrain et al., 2014, Jennings and van der Molen, 2015). However, isotopic ratios are influenced by both trophic feeding position, and spatial feeding location. To successfully use isotopes to determine the trophic level of an organism, spatial isotopic baseline variability must be known, so diet differences can be distinguished from foraging area segregation (Post, 2002, Roscales et al., 2011, Lorrain et al., 2014, Jennings and van der Molen, 2015, Kopp et al., 2015, Mackey et al., 2015). Isoscapes can provide measures of this baseline variation over large spatial scales, so consumer isotope ratios can be spatially corrected before trophic levels are calculated (Olson et al., 2010, Watt et al., 2013, Kopp et al., 2015, Young et al., 2015). In addition isoscape models that provide an explicit measure of baseline isotope uncertainty allow expected uncertainty in trophic level estimates to also be reported (Jennings and van der Molen, 2015). Although attempts have been made to extrapolate these baseline calibrations over past decadal time scales to infer historical changes in trophic feeding position (Hanson et al., 2017), it must be noted mechanistic drivers controlling isotopic variability in space, do not necessarily translate to isotopic variability in time.

2.4.2 Assignment to origin

Tissue isotope ratios act as intrinsic tags, providing information on feeding and migratory ecology (Jaeger et al., 2010). Comparisons of consumer isotope ratios with baseline isoscapes, provides information on residency and migration of the individual, allowing movements to be tracked, and origins to be distinguished, across isotopic gradients (Royle and Rubenstein, 2004, Graham et al., 2010, Hobson et al., 2010, Trueman et al., 2012a). The first studies into isotope geolocation began with the assignment of butterflies (Hobson et al., 1999b) and migratory songbirds using terrestrial precipitation isoscapes (Chamberlain et al., 1997, Royle and Rubenstein, 2004, Hebert and Wassenaar, 2005, Wunder and Norris, 2008b), and remain the dominant application for terrestrial isoscapes. Within the marine environment, several studies have compared tissue isotope ratios of consumers to spatial isotope gradients (Schell et al., 1989, Cherel and Hobson, 2007, Cherel et al., 2009, Graham et al., 2010, Quillfeldt et al., 2015, Matthews et al., 2016), however there are few examples of explicit probabilistic assignment of location based on marine isoscapes (Vander Zanden et al., 2015, Torniainen et al., 2017, Trueman et al., 2017).

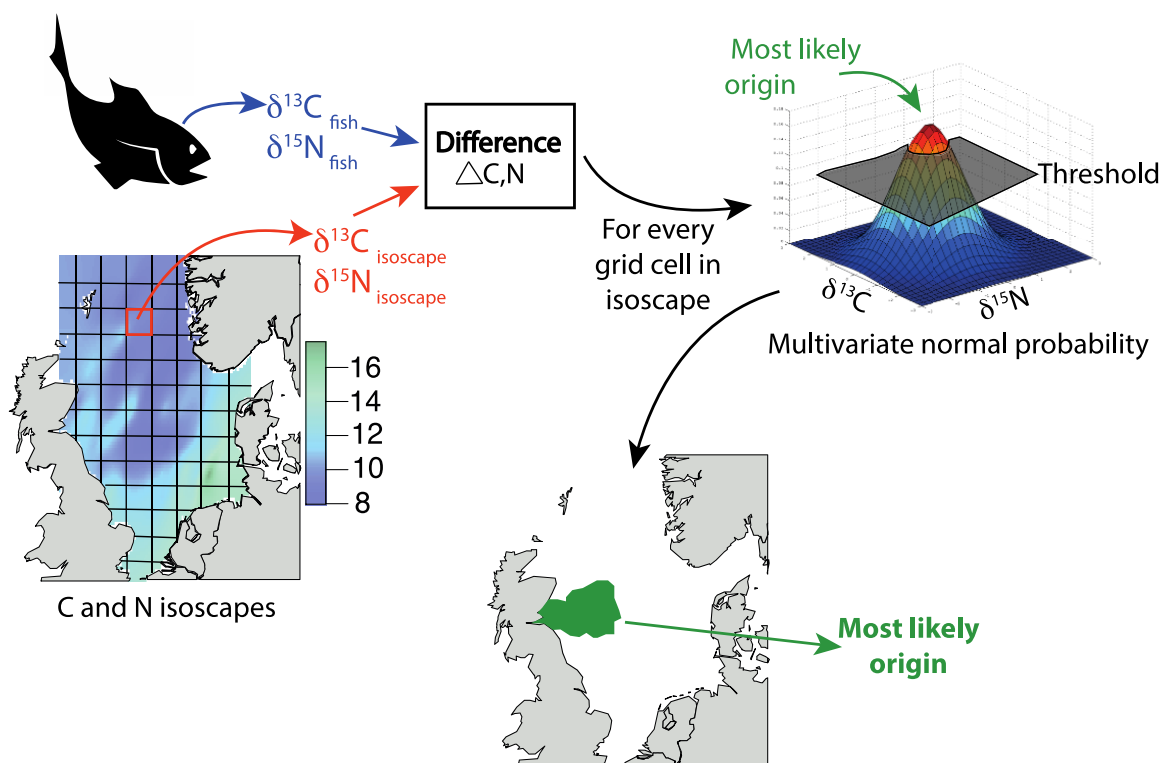


Figure 2.5 Schematic illustrating the multivariate normal probability isoscape assignment methodology. The difference between the isotope values of the assignment individual and every cell within the isoscape is calculated. Likelihood of origin is estimated for each cell using the multivariate normal probability equation and incorporating all known sources of uncertainty. The cells with the least difference between cell and sample isotope values, with the least variation compared to all other cells in the isoscape and with likelihood values above a predefined threshold, are classed as the most likely origin for the sample.

To probabilistically determine an individual's origin, the organism is assigned to a location within the isoscape using a calibrated model, such as the likelihood assignment approach (Wunder and Norris, 2008a, Van Wilgenburg et al., 2012, Wunder, 2012), which can be used with both discrete and continuous isoscapes. Discrete assignments probabilistically geolocate an animal to the most likely predetermined region, based on the similarity of isotope values. For the continuous assignment approach, the difference between every cell within the isoscape, and the sample isotope value is calculated (Wunder, 2012, Veen et al., 2014)(Fig. 2.5). A normal probability density curve is created using the mean and standard deviation isotope values of the isoscape and is applied to each grid cell in the range (Van Wilgenburg and Hobson, 2011, Van Wilgenburg et al., 2012, Bowen et al., 2014). The cells with the least difference between cell and sample isotope values, with the least variation compared to all other cells in the isoscape, are the most likely origin for the sample (Fig. 2.5). The animal is assigned to the grid cells with the highest maximum likelihood (Royle and Rubenstein, 2004, Wunder and Norris, 2008a, Veen et al., 2014) using the bivariate normal probability function as follows:

$$\mathcal{f}(x_C, x_N | l_i; (\sigma_C, \sigma_N, r)) = \frac{1}{2\pi\sigma_C\sigma_N\sqrt{1-r^2}} e^{-\frac{1}{2(1-r^2)}\left(\frac{(x_C-\mu_C)^2}{\sigma_C^2} + \frac{(x_N-\mu_N)^2}{\sigma_N^2} - \frac{2r(x_C-\mu_C)(x_N-\mu_N)}{\sigma_C\sigma_N}\right)}$$

where $\mathcal{f}(\delta C, \delta N | l_i; R)$ is the likelihood of the sample individual, with measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, originating from a particular grid cell l_i with mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values which are equal to the components in vector l_i with a variance-covariance matrix R . x_C and x_N are the isotopic compositions of the sample tissue plus or minus the offset between the baseline organism and the assignment individual, μ_C and μ_N are the isotopic compositions of the isoscape, r is the correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values calculated from the sample data set and σ_C and σ_N are the variances associated with the isoscape model and the multivariate normal probability function. Total assignment uncertainty is given by:

$$\sigma_{C,N} = \sqrt{(\sigma_{iso(C,N)}^2 + \sigma_{assign(C,N)}^2 + \sigma_{calib-off(C,N)}^2)}$$

where σ_{iso} is a sum of the variance associated with the isoscape interpolation process, the individual difference between the baseline organisms and any measurement error of the baseline organisms, σ_{assign} is a sum of the variance associated with measurement error and between individual difference of the assignment individual and $\sigma_{calib-off}$ is the variance associated with the calibration-offset calculation between the baseline and assignment organisms (Wunder, 2010, Vander Zanden et al., 2015, Trueman et al., 2017).

Likelihoods can be converted to probabilities using Bayes' rule,

$$P(A_i | B) = \frac{P(B | A_i)P(A_i)}{\sum P(B | A_i)}$$

where $P(A_i | B)$ is the posterior probability distribution of an individual originating from location i , given the measured isotope values. $P(B | A_i)$ is the probability distribution of observing the measured isotope values, given the geographic locations, and $P(A_i)$ is the prior probability distribution of the individual originating from location i , based on prior knowledge or assumptions. Bayes' rule inverts conditional probabilities (Wunder and Norris, 2008a) and enables the evaluation of the probability that a hypothesised grid cell is the true location of origin for a sample over all other possible grid cells in the region (Bowen et al., 2014). This calculation is based on the interpolated baseline isotopic composition of that region, information on fractionation between the baseline and sample isotopic values and estimates of the variance associated with these factors, providing information on the relative strength of the assignment (Wunder and Norris, 2008a, Wunder, 2012, Bowen et al., 2014).

2.4.2.1 Examples of probabilistic assignments to marine isoscapes

Discrete assignments within the marine environment, where individuals are allocated to regions with distinct isotopic ratios have been used to discriminate between different populations, stocks and nursery grounds in marine fish, invertebrates and mammals (Rooker et al., 2008a, Rooker et al., 2008b, Barnett-Johnson et al., 2010, McMahon et al., 2012, Seminoff et al., 2012, Vander Zanden et al., 2015, Zupcic-Moore et al., 2017). The first example of continuous probabilistic assignment to a marine isoscape was carried out by Vander Zanden et al. (2015), where loggerhead turtle samples were assigned to carbon and nitrogen isoscapes produced from the same species, within the Gulf of Mexico. Individual foraging areas were accurately determined, demonstrating the potential of marine isoscape assignment in determining origin and movement patterns. Trueman et al. (2017)(Chapter 3 of this thesis) used the same techniques to probabilistically assign scallop and Atlantic herring individuals to a North Sea jellyfish isoscape. As the scallop samples were from known catch locations, the accuracy and precision of assignment could be determined over a range of probability thresholds. Assignment accuracy and precision was equivalent to light based geolocator tags, supporting the use of isoscape assignment in conservation and fisheries management (Trueman et al., 2017)(Chapter 3 of this thesis). Continuous assignment has further been applied to salmon otolith isotope measurements within carbon and oxygen Baltic Sea isoscapes, to determine individual locations during different seasons (Torniainen et al., 2017).

2.4.2.2 Assignment requirements and limitations

To perform probabilistic assignments, the isoscape must have evenly distributed data with measured and quantified homogenous uncertainty values across space. The isoscape must also be relatively heterogeneous, with isotope gradients larger than observed individual differences. If isotope ratios are too similar, or too many isotopically similar areas exist, assignment accuracy and precision will be low.

Individual assignment potential is largely dependent on tissue choice (Graham et al., 2010, Hobson et al., 2010) and migratory behaviour (Hobson, 2005), as individual residency will determine the turnover time required for the isotopic value to be incorporated into the measured tissue (Graham et al., 2010, Seminoff et al., 2012, McMahon et al., 2013a, Tucker et al., 2014). Inert tissues, which remain metabolically inactive once formed, such as calcified and keratinous material (feathers, hair, baleen, otoliths etc.) are the most reliable for assignment (Hobson, 2005, Hobson et al., 2010, Ramos and Gonzalez-Solis, 2012), as animal movements can be determined during a known period of time when that tissue was formed (Hobson et al., 2010).

It must also be noted that when geolocating a predator to its foraging location using carbon and nitrogen isotopes (i.e. chemical tracers inherited through predator-prey interactions), prey are usually assumed to be static, however this is rarely true in the marine environment. Individual isotope ratio does not necessarily represent foraging location; it indicates where the nutrients, at the base of the food web, originate from for that consumer. This information enables specification of important nutrient contributing areas, however unless prey items are significantly less mobile than the predator, foraging area cannot be assumed.

2.4.2.3 Assignment applications

The study of marine animal migratory patterns, foraging area locations and origin is essential for conservation and management, both at the individual and ecosystem level (Hobson, 1999, Cherel and Hobson, 2007, Seminoff et al., 2012, Ceriani et al., 2014, Ceia et al., 2015). Understanding where populations are located, and the regions they travel between, is vital in understanding life history patterns, preferential foraging areas and diets, connectivity, population mixing, disease transfer and ability to adapt to environmental and anthropogenic change (Cherel and Hobson, 2007, Witteveen et al., 2009, Ramos and Gonzalez-Solis, 2012, McMahon et al., 2013a, Pethybridge et al., 2015, Vighi et al., 2015). At the ecosystem level, distinguishing important foraging, breeding and nursery areas enables effective spatial management and protection from over fishing, pollution and marine development (McMahon et al., 2013a, Ceia et al., 2015, Garza, 2016).

To date, migratory information has largely been provided by extrinsic tags (McMahon et al., 2013a), with mark and recapture and satellite tagging studies (Hobson, 2005, Hobson et al., 2010), which are highly effective for fine spatial scale information (Graham et al., 2010). Although continuously developing, tags remain relatively expensive and tend to be limited to studies consisting of a small number of large individuals, with a high probability of recapture (Graham et al., 2010, Seminoff et al., 2012), discriminating against small and rare individuals, which are difficult to recapture, but may require the highest conservation efforts (Trueman et al., 2012a, McMahon et al., 2013a). In addition, tags are not able to provide behavioural information of the individual at the determined location. Advances in the ecological use of naturally occurring stable isotopes and the production of marine isoscapes provides an alternative technique for studying juvenile, elusive and highly migratory animals, which may only be sited during selective periods of their life history (Hobson, 2005, West et al., 2006, Wunder and Norris, 2008b, McMahon et al., 2013a). Going forward, the combined use of both tagging based and isotope-based geolocation techniques will enable more refined and precise origin estimations (Seminoff et al., 2012, Vander Zanden et al., 2015, Cherel et al., 2016).

Marine isoscapes show great promise in ecological and biogeochemical research, providing cheaper, more efficient and therefore broader scaling solutions to traditional methods in many situations. This review goes as far as highlighting the benefits of regional *in situ* sample based isoscapes, preferentially using primary consumer tissues, with an even spatial coverage of data. Where sufficient *in situ* sampling is limited, work must focus on coupled models incorporating both *in situ* measurements and biogeochemical data, as well as explicitly quantifying variability across space. Also, increased research into less well studied isotopic elements, such as sulfur, and the combined use of isotope analysis and alternative geolocation techniques is required. Isoscapes are powerful tools with a wide scope of ecological applications, particularly when used in conjunction with other methods, however limitations must be fully understood to ensure appropriate interpretation and application of results.

Chapter 3 Stable-isotope based location in a shelf sea setting: accuracy and precision are comparable to light-based location methods

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3.1 Summary

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 <20% of the North Sea, with a mean linear error on the order of 10^2 km. 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.

3.2 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, Cawthorn et al., 2012, Nielsen 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 and 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 et al., 1999a, Graham et al., 2010, Seminoff et al., 2012, McMahon et al., 2013a). In recent years, stable isotope location has proven effective at reconstructing long-distance migrations in terrestrial, particularly avian, ecology (Rubenstein and Hobson, 2004, Wunder and Norris, 2008b, Hobson et al., 2012). Statistical models of spatial variation in the isotopic composition of precipitation (Bowen, 2010a), vegetation (West et al., 2007, Still and 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 and Norris, 2008b). 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 and Norris, 2008b)). Considerable debate remains around the most effective way to incorporate error and uncertainty into stable isotope-based geographic assignment methods (Wunder and Norris, 2008b, Van Wilgenburg and Hobson, 2011, Wunder, 2012, Bowen et al., 2014, Vander Zanden et al., 2015). Wunder (2012) 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., 2012a). 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., 2013a, Radabaugh et al., 2013, Jennings and 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 across marine isotopic gradients (Hobson and Schell, 1998, Jaeger et al., 2010, MacKenzie et al., 2011).

Marine carbon and nitrogen isoscape models are generated by interpolation from spatially explicit samples (Schell et al., 1998, McMahon et al., 2013a). Sessile invertebrates such as filter feeding bivalves have often been used to produce spatial isotope models (e.g. Jennings and 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 and Warr, 2003, Barnes et al., 2009b, 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 and Revenaugh, 2003), and has not been fully attempted for marine isoscapes. An alternative approach lies in relaxing the requirement for sessile reference organisms and instead selecting reference organisms that are widely distributed, but may have larger, but easily quantified, between-individual variance associated with diet or movement 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 relatively 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 isoscapes from lion's mane jellyfish (*Cyanea capillata*) expanding on the dataset and methods outlined in MacKenzie et al. (2014). 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 and van der Molen, 2015). We then identify feeding locations of 351 herring (*Clupea harengus*) caught at known locations throughout the North Sea.

3.3 Materials and methods

3.3.1 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 for jellyfish are illustrated in Fig. 3.1.

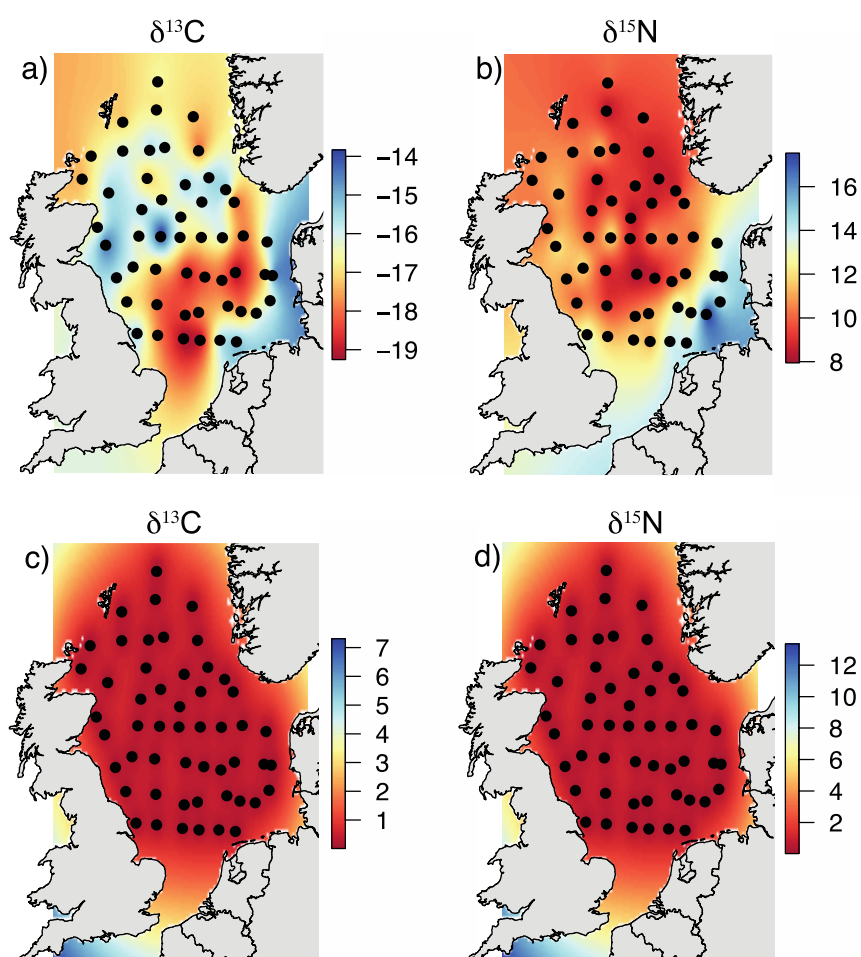


Figure 3.1 North Sea isoscape models (a,b) and associated variances (c,d) for $\delta^{13}\text{C}$ (a,c) and $\delta^{15}\text{N}$ (b,d) values based on *Cyanea capillata* sampled in 2015. Sample stations are indicated with filled circles.

A total of 351 individual herring were captured at 41 known locations within the North Sea during September 2011 as part of the International Bottom Trawl Survey. Fishing was

conducted from the RV 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, 2012b). Muscle samples were freeze-dried, ground to a powder and analysed for carbon and nitrogen isotopic composition. Capture locations for herring muscle are illustrated in Fig. 3.2.

Analyses were performed by either OEA laboratories or Elementex 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values was within 0.1‰ of long-term average values for this standard, and precision was 0.2‰ for $\delta^{13}\text{C}$ and 0.17‰ $\delta^{15}\text{N}$ values.

Jellyfish bell tissue $\delta^{13}\text{C}$ values showed a significant negative linear relationship with C:N ratios ($p = 4.54\text{e-}05$, 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 $\delta^{13}\text{C}$ values, measured $\delta^{13}\text{C}$ values were adjusted to those predicted for a lipid-free protein (atomic C:N ratio of 3.4) using linear regression between $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ values of jellyfish show a positive correlation with bell diameter, accordingly they were normalised to the median diameter (107mm):

$$\delta^{13}\text{C}_{S.cor} = \delta^{13}\text{C}_{cor} + (\text{Diameter} - 10.73) * 0.19$$

Herring muscle contained varying C:N ratios, and $\delta^{13}\text{C}$ values were corrected for lipid content arithmetically (Kiljunen et al., 2006).

Isotopic data from queen scallops were recovered from Jennings and Warr (2003) and Barnes et al. (2009b) for scallops sampled between 25 July and 29 September 2001, and from S. Jennings (Barnes et al., 2009a) for scallops sampled in similar locations in the summer of 2010 (Jennings and van der Molen, 2015). Up to seven individual scallops were sampled in each area. Locations of capture sites are shown in Fig. 3.2, Details of sampling, preparation and analytical methodologies are provided in Jennings and Warr (2003), Barnes et al. (2009b) and Jennings and van der Molen (2015).

3.3.2 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. 3.1 together with the associated spatial variances, and locations of jellyfish sampled to create the isoscapes.

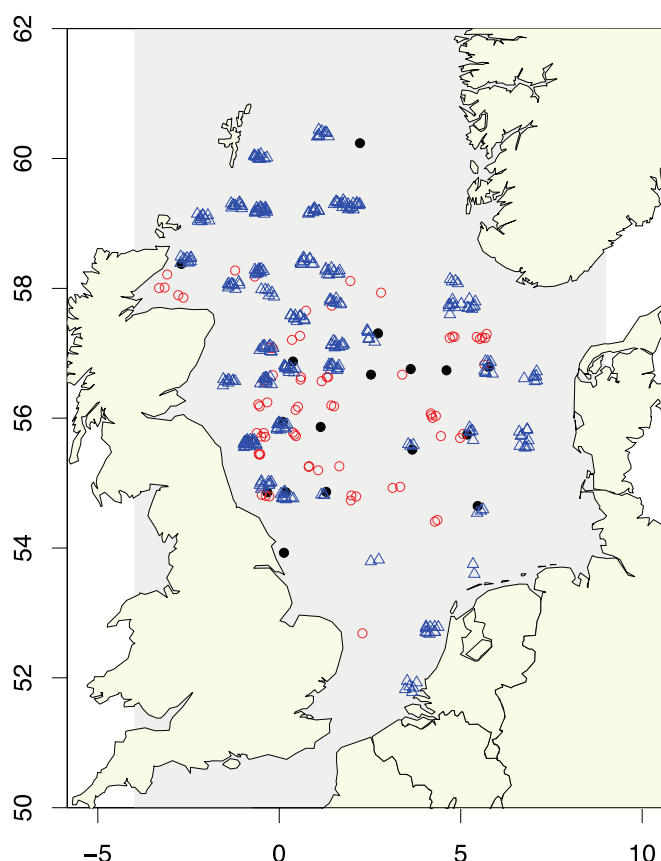


Figure 3.2 Locations of herring and scallop North Sea samples. Herring samples are represented by open triangles and scallop samples are represented by filled circles (2001 data) and open circles (2010 data).

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 $\delta^{15}\text{N}$ and 0.1‰ for $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}_{\text{cor}}$ and $\delta^{15}\text{N}$ 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:

$$\sigma_{iso(x,y)}^2 = \sigma_{k.iso(x,y)}^2 + \sigma_{m.iso(x,y)}^2 + \sigma_{bi.iso(x,y)}^2$$

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

Measurement error associated with $\delta^{15}\text{N}$ 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* $\delta^{15}\text{N}$ values (Jennings and Warr, 2003, Jennings and van der Molen, 2015). We estimate associated measurement precision associated with $\delta^{13}\text{C}$ values in scallop tissues as 0.2‰, similar to measurement errors associated with $\delta^{13}\text{C}$ analyses of *C. capillata*. Between-individual variance in lipid-corrected $\delta^{13}\text{C}$ values of scallops across 22 stations sampled in 2010 was 0.21‰ (Barnes et al., 2009a). Pooled error associated with the measurement of scallop stable isotope compositions is therefore given by:

$$\sigma_{assign(x,y)}^2 = \sigma_{m.assign(x,y)}^2 + \sigma_{bi.assign(x,y)}^2$$

where $\sigma_{assign(x,y)}^2$ is the pooled variance associated with the assignment, $\sigma_{m.assign(x,y)}^2$ is the variance associated with measurement error and $\sigma_{bi.assign(x,y)}^2$ 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 and Norris (2008b)). Trophic separation between jellyfish and scallops was constrained from known diet preferences. Scallops are filter-feeding molluscs sustained primarily on detrital phytoplankton and micro-zooplankton. 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 and 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 and 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values contained in the isoscape, and apply the smallest common 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 +0.85‰ ($\sigma = 0.5$) for $\delta^{15}\text{N}$ and -0.75‰ ($\sigma = 0.5$) for $\delta^{13}\text{C}$ values. The final tissue correction also corrects for any systematic under or over-estimation of trophic differences or isotopic fractionation. The estimated variance associated with calibration between scallop and jellyfish tissues σ^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,

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

where x and y refer to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values respectively, TD is the distribution of trophic difference values, TF is the distribution of isotopic fractionation values and σ^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 and 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 $\delta^{15}\text{N}$ and 0.39‰ for lipid-corrected $\delta^{13}\text{C}$ values. Minimum tissue offset values between herring and jellyfish were estimated as described above as +2‰ ($\sigma=0.5$) for $\delta^{13}\text{C}$ and +0.5‰ ($\sigma=0.5$) for $\delta^{15}\text{N}$. A summary of assignment conditions is provided in Table 3.1.

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

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

where $f(x, y | \mu_i, \Sigma)$ represents the probability that an individual with adjusted isotopic composition ($\delta^{13}\text{C}=x$ and $\delta^{15}\text{N}=y$) originates from a given cell (i) within the isoscape with mean isotopic composition equal to the components of vector μ_i , and variance co-variance matrix Σ . ρ is the correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values throughout the isoscape, σ_x and σ_y are the pooled standard deviations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values respectively given by the sum of the variances:

$$\sigma_{(x,y)} = \sqrt{(\sigma_{iso(x,y)}^2 + \sigma_{assign(x,y)}^2 + \sigma_{calib(x,y)}^2)}$$

The range in pooled error terms across the isoscape for scallop assignment was 3.5-12.4‰ for $\delta^{13}\text{C}$ values and 5.5-11.6‰ for $\delta^{15}\text{N}$ 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.

Table 3.1 Assignment conditions adopted for stable isotope-based location of scallops and herring against isoscapes derived from jellyfish tissues

Variable	Isoscape jellyfish	Scallop calibration	Herring calibration
Measurement error (σ)	$\delta^{13}\text{C}$: 0.1, $\delta^{15}\text{N}$: 0.2: measured	$\delta^{13}\text{C}$: 0.2, $\delta^{15}\text{N}$: 0.2: estimated / measured	$\delta^{13}\text{C}$: 0.2, $\delta^{15}\text{N}$: 0.2: measured
Between-individual variance	$\delta^{13}\text{C}$: 1.69, $\delta^{15}\text{N}$: 1.04: measured	$\delta^{13}\text{C}$: 0.2, $\delta^{15}\text{N}$: 0.7: estimated / measured	$\delta^{13}\text{C}$: 0.2, $\delta^{15}\text{N}$: 0.2: measured
Trophic distance	NA	1 ($\sigma = 0.25$): literature estimate	-0.2 ($\sigma = 0.25$): literature estimate
Isotopic trophic fractionation	NA	$\delta^{13}\text{C}$: 1($\sigma = 0.5$), $\delta^{15}\text{N}$: 3.4($\sigma = 0.5$): literature compilation	$\delta^{13}\text{C}$: 1($\sigma = 0.5$), $\delta^{15}\text{N}$: 3.4($\sigma = 0.5$): literature compilation
Tissue specific fractionation	NA	$\delta^{13}\text{C}$: -0.75($\sigma = 0.25$), $\delta^{15}\text{N}$: +1($\sigma = 0.25$): graphical estimate	$\delta^{13}\text{C}$: +2($\sigma = 0.25$), $\delta^{15}\text{N}$: +0.5($\sigma = 0.25$): graphical estimate
Threshold odds ratio	NA	1.33	1.5

3.3.3 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), and explore the trade-off between accuracy and precision. 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:

$$\text{Odds Ratio} = \frac{\frac{P}{1-P_i}}{\frac{P}{1-P_{i,max}}}$$

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 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 $2^{-1} = 50\%$ 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).

3.4 Results

3.4.1 Isoscapes

The spatial isotope models (isoscapes) derived from *C. capillata* are shown in Fig. 3.1a,b. Broad spatial patterns are similar to those shown in Jennings and Warr (2003), Barnes et al. (2009b), MacKenzie et al. (2014) and Jennings and 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 isoscape models is relatively low and constant across the region Fig. 3.1c,d.

3.4.2 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. 3.3. The assignment method provides better than random accuracy at all odds ratio thresholds (Fig. 3.3). 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 ($\sigma = 137$) km for the 2001 scallop data and 318 ($\sigma = 114$) km for the 2010 scallop data.

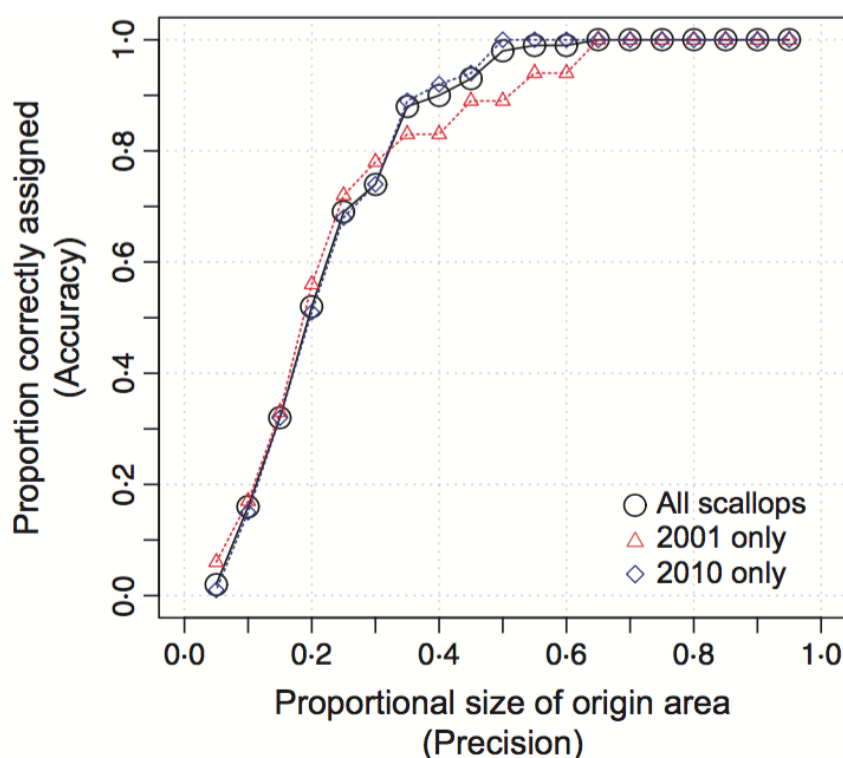


Figure 3.3 Accuracy and precision of assignment for the combined 2001 and 2010 scallop data. Precision is defined by the probability threshold and expressed as the proportion of data (i.e. cells) considered as likely. Accuracy is assessed as the proportion of individual scallops where the threshold area contained the known sample location.

3.4.3 Herring assignment

Herring were assigned to likely feeding areas using the assignment parameters outlined in Table 3.1. To report pooled results, individual herring areas were grouped according to body size. Following Van Wilgenburg and Hobson (2011), for each fish, cells designated as likely feeding areas were assigned a value of 1 and all other cells assigned a value of 0. Values were then summed for each cell across the total number of individual fish (Fig 3.4). Irrespective of capture location, larger fish are assigned to feeding areas in the central northern North Sea (Fig. 3.4a), consistent with summer fishery catches (ICES 2012, Fig. 3.4c). Smaller (juvenile) herring are assigned to feeding areas in the southern North Sea particularly around the German Bight (Fig. 3.4b), again consistent with locations of juvenile herring inferred from acoustic surveys (ICES 2012, Fig. 3.4d).

3.5 Discussion

3.5.1 Isotope based location accuracy and precision

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 4 independent sampling dates, although the exact location of boundaries between areas of high and low isotopic values varies slightly between sample suites. Consequently, assignment accuracy is relatively consistent between the two data sets (Fig. 3.3). 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 and 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 and van der Molen, 2015). The similarity between jellyfish and scallop isoscapes 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., 2013b), and the spatial isotope models presented here are unlikely to perform well.

3.5.2 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. 3.4), implying that isotope based location offers a promising additional tool for marine spatial ecology and management.

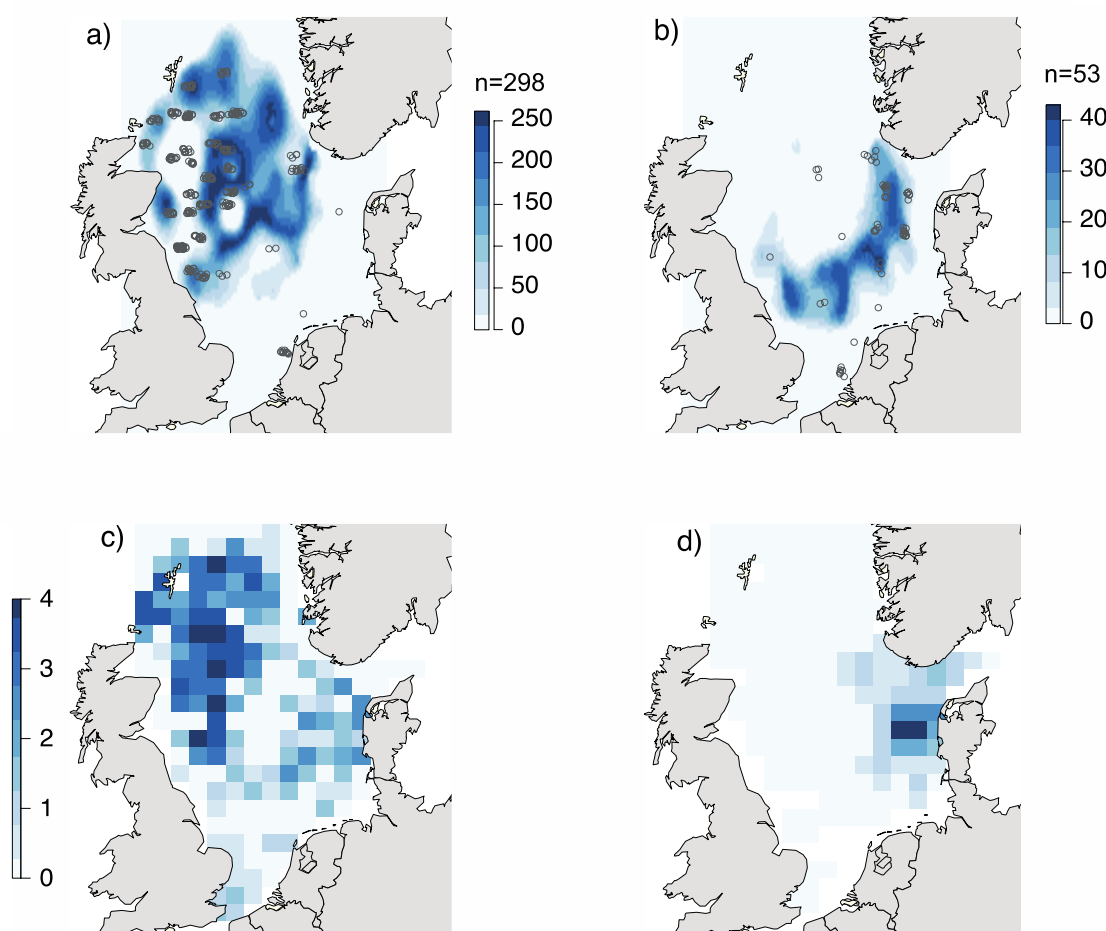


Figure 3.4 Comparison of isotope-based feeding area assignments and fisheries survey data (a,b). Most likely feeding areas for 351 North Sea herring sampled in September 2011 as derived from stable isotope-based location. Colours represent the number of individual herring assigned to each grid square. 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 (2012b). d) Estimated biomass of immature herring in June- July 2011 from combined acoustic surveys (figure modified from ICES (2012b)).

3.5.3 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 and Daskalov, 2007) fish, and isotopic equilibration is likely to occur with a half life on the order of c.50 days (Miller, 2006, 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.

Chapter 4 Moulting location and diet of auks in the North Sea, inferred from coupled light-based and isotope-based geolocation

This chapter is a reproduction of text currently in press with *Marine Ecology Progress Series*, and as such is written in the style of the journal.

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4.1 Abstract

Many pelagic seabirds moult their feathers while at sea, which is an energetically costly behaviour. Mortality rates during moult can be high, so spatial and trophic ecology during this critical period is important for understanding demographic patterns. Unfortunately, individual foraging behaviours specifically linked to at-sea moult are commonly unclear. This paper combines two different approaches to geolocation: data from bird-borne geolocation loggers and stable-isotope assignment using carbon and nitrogen isotope maps (isoscapes). Coupling two geolocation processes allows some uncertainties associated with isotope-based assignment to be constrained. We applied this approach to quantify species-specific foraging locations and individual trophic variability during feather regrowth in three sympatric auk populations breeding on the Isle of May, Scotland (common guillemot (*Uria aalge*), razorbill (*Alca torda*) and Atlantic puffin (*Fratercula arctica*)). Inferred foraging areas during moult differed between species and feather types. Guillemots likely underwent moult within the southern North Sea, razorbills along the east coast of England and into the southern North Sea and puffins off the east coast of Scotland. Estimates of individual trophic position varied considerably within feather types (up to 1 trophic level difference between individuals), among feather types grown during different time periods, and across the three species, with guillemots consistently foraging at higher trophic positions than razorbills and puffins. Used in combination, these methods better constrain foraging areas during moult, as well as providing a technique to explore individual differences and flexibility in foraging strategy, which is valuable information for both seabird conservation and marine spatial planning.

4.2 Introduction

The widespread use of miniaturised, ring-mounted data loggers that use light levels in conjunction with a time base to provide positional data, has greatly increased our knowledge

of important foraging grounds for pelagic seabirds. Tag-based geolocation data are particularly useful in time periods when birds are away from the breeding colonies, for example by identifying migration routes and locations of wintering hot spots (Guilford et al., 2009, Jessopp et al., 2013, Frederiksen et al., 2016). Birds must replenish feathers to maintain feather function. In marine birds, feather moult typically occurs outside the breeding season and potentially has major fitness consequences because flight feathers are crucial for long distance migration while body feathers play an important role in thermoregulation, particularly for diving species (Daunt et al., 2006). In most alcid species, there is typically one period of the non-breeding season lasting a few weeks when moult of flight feathers is focused, known as the main feather moult. At this time individuals have fewer foraging options making them less able to cope with adverse environmental conditions or reduced prey availability (Sandvik et al., 2005, Harris et al., 2014). Environmental sensitivity during feather moult is particularly acute in species such as auks and ducks that are flightless while replacing their regimes. Given the increasing pressures on the marine environment from multiple anthropogenic activities, quantification of foraging location and diet whilst birds are moulting is urgently required for effective conservation and marine spatial planning (Grecian et al., 2010, Lewison et al., 2012, JNCC, 2015, MMO, 2015).

Geolocator derived data alone cannot identify specific foraging locations during moult as individual moult timing is unconstrained therefore location and foraging behaviours during winter feather moult are relatively poorly known (Sandvik et al., 2005, Harris and Wanless, 2011, Breton et al., 2014). The stable isotope compositions of animal tissues provide a complimentary approach for geolocating animals, particularly when combined with isoscapes (spatially explicit predictive maps of isotope ratios derived from process level models of isotope fractionation or distribution (Royle and Rubenstein, 2004, Hobson et al., 2010)).

Stable-isotope-based geolocation has been widely used for migratory terrestrial birds utilising well-established hydrogen isoscapes (Wunder and Norris, 2008b, Wunder, 2010, Hobson et al., 2012, Hobson et al., 2014). In the marine environment, the ocean is spatially homogenous with regard to hydrogen isotope ratios, so $\delta^2\text{H}$ cannot be used to assign marine animals to a likely geographic origin (Trueman et al., 2012a). The isotopic composition of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in phytoplankton varies spatially, largely reflecting differences in the isotopic composition of nutrients and phytoplankton taxonomic composition. Spatial variations in the isotopic compositions of phytoplankton are passed through the food chain to higher trophic level organisms (Jennings and Warr, 2003, Some et al., 2010, McMahon et al., 2013a) and can be used to infer animal movement patterns across marine isotopic gradients (Quillfeldt et al., 2005, Cherel and Hobson, 2007, Cherel et al., 2008,

MacKenzie et al., 2011). In the context of feather moult in seabirds, the isotopic composition of a regrown feather provides a chemical record of the area from which resources used in feather growth are derived.

To date, the limited number of detailed and spatially explicit, regional and *in situ* sample based marine isoscapes (Jaeger et al., 2010, MacKenzie et al., 2014, Radabaugh and Peebles, 2014, Vander Zanden et al., 2015) has limited the application of these techniques in the marine environment. Geolocation using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of animal tissues is further complicated as the isotopic composition of a consumer's diet reflects both the spatial origin of the prey items and their trophic level. Here we address this limitation by combining light- and stable-isotope-based geolocation approaches.

Bird-borne data loggers can indicate the general location of an animal at known points in time (with an error on the order of $\pm 200\text{km}$ (Phillips et al., 2004, Lisovski et al., 2012)), whereas $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements depict the most likely origin of the resources assimilated during tissue growth and are influenced by both the location of foraging and composition of the diet. Isotope-based geolocation is generally performed by comparing the isotopic composition of a consumer's tissue to a spatial model (isoscape). If the reference isoscape is created using tissues from species other than the species to be assigned, then some form of isotopic offset or calibration is needed (e.g. Bowen et al. (2014) and Trueman et al. (2017)). Consumer tissues are typically enriched in ^{15}N by 3.4‰ and in ^{13}C by 1‰, relative to their prey (Kelly, 2000, Cherel and Hobson, 2007, Ramos and Gonzalez-Solis, 2012). Hence, accurate geolocation using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios requires prior knowledge of the isotopic difference between the reference isoscape and the measured tissue attributed to differences in tissue composition, trophic level and individual physiology. Individual differences in trophic geography (the location and composition of a consumer's diet (Bird et al., 2018)) may, however, complicate the use of a single calibration value applied to all individuals within a population. Population-level isotopic calibration-offsets between reference isoscapes and tissues of interest can be estimated if the region of foraging is known *a priori*. Population average tissue isotopic compositions can be compared to spatially averaged isotope compositions across the known foraging area extracted from isoscapes (Fig. 4.1). This approach assumes that individual differences in isotopic calibration effects are normally distributed.

Here we studied individual variation in location and diet during winter moult in three species of auks: common guillemot (*Uria aalge*, hereafter guillemot), razorbill (*Alca torda*) and Atlantic puffin (*Fratercula arctica*, hereafter puffin) from the Isle of May (Fig. 1), a major colony in the western North Sea. We draw on light-based geolocator data from breeding individuals for which we also had stable isotope data for flight and body feathers. We

interpreted isotope data with reference to recently developed $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes for the North Sea (MacKenzie et al., 2014, Trueman et al., 2017). Our primary aims were to determine population-level foraging locations and quantify individual diet variability during annual moult by combining two complimentary geolocation techniques.

4.3 Methods

4.3.1 Study area and species

Fieldwork for this study was carried out on the Isle of May National Nature Reserve, southeast Scotland ($56^{\circ}11'\text{N}$, $2^{\circ}34'\text{W}$) (Fig. 4.1) which is a major breeding colony for puffins (*c.*40,000 pairs), guillemots (*c.*16,000 pairs) and razorbills (*c.*3,000 pairs) in the western North Sea. Data from ringing recoveries of all three species indicate that most individuals from the Isle of May winter and moult (shed and replace their feathers) within the North Sea (Wernham et al., 2002). However, information from geolocators deployed in the Isle of May colonies indicates that some puffins travel around the north of Scotland into the northeast Atlantic and a single, probably atypical, guillemot had replaced its wing feathers while in the Barents Sea in three consecutive winters while all other guillemots appear to have moulted in the North Sea (Harris et al., 2010, Harris et al., 2015b).

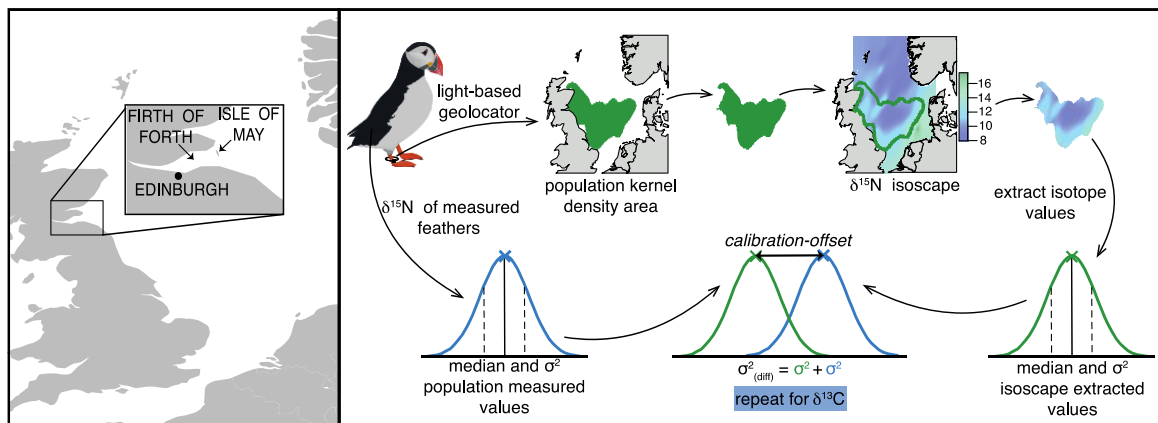


Figure 4.1 Schematic depicting the location of the Isle of May and the methodology used to determine calibration-offset values between seabird measured feather isotope values and isoscape isotope values within the over winter locations indicated by light-based data loggers. This method was repeated for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for all species and feather types.

Evidence from beached birds and observations at sea suggest that timing of moult differs somewhat between the species although information at the colony level is largely lacking (Gaston and Jones, 1998). Guillemots undergo a complete post-breeding moult soon after they leave the colony in July, emerging into the winter plumage when birds have white

cheek and neck feathers. The primary wing feathers are shed first followed soon by the secondaries resulting in birds becoming flightless for between 25 and 80 days (Thompson et al., 1998) and regrowth is complete by the end of September. In October birds from the Isle of May immediately start a partial pre-breeding moult back into summer plumage when the white feathers of the head and cheek are replaced by dark ones; this moult is completed by the end of December (Harris and Wanless, 1990). Body feathers are not replaced during the pre-breeding moult (Gaston and Jones, 1998). Razorbill moult is similar except that the post-breeding moult starts slightly earlier and pre-breeding moult starts later so that cheek and neck feathers are not replaced until late winter/early spring, meaning that adults spend several months longer in winter plumage compared to guillemots (Harris and Wanless, 1990, Wernham et al., 2002). Puffins undergo a complete moult of the feathers of the body and head (when the white cheek feathers are replaced by black ones) soon after leaving the colony in mid- to late July. The timing of the replacement of the flight feathers, and hence the flightless period, appears to be much more variable compared to guillemots and razorbills and can occur any time between September and March, with peaks in October and March (Harris et al., 2014). The partial pre-breeding moult during which the black feathers of the winter face are replaced by white ones occurs immediately prior to the birds returning to the colony in March (Harris and Wanless, 2011).

Small fish, mainly sandeels (Ammodytidae), juvenile herring (*Clupea harengus*) and sprat (*Sprattus*) and various species of juvenile Gadidae, make up the bulk of the diet of all three auks during winter. Some crustacea, polychaete worms and other pelagic invertebrates are also eaten, the proportion of invertebrates in the diet appearing to be higher in the puffin (45% by biomass) than in the guillemot (<5%) (Blake, 1984, Blake et al., 1985, Sonntag and Hüppop, 2005, Harris et al., 2015a). The limited data available describing winter diet for razorbills in the North Sea suggest that few invertebrates are eaten but elsewhere crustacea can dominate the winter diet (Hipfner and Chapdelaine, 2002, Lilliendahl, 2009).

4.3.2 Data Loggers

In June and July 2014 breeding birds were caught and each was equipped, under British Trust for Ornithology licence, with a data logger (Migrate Technology, UK: model C250 on guillemots, model C65 on razorbills, model W65 on puffins) attached to a plastic leg ring (combined mass of logger and ring < 0.4 % the mass of the adults on which they were deployed). Birds were recaptured in June and July 2015, the data loggers removed and the data downloaded. Details of the field protocols for guillemots and puffins are given in Harris et al. (2010) and Harris et al. (2015b). The methods for razorbills were the same as those for guillemots.

The data loggers measured light intensity at 60 second intervals and recorded the maximum value in each 10 minute interval. This allowed the determination of dawn and dusk which when linked to a time base enabled the determination of latitude from the duration of night and day, and longitude from timing of local midnight or mid-day. Details of data-handling and processing are given in Hanssen et al. (2016). Latitudes are unreliable around the equinoxes so locations during the periods 10 September to 18 October, and 20 February to 5 April were excluded, as were latitudes that were clearly unreliable on visual inspection. The average error of locations has been estimated to be ± 200 km (Phillips et al., 2004) so for birds with coastal distributions many positions occurred over land but such positions were not filtered out.

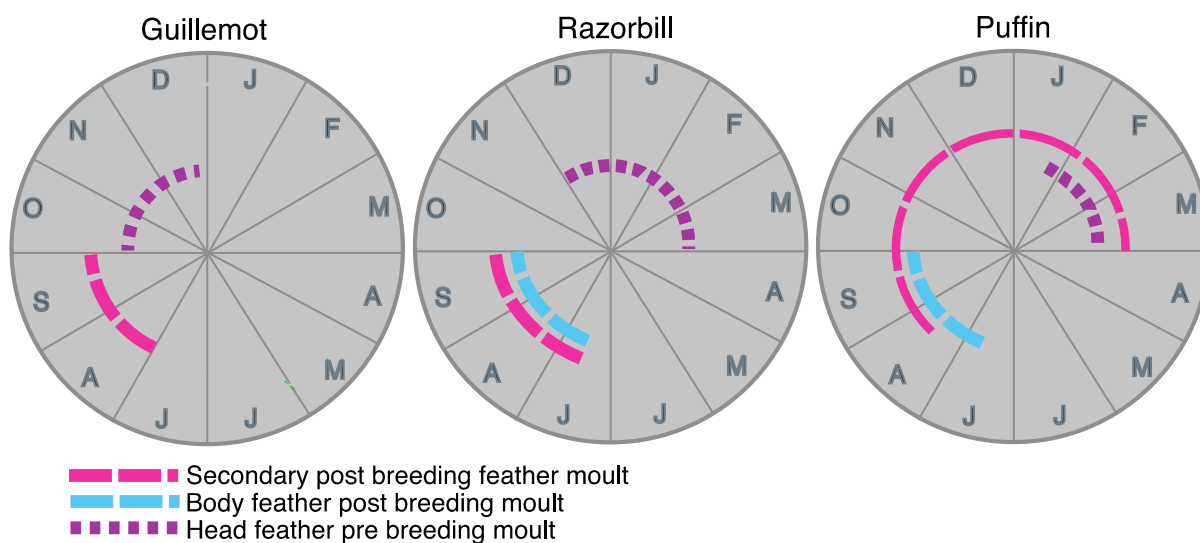


Figure 4.2 Graphical representation of assumed moult timings used for subsetting data logger-based geolocation data in guillemots, razorbills and puffins. The prolonged period for secondary moult of puffins is due to the extreme inter-bird variability in the timing of this moult.

To assess usage of the North Sea by each species, temporally specific kernel density maps were produced in R 3.1.2 (R Core Development Team, 2016). Kernel density data were subset for each population for the known moulting periods of each feather type (Fig. 4.2). As secondary feather moult timing of puffins is extremely variable, in this case a kernel density area over the entire winter period was produced. Temporally specific kernel density maps provide a visual representation of the likely location of populations of birds at broadly known moulting seasons throughout the winter period, but locations are not necessarily specific to the individual short term feather moult and/or regrowth periods. Utilization distributions were estimated using Kernel density distribution with the 'bkde2d' function in the 'KernSmooth' package (Wand and Jones, 1995). All grid cells (0.1 decimal degrees square) with a population density value greater than 0.01 were interpreted as a possible location, and

each cell within this area was given a value of 1. This was done in order to compare geolocation results with isotopic assignment areas, which represents all likely moulting areas within the isoscape. Individuals with inferred kernel density areas outside the North Sea (1 guillemot secondary feather out of 20 samples, and 1 razorbill cheek feather out of 8 samples) were excluded from the population kernel density areas and the rest of the study, as the isoscape range is currently limited to within this region.

4.3.3 Stable isotope data collection and analysis

On recapture of each data logger-equipped bird, samples of feathers were collected, under UK Home Office licence. The distal two-thirds of a central secondary wing feather was snipped off each individual. Secondary feathers moult synchronously in the study species, therefore the specific secondary feather sampled was not specified. In addition 2-5 ventral body feathers and 2-5 head feathers were taken from the sides of the neck (guillemot) or cheeks (puffin and razorbill) (Table 4.1). Feathers were stored in paper envelopes and deep-frozen until needed.

Table 4.1 Sample sizes of feathers collected from data logger-equipped birds known to spend the winter within the North Sea.

	Guillemot		Razorbill			Puffin		
Feather Type	Neck	Secondary	Cheek	Body	Secondary	Cheek	Body	Secondary
Sample size (Birds)	18	19	7	9	9	3	12	12

Feathers were cleaned of surface contaminants using 0.25M NaOH and rinsed with milliQ water, dried in a 60°C oven for 12 hours, then cut into small fragments avoiding the quill and shaft. For secondary, body and neck feathers only one feather sample was analysed per individual; however, cheek feathers were pooled to obtain enough material for analysis. A 0.5-0.7mg sample was weighed into a tin capsule and bulk $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were measured by Elementex Laboratories, Cornwall, UK.

4.3.4 Population level assignment

The carbon and nitrogen isoscapes for the North Sea produced by Trueman et al. (2017) were used to assign seabirds to likely foraging areas. Although these isoscapes were produced from samples collected in summer 2015, the spatial distribution $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

values in consumers within the North Sea are relatively stable over time (MacKenzie et al., 2014, Trueman et al., 2017). Seasonal variations in isotopic compositions of primary producers and low trophic level consumers are probable (Kürten et al., 2013b) but are likely attenuated during successive predator-prey interactions so that higher trophic level consumers (such as the jellyfish used to construct the isoscape and seabirds) inherit isotopic compositions that reflect a seasonal average value. Therefore we assume that the time difference between isoscape construction and collection of bird samples is unlikely to significantly influence geographic assignments at the broad spatial scales considered here. Isotope values for each species and feather type were used to identify a most likely spatial origin within the North Sea using continuous assignment methods following Trueman et al. (2017) and Vander Zanden et al. (2015). Assignments were made by estimating the likelihood that each raster cell of the North Sea carbon and nitrogen isoscapes represented the foraging area of each individual, using the bivariate normal probability function (Vander Zanden et al., 2015, Trueman et al., 2017). Assignment results indicate the likely origin of food resources the birds utilised during the period of feather growth. As auks are flightless during flight feather moult, these locations were determined as the likely location of shedding and regrowth of secondaries.

The North Sea isoscape models were derived from jellyfish bell tissues, therefore a calibration was required to refer the isotope values to bird feathers, accounting for systematic isotopic offsets arising from differences in the amino acid compositions of tissues, and combined effects of trophic level and physiology. Data loggers provided an independent estimate of location from which a population-average calibration-offset value was derived for each species and feather type. The calibration-offset value combines any isotopic offset between jellyfish tissue and feather keratin associated with differences in amino acid composition, which is assumed to be consistent across all species and feather types (however specific alcid offset values have yet to be explicitly determined), and isotopic offsets associated with differences in trophic level between jellyfish and seabirds. Any trophic offset (trophic enrichment factor) may vary among individuals, species and feather types due to differences in diet compositions and diet quality during different time periods. To estimate calibration-offset values, the most likely spatial location of birds at the population level was estimated from light-based geolocation data as the average temporally specific kernel density areas for the population. Population-level likely locations were then overlaid on the carbon and nitrogen isoscapes and spatial average (median and standard deviation) isotope values were extracted at the corresponding coordinates (Fig. 4.1). Median extracted values represent the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ composition the birds would display foraging within these estimated regions during moult, if tissue and diet correction factors were not required. By comparing the population median expected values in regions prescribed by light-based

geolocation, to the population median measured isotope values, population level calibration-offset values can be estimated (Fig. 4.1);

$$\text{Calibration-Offset}_{(C,N)} = \text{Median Measured Value}_{(C,N)} - \text{Median Extracted Value}_{(C,N)} (+/- \text{error terms})$$

Combined variance values were calculated by summing the isoscape-extracted isotope variance value and the measured isotope variance value from each feather type and species;

$$\sigma^2_{(Combined)} = \sigma^2_{(isoscape \text{ extracted values})} + \sigma^2_{(feather \text{ measured values})}$$

Population level calibration-offset values were applied to each individual within a population and of the same feather type to compare feather and jellyfish isotopic compositions. Individual birds were then assigned to likely foraging areas as per Trueman et al. (2017), using assignment conditions summarised in Table 4.2. A threshold odds ratio was set at 1.42 (representing approximately 70% of the probability values and 70% of the isoscape area), meaning any grid cells with a probability value greater than this threshold (the highest 30%) were classed as likely, and all other grid cells were classed as unlikely (Trueman et al., 2017). The total number of individual birds assigned to each grid cell was summed for each species and feather type, to depict the most likely population-level foraging locations.

Isotope-based likely foraging locations during moult, and light based kernel density areas, representing possible locations visited during the assumed seasonal feather moult period, were mapped together. The resulting overlapping locations were inferred as the most likely predicted moult locations based on two alternative geolocation techniques.

Table 4.2 Assignment conditions adopted for stable isotope-based location of guillemots, razorbills and puffins against isoscapes derived from jellyfish tissue.

Variable	Isoscape Jellyfish	Seabird Assignment		
		Guillemot	Razorbill	Puffin
Measurement error (σ) (measured)(‰)	$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$: 0.2	$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$: 0.2		
Between individual variance (measured)(‰)	$\delta^{13}\text{C}$: 0.78, $\delta^{15}\text{N}$: 1.02	$\delta^{13}\text{C}$: 0.52, $\delta^{15}\text{N}$: 0.91	$\delta^{13}\text{C}$: 0.51, $\delta^{15}\text{N}$: 0.58	$\delta^{13}\text{C}$: 0.53, $\delta^{15}\text{N}$: 0.56
Calibration-Offset and variance values	NA	<i>Derived – see Results Table 4.3</i>		
Threshold odds ratio	NA	1.42		

4.3.5 Isotope variation among individuals

Individual differences in foraging behaviour within a population were then investigated to determine the extent of deviation from an average population diet. It was assumed that any isotopic differences associated with the difference in protein compositions between jellyfish and feather proteins are consistent within species and feather types. Thus, individual variability between feather isotopic values within a population moulting in the same region at the same time are assumed to largely represent isotopic differences associated with diet. To assess individual-level foraging behaviour, a similar approach was adopted as used to determine population calibration-offset values (Fig. 4.1).

Individual-level light-based logger kernel density areas (rather than population kernel density areas) were overlaid on the carbon and nitrogen isoscapes, and the corresponding spatial median and variance isotope values were extracted (Fig. 4.1). To calculate the expected feather isotope values, the population-level isotopic calibration estimate was applied to each individual. If the individual was foraging at the same trophic position as the average value for the population, the expected and extracted isotope values would match. Individual differences in isotopic compositions associated with diet were therefore quantified as:

$$\text{Ind. Expected value}_{(C,N)} = \text{Median Extracted Ind. value}_{(C,N)} + \text{Pop. Calibration-Offset}_{(C,N)}$$

$$\text{Residual Ind. Variability}_{(C,N)} = \text{Ind. Expected value}_{(C,N)} - \text{Ind. Measured Value}_{(C,N)}$$

Residual individual variability measures indicate the deviation of each individual from the average population calibration-offset value, reflecting the range of trophic levels that individuals from the same population are feeding over and any individual variability in isotopic fractionation due to differences in diet quality and physiological stress (McMahon et al., 2015). The extent of residual individual isotopic variability associated with diet within species and feather types were displayed graphically using density histogram plots produced in R 3.1.2 (R Core Development Team, 2016).

4.4 Results

4.4.1 Population moult location

In all species and feather types the population median isotope-based assignment areas overlapped light-based estimates of likely location during the moulting period, indicating consistency of methods and allowing refinement of the most likely foraging areas during moult (Fig. 4.3). The refined most likely foraging area, determined from the coupled geolocation approach, shows greater precision in comparison to either geolocation method used alone, as depicted by the reduced surface area (Fig. 4.3). The guillemots most likely grew their secondary and neck feathers while in the mid to southern North Sea (Fig. 4.3a,b). Razorbills also most likely grew cheek feathers in the southern North Sea (Fig 4.3c), whereas body and secondary feather moult likely occurred off east England or north of the Firth of Forth (Fig. 4.3d,e). For puffins, the most likely location of foraging prior to feather moult was off northeast Scotland or east England across all feather types (Fig. 4.3f-h).

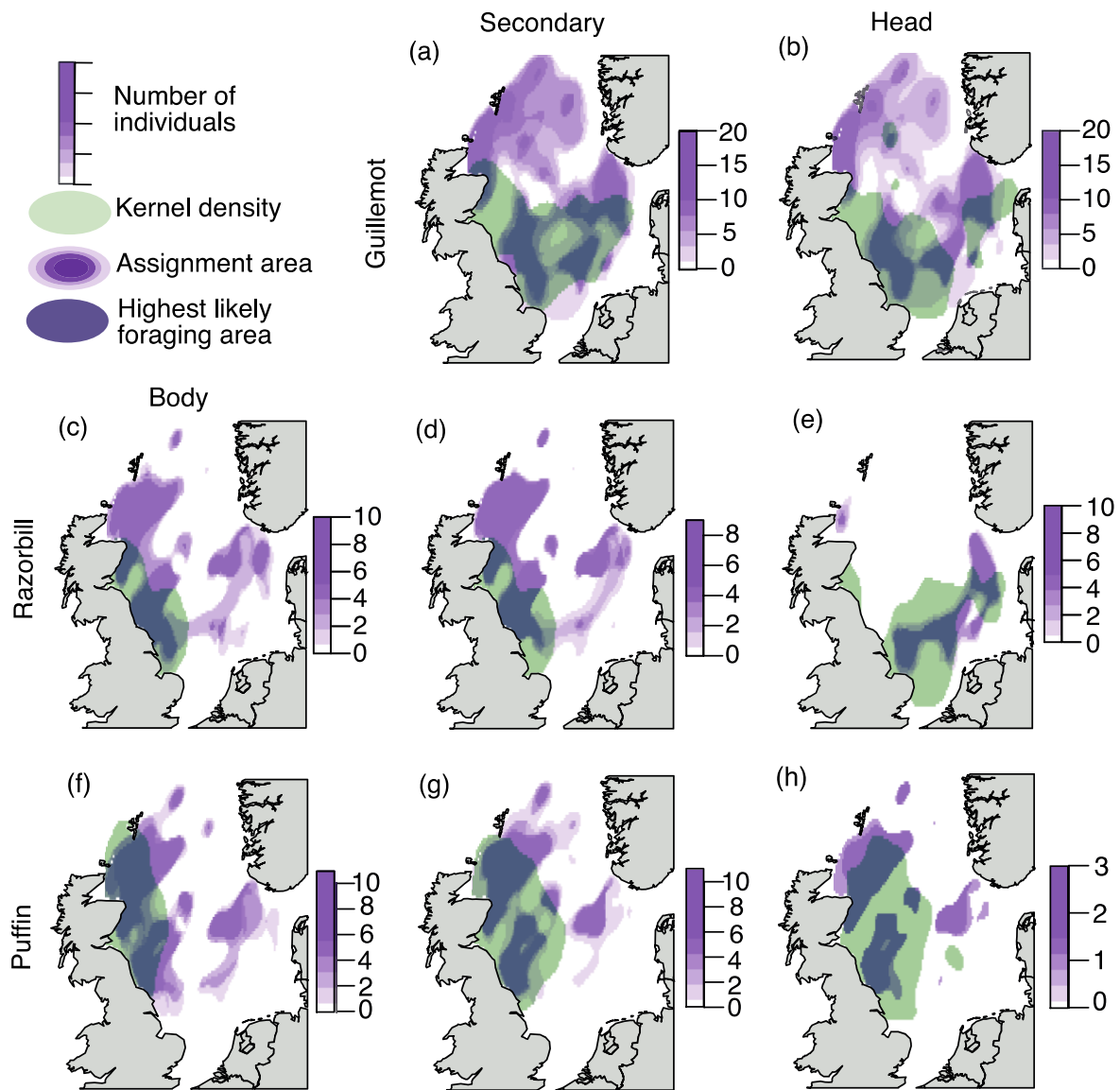


Figure 4.3 Population isoscape assignment areas and corresponding kernel density areas. Isoscape assignment areas (purple: darker colours indicate more individuals consumed food resources from this area) and kernel density areas (green: where density values were >0.01) for the months when moult of each feather type for each species (guillemots, razorbills and puffins) is known to occur. Guillemot (a,b) secondary and neck feathers, razorbill (c,d,e) body, secondary and cheek feathers and puffin (f,g,h) body, secondary and cheek feathers. Overlap area, and therefore the highest likely foraging area during feather moult is indicated in dark violet

4.4.2 Population and individual offsets

Isotopic offsets between jellyfish and feather tissues for carbon ($\delta^{13}\text{C}_{\text{f-j}}$) were relatively similar across all species and feather types, with specific species and feather type calibration-offset values falling within 2‰ and ranging between -0.77 and 0.91‰ (Table 4.3, Fig. 4.4). Guillemot feather isotope values had consistently higher $\delta^{13}\text{C}$ values than jellyfish tissue whereas puffins had lower $\delta^{13}\text{C}$ values and razorbill feathers showed mixed results (Table 4.3, Fig. 4.4). Isotopic offsets between feather keratin and jellyfish bell tissues for nitrogen

($\delta^{15}\text{N}_{\text{fj}}$) ranged from 4.53-7.23‰. $\delta^{15}\text{N}_{\text{fj}}$ values were highest for guillemots, followed by razorbill cheek feathers. Razorbill body and secondary feathers and all puffin feather types had similar $\delta^{15}\text{N}_{\text{fj}}$ values (Table 4.3, Fig. 4.4).

Table 4.3 Population calibration offset values and combined variances calculated from the difference between median extracted isotope values and median measured isotope values. Individual isotope difference values are the difference between individual expected isotope values (individual extracted + population offset) and individual measured values and represent individual diet differences.

	Guillemot		Razorbill			Puffin		
Feather	Neck	Secondary	Cheek	Body	Secondary	Cheek	Body	Secondary
$\delta^{13}\text{C}$ Cal. Offset (‰)	0.75 ±1.68	0.47 ±1.33	0.91 ±1.02	-0.33 ±1.07	-0.08 ±1.18	-0.2 ±0.84	-0.65 ±0.98	-0.77 ±0.73
$\delta^{15}\text{N}$ Cal. Offset (‰)	7.23 ±2.81	6.74 ±2.20	6.30 ±2.70	4.64 ±1.11	4.86 ±0.74	5.87 ±1.02	4.53 ±0.97	5.21 ±0.93
$\delta^{13}\text{C}$ Ind. Diff. (‰)	-0.62 – 2.51	-0.65 – 0.91	-0.25 – 1.86	-0.33 – 1.00	-0.21 – 1.41	-0.15 – 0.59	-0.87 – 1.31	-1.47 – 0.83
$\delta^{15}\text{N}$ Ind. Diff. (‰)	-1.67 – 2.06	-1.70 – 1.20	-0.72 – 0.71	-1.18 – 0.76	-0.21 – 0.89	-0.84 – 0.08	-1.28 – 1.50	-0.75 – 0.75

A large degree of residual individual variability in diet-related isotopic compositions was observed within species and feather types (individual $\delta^{13}\text{C}$ values ranged from 0.74-3.13‰ and $\delta^{15}\text{N}$ values ranged from 0.92-3.73‰), likely representing up to 1 trophic level difference between individuals within a population (Fig. 4.4). In general, guillemots showed the greatest among individual residual isotopic variability in both feather types, with greater variability observed in $\delta^{15}\text{N}$ values. Razorbill and puffin body feathers also displayed greater among-individual residual variation in $\delta^{15}\text{N}$ values, compared to $\delta^{13}\text{C}$ values, whereas the opposite was observed within secondary feathers (Fig. 4.4). The small number of individual puffin cheek feather samples prevents reliable interpretation of results.

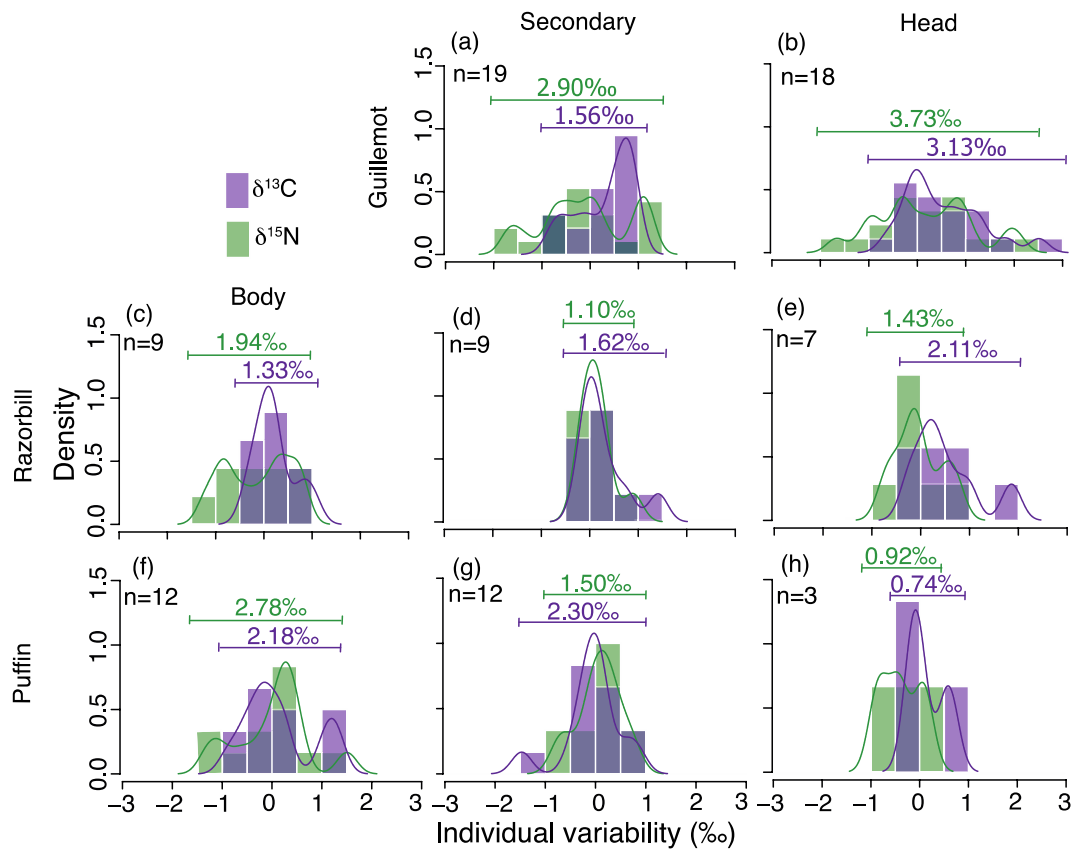


Figure 4.4 Individual carbon and nitrogen isotope variability of guillemot, razorbill and puffin feathers displayed in density histograms. Individual variability is calculated by determining the difference between the individual expected and measured isotope values, where expected values are individual extracted isotope values plus/minus the population calibration-offset. Guillemot (a,b) secondary and neck feathers, razorbill (c,d,e) body, secondary and cheek feathers and puffin (f,g,h) body, secondary and cheek feathers. Numbers in green ($\delta^{15}\text{N}$) and purple ($\delta^{13}\text{C}$) are the isotope variability ranges for each feather type and species, n = number of individuals.

4.5 Discussion

Both bird-borne data loggers and isoscape assignment geolocation methods produce relatively accurate location and resource origin information with quantifiable degrees of error (Phillips et al., 2004, Trueman et al., 2017), but both methods currently lack precision. Combining the two techniques begins to address some of complications surrounding isoscape assignment, particularly calibration-offsets when the species of interest is different to the organism used to define the isoscape. Combining geolocation approaches may improve assignment precision whilst maintaining accuracy and provides additional information on individual dietary variation during the annual moult. Here we combined light-based and stable-isotope based geolocation methods to compare foraging location and trophic position in three species of auk while they were growing wing and body feathers, but the approach

could readily be applied to other populations of seabird that winter in the North Sea and can be fitted with data loggers.

4.5.1 Feeding locations during moult

The most likely foraging region of Isle of May guillemots during post-breeding secondary feather growth and pre-breeding neck feather growth in guillemots was in the southern North Sea. This area has previously been identified as a major wintering area for guillemots including those from the Isle of May (Stone et al., 1995, Harris et al., 2015b), but our results emphasise its importance for moult. To our knowledge, the light based data logger results presented here are the first for razorbills from a North Sea colony. Taken together with the stable isotope data they indicate that foraging during the post-breeding body and secondary feather growth most likely occurred in waters off the east coast of England, whereas pre-breeding cheek feather growth primarily occurred in the southern North Sea suggesting a shift in foraging location during different feather moults. Individual puffin overwintering areas vary greatly, with locations of birds from the Isle of May sometimes extending beyond the North Sea into the northeast Atlantic (Harris et al., 2010, Harris et al., 2013). In our study, puffin foraging areas during moult of individuals that remained in the North Sea were relatively consistent across the three feather types, with the most likely foraging areas during autumn and spring restricted to waters off the east coast of Scotland and northeast England.

Body feathers of pelagic species are not always grown outside the breeding season (Graña Grilli and Cherel, 2016), which could affect interpretation of the results. However, neck and cheek feather moult of these auk species definitely occurs outside the breeding season as indicated by the change in feather colour between the summer and winter plumage. Isotope-based geolocation is currently only possible within the North Sea; a more extensive isoscape is required to investigate foraging location during moult of populations with winter distributions that extend beyond the North Sea into the east Atlantic.

4.5.2 Diet during moult

In the absence of a dataset of tissue samples from animals from known spatial origins, accurate spatial assignment to an isoscape relies on determining trophic level and tissue offset estimates for each individual and species (e.g. (Trueman et al., 2017)). In our study, locations of individual auks estimated via light-based data loggers were used to calibrate isotopic offsets between the reference isoscape and feather tissues, and therefore derive population level (combined tissue and trophic) calibration-offset values. By then assuming that the isotopic contrast associated with differing protein composition of jellyfish bell tissue

and species and feather specific keratin is constant, between-individual residual variations in isotopic composition should reflect differences in individual dietary effects.

In general, inferred trophic positions were in line with previous studies of winter diet, i.e. with guillemots feeding at a higher trophic level than razorbills (Blake, 1984, Blake et al., 1985). The trophic niche of puffins has been found to decrease from a highly specialised high trophic level diet in summer to a more generalized lower trophic level diet, consisting of more invertebrate prey items, in winter (Hedd et al., 2010, Harris et al., 2015a). Our results add to this picture and suggest that within the North Sea auk community guillemot, razorbill and puffin populations have different winter diets during different feather moult periods and trophic level segregation could be a mechanism to reduce interspecific competition outside the breeding season.

The high degree of residual individual variability (Table 4.3, Fig. 4.4) between and within feather types and species indicates flexible and generalist diets during the moulting periods. Such flexibility in winter diet has been found in many other species, with individuals also displaying as much as one trophic level difference between diets (Phillips et al., 2009, Young et al., 2010, Grecian, 2011, Phillips et al., 2017). Guillemots were observed to have the greatest residual individual variability, potentially representing a flexible and adaptive winter diet (Blake et al., 1985, Smout et al., 2013). Both puffin and razorbill individuals displayed reduced isotopic residual variability for nitrogen during secondary feather moult, indicating a more uniform population behavioural response during flight feather regrowth. Isotopic residual variability differed between feather types and between species, suggesting different population and individual scale foraging strategies, during different feather moult time periods. Whilst observed differences in residual isotopic variability seem biologically plausible in terms of dietary differences, variability could also be a result of spatially diverse foraging locations, different body conditions or potentially different melanin content across the different feather types (Michalik et al., 2010, McMahon et al., 2015, Phillips et al., 2017). Further work is needed to clarify these sources of variation.

4.5.3 Method constraints

Our approach of combining light based and isotope-based geolocation methods has some limitations. In the absence of sampling constraints, the calibration-offset values for each population and feather type would be calculated from an additional training data set covering the range of all possible locations. However, the volume of data required is beyond the scope of many tracking studies. We appreciate that there is circularity in our approach: we use the estimated population-level calibration-offset values to infer location and individual level diet within the same individuals. In addition, although we have demonstrated an increase in

method precision by combining two techniques we are currently unable to explicitly quantify the cost in terms of accuracy. Without a further third, independent measure of individual seabird location such as satellite or immersion tags (e.g. (Cherel et al., 2016), we are unable to quantify the proportion of birds truly undertaking moult within the inferred most likely foraging region, or how this differs to the accuracy achieved for each method alone. As satellite tags get smaller and less expensive to deploy, we suggest further research quantifying the relative cost of accuracy corresponding to the increased precision identified by this technique.

4.5.4 Conclusions

Both bird-borne data loggers and isoscape assignment geolocation techniques have their limitations and associated errors. However, used in combination they provide a powerful approach to better constrain foraging areas and diet, as well as facilitating exploration of individual differences and flexibility in foraging behaviour during moult. Such information is urgently needed for the North Sea both to inform marine spatial planning decisions, for example the positioning of offshore renewable energy developments, and to develop effective conservation strategies by assessing regions of importance and therefore vulnerability to anthropogenic activities, such as increased fishing efforts.

Chapter 5 Sympatric auk species show contrasting foraging responses to adverse marine conditions during winter moult

This chapter is currently in preparation for submission.

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5.1 Abstract

Marine environments vary in conditions among years, and top predators inhabiting these areas must adapt to these changes to survive. To date, the majority of seabird research has focussed on changes during the breeding season, with fewer studies investigating effects outside the breeding period, for example during the annual feather moult when individuals may be particularly vulnerable to adverse conditions. This paper compares the foraging location and trophic position of two auk populations breeding on the Isle of May, Scotland (Atlantic puffins (*Fratercula arctica*) and razorbills (*Alca torda*)) using a combination of data logger and stable isotope approaches. Population distribution and trophic position were compared over two winters when adult survival rates differed markedly and the abundance and distribution of potential high and low quality prey species varied. Puffins used broadly the same foraging locations in the northwestern North Sea during moult in both winters. However, diet differed, with a lower average trophic position in the poor survival winter. In contrast, razorbills maintained trophic position but used different foraging areas in the two winters, occurring in the northwestern North Sea when survival was high and further south when survival was lower. With environmental conditions and prey availability in the North Sea predicted to change, research into how different seabird populations adapt their foraging behaviours has important implications for effective conservation and spatial based management.

5.2 Introduction

High trophic level predators must respond to the impacts of environmental variation cascading through food webs. Mobile predators may potentially respond to changes in diet availability by moving to find new resources or switching diets (Moody and Hobson, 2007, Montevecchi et al., 2009, Hedd et al., 2010, Garthe et al., 2011). The nature of any such

response must reflect a balance of risk /reward associated with costs and physiological demands. The ability of two sympatric species with similar ecological niches to respond to environmental variation, and the relative nature of their responses could profoundly influence their relative abundances and abilities to persist thereby shaping patterns of species distribution, particularly when environmental disturbances occur during critical life history stages.

Members of the auk family (Alcidae) dominate the avian community wintering in the North Sea (Stone et al., 1995). They forage by pursuit diving and the medium and larger sized species undergo moult of their flight and body feathers in winter. During this period of annual feather moult energy demands are high and some species become flightless making them potentially vulnerable to reduced prey availability and severe weather conditions (Nettleship and Birkhead, 1985, Sandvik et al., 2005). Like many other seabirds, annual survival rates of auks are typically high (c.0.90) with most mortality occurring in winter (Nettleship and Birkhead, 1985, Igual et al., 2009). However, the majority of studies on environmental influences on diet and distributions of seabirds have focussed on impacts during the breeding season (Russell et al., 2015, Howells et al., 2017), and considerably less information is available regarding changes occurring outside the breeding season.

Long-term demographic studies of three auk species breeding at a colony in the northwestern North Sea, found that annual survival rates varied synchronously across the species and in association with the same environmental proxies suggesting shared wintering areas and/or prey species (Lahoz-Monfort et al., 2011). Until recently testing such hypotheses has been impossible but the development of bird-borne, light-based geolocators (Guilford et al., 2009, Cherel et al., 2016, Fayet et al., 2017) and stable isotope analysis to retrospectively determine nutrient acquisition and trophic positioning (Hedd et al., 2010, Roscales et al., 2011), now allow the collection of data reflecting foraging locations and diet during at-sea winter moult.

In this study we combine data logger and stable isotope data and show how the combination of methods reveals spatial and trophic information that cannot be retrieved from either approach in isolation. In a previous study (St John Glew et al., In Press)(Chapter 4) we reconstructed at-sea locations and diet in two species of auk (razorbill (*Alca torda*) and Atlantic puffin (*Fratercula arctica*), hereafter puffin) during the winter moult period of 2014/15, a period characterised by relatively high overwinter survival rates of these auk populations (see results), and presumably favourable environmental conditions. Here we compare locational and isotopic data from puffins and razorbill during the winter moult period of 2014/15 with similar data from the winter moult period of 2007/08, when overwinter survival was markedly lower implying that conditions were less favourable.

Our specific aims were to 1) test whether differences in overwinter survival were associated with changes in moulting locations and/or trophic position, 2) assess whether such responses were similar in the two species and 3) investigate whether any observed changes in moult locations and diet are related to the abundance and distribution of potential prey species. For the final test we collated data on the distribution and abundance of lesser sandeel (*Ammodytes marinus*), sprat (*Sprattus sprattus*) and herring (*Clupea harengus*), species that are known to be common in the diet of puffins and razorbills in this region during the breeding season across the North Sea (Harris and Wanless, 2011). We also extracted distribution and abundance data, as well as isotope measurements for snake pipefish (*Entelurus aequoreus*), as this normally rare species showed a dramatic and short-lived population explosion in 2007 and 2008, and was found in the diets of a wide range of marine predators (Harris et al., 2007).

5.3 Methods

5.3.1 Survival rates

Fieldwork was carried out on the Isle of May National Nature Reserve, south-east Scotland (56°11'N, 2°34'W) where annual survival rates of adult puffins and razorbills have been estimated each year since 1984 from resighting of 694 and 215 individually colour-ringed puffins and razorbill, respectively. Annual survival estimates were taken from a standard Cormack-Jolly-Seber mark-recapture model, fitted to recaptures of colour-ringed birds using a Bayesian method as outlined in Freeman et al. (2014), and updated to include subsequent years' data.

5.3.2 Data logger deployments and data analysis

During June and July 2007 and 2014, razorbills were caught at their breeding sites using a 7m noose pole and puffins were hand caught in their breeding burrows. Captured birds were equipped, under British Trust for Ornithology licence, with a plastic leg ring and data logger (2007: British Antarctic Survey Mark 14; 2014: Migrate Technology, UK: model w65 for puffins and c65 for razorbills; combined mass of ring and device < 0.4% body mass of both species in both study seasons). Birds were recaptured the following summer (2008 and 2015 respectively), the data loggers removed and the data retrieved. Post-processing of data logger results followed the protocols described in detail in St John Glew et al. (In Press) (Chapter 4). Location data for each bird during the moult periods for each feather type in these populations (Table 5.1) and population kernel density maps of daily locations during the relevant moult periods were produced using the 'bkde2D' function in the 'KernSmooth'

package (Wand and Jones, 1995) in R 3.1.2 (R Core Development Team, 2016). As our reference stable isotope data is currently limited to the North Sea (Trueman et al., 2017), only cases where data logger points indicated that the moult period of a given feather type occurred in the North Sea were included in the analysis, resulting in the removal of all feather samples from 2 puffins and 1 razorbill for 2007/08 and 1 razorbill cheek feather sample for 2014/15.

5.3.3 Stable isotope data collection and analysis

Feather samples were collected, under UK Home Office licence, from recaptured birds. The distal two-thirds of a central secondary wing feather was removed and 2-5 ventral body feathers and 2-5 feathers taken from the neck or cheeks (Table 5.1). Body feathers of both puffins and razorbills are moulted and regrown in autumn, whereas head feathers are grown in spring. Secondary feathers of razorbills are moulted and regrown in autumn, however puffins are recorded to moult their secondary feathers any time between autumn and spring. Feathers were stored in paper envelopes and deep-frozen until analysed.

Feathers were cleaned of surface contaminants using 0.25M NaOH and rinsed with MilliQ water, oven-dried (60°C, 12 hours), then cut into small fragments avoiding the quill and shaft. A single secondary and body feather was analysed per individual, whereas cheek feathers were pooled to obtain enough material for analysis. A 0.5 – 0.7mg sample was weighed into a tin capsule and bulk $\delta^{13}\text{C}$ and $\delta^{14}\text{N}$ values were measured. All razorbill feather samples and puffin samples for 2014/15 were processed at the University of Southampton and analysed by Elementex Laboratories, Cornwall, UK. Accuracy and precision were monitored through laboratory internal standards (USGS 40 and USGS 41) and an in-house comparison standard (ARCOS glutamic acid). Accuracy was within 0.1‰ in comparison to long term standard averages of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and precision was 0.1‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Puffin feathers from 2007/08 were processed at the Centre for Ecology & Hydrology and analysed at the University of Aberdeen, using an elemental analyser on the front end of a dual inlet gas source isotope ratio mass spectrometer (Micromass Ltd, Manchester, UK) but using a different set of in house standards referenced to IAEA international reference materials. Differences in isotopic variation between species and years were compared using ANOVA statistical tests in R 3.1.2.

Snake pipefish (subsequently referred to as pipefish) isotopic data were obtained from 62 samples opportunistically collected across the North Sea between July and November 2007 during the ICES 3rd quarter International Bottom Trawl Surveys (IBTS) on board RV *Cefas Endeavour* and in the framework of the Marine Ecosystem Connections MEC project of Cefas (Kürten et al., 2013a). White muscle tissue samples were taken from each individual,

and then samples were freeze-dried, ground and weighed (~1mg) into tin capsules. The majority of stable isotope analyses were carried out using a ThermoElectron Delta XP Plus connected to a Costech ECS 4010 elemental analyser by NERC Life Sciences Mass Spectrometry Facility, East Kilbride, UK. Accuracy and precision were monitored through international standards (ammonium sulphate, USGS 25, IAEA-N1, IAEA-N2 for nitrogen and polyethylene (IAEA-CH-7), graphite (USGS 24) and sucrose for carbon). Precision was 0.3‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. A subset of pipefish samples were analysed on a ThermoElectron Delta V IRMS at Leibniz Institute for Research on Evolution and Biodiversity, FRG, using peptone as an internal standard. Precision was <0.2‰ for both isotopes. Carbon isotope values were lipid corrected as per Kiljunen et al. (2006).

Table 5.1 Sample sizes, median and standard deviation $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ feather values of puffins and razorbills for which feathers were collected from geolocator equipped birds known to spend the winters of 2007/08 and 2014/15 within the North Sea. The timing of growth for the different feather types for the two species is also shown.

	Puffin 07/08			Puffin 14/15			Razorbill 07/08		Razorbill 14/15		
Feather Type	Cheek	Body	Sec	Cheek	Body	Sec	Body	Sec	Cheek	Body	Sec
Sample Size	4	8	5	3	12	12	16	15	7	9	9
Feather s likely grown	Jan-Mar	Jul-Sep	Autumn /Spring	Jan-Mar	Jul-Sep	Autumn /Spring	Jul-Sep	Jul-Sep	Dec-Mar	Jul-Sep	Jul-Sep
$\delta^{13}\text{C}$: Median & (σ)	-15.65 (0.33)	-15.87 (0.38)	-16.73 (1.15)	-16.46 (0.27)	-16.81 (0.72)	-16.99 (0.39)	-16.47 (0.71)	-16.53 (0.71)	-16.77 (0.31)	-16.48 (0.68)	-16.23 (0.76)
$\delta^{15}\text{N}$: Median & (σ)	14.52 (1.95)	12.58 (2.94)	12.64 (1.59)	16.43 (0.09)	15.15 (0.58)	15.72 (0.41)	16.44 (1.40)	16.44 (1.38)	17.79 (0.46)	15.76 (0.65)	15.98 (0.24)

5.3.4 Population level assignment and calibration-offset derivation

Puffin and razorbill feather samples collected following both winters were assigned to the North Sea isoscapes produced by Trueman et al. (2017) to estimate foraging locations during moult. Although the isoscapes were produced from samples collected in summer 2015, the broad spatial pattern in isotopic variability across the North Sea is largely consistent, reflecting long-term stability in oceanographic and associated pelagic biogeochemical conditions conserved over time (MacKenzie et al., 2014). The North Sea isoscapes were produced using lion's mane jellyfish (*Cyanea capillata*) as reference

organisms. Therefore, to carry out the assignment of seabird feathers to the likely foraging location during moult, a calibration was required to account for isotopic differences between jellyfish baseline values and seabird feathers as a result of different tissue compositions and trophic levels of the organisms.

The degree of isotopic offset between jellyfish and bird feathers was estimated at the population-scale (i.e. a median value for all individuals within a population) by aligning North Sea isoscapes and population kernel density areas (independent location estimates) for both puffin and razorbill populations for each feather type in both years as per St John Glew et al. (In Press) (Chapter 4)(Fig. 4.1).

Coordinates from population kernel density areas (with density values greater than 0.01), representing all of the locations within the North Sea visited by the birds during the seasonal moult periods, were recovered and used to extract isotope values from the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes at the corresponding locations. Population median and standard deviation isoscape $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were then calculated. The population calibration-offset was derived as the isotopic difference between the median values within the isoscape area and the median values measured in the population of feathers (Fig. 4.1). Combined variance values were calculated by adding the isoscape-extracted and measured variance values. Isotopic differences associated with the difference in protein compositions between jellyfish and feather proteins were assumed to be constant within species and feather type. We assume that any remaining physiological differences potentially influencing isotopic compositions such as differences in dietary protein quality or physiological stress (McMahon et al., 2015) are relatively minor compared to effects of foraging at different levels (at least at the population level). Thus, differences in calibration-offset values between species and feather types were assumed to represent differences in isotopic trophic level.

We estimated the most likely feeding areas during winter moult at the population level using the methodology described in Trueman et al. (2017) and St John Glew et al. (In Press)(Chapter 4) with the addition of a Bayesian framework to include independent prior information from data logger-derived location estimates (e.g. Wunder (2010) and Vander Zanden et al. (2015)). Assignments were performed using the derived calibration-offset and combined variance values (the assignment conditions are summarised in Table 5.2). Likely feeding locations were identified by estimating the likelihood that each raster cell of the North Sea carbon and nitrogen isoscapes represented the foraging area of each individual, using the bivariate normal probability function and prior knowledge of known winter locations using the population kernel density areas. Probability raster surfaces derived from data loggers were treated as prior probabilities and were multiplied with the isotope

assignment probability raster surfaces to obtain the posterior probability density surface. All cells with probabilities exceeding a defined threshold likelihood were considered likely areas.

The North Sea assignment regions of puffins and razorbills, based on feather isotope values and moult timing specific kernel density priors were classed as the highest likely foraging locations during moult. Foraging locations were compared between years by overlaying assignment surfaces and calculating percentage overlap of likely foraging areas. Species and feather type Bayesian probability assignment surfaces were mapped in R 3.1.2 (R Core Development Team, 2016).

Table 5.2 Assignment conditions adopted for stable isotope-based location of puffins and razorbills against isoscapes derived from jellyfish tissue.

Variable	Isoscape Jellyfish	Seabird Assignment			
		Puffin		Razorbill	
		2007/8	2014/5	2007/8	2014/5
Measurement error (σ)	$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$: 0.2	$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$: 0.2			
Between individual variance (measured)	$\delta^{13}\text{C}$: 0.78, $\delta^{15}\text{N}$: 1.02	$\delta^{13}\text{C}$: 0.60, $\delta^{15}\text{N}$: 2.74	$\delta^{13}\text{C}$: 0.52, $\delta^{15}\text{N}$: 0.91	$\delta^{13}\text{C}$: 0.63, $\delta^{15}\text{N}$: 2.10	$\delta^{13}\text{C}$: 0.51, $\delta^{15}\text{N}$: 0.58
Calibration-Offset and variance values	NA	<i>Derived – see Results Table 5.3</i>			
Threshold odds ratio	NA	1.42			

5.3.5 Prey abundance

Data on the distribution and abundance of sandeels, sprat and herring, high quality prey, that are known to be taken by puffins and razorbills (Hislop et al., 1991, Harris et al., 2008, Harris and Wanless, 2011) were extracted from North Sea IBTS data ((ICES, 2012a), <https://datras.ices.dk>) . Hourly catch per unit effort data (CPUE) for each ICES statistical rectangle, were extracted for herring, sprat and sandeel that were small enough to be eaten by these seabirds (defined as individuals <160mm (ICES, 2006), <80mm (ICES, 2006), and <120mm (Boulcott et al., 2007)). Data were obtained for January to March 2008 and 2015. Prey abundance data were not available for October to December. CPUE data were averaged

for each ICES rectangle and displayed as $\log_{10}(\text{CPUE}/\text{hr} + 1)$ to graphically display differences in abundance across orders of magnitude. Snake pipefish CPUE data were obtained for each ICES statistical rectangle for quarter 1 in 2008 and 2015.

5.3.6 Snake pipefish isotopic variability

Estimates of likely spatial variations in the isotopic compositions of pipefish across the wider North Sea were produced from known origin data using ordinary kriging in R 3.1.2 (R Core Development Team, 2016). Estimated pipefish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were extracted for coordinates matching the most likely feeding areas as estimated from combined geolocator and isotope results (e.g. Fig. 5.3). Coordinates of likely foraging location during each feather type regrowth were combined as pipefish samples were collected across the winter period and likely foraging locations were consistent between feather types during 2007/08 moult.

5.4 Results

5.4.1 Adult survival

Survival rates of adult puffins and razorbills breeding on the Isle of May are normally high (means of 33 years; puffin 0.921 (95% Credible Interval 0.900 – 0.939); razorbill 0.923 (0.897 – 0.945)), but survival over the 2007/08 winter was much reduced (puffin 0.721 (0.643 – 0.791); razorbill 0.828 (0.704 – 0.923)). In contrast, over the 2014/15 winter, the survival of puffins was higher than average (0.945 (0.904 – 0.977)) and that of razorbills was average (0.894 (0.788 – 0.964)).

5.4.2 Stable isotope results

Population median isotopic values were broadly comparable between the two species in 2014/15, but differed markedly in 2007/08 (Fig. 5.2)(Table 5.1). During 2014/15 when survival rates were high puffin feather median isotope values ranged between -16.46‰ and -16.99‰ for $\delta^{13}\text{C}$ and between 15.15‰ and 16.43‰ for $\delta^{15}\text{N}$ for different feather types, similar to razorbill feather median isotope values (-16.23 – -16.77‰ and 15.76 – 17.79‰)(Table 5.1). During 2007/08 when survival rates were poor, median carbon isotope values remained comparable between puffin feathers (-15.65 – -16.73‰) and razorbill feathers (-16.47 – -16.53‰) but median puffin nitrogen isotope values were notably decreased (12.64 – 14.52‰), whereas razorbill median isotopes values increased (16.44‰)(Table 5.1) compared to the better survival winter. Within population and feather isotopic variability differed between years. In the good survival winter, within population

isotope variability was relatively constant between species with $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ standard deviations ranging from 0.09 – 0.58‰ and 0.27 – 0.72‰ respectively across feather types in puffins, and 0.24 – 0.65‰ and 0.31 – 0.76‰ in razorbills. When survival rates were lower, carbon isotope variability in puffin feathers (0.33 – 1.15‰) was similar to razorbill feathers (0.71‰) and between sample years, but nitrogen isotope variability increased in both species (1.59 – 2.94‰ in puffins and 1.38 – 1.40‰ in razorbills). Year had a significant effect on $\delta^{15}\text{N}$ variability ($F = 28.24$, $P < 0.001$), whereas no significant year effect was apparent in $\delta^{13}\text{C}$ variability in either species ($F = 1.73$, $P > 0.05$). Feather type and species had no significant effect on isotope values. All isotope data are displayed in Fig. 5.1, summary data are provided in Table 5.1.

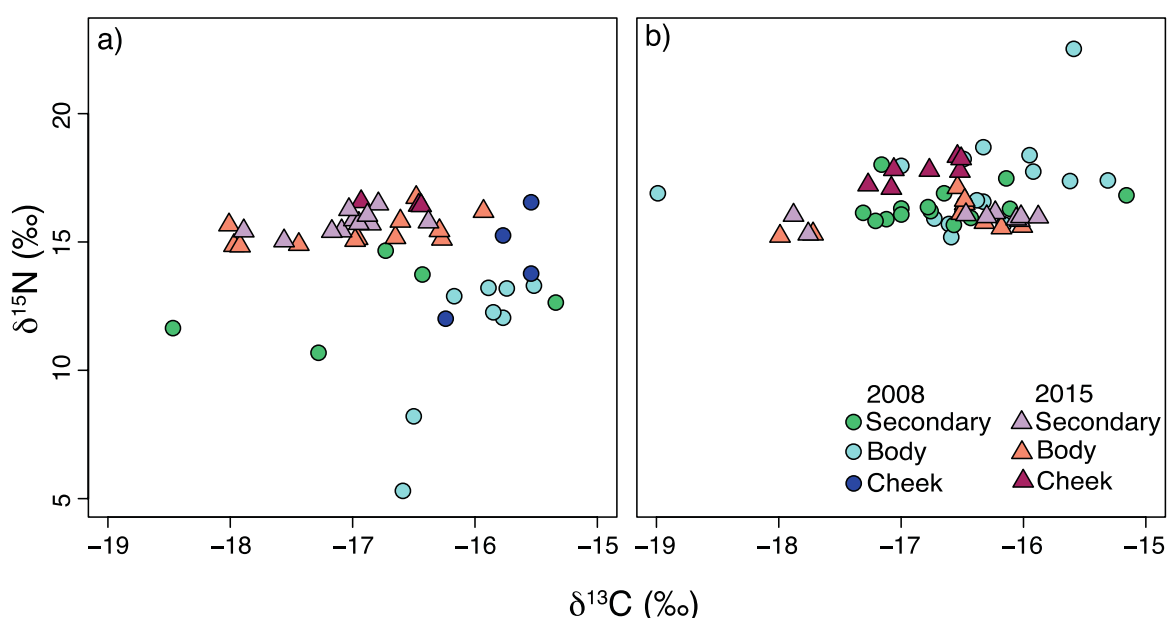


Figure 5.1 Biplots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of puffin (A) and razorbill (B) feathers grown in the winters of 2007/2008 (poor survival year)(circles) and 2014/2015 (high survival year)(triangles).

5.4.3 Wintering and moulting locations

Data loggers show that during both winters puffins occurred mainly in the northwest North Sea with the highest kernel densities within approximately 400 km of the Isle of May (Fig. 5.2a,c). In both years individuals were also recorded leaving the North Sea at varying time periods during winter (6/10 individuals in 2007/08 and 6/14 individuals in 2014/15). Numbers of individuals leaving the North Sea did not differ significantly between years ($X^2 = 0.17$, $P = 0.67$). Individual razorbills were also recorded leaving the North Sea in both years

(3/17 individuals in 2007/08 and 5/9 individuals in 2014/15), but again no significant differences were observed between years ($X^2 = 2.38$, $P = 0.12$). In contrast to the situation in puffins, data logger-defined wintering areas used by razorbills differed markedly between winters (Fig. 5.2b,d). In 2007/08, birds were predominantly located in the southern North Sea, whereas in 2014/15 the majority of birds used areas off southeast Scotland, with less use being made of the southern North Sea. The highest densities were within approximately 120km of the Isle of May (Fig. 5.2).

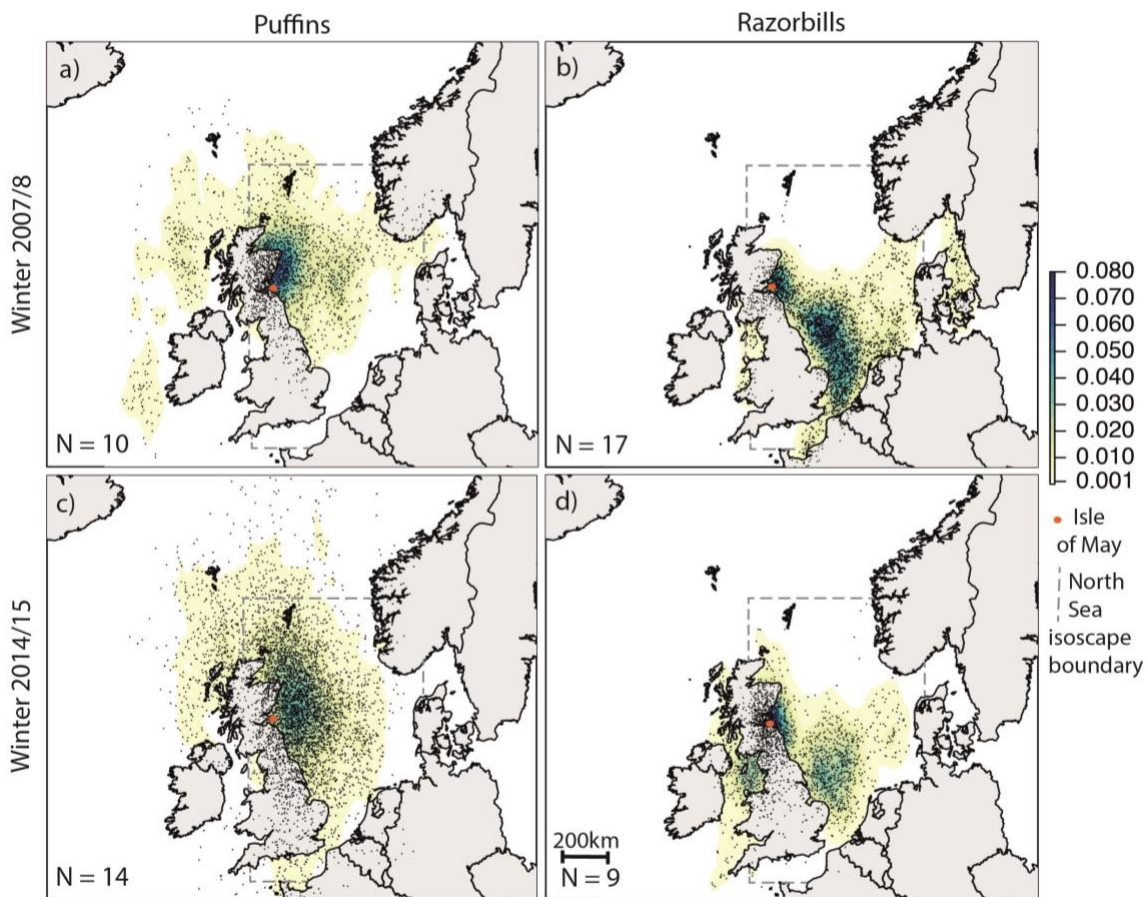


Figure 5.2 Kernel density surface depicting population spatial usage around the UK using coordinate data collected from light-based geolocators attached to populations of puffins (A = 2007/08, C = 2014/15) and razorbills (B = 2007/08, D = 2014/15) during the entire non-breeding period (Jul-March). Kernel is calculated as the standard bivariate normal density, with higher values representing greater use regions. Individual data points are also overlaid. All individuals for which geolocator data was obtained were included.

In 2007/08, data loggers indicated that 8 out of 10 puffins were in the North Sea during the months when moult occurred and could therefore be assigned to the North Sea isoscape to infer moult locations. In 2014/15, moult locations could be estimated for all individuals. As with the overall wintering distribution, regions identified as likely areas used by puffins for

moulting were similar in the two winters (Fig. 5.3a-f). During winter 2007/08 cheek, body and secondary feather moult of puffins most likely occurred in the northwestern North Sea, close to the Isle of May (Fig. 5.3a-c). In 2014/15 moult also occurred in the northwestern North Sea, but cheek and secondary feather moult occurred further offshore and body moult occurred slightly further north and offshore compared to 2007/08 (Fig. 5.3d-f). The areas identified as most likely foraging regions in winter 2007/08 overlapped those for the winter of 2014/15, with an overlap of 64% of the total likely area during body feather moult and 52% during secondary feather moult. Cheek feather moulting locations did not overlap between years.

Most razorbill individuals remained within the North Sea during known moulting months (94% in 2007/08 and 87.5% in 2014/15) and were therefore assigned to the North Sea isoscape. The most likely moult locations for razorbill differed between the two studied winters (Fig. 5.3g-k). Body and secondary feather growth in 2014/15 likely occurred in the northwestern North Sea, close to the Isle of May, whereas body and secondary feather growth in 2007/08 likely occurred in the central North Sea, in a similar region to cheek feather growth in 2014/15.

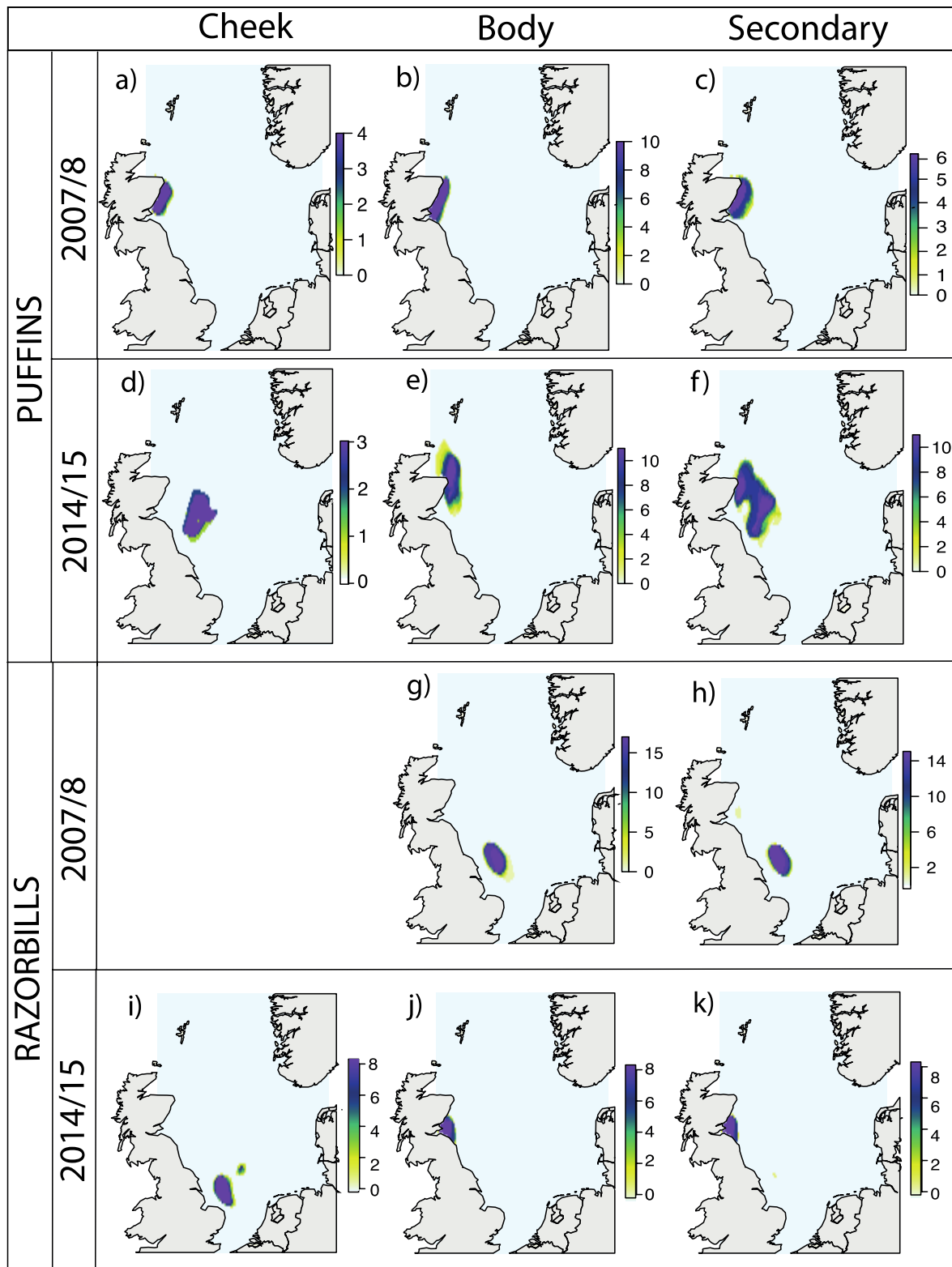


Figure 5.3 Bayesian probability assignments using derived calibration-offsets and season specific kernel density areas as prior probability surfaces of puffin and razorbill feathers collected in 2008 and 2015. Regions identified represent the most likely foraging regions during moult.

5.4.4 Trophic position

The isotopic calibration-offset between puffin feathers and the jellyfish isoscape ($\Delta^{15}\text{N}_{\text{f-j}}$) in the likely foraging area was markedly lower in the (high mortality) winter of 2007/08 compared to the (low mortality) winter of 2014/15 (Table 5.3). In 2014/15, the range in puffin nitrogen calibration-offset values ($\Delta^{15}\text{N}_{\text{f-j}}$) was 4.53-5.87‰, similar to that of razorbills (4.64 – 6.30‰). However, in 2007/08 puffin nitrogen calibration-offset ($\Delta^{15}\text{N}_{\text{f-j}}$) was markedly lower during both secondary and body feather moult (2.02‰ and 1.65‰ higher than jellyfish) (Table 5.3). In contrast, razorbill nitrogen calibration-offset ($\Delta^{15}\text{N}_{\text{f-j}}$) values were similar in both winters, with the population consistently displaying values of 4.64 – 6.30‰ higher than jellyfish (Table 5.3). Razorbill carbon calibration-offset values ($\Delta^{13}\text{C}_{\text{f-j}}$) also remained relatively consistent between years, whereas in puffins values decreased in 2014/15 compared to 2007/08. In both species and across all feather types, individual isotopic variance was greater in the poor survival winter compared to the higher survival winter (Table 5.3).

Table 5.3 Population calibration-offset values and difference values calculated from the difference between median isoscape extracted isotope values within the population kernel density areas for each feather type and median measured feather isotope values of razorbills and puffins in winters 2007/8 and 2014/15.

	Puffin 07/08			Puffin 14/15			Razorbill 07/08		Razorbill 14/15		
Feather Type	Cheek	Body	Sec	Cheek	Body	Sec	Body	Sec	Cheek	Body	Sec
$\delta^{13}\text{C}_{\text{f-j}}$: Cal. offset & (σ)	0.56 (0.82)	0.13 (0.46)	-0.38 (1.84)	-0.2 (0.84)	-0.65 (0.98)	-0.77 (0.73)	0.37 (1.88)	-0.15 (1.37)	0.91 (1.02)	-0.33 (1.07)	-0.08 (1.18)
$\delta^{15}\text{N}_{\text{f-j}}$: Cal. offset & (σ)	4.08 (4.71)	1.65 (8.91)	2.02 (14.19)	5.87 (1.02)	4.53 (0.97)	5.21 (0.93)	6.17 (4.19)	5.32 (11.61)	6.30 (2.70)	4.64 (1.11)	4.86 (0.74)

5.4.5 Prey availability

The abundance and spatial distributions of sprat, sandeel and herring differed markedly in the two winters. In 2014/15, CPUE of all three fish species was higher in the northwestern North Sea, and all three species had a more northerly distribution compared to 2007/08, when prey populations were largely concentrated within the southern North Sea

(Fig. 5.4). The abundance of pipefish was dramatically higher across the entire North Sea in 2007/08 and pipefish were more widely distributed compared to 2014/15 (Fig. 5.4).

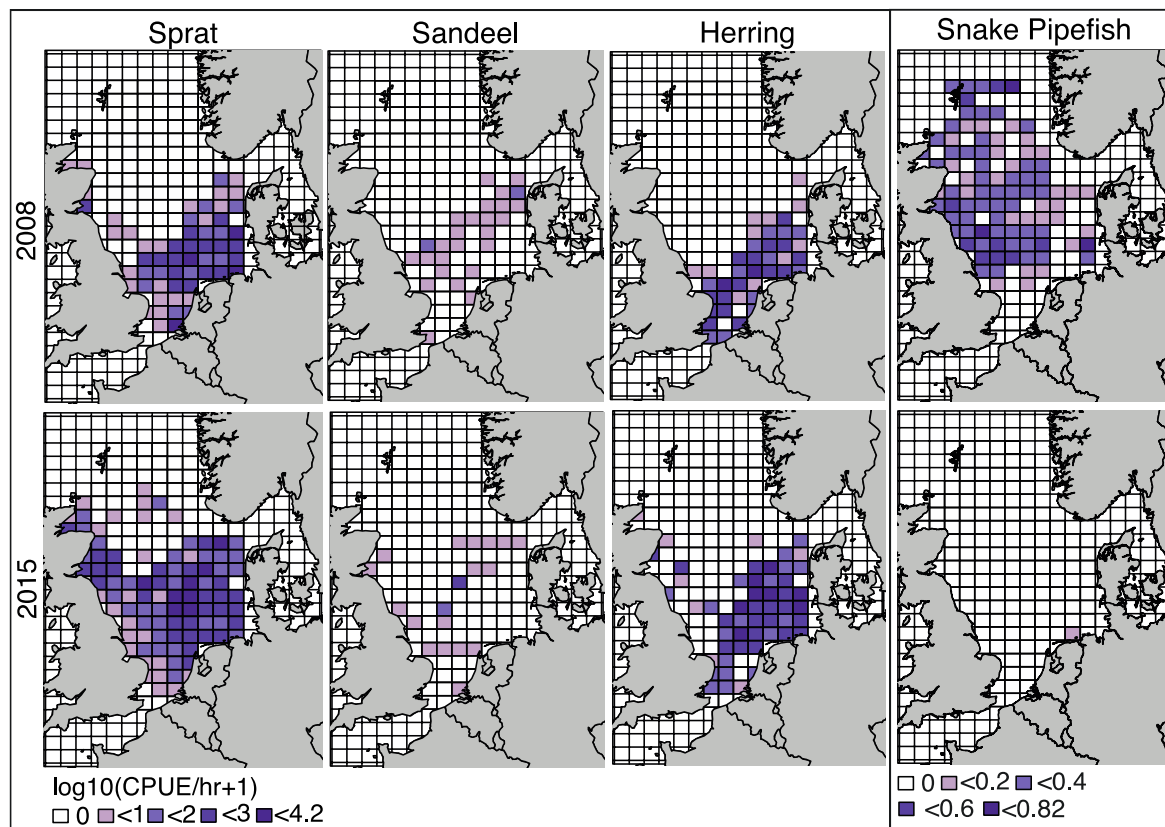


Figure 5.4 Catch per unit effort (CPUE) per hour data, per ICES statistical rectangle for age class 0 sprat, sandeel, herring and snake pipefish, in quarter 1 in 2008 and 2015. CPUE/hr values were averaged in each ICES rectangle and displayed as $\log_{10}(\text{CPUE/hr} + 1)$. Data were obtained from ICES North Sea IBTS database.

The isotopic difference between pipefish muscle tissue and puffin and razorbill feather tissues in the most likely moulting regions was 2.19‰ and 1.52‰ for carbon and 3.49‰ and 7.44‰ for nitrogen, respectively (Table 5.4).

Table 5.4 Average isotopic differences between seabird feathers and pipefish muscle. Average puffin and razorbill feather carbon and nitrogen isotope values across all feather type samples collected in winter 2007/8, average pipefish isotope values extracted from pipefish carbon and nitrogen isoscapes, produced from samples collected in winter 2007/8, within the highest likely foraging regions during moult of both puffins and razorbills, and the difference between these values.

	Puffin		Razorbill	
	Carbon	Nitrogen	Carbon	Nitrogen
Average feather isotope values (‰)	-16.03	13.37	-16.60	17.40
Average pipefish isotope values in most likely foraging locations during feather moult (‰)	-18.22	9.88	-18.12	9.96
Difference between pipefish isotope values and seabird isotope values (‰)	2.19	3.49	1.52	7.44

5.5 Discussion

We compared location and trophic level during at-sea winter moult in two sympatric auk species across two winters of contrasting mortality levels. Overwinter survival of puffins and razorbills from the Isle of May was lower in 2007/08 (0.721% and 0.828%) compared to 2014/15 (0.945% and 0.894%) and survival of a third species of auk, the common guillemot (*Uria aalge*), at this colony was also depressed in 2007/08 (Lahoz-Monfort et al., 2011). Such variations in survival are likely to be associated with changes in environmental conditions, particularly prey availability outside the breeding season (Sandvik et al., 2005, Breton et al., 2014). Our data logger and isotopic data indicated that puffins and razorbills from the Isle of May showed opposite responses to differing environmental conditions during the moult period. Puffins used broadly similar foraging areas during both winters but fed at a markedly lower trophic level during the poor survival year, while razorbills maintained a high dietary trophic level in both winters but altered foraging areas having a more southerly (distant) distribution in the winter with increased mortality.

Lahoz-Monfort et al. (2011) speculated that observed synchrony in survival rates of auks from the Isle of May colony might be associated with shared wintering areas and/or shared prey species. The abundance and distribution of potential prey species of auks suggest that conditions in the two winters did indeed differ such that in 2007/08 when auk survival rates were low, the abundance of high-quality prey was lower and fish were mainly distributed in the southern North Sea. The 2007/08 winter was also characterised by an unusually high abundance and widespread distribution of snake pipefish which are of low nutritional value and difficult for small to medium sized seabirds like puffins and razorbills to handle and digest (Harris et al., 2008).

The two species of auk therefore appeared to demonstrate contrasting responses to reduced availability of these high quality prey fish in the regions adjacent to the Isle of May colony. Puffins remained in the same foraging areas used during winters of high survival, and this residency forced them to consume lower quality prey, potentially including pipefish. Razorbill by contrast responded to the prey availability challenge by moving to more distant wintering areas where they were able to maintain higher trophic level diets.

Razorbills have previously been recorded altering their range during summer months in response to changing prey availability (Gaston and Woo, 2008, Thaxter et al., 2010), whilst puffins were observed to maintain overwintering locations between contrasting environmental condition years (Harris et al., 2013) and are known to be able to lower their trophic position and adopt a more opportunistic foraging strategy during the harsher winter months (Hedd et al., 2010, Harris et al., 2015a). Foraging flexibility is important to survival when prey abundance and distribution varies (Hamer et al., 2007) with recent evidence suggesting reduced foraging site fidelity may be ecologically advantageous in variable climatic conditions (Abrahms et al., 2018). However, a trade-off is likely to exist between expending greater energy migrating to distant locations with potentially greater food resources or maintaining position and adapting to a lower trophic level diet (Harris et al., 2013). Survival of puffins in the winter of 2007/8 was depressed to a greater extent compared to that of razorbills implying that for this winter, the strategy pursued by razorbills appeared to be more successful.

Although we demonstrate clear differential winter resource use by razorbills and puffins, only one colony was investigated with relatively few individuals sampled and high individual variability. Therefore our results cannot be extrapolated to infer adaptive responses in populations from other breeding colonies or over alternative years as different populations are shown to demonstrate variable foraging strategies (Fayet et al., 2017, Votier, 2018). We also stress the Bayesian assignment approach does not depict the specific locations that all individuals within the population carried out their moult. It is rather an indication of the most likely population foraging location, compared to the surrounding North Sea range, when taking into account isoscape assignment and data logger geolocation. An additional independent geolocation technique is required to verify whether individual birds are in fact moulting within these defined regions. In addition we appreciate the limitations of the assignment methods due to limited sampling feasibility, as outlined by St John Glew et al. (In Press)(Chapter 4). Where data are collected opportunistically, as is often the case in ecological research, this methodology displays a logical representation as to where different populations likely forage during moult and the most probable trophic position and individual variability in diet.

With combined effects of reduced prey availability (Harris et al., 2005, Frederiksen et al., 2007, Grémillet and Boulinier, 2009) and increased offshore development, research into the ability of seabird populations to adapt their foraging behaviours is critical to providing effective long-term conservation strategies that will withstand changing future climates. The establishment of Marine Protected Areas is considered fundamental to future seabird conservation and there is a call to better define at-sea foraging locations and spatial ecology of seabirds, so appropriate and effective management areas can be designated (Lewison et al., 2012, MMO, 2015, Perrow et al., 2015, Votier, 2018). Our study highlights how the combination of light based data logger geolocation and stable isotope assignment techniques can be an effective tool to study seabird foraging strategies and adaptations to changing environmental conditions.

Chapter 6 Spatial models of carbon, nitrogen and sulfur stable isotope distributions (isoscapes) across a shelf sea: an INLA approach.

6.1 Abstract

Maps of marine spatial isotopic variability (isoscapes) offer information on physical and biogeochemical processes occurring across space and provide a tool for retrospective assignment of animals or animal products to their foraging area or geographic origin. However, marine isoscape predictions are currently constrained by the practical difficulty of obtaining sufficient reference organisms of the same species, evenly-distributed across large spatial scales, and the associated difficulty of producing statistically valid spatially explicit uncertainty surfaces accompanying isoscape models. We address this issue by modelling isoscapes using multiple species and explicitly addressing the additional isotopic variation introduced by including multiple reference species. We introduce the use of Integrated Nested Laplace Approximation (INLA) based approaches to predict isoscapes, and present carbon, nitrogen and sulfur isoscapes across the UK shelf seas. We draw on 7 different species of jellyfish as spatial reference data and develop predictive models through a range of environmental correlates. We briefly discuss the likely biogeochemical explanations for the observed spatial distributions of isotope compositions. We show for the first time that sulfur isotopes display systematic spatial variation across open marine shelf seas and may therefore be a useful additional tool for marine spatial ecology. We compare the spatial distribution and associated variances between INLA and kriging based approaches, and explore the relative accuracy and precision associated with assigning organisms to their known origin. Compared to alternative isoscape prediction methods, INLA-spatial isotope models show high spatial precision and reduced variance. Accuracy and precision of sessile marine organism assignments to INLA and more familiar kriging-based isoscape models were comparable, with 90% assignment accuracy to 40% of the isoscape area and ~80% assignment accuracy to known ICES subareas. The INLA technique provides a promising tool for isoscape predictions across numerous isotope systems and environments, benefitting future ecological research at management appropriate scales.

6.2 Introduction

Spatial based management is increasingly being recommended to address a host of marine issues such as reducing fisheries discards and bycatch (Little et al., 2015), designating Marine Protected Areas (MPAs) (Fulton et al., 2015), planning marine development and aquaculture (Gimpel et al., 2015) and managing the cumulative impact of human demands on the world's oceans (Halpern et al., 2015). Spatial based management capitalizes on regional differences to ensure effective management across appropriate range and also time scales (Maxwell et al., 2015). To effectively implement these strategies knowledge on the spatial structure of ecosystems including underlying spatial variability in productivity, nutrient cycling and circulation and their impacts on species foraging locations, movements and migrations, population dynamics and connectivity are critical.

The spatial distribution of naturally occurring stable isotopes of carbon, nitrogen and sulfur, measured within animal tissues, integrate spatio-temporal variations in physio-chemical influences such as nutrient source, growth rate and taxonomy on primary production (Trueman et al., 2012a, McMahon et al., 2013a). Mapping spatial isotopic variability in low trophic level or baseline organisms (isoscaples) therefore provides information on physical, chemical and biological processes occurring across space. Carbon and nitrogen stable isotopes also act as intrinsic tags providing information on feeding and migratory ecology (Jaeger et al., 2010). The isotopic composition of animal tissues is passed through trophic links, with consumer isotope ratios reflecting the isotopic composition of prey foraging within a particular area (Ramos and Gonzalez-Solis, 2012). Statistical comparisons between consumer tissue isotope ratios and marine isoscape predictions enable retrospective predictions of an individual's origin or foraging location (Royle and Rubenstein, 2004, Hebert and Wassenaar, 2005, Norris et al., 2006, Vander Zanden et al., 2015, Trueman et al., 2017). Isotope-based geolocation can then be used to track migration between isotopically distinct regions, monitor population connectivity, distinguish between nursery habitats and identify important hot spots for foraging. Marine isoscaples also provide a measure of baseline isotopic variability, to calibrate the use of stable isotopes in trophic positioning research (Jennings and Warr, 2003, Lorrain et al., 2014, Jennings and van der Molen, 2015, Kopp et al., 2015). Marine isoscaples provide a promising tool for spatial based management within marine environments.

Spatial variations in stable isotope compositions have been extensively researched in terrestrial environments, particularly environmentally-linked spatial variations in hydrogen and oxygen isotopes of precipitation (Bowen, 2010a). Spatial variation in isotopic compositions in marine systems have also been explored (Schell et al., 1989, Cherel and Hobson, 2007, Ramos and Gonzalez-Solis, 2012). However relatively few continuous-surface

isoscapes have been published in marine compared to terrestrial systems, probably due to the difficulty in obtaining sufficient reference samples over appropriate spatial and temporal scales.

Shelf seas are physically, chemically and biologically diverse regions, which account for 20% of global marine primary productivity (Ciavatta et al., 2018), a fifth of global atmospheric CO₂ uptake (Borges, 2011) and over 90% of fisheries catches (Pauly et al., 2002). Shelf seas are under increased pressure from marine development, recreational and shipping use and are in great need for effective spatial based management. Shelf seas also provide suitable regions to model isoscapes due to the relative ease of sample collection and broad scale temporal stability (MacKenzie et al., 2014) but display large ranges of isotopic variability; ideal for geolocation (MacKenzie et al., 2014, Trueman et al., 2017).

6.2.1 Previous isoscape models

To construct a continuous surface isoscape model, isotopic compositions of reference organisms are typically projected across space using either spatial interpolation methods (Vander Zanden et al., 2015, Kurle and McWhorter, 2017, Rodríguez-Pérez et al., 2018), by statistical inference based on correspondence between measured data and environmental correlates (Bowen and Wilkinson, 2002, Jennings and Warr, 2003, Barnes et al., 2009b, Mackey et al., 2015, Young et al., 2015) or a combination of both (Wunder, 2010, MacKenzie et al., 2014). Probabilistic assignment of an unknown animal to a likely origin also requires a spatially explicit variance surface. Isoscape model variance is a measure of how well the isoscape accurately represents true isotopic composition across space. In simple interpolation models, variance increases with distance from sampling points. For non-biased animal assignments evenly spaced samples are therefore required to produce spatially homogenous variance surfaces. When environmental correlates are introduced, variance measures become more complex with values dependent on the sampling locations of both the isotope measurements and environmental variables and the strength and nature of the relationship between isotope ratio and the selected variables across space.

Carbon and nitrogen isoscape models have previously been produced for UK Shelf Seas. Barnes et al. (2009b) and Jennings and Warr (2003) and Jennings and van der Molen (2015) used queen scallops (*Aequipecten opercularis*) from known catch locations as reference samples, coupled with more widely measured environmental variables, however explicit variance surfaces were only calculated for $\delta^{15}\text{N}$ predictions by Jennings and van der Molen (2015). In addition, queen scallops are rarely found in muddy substrates resulting in a spatially systematic patchy sample distribution, unsuitable for modelling isoscapes for animal assignments. High resolution, *in situ* sample based isoscapes have been modelled for the

North Sea using widely distributed lion's mane jellyfish (*Cyanea capillata*) as reference organisms through ordinary kriging of evenly spaced samples (Trueman et al., 2017), and with the addition of environmental variables (MacKenzie et al., 2014). Spatially explicit variance surfaces were calculated in both examples and initial assignments of invertebrate, fish and seabird samples have proven successful (Trueman et al., 2017, St John Glew et al., In Press). However this approach is constrained by the availability and distribution of a single reference species across the region of interest, limiting marine isoscape modelling capabilities across larger spatial scales. In addition, barrier effects (e.g. uneven coastlines) are particularly important in basin scale marine isoscape predictions, yet many existing techniques do not enable easy incorporation of coastlines and boundaries.

We aim to address the common issue of limited sample availability, by modelling isoscapes using multiple species and explicitly addressing spatial isotopic variation due to mixed sample sources. We explore the use of novel Bayesian hierarchical modelling techniques, using INLA to firstly produce single species North Sea isoscapes, and compare the assignment accuracy and precision associated with INLA-produced and alternative North Sea isoscapes (Trueman et al., 2017). Secondly we predict UK shelf sea carbon, nitrogen and the first marine sulfur isoscapes using multiple reference species and quantify assignment accuracy and precision in the context of the requirements of spatial based management.

6.3 Methods

6.3.1 Data collection and stable isotope analysis

Shelf seas around the British Isles (UK and Ireland) comprise an area of c. $1 \times 10^6 \text{ km}^2$, the largest contiguous stretch of shallow shelf seas in Europe outside of the Arctic. UK shelf seas host some of the most globally productive fisheries, regionally significant oil, gas and renewable energy resources and infrastructure and intensive shipping activity. Spatial management of UK shelf seas is therefore complex, reflecting multiple stakeholders and interests (Eastwood et al., 2007, Stelzenmüller et al., 2008, Foden et al., 2011, Spro et al., 2015, Jansen et al., 2016, van den Burg et al., 2016).

To construct isoscape models of UK shelf seas, 627 jellyfish samples of 7 different species (Barrel (*Rhizostoma pulmo*), Blue (*Cyanea lamarckii*), Compass (*Chrysaora hysoscella*), Crystal (*Aequorea victoria*), Lion's Mane (*Cyanea capillata*), Mauve stinger (*Pelagia noctiluca*) and Moon (*Aurelia aurita*)) were collected from 308 stations across the UK shelf seas between August 2015 and December 2016 (Fig. 6.1). Samples were opportunistically collected on board the RV Cefas Endeavour (Cefas), MRVs Scotia (Marine Scotland), Thalassa (Ifremer) and RV Celtic Explorer (Marine Institute) during annual fisheries surveys. In

addition further samples were opportunistically collected from small commercial fisheries, research and private vessels. Jellyfish were collected, identified, weighed and measured on-board before thorough washing with salt water and immediately freezing to -20°C . In the laboratory, samples were thawed, washed repeatedly (Mackenzie et al., 2017) and a section of bell tissue (mesoglea) removed and refrozen prior to freeze-drying for 24 hours, subsampling and submission for isotopic analysis.

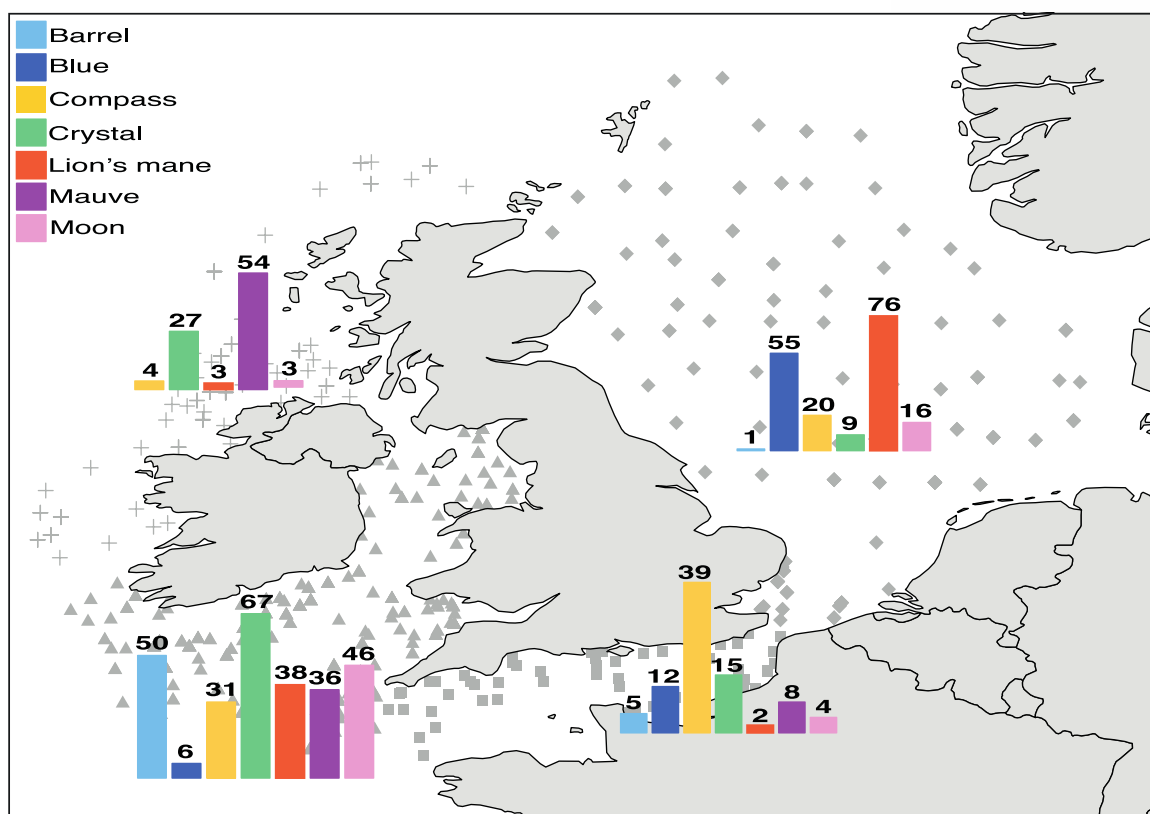


Figure 6.1 Jellyfish sampling locations around the UK shelf seas and a summary of species collected within the North Sea, English Channel, Celtic and Irish Seas and off West Scotland and Ireland.

A subset of North Sea lion's mane jellyfish samples (57) from 51 stations were analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at Elementex laboratories, Cornwall, UK in Autumn 2015. All 627 samples across the UK shelf sea (including repeats of North Sea samples) were analysed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ by Life Sciences Mass Spectrometry Facility (LSMSF), East Kilbride, UK in Autumn 2017. Accuracy and precision were monitored through laboratory internal standards (LSMSF = MSAG, M2 and SAAG2) and an in house comparison standard (ARCOS glutamic acid) nested within samples. North Sea samples analysed in both laboratories were compared for consistency and no significant differences were observed between analysis results.

Jellyfish bell tissue $\delta^{13}\text{C}$ values showed a significant negative linear relationship with C:N ratios ($P < 0.005$, slope = -2.22 , adjusted $R^2 = 0.06$). To correct for potential lipid-related

variance in $\delta^{13}\text{C}$ values, the Kiljunen et al. (2006) correction equation was applied. Queen scallop lipid corrected carbon and nitrogen isotopic data from known location individuals were taken from Jennings and Warr (2003) and Barnes et al. (2009b), for scallops collected between 25th July and 29th September 2001 and from Barnes et al. (2009a) for scallops collected in 2010.

Within-species variation in jellyfish stable isotope compositions was estimated by calculating the standard deviation among individuals of the same species occurring at the same sampling location. Standard deviation values were then averaged across locations to obtain a mean within-species standard deviation. Among-species isotopic difference values were estimated by calculating the difference between species mean isotope values occurring at the same location. Difference values were then averaged across locations to obtain the average isotopic difference among species.

6.3.2 Environmental data

Chlorophyll and sea surface temperature (SST) level three (instrument calibrated data projected onto a well-defined spatial grid over a well-defined time period) monthly average data were downloaded from the MODISA satellite (NASA Goddard Space Flight Center, 2014) between March and September in 2015 and 2016 over the spatial range of the UK shelf seas (Fig. 6.2). Chlorophyll-a and night-time short wave length SST (SST4) data were downloaded at 4km resolution. Bottom temperature, surface salinity and mixed layer depth daily mean data were downloaded from the Forecasting Ocean Assimilation Model 7km Atlantic Margin model (FOAM AMM7) at a 0.11 by 0.07 degree resolution across the UK shelf seas between March and September in 2015 and 2016 (CMEMS, 2017). Two year median spring-summer raster surfaces were then produced for each variable (Fig. 6.2). A temperature difference (Tdiff) surface was also calculated by subtracting the bottom temperature raster surface from the SST raster surface. Water column depths were acquired from NOAA bathymetry database at a resolution of 1 degree (Fig. 6.2). All raster surfaces were resampled to a resolution of 0.1 x 0.1 degrees over the coordinates (-13, 8, 48, 62). Covariate values at jellyfish sampling point locations were extracted and all environmental variable values were scaled, by subtracting the variable mean from each value and dividing by the variable standard deviation, to have comparable mean and standard deviation values across all environmental variables (Fig. 6.2).

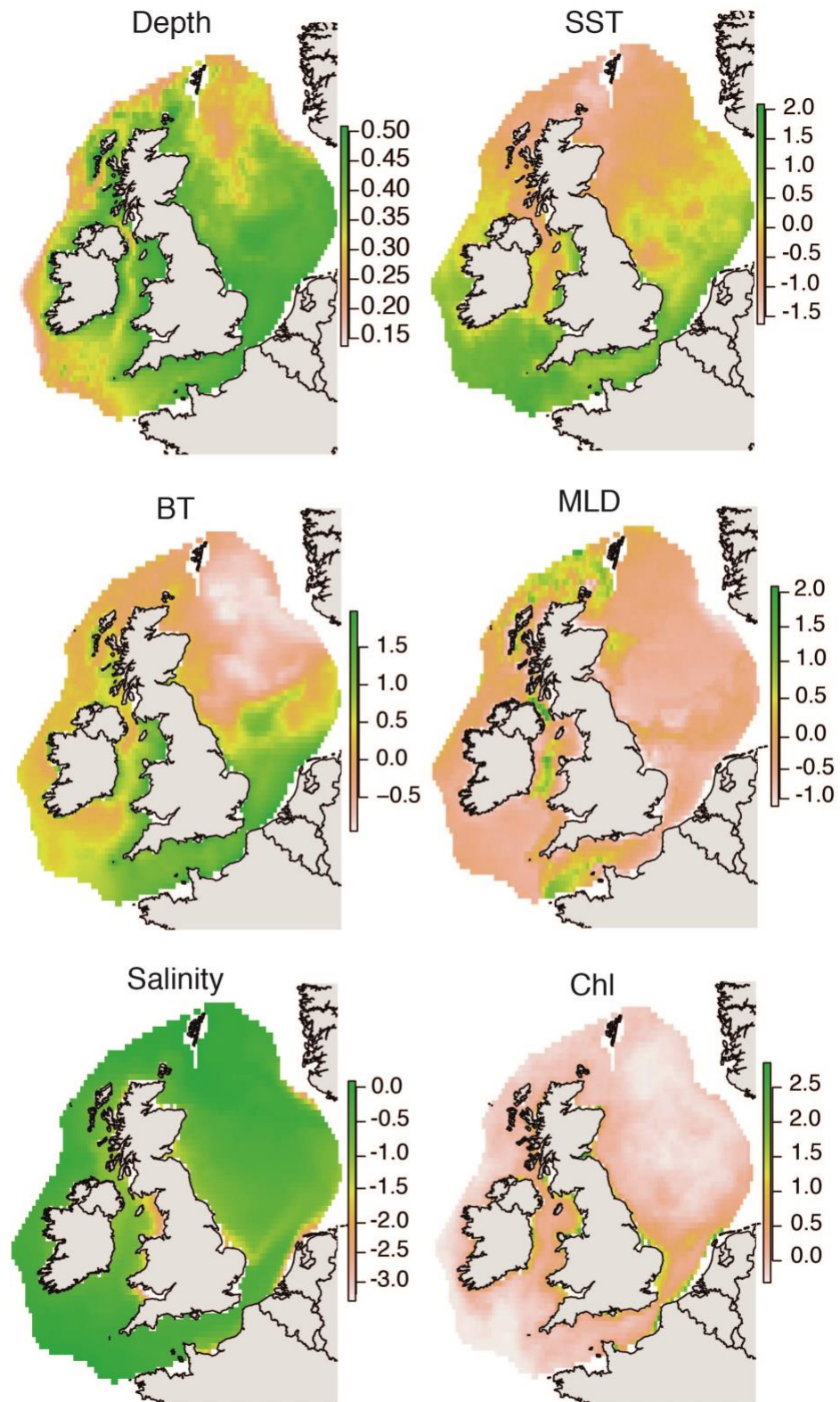


Figure 6.2 Environmental covariate raster surfaces (depth, sea surface temperature (SST), bottom temperature (BT), mixed layer depth (MLD), salinity and chlorophyll (Chl)). All covariates were scaled (subtracting the mean value and dividing by the standard deviation) so all variables fall over a similar and comparable range.

6.3.3 Model Formation

We present a Bayesian hierarchical spatial modelling framework for the shelf seas, predicting $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ as response values using integrated nested Laplace approximations (INLA) via the R-INLA package (<http://www.r-inla.org>) (Rue et al., 2009). R-INLA includes a stochastic partial differential equation (SPDE) approach that allows the fast modeling of Gaussian Random Fields (GRFs) similar to kriging approaches, but is better adapted to handling data with complex spatial structures (Lindgren et al., 2011).

When modelling across a spatial range, ordinary linear regression ignores spatial dependency between sampling locations. R-INLA provides a means to explicitly incorporate the spatial dependency term:

$$y_{(si)} \sim N(x_{(si)}, \sigma^2), \quad x_{(si)} = \text{covariates}_{(si)} + u_{(si)}$$

where $y_{(si)}$ are the response values at all sampling locations which are assumed to be normally distributed (continuous Gaussian Field) with mean $x_{(si)}$ and variance σ^2 . $u_{(si)}$ is the spatial dependency random effect. The SPDE approach enables the covariance matrix of the Gaussian Field to be approximated as a Gaussian Markov Random Field (GMRF) using a Matérn covariance structure.

The SPDE model makes use of Delaunay triangulation in order to create prediction locations. Triangulation is the partitioning of the region of interest into triangles. Observations are treated as initial vertices for the triangulation, then further vertices are added with the aim of reducing the number of triangles required to cover the region, but taking into account all observations in denser sampled areas. The added vertices act as prediction locations and the partitioning surface is termed a mesh. Shape files of the UK and Europe were used to create a spatial mesh with a boundary effect, taking into account landmasses, so isotope values would be modelled around rather than through land. Different mesh designs were evaluated to investigate their effects during model selection. The final shelf sea mesh design is displayed in Fig. 6.3.

To allow a comparison between isoscape models created through INLA and kriging approaches, the INLA North Sea model was developed using reference data from lion's mane jellyfish only. Isotope data from all 7 sampled jellyfish species were incorporated into the shelf sea model and species identity included as a random effect. Models were specified as:

$$Y \sim 1 + X + f(U, \text{model} = \text{iid}) + f(W, \text{model} = \text{spde})$$

where Y is the isotope value (for each of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$), 1 is the intercept term, X is a vector containing the environmental covariates as linear fixed effects, U is the species

random effect with assumed Gaussian distribution and W represents the smooth spatial effect, linking each observation with a spatial location. Appropriate models were selected for both the North Sea and shelf sea isoscapes using backwards stepwise model selection using the function “INLAstep” within the “INLAutils” package (Redding et al., 2017), specifying both no interactions and then first order interactions. Environmental variable combination and inclusion or exclusion of interactions was based on deviance information criterion (DIC) and model fit (by assessing the Pearson product moment correlation coefficient between model predicted values and observed values). Within the North Sea, models with and without a spatial effect were both tested, and DIC and correlation coefficient values compared. When similar DIC values were observed (within 2 of each other), the simpler model (without the spatial random effect component) was selected (Burnham and Anderson, 2003). Only models including the spatial effect were used for the shelf seas. The purpose of this was to capture and model spatial variation in the response variable that was driven both by the larger spatial extent of the UK shelf study area, and additional potential variance introduced by using multiple species of jellyfish as reference samples. Non-informative default priors were used for each model (Gaussian fixed effects and iid latent model = log gamma prior with parameters (1,0.01)). For the SPDE model, the default priors take the form of Gaussian distributions with mean and variance values calculated based on the size of the study area.

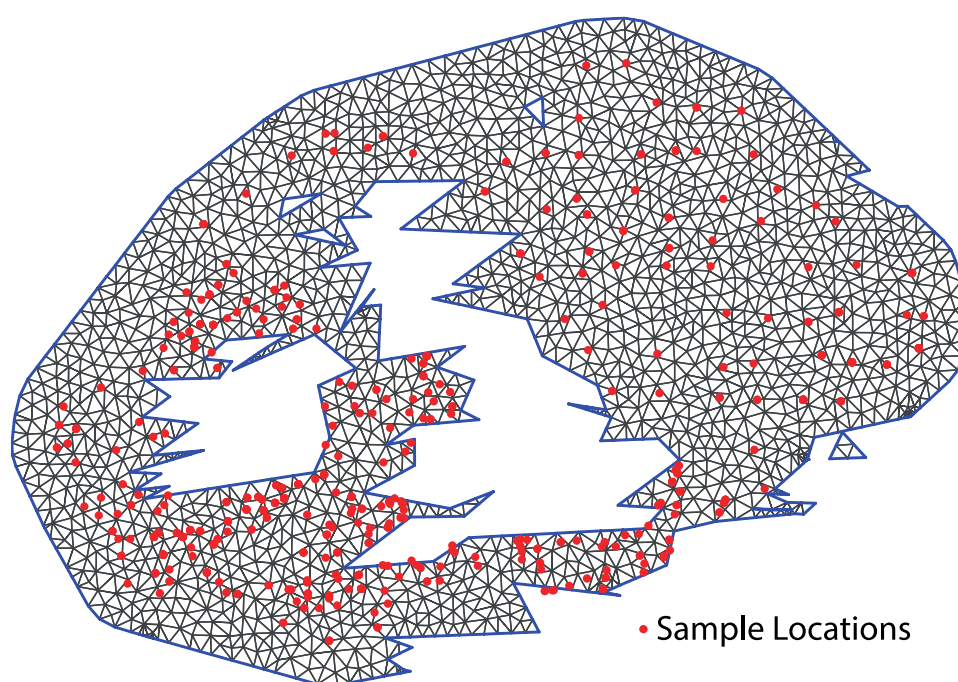


Figure 6.3 Delaunay triangulation mesh designs for the UK shelf sea model. Sampling locations are indicated in red.

The best model for each isotope was used to estimate isotope values across the whole spatial domain using the continuous raster surfaces of scaled environmental variables.

Including the spatial random effect means this method is a Bayesian equivalent of kriging, while also accounting for variability due to environmental factors. Response variables were estimated at all mesh vertices, which were then linearly interpolated within each triangle into a finer regular grid. A lattice of 0.2 x 0.2 degree grid cells was produced for the North Sea and UK shelf seas, and grid cells were masked where predictor covariate values fell largely outside the range of values observed at the jellyfish sampling locations to avoid issues with extrapolating beyond known values of the environmental covariates. Estimates of the mean and variance values of each isotope were obtained for each grid cell and mapped to produce isoscapes and model variance surfaces.

Carbon and nitrogen isoscapes and variance surfaces were produced for the North Sea using lion's mane jellyfish samples analysed by Elementex laboratory (the same data used in Trueman et al. (2017)). Multi species carbon, nitrogen and sulfur isoscapes were produced for the shelf seas using jellyfish data analysed by LSMSF. All models were run through R 3.4.2 (R Core Development Team, 2016).

6.3.4 Statistical assignment methods and accuracy and precision comparisons

Following the methods described in Trueman et al. (2017) scallops were assigned to their most likely spatial origin within the North Sea (Fig. 6.4) and UK shelf seas (Fig. 6.7) based on similarity between measured isotopic compositions and isoscape predictions using multivariate normal probability distributions. Assignment conditions are displayed in Table 6.1. Assignment accuracy and precision results were displayed as per Trueman et al. (2017) using odds ratios to set probability threshold values to differentiate between cells of likely and unlikely origin. Assignment precision is defined by the odds ratio threshold and represents the proportion of the surface area with probability values above this set threshold, and assignment accuracy is defined as the proportion of individuals where the true location falls within the assigned area (Vander Zanden et al., 2015, Trueman et al., 2017). The accuracy and precision achieved when assigning known-origin scallops sampled in 2001 and 2010 to the INLA predicted North Sea isoscapes were compared with assignments to the ordinary kriging isoscapes produced by Trueman et al. (2017). When assigning known-origin scallops across the wider UK shelf seas, scallops were split into North Sea, English Channel and Irish and Celtic Sea samples to enable assignment accuracy and precision comparisons between basins.

Table 6.1 Assignment conditions adopted for stable isotope based location of known location scallops to the UK shelf sea mixed jellyfish species carbon and nitrogen isoscapes. Between individual jellyfish variance value is the greatest between individual variance value of all sampled species.

Variable	Isoscape Jellyfish	Scallop Assignment	
		2001	2010
Measurement error (σ) (measured)(‰)	$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$: 0.2	$\delta^{13}\text{C}$ & $\delta^{15}\text{N}$: 0.2	
Between individual variance (measured)(‰)	$\delta^{13}\text{C}$: 1.08, $\delta^{15}\text{N}$: 1.65	$\delta^{13}\text{C}$: 0.2, $\delta^{15}\text{N}$: 0.7	
Trophic distance	NA	1 ($\sigma = 0.5$)	
Isotopic trophic fractionation (‰)	NA	$\delta^{13}\text{C}$:1 ($\sigma = 0.5$), $\delta^{15}\text{N}$: 3.4 ($\sigma = 0.5$)	
Calibration offset (‰)	NA	$\delta^{13}\text{C}$: - 1.5, $\delta^{15}\text{N}$: -0.5	$\delta^{13}\text{C}$: - 0.5, $\delta^{15}\text{N}$: -0.5

The ability to assign scallops to known ICES subarea locations was also investigated. Scallops collected within each ICES subarea were individually assigned to the UK shelf sea isoscapes at both a high precision odds ratio threshold where assignment areas represented approximately 17% of the isoscape on average (2001 threshold value = 1.25, 2010 threshold value = 1.33) and at a high accuracy odds ratio threshold where over 90% of scallops were accurately assigned to an area covering approximately 50% of the isoscape surface on average (2001 threshold value = 1.66, 2010 threshold value = 1.33). If the assignment surface overlapped the ICES subarea, the individual could have originated from within the correct subarea and was therefore labelled as “in”. If the assignment surface did not overlap the correct ICES subarea, the individual was unable to be assigned to the correct subarea and was labelled as “out”. The average proportion of individuals assigned to the correct subarea (in) and those unable to be correctly assigned (out) were calculated for both 2001 and 2010 scallop data sets at both thresholds.

Multivariate assignment using three isoscape surfaces was unable to be trialled due to a lack of sulfur isotope measurements of scallops from known locations. All assignments were performed using R 3.4.2.

6.4 Results

6.4.1 Within and between species variability

Table 6.2 Within species isotopic variation (red) and between species isotopic differences (black). Calculated at locations where more than one individual of the same species or multiple species occur. Values were then averaged across locations. Between species differences are read as those species in the first row subtracted from those species in the first column. Blue numbers indicate the number of locations where pairs of species were observed.

	Barrel	Blue	Compass	Crystal	Lion's mane	Mauve	Moon
Carbon (‰)							
Barrel	0.37	NA	-3.0	-2.16	-2.73	-0.93	-2.34
Blue	0	0.62	1.14	-0.40	0.26	2.22	2.27
Compass	5	11	0.59	0.74	0.63	-0.03	0.63
Crystal	8	5	16	1.08	1.05	0.71	0.06
Lion's Mane	4	24	9	10	0.60	0.84	0.39
Mauve	1	1	7	30	1	0.48	-0.71
Moon	5	9	11	21	8	14	0.46
Nitrogen (‰)							
Barrel	0.79	NA	-2.08	-1.85	-1.37	7.10	-0.60
Blue		0.44	0.49	-1.47	0.02	1.63	0.78
Compass			0.52	-3.55	-0.30	-0.19	-3.68
Crystal				1.65	1.34	5.22	2.54
Lion's Mane					0.62	2.97	0.32
Mauve						0.95	-5.25
Moon							0.63
Sulfur (‰)							
Barrel	0.45	NA	0.13	0.37	0.83	1.45	0.88
Blue		0.62	-0.74	0.65	-0.1	-0.01	-0.49
Compass			0.41	0.15	0.53	-0.19	-0.39
Crystal				0.75	-0.62	0.50	-0.34
Lion's Mane					0.44	0.29	-0.47
Mauve						0.59	-0.03
Moon							0.63

The variations in average stable isotope compositions within species sampled at the same locations, were found to be relatively consistent across species in both carbon (0.37-0.62‰) and sulfur (0.41-0.63‰) apart from in crystal jellyfish where among-individual variation was higher for both carbon (1.08‰) and sulfur (0.75‰) isotopes (Table 6.2). Within species differences in nitrogen were more variable ranging from 0.44‰ in blue jellyfish to a greater variability of 1.65‰ again observed in crystal jellyfish (Table 6.2). Among species differences ranged considerably between species and isotope (Table 6.2). Sulfur isotopic differences among species were relatively constrained with differences ranging from 0.01 to 1.45‰. Carbon isotopic differences among species ranged from 0.03‰ (between mauve stingers and compass jellyfish) to 3‰ (between barrel and compass jellyfish). Among species differences in nitrogen isotopic ratio varied over the largest range, from a 0.02‰ difference between lion's mane and blue jellyfish up to a 7.1‰ difference between barrel and mauve jellyfish.

6.4.2 INLA isoscape models

6.4.2.1 The North Sea

6.4.2.1.1 Isoscape models

The best-fit carbon isoscape model following backwards stepwise model selection for the North Sea was:

$$\delta^{13}\text{C} \sim -1 + \text{Intercept} + \text{SST} + \text{BT} + \text{Depth} + \text{Salinity} + \text{Chl} + \text{Tdiff},$$

with a DIC value of 174.4 and a significant positive correlation between the observed and model fitted values ($R = 0.59$, $p < 0.05$). The best-fit nitrogen isoscape model for the North Sea was:

$$\delta^{15}\text{N} \sim -1 + \text{Intercept} + \text{SST} + \text{BT} + \text{Depth} + \text{MLD} + \text{Chl} + \text{Tdiff},$$

with a DIC value of 205.4 and a significant positive correlation between observed and fitted values ($R = 0.74$, $p < 0.05$). Interaction terms and spatial models with the additional spatial random effect were trialled for both carbon and nitrogen, however DIC values and model fit did not improve, therefore the simpler non spatial models without interaction terms were selected. Broad spatial patterns in carbon and nitrogen isotopic ranges are consistent with those modelled in Trueman et al. (2017) (Figure 6.4.a and c, respectively) and Jennings and van der Molen (2015), indicating INLA isoscape prediction surfaces are comparable with more traditional ordinary kriging prediction surfaces. Associated variance surface values for carbon and nitrogen (Fig 6.4.b & d) are considerably lower than those calculated by Trueman

et al. (2017) using ordinary kriging methods, with both carbon and nitrogen variance values predominately below 1‰ across the range of the isoscape.

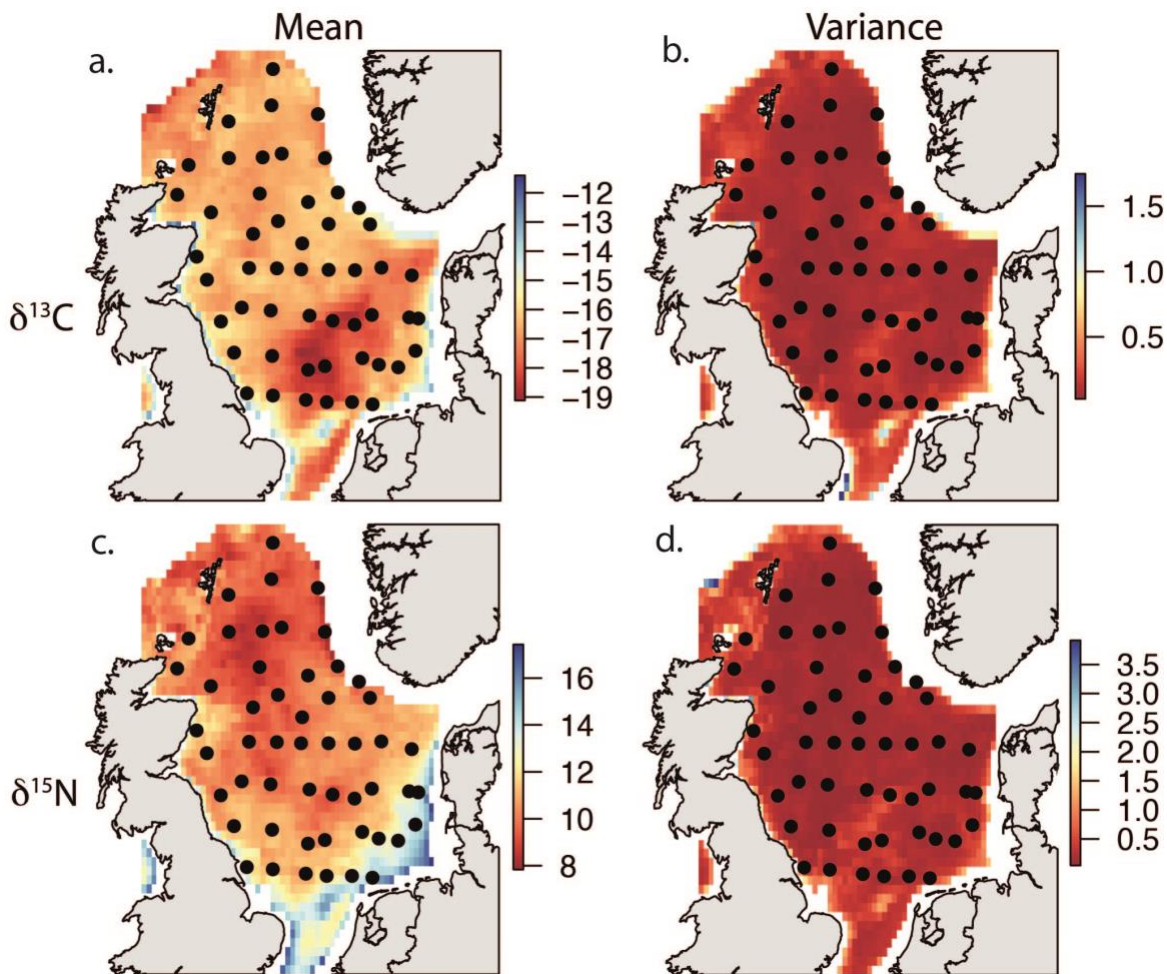


Figure 6.4 North Sea carbon (a) and nitrogen (c) isoscape models and associated variance surfaces (b, d). Values based on *Cyanea capillata* sampled in August 2015. Filled circles represent sampling locations.

6.4.2.1.2 Scallop assignment and method comparison

We compared the accuracy and precision associated with assigning scallops to their known origin using both the original Trueman et al. (2017) North Sea isoscapes and the INLA modelled isoscapes over a range of odds ratio threshold values (Fig. 6.5). Assignment to the new INLA modelled isoscapes shows better than random accuracy at all precision values (Fig. 6.5). Assignment accuracy for scallops sampled in 2001 and 2010, is over 90% when assigning to areas representing on average over 40% of the total North Sea isoscape area. When precision is increased to an area representing 20% of the isoscape, assignment accuracy is still relatively high with >70% accuracy both years scallop data sets. At higher

precision values, assignment of the 2001 data set to the INLA modelled isoscapes appears more accurate than assignment to the kriging isoscapes by Trueman et al. (2017), whereas the opposite is observed with the 2010 scallop data set. Overall assignment to the original kriging isoscapes is slightly more accurate than to the INLA modelled isoscapes, but both methods of isoscape prediction are largely comparable.

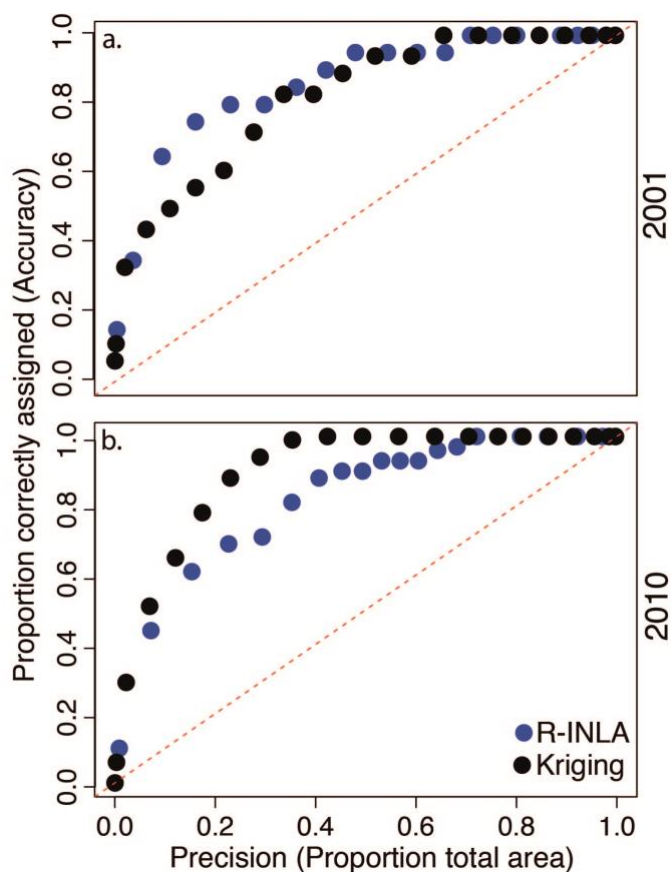


Figure 6.5 Accuracy (the proportion correctly assigned) and precision (proportion of the total surface area) of assignment to both the original North Sea kriging isoscape models (Trueman et al. 2017) shown in black, and the new INLA modelled carbon and nitrogen North Sea isoscapes shown in blue for the 2001 and 2010 scallop data sets. The red line represents the accuracy and precision values if assignments were no better than random.

6.4.2.2 UK shelf seas

6.4.2.2.1 Isoscape models

Spatial random effect terms were included in all UK shelf models to capture and model spatial variation in the response variable that was driven by both the wider study extent and the within-species variations. The models with the smallest DIC values, largest Pearson product moment coefficient value between observed and fitted values and the most appropriate predicted isotopic range were selected. The best-fit carbon isoscape model for the UK shelf seas was:

$$\begin{aligned} \delta^{13}\text{C} \sim & -1 + \text{Intercept} + \text{SST} + \text{Salinity} + \text{MLD} + \text{Depth} + \text{Chl} + \text{Tdiff} + \\ & \text{SST:BT} + \text{SST:Salinity} + \text{SST:MLD} + \text{SST:Depth} + \text{SST:Chl} + \text{SST:Tdiff} + \\ & \text{BT:Salinity} + \text{BT:MLD} + \text{BT:Depth} + \text{BT:Chl} + \text{BT:Tdiff} + \text{Salinity:MLD} + \\ & \text{Salinity:Depth} + \text{Salinity:Chl} + \text{Salinity:Tdiff} + \text{MLD:Depth} + \text{MLD:Chl} + \\ & \text{MLD:Tdiff} + \text{Depth:Chl} + \text{Depth:Tdiff} + \text{Chl:Tdiff} + f(\text{Species}, \text{model} = \text{iid}) + \\ & f(W, \text{model} = \text{spde}), \end{aligned}$$

with a DIC value of 2011.5 and a significant positive correlation between the observed and model fitted values ($R = 0.57$, $p < 0.05$). The best-fit nitrogen isoscape model for the UK shelf sea was:

$$\begin{aligned} \delta^{15}\text{N} \sim & -1 + \text{Intercept} + \text{SST} + \text{BT} + \text{Salinity} + \text{Depth} + \text{Chl} + \text{Tdiff} + \\ & \text{SST:BT} + \text{SST:Salinity} + \text{SST:MLD} + \text{SST:Depth} + \text{SST:Chl} + \text{SST:Tdiff} + \\ & \text{BT:Salinity} + \text{BT:MLD} + \text{BT:Depth} + \text{BT:Chl} + \text{BT:Tdiff} + \text{Salinity:MLD} + \\ & \text{Sal:Depth} + \text{Salinity:Tdiff} + \text{MLD:Depth} + \text{MLD:Tdiff} + \text{Depth:Chl} + \\ & \text{Depth:Tdiff} + \text{Chl:Tdiff} + f(\text{Species}, \text{model} = \text{iid}) + f(W, \text{model} = \text{spde}), \end{aligned}$$

with a DIC value of 2394.4 and a significant positive correlation between observed and fitted values ($R = 0.80$, $p < 0.05$). The best-fit sulfur isoscape model for the UK shelf sea was:

$$\begin{aligned} \delta^{34}\text{S} \sim & -1 + \text{Intercept} + \text{SST} + \text{MLD} + \text{Depth} + \text{Chl} + \text{Tdiff} + f(\text{Species}, \text{model} = \text{iid}) \\ & + f(W, \text{model} = \text{spde}), \end{aligned}$$

with a DIC value of 1449.3 and a significant positive correlation between observed and fitted values ($R = 0.47$, $p < 0.05$).

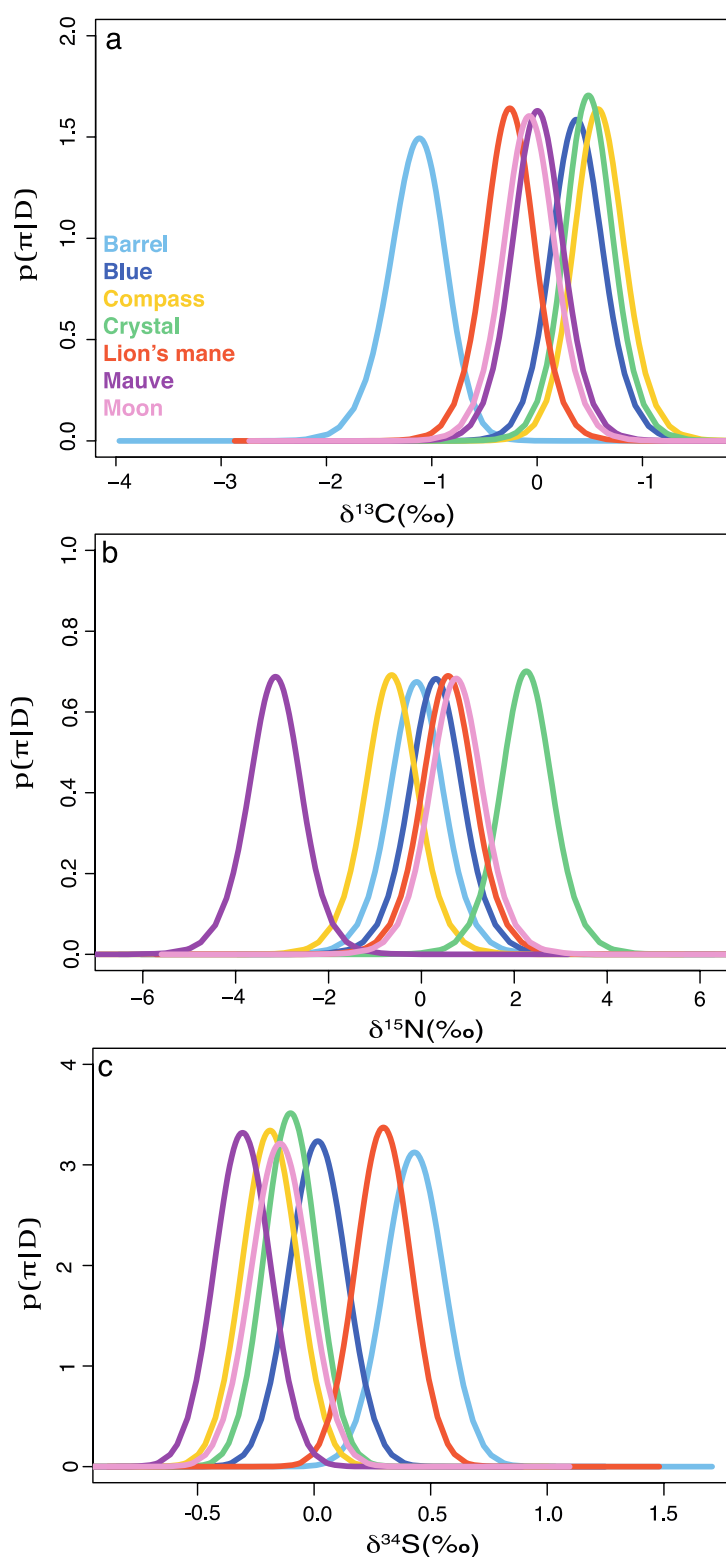


Figure 6.6 Marginal posterior distributions of the species random effect for the chosen carbon, nitrogen and sulfur isoscape prediction models. π is the species-level deviation from the overall mean isotope value, and D is the data. Distributions represent the probability of a given isotopic difference, given the data and represents species differences that remain after the models have been applied. Differences between species represent isotopic differences unable to be explained by environmental variables.

Marginal posterior distributions of the species random effect for the chosen carbon, nitrogen and sulfur models (Fig. 6.6) represent the isotopic differences between species still present after the variability accounted for by the covariates in the model and has been removed. Residual carbon isotopic variability between species ranges over approximately 3‰, with barrel jellyfish the most isotopically distinct from the other species in terms of carbon (Fig. 6.6a), also depicted in Table 6.2, where barrel jellyfish show low $\delta^{13}\text{C}$ values compared to all other species. Residual nitrogen isotopic variability has a larger range between species ($\sim 6\text{‰}$), with mauve stinger jellyfish depleted in ^{15}N and crystal jellyfish displaying relatively high $\delta^{15}\text{N}$ values (Fig. 6.6b, Table 6.2). Sulfur isotopic variability between species has a markedly smaller range ($\sim 1\text{‰}$), with lion's mane and barrel jellyfish displaying slightly higher $\delta^{34}\text{S}$ values than the other species (Fig. 6.6c).

Spatial distributions of $\delta^{13}\text{C}$ values within the North Sea are consistent with previous findings showing relatively low $\delta^{13}\text{C}$ values (-18 to -17‰) in the central North Sea and higher $\delta^{13}\text{C}$ values in the northern and southern North Sea (Fig 6.7a). Similar $\delta^{13}\text{C}$ values of between -17 to -16‰ are predicted within the western English Channel and into the Celtic and Irish Seas. Higher $\delta^{13}\text{C}$ values (-15.5 - -14.5‰) are predicted along the French and Belgian coasts of the English Channel and southern North Sea, off the southwest coasts of Cornwall and north of the Irish Sea (Fig 6.7a). Spatial distributions of $\delta^{15}\text{N}$ values are also consistent with previous North Sea predictions, with a strong isotopic gradient between the northern (8 - 10‰) and southern (11 - 13‰) North Sea (Fig 6.7c). Higher $\delta^{15}\text{N}$ values are also observed into the English Channel and within the Irish Sea, whereas lower $\delta^{15}\text{N}$ values are predicted around north and west Scotland and Ireland (Fig 6.7c). The isotopic range in sulfur is relatively small across the shelf (20.5 - 22‰) in comparison to the large variability observed in carbon and nitrogen isotope ratios (Fig 6.7e). The highest $\delta^{34}\text{S}$ values ($>21.5\text{‰}$) are observed in the northern North Sea, north Scotland and Ireland into the northwest Irish Sea (Fig 6.7e). A clear isotopic gradient is predicted between the northern and southern North Sea, with decreasing values into the southwest North Sea. Lowest $\delta^{34}\text{S}$ values are predicted off the southwest coast of the UK and into the Celtic Sea (Fig 6.7e).

Variance surfaces show broadly similar patterns for each isotope element, with low variance values ($<2\text{‰}$) across the majority of the shelf, and increased variance values observed within the eastern English Channel, eastern Irish Sea and in coastal regions. The latter coincide with increased freshwater inputs, water body mixing and likely isotopic variability across smaller spatial scales (Figs 6.7b, d, & f).

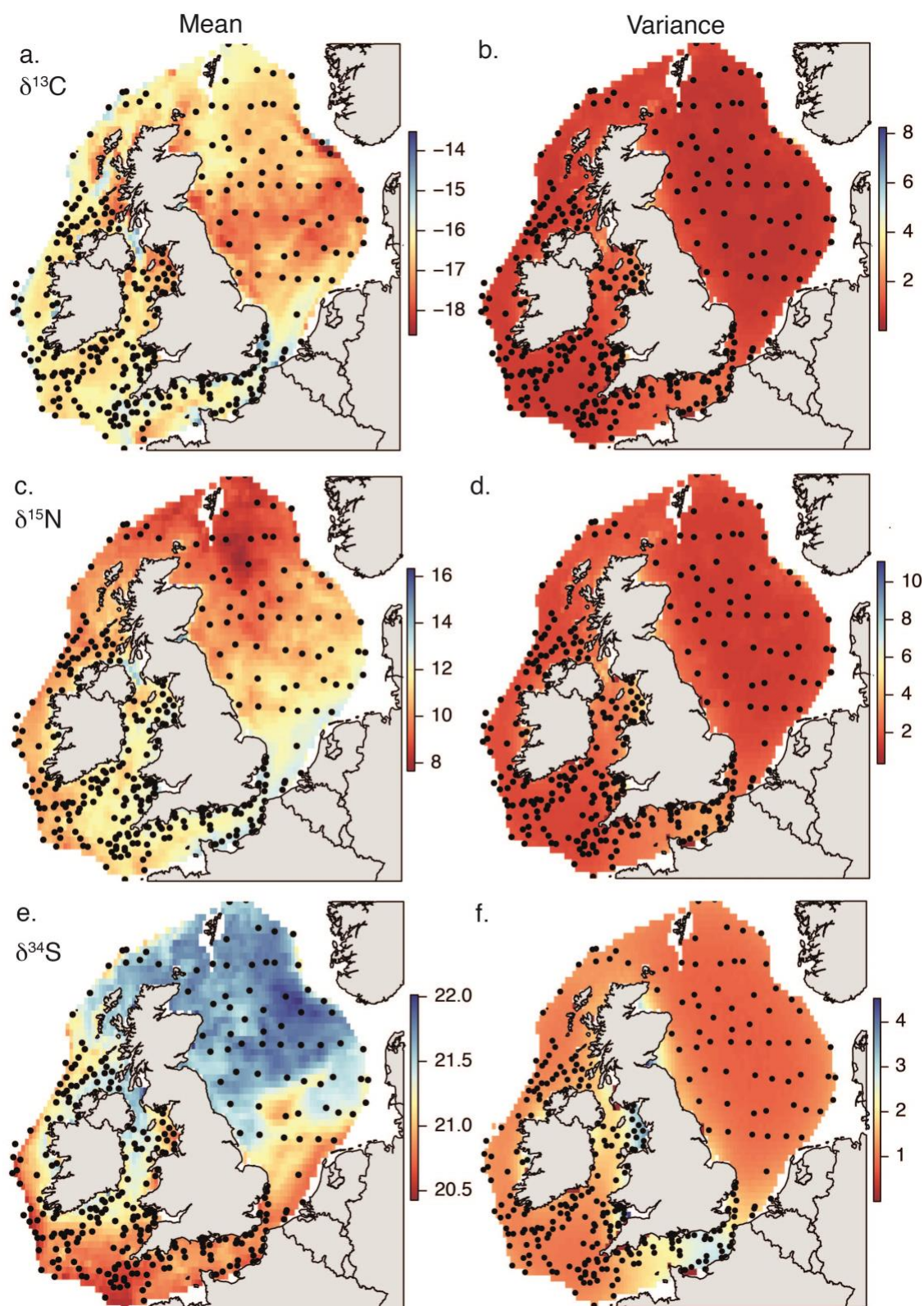


Figure 6.7 UK Shelf Sea carbon, nitrogen and sulfur isoscape models (a, c, e) and associated variance surfaces (b, d, f). Values based on 7 species of jellyfish (Barrel, Blue, Crystal, Compass, Lion's mane, Mauve and Moon) sampled between August 2015 and December 2016. Filled circles represent sampling locations.

6.4.2.2.2 Scallop assignment

Geographic assignment of scallops to the UK shelf isoscapes shows better than random accuracy at all precision values (Fig 6.8). Assignment of scallops collected in both 2001 and 2010 was over 90% accurate to an area covering approximately 40% of the UK shelf sea isoscape, when assigning to each region within the UK shelf. When precision is increased to an area representing approximately 20% of the isoscape, assignment accuracy of both 2001 and 2010 scallops to the North Sea and Irish Sea was over 65%. Assignment accuracy for 2010 scallops to the English Channel at a precision of approximately 20% of the isoscape area was slightly lower at approximately 50% (Fig. 6.8e). Assignment to the North Sea isoscape was notably better, with over 90% assignment accuracy for 2001 and 2010 scallop samples at precision values of 24% and 32% of the isoscape, respectively (Fig. 6.8a&d).

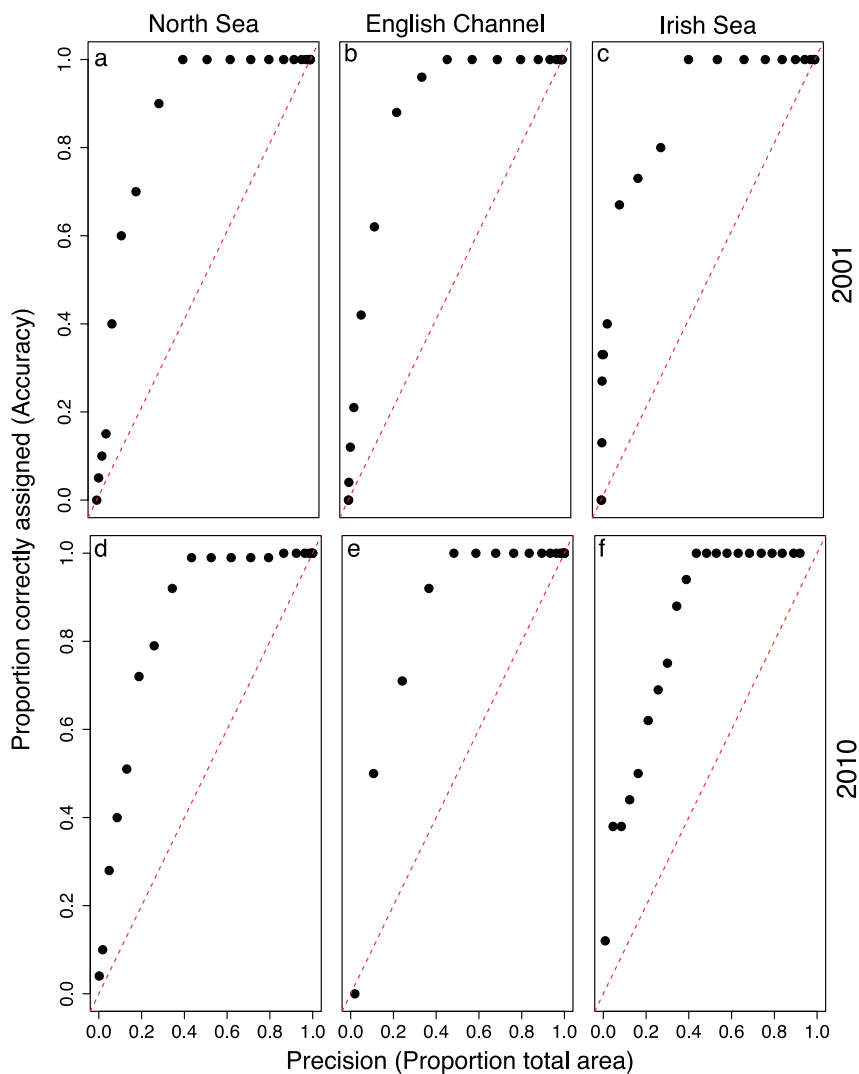


Figure 6.8 Accuracy (the proportion correctly assigned) and precision (proportion of the total surface area) of assignment to both the INLA modelled carbon and nitrogen UK Shelf sea isoscapes of the 2001 (a, b, c) and 2010 (d, e, f) scallop data sets. Assignments are separated into North Sea (a, d), English Channel (b, e) and Irish Sea (c, f) scallop samples. The red line represents the accuracy and precision values if assignments were no better than random.

Scallop assignment was also assessed in terms of accuracy of assignment to the correct ICES subarea (Fig. 6.9). At the high precision threshold (~17% of the isoscape area) 40.7% of scallops from the 2001 data set could be assigned to the correct ICES subarea, whereas 79.7% of the 2010 scallop data set could be accurately assigned (Table 6.3). At the high accuracy and lower precision threshold (>90% accuracy to an area approximately 50% of the isoscape area), 88.1% and 97.8% of the 2001 and 2010 scallop samples could be accurately assigned to the correct ICES subareas (Table 6.3). At the higher precision threshold all scallop individuals could be assigned to the North Sea subareas (IVa and IVb). However accurate assignment to the Irish Sea (VIIa) was more challenging at the higher precision threshold but accuracy increased with decreased precision.

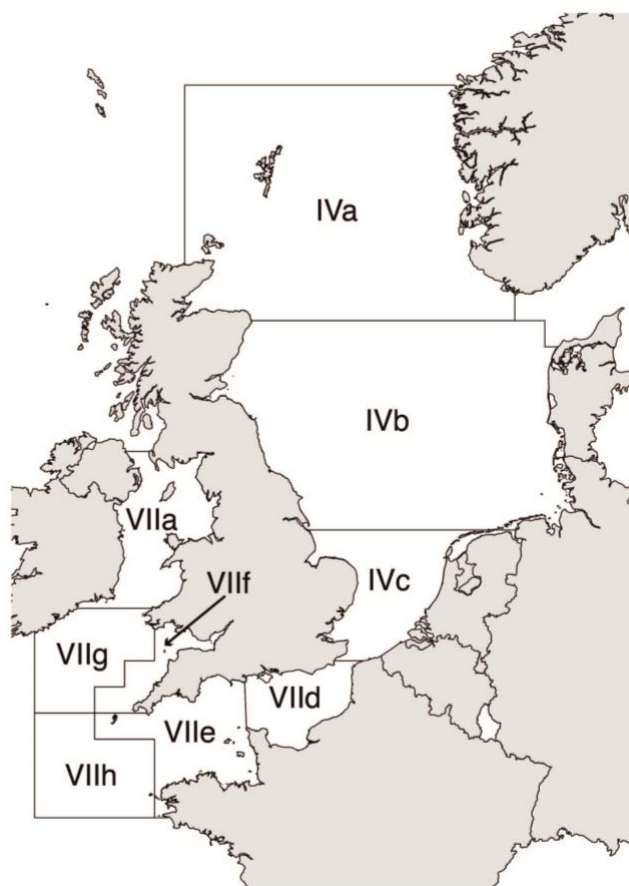


Figure 6.9 ICES subareas around the UK shelf seas where scallops data were collected and assigned to the UK shelf sea isoscapes.

Table 6.3 Number of scallops correctly assigned to the known ICES subarea using 2001 and 2010 scallop isotope data and assigning to the carbon and nitrogen INLA modelled isoscapes. High precision assignments used threshold values that represented an area measuring approximately 17% of the UK shelf sea surface area (threshold value = 1.25 for 2001 samples and 1.33 for 2010 samples). High accuracy assignments used threshold values that represented areas covering approximately 50% of the UK shelf sea surface area, where over 90% of scallops were correctly assigned within this area (threshold value = 1.66 for 2001 samples and 1.82 for 2010 samples). Where assignment area overlapped correct ICES subarea, scallops were classed as “In”, where assignment area and ICES subarea did not overlap, scallops were classed as “Out”.

	2001				2010			
	High precision		High accuracy		High precision		High accuracy	
Subarea	In	Out	In	Out	In	Out	In	Out
IVa	2	0	2	0	11	0	11	0
IVb	16	0	16	0	60	0	60	0
IVc	0	2	2	0	0	1	1	0
VIIId	4	19	16	7	41	23	60	4
VIIe	NA				2	1	3	0
VIIIf	0	1	1	0	6	0	6	0
VIIg	NA				3	3	4	2
VIIh	NA				2	0	2	0
VIIa	2	13	15	0	95	28	123	0
TOTAL (%)	40.7	59.3	88.1	11.9	79.7	20.3	97.8	2.2

6.5 Discussion

Creating isoscapes in regions where evenly spaced sample collection is impossible is complicated: sampling practicalities may mean that reference samples must be compiled from a range of taxa, and uneven sample distribution may require the use of environmental correlates to expand predictions into un-sampled areas. Simple spatial interpolation methods for generating isoscapes do not consider barriers (such as coastlines) when generating continuous prediction surfaces. Spatial modelling using the INLA approach addresses these common constraints. Here we have produced carbon and nitrogen isoscapes within the North Sea based on measured known-origin reference data from a single species, and obtained broadly similar patterns of isotopic distribution to previous research (MacKenzie et al., 2014, Trueman et al., 2017). Spatial assignment using INLA-produced isoscape models also yielded comparable accuracy and precision to previous kriging-based isoscape models (Trueman et al., 2017). Using the same techniques and incorporating seven different species of jellyfish, environmental variables and additional spatial effects, we have

modelled the spatial distribution of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values across the shelf seas. Known location sessile invertebrate samples were assigned to the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes with relatively high accuracy over a range of precision thresholds, demonstrating the potential for UK Shelf Sea isoscape assignment within spatial based management decisions.

6.5.1 The North Sea: method comparison

The INLA predicted North Sea $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes (Fig. 6.4) are broadly similar to Trueman et al. (2017) isoscapes produced from ordinary kriging of identical lion's mane jellyfish data. Spatial random effect components were not added to either "best fit" model, as the included environmental variables were sufficient to account for isotopic spatial variability, with temperature and depth key components in both models. Temperature and depth were significant drivers of isotopic variability in previous research (Jennings and Warr, 2003, Barnes et al., 2009b, MacKenzie et al., 2014), with temperature likely influencing rate of production and depth influencing nutrient mixing and prevalence of terrestrial inputs, indirectly controlling isotopic ratio.

Scallop assignment accuracy and precision were comparable to that demonstrated by Trueman et al. (2017) (Fig. 6.5). The scallops collected in 2010 were more accurately assigned to the Trueman et al. (2017) isoscapes across all precision thresholds (Fig. 6.5b). Whereas assignment of 2001 sampled scallops at higher precision thresholds, representing smaller surface areas, was slightly better using the R-INLA predicted isoscapes (Fig. 6.5a). In general it is expected that assignment to simple interpolated isoscapes would be better due to the more accurate nature of the isoscapes, providing reference samples are evenly spaced throughout the modelled region. Where evenly-spaced reference samples cannot be recovered across the entire region of interest, or where spatial variation in isotope values is expected to occur at smaller spatial scales than the spacing between reference samples, isoscapes drawn from environmental predictors may produce more accurate and precise assignments.

6.5.2 Isotopic variability across the UK shelf seas

Physical conditions have a significant impact on spatial isotopic variability. Carbon and nitrogen isotope ratios are influenced by the isotopic composition of primary nutrients, and the extent of isotopic fractionation during photosynthesis, which is itself influenced by species composition, light and nutrient availability. Many of these variables are dependent on, or co-vary with, physical characteristics of the environment (van Leeuwen et al., 2015). Stratification and mixing extent are strong drivers of spatial isotopic variability, with front locations closely matching isotope ratio boundaries in carbon, nitrogen and sulfur (Miller and

Christodoulou, 2014, van Leeuwen et al., 2015). In shallow well-mixed regions, such as the Irish Sea and southern North Sea, sediments become re-suspended releasing nutrients into the water column (MacKenzie et al., 2014, Miller and Christodoulou, 2014), such re-suspended nutrients may be isotopically heavy (especially in the case of nitrogen) reflecting greater trophic distance from initial fixation (Fig. 6.7). In deeper, more seasonally stratified regions such as the northern North Sea and Celtic Sea, nutrients become more limited, reducing the preferential uptake of the lighter ^{12}C isotope (Goericke and Fry, 1994), resulting in regions characterised by higher $\delta^{13}\text{C}$ values (Fig. 6.7). Production also becomes more dependent on either newly fixed nitrogen or ^{15}N -depleted ammonia excreted by zooplankton, reducing $\delta^{15}\text{N}$ values, as seen in the northern North Sea (Jennings and Warr, 2003). The English Channel front between the northern and southern English Channel (Miller and Christodoulou, 2014), also divides isotopically distinct waters with higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values observed in the south (Fig. 6.7).

Isotopic composition is also strongly influenced by freshwater and terrestrial inputs. Freshwater has a lower $\delta^{34}\text{S}$ ratio compared to seawater (Fry, 2002), reducing $\delta^{34}\text{S}$ values in regions with high freshwater input such as the eastern Irish Sea, southern North Sea and English Channel, particularly along the Dutch coastline, and areas off West Scotland (Greenwood et al., 2011, Painting et al., 2013, van Leeuwen et al., 2015)(Fig. 6.7). Limited freshwater inputs into the northern North Sea potentially explain the higher sulfur isotope values observed ($\sim 21.5\text{--}22\text{‰}$). Anthropogenic nutrient sources (fertilizers, animal feed and sewage) are introduced to the marine environment through estuaries (Howarth, 1998) and influence productivity causing increased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in coastal and estuarine environments (Oczkowski et al., 2018). Enhanced nutrient inputs to the southern North Sea and eastern English Channel due to extensive agriculture within eastern England and riverine runoff from continental Europe (Painting et al., 2013, Ciavatta et al., 2018) result in higher isotope values ($\delta^{15}\text{N} = \sim 14\text{‰}$, $\delta^{13}\text{C} = \sim -15\text{‰}$) extending across the southern North Sea (Bristow et al., 2013)(Fig. 6.7). Historical pollution inputs to Liverpool Bay (Greenwood et al., 2011) have increased productivity and potentially $\delta^{15}\text{N}$ values within the eastern Irish Sea. Increased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values predicted north of the Irish Sea could be due to the northward flow through the Irish Sea, transporting nutrients and higher isotope ratios to this area (Elliott et al., 1992, Greenwood et al., 2011)(Fig. 6.7). Nutrients are also introduced through aquaculture waste products, concentrated along the western Scottish coast (Painting et al., 2013) possibly explaining the patches of increased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in comparison to surrounding waters.

Phytoplankton species composition also influences isotopic variability, with a distinct split in community structure between the northern and southern North Sea (Ford et al.,

2016). The southern North Sea and English Channel are dominated by diatoms (Ciavatta et al., 2018), which are large and fast growing with higher $\delta^{13}\text{C}$ values. Cyanobacteria have been recorded within the western English Channel (Rees et al., 2009), possibly explaining the patches of lower $\delta^{15}\text{N}$ values off the southwest coast of England due to nitrogen fixation (Fig. 6.7). Cyanobacteria have higher $\delta^{13}\text{C}$ values than other marine phytoplankton (Levitan et al., 2007), also possibly explaining the corresponding areas of higher $\delta^{13}\text{C}$ values. Carbon sources other than phytoplankton also influence isotopic variability. Higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values observed within the East Anglian Plume may also be linked to macroalgae found within salt marshes around the East Anglian coast (Bristow et al., 2013).

Variance surfaces are similar for each isoscape, with uniform variance across the majority of the UK shelf, but greater values found within the eastern English Channel and eastern Irish Sea. These regions coincide with high freshwater inputs and extensive mixing resulting in increased isotopic variability and greater uncertainty.

6.5.3 Benefits and current limitations of the INLA approach

One significant benefit of the spatial INLA approach is the ability to incorporate the large among species isotopic differences, ranging between 0.03-3.0‰ in $\delta^{13}\text{C}$, 0.02-7.1‰ in $\delta^{15}\text{N}$ and 0.01-1.45‰ in $\delta^{34}\text{S}$ (Table 6.2) into the spatial model. The residual differences unaccounted for by the environmental predictors within the final models are displayed as marginal distributions in Fig. 6.6. Carbon isotope ratios measured within barrel jellyfish were consistently depleted in ^{13}C (Fig. 6.6a), possibly due to the different tissue composition compared to all other sampled species. Mauve stinger and crystal jellyfish had markedly different $\delta^{15}\text{N}$ values with mauve stingers displaying consistently low and crystal jellyfish consistently high $\delta^{15}\text{N}$ values. All species have similar $\delta^{34}\text{S}$ values with lion's mane and barrel jellyfish slightly higher. Deciphering the reasons behind these species isotopic differences is beyond the scope of this study, but we emphasize the importance of treating gelatinous zooplankton as separate species in any isotopic study. In this example we use INLA to incorporate isotopic differences between species, however the same concept applies whenever data with known, or assumed, differences must be combined. For example, in isoscape models where plankton or zooplankton are sampled and grouped (Schell et al., 1998, McMahon et al., 2013a); where data have been collected from multiple sources (McMahon et al., 2013a); or where different sampling techniques have been adopted. The same approach could also be used to incorporate temporal variability in sample collection. Although here samples were collected over two years, sampling locations did not overlap across different times, so temporal effects could not be explicitly quantified.

INLA models can produce precise prediction surfaces but ultimately any model-based approach relies upon the information supplied. The covariates need to be included appropriately, whilst taking into account the purpose of the isoscape. Our study aimed to produce isoscapes for use in animal assignments. Therefore interaction terms and complex models were selected which increased prediction accuracy, but limit the ability to make inferences from the included covariates regarding biological, chemical or physical factors within the environment directly controlling isotopic patterns. Model outputs are also influenced by the structure of the dependant variable. In this example, for each isotope the variance observed within each species was not homogenous between species. Variability between species outweighed mean species differences, resulting in potential shrinkage effects, where the model struggles to predict across the entire range of measured values. By incorporating numerous interaction terms the shrinkage effect was lessened, but appropriate mechanisms to deal with high variance between sample groups (as is often the case in isotopic research) should be explored.

In addition, although the INLA approach enables precise isoscape model predictions with low variance values, these prediction surfaces are likely unrepresentative of isotopic spatial variability through time. Isoscape models with higher uncertainty associated with each cell prediction may be better able to accommodate unknown temporal variation. Assigning scallops to the INLA isoscape surface was much less accurate for scallops sampled in 2001 compared to 2010, potentially indicating higher temporal specificity associated with the isotopic patterns thought to be relatively consistent over time (MacKenzie et al., 2014, Trueman et al., 2017). UK shelf sea physical properties are relatively stable (van Leeuwen et al., 2015), but in more dynamic environments, the uncertainty associated with temporal mismatch between collection of reference samples used to generate the isoscape and collection of unknown origin samples must certainly be considered.

6.5.4 Geographic assignment and management implications

Scallops collected in 2001 and 2010 could be assigned to their likely origin with over 90% accuracy to regions representing approximately 40% of the total possible isoscape area (i.e. assigned into regions of $\sim 340,000\text{km}^2$) (Fig. 6.8). When precision was increased to 20% of the isoscape ($\sim 155,000\text{km}^2$), assignment accuracy of North Sea and Irish Sea samples remained above 65% (Fig. 6.8). Assignment accuracy and precision is reduced compared to North Sea isoscape assignments (as 40% surface area represents a significantly larger region), however this is expected due to the increased number of grid cells sharing the same isotope values resulting in larger assignment areas and reduced precision. Accuracy of assignment back to known ICES subareas varied between scallop data collection years and

different precision thresholds. Overall, the majority of individuals could be assigned to the correct ICES subarea when looking at an area covering approximately 50% of the isoscape (Table 6.3). At high precision thresholds (approximately 17% of the isoscape surface area) 2010 data could be assigned to the correct subarea with nearly 80% of individuals correctly assigned. This has useful implications for spatial based management schemes within the UK shelf seas, where decisions are often made at management area scales.

Scallops from the North Sea can be assigned with better accuracy and precision than those sampled from the English Channel and Irish Sea (Table 6.3), due to the increased variability and less distinct carbon and nitrogen isotope values characterising the English Channel and Irish Sea regions. For management purposes a uniform variance surface could be applied to prevent regional discrimination effects. For example, applying an even variance surface ($\delta^{15}\text{N} = 4\text{‰}$ and $\delta^{13}\text{C} = 2\text{‰}$) increased assignment accuracy for the 2010 scallops within the Irish Sea to 85% to an area representing approximately 11% of the shelf area, in comparison to 78% accuracy to 17% of the shelf sea area with modelled variance surfaces. To further improve assignment accuracy and precision, an additional isotope tracer could be included such as sulfur. In regions with indistinct carbon and nitrogen isotope values, where $\delta^{34}\text{S}$ displays strong gradients, such as between the Irish and Celtic Seas, the addition of sulfur isotopes is likely to improve assignment accuracy and precision. However, there is currently limited research on marine sulfur spatial and temporal variability, indeed, we believe this study is the first to demonstrate systematic spatial variation in $\delta^{34}\text{S}$ values in open marine settings, or to produce marine $\delta^{34}\text{S}$ isoscapes.

6.5.5 Conclusion

Where sufficient evenly spaced and high-resolution sample collection is possible over the entire target region, an ordinary kriging isoscape prediction approach is favoured. Where samples are limited, collected from multiple sources with different levels of variation, boundary effects are important and inclusion of environmental data is required, the INLA technique provides a promising approach. Here we demonstrate highly precise shelf-scale isoscape predictions, comparable to more traditional isoscape modelling techniques. Increased precision likely reduces applicability over different temporal scales, however shelf wide assignments of organisms collected 5 and 15 years prior to isoscape sample collection, both display useful levels of accuracy and precision. This approach has great potential as a tool to aid future ecological research and spatial based management plans into understanding space use at management appropriate scales. Although our study focuses on marine carbon and nitrogen and the newly introduced sulfur isotopes, the same methods and benefits and limitations are applicable across all environments and isotope systems.

Chapter 7 Summary Discussion

7.1 Summary of Findings

Marine isoscape development has expanded rapidly over the past two decades with 27 regional marine isoscape models published, varying in terms of baseline organism, interpolation method, resolution, degree of variance quantified and spatial and temporal scale (Fig. 2.4, Table 2.1). Marine isoscapes have been used to answer a broad range of spatial and trophic ecological questions (Chapter 2). However, the use of isoscapes to assign animals probabilistically to a likely origin (arguably the principle use of isoscapes in terrestrial ecology) remains limited (Vander Zanden et al., 2015, Tornaiainen et al., 2017). Many existing marine isoscape models lack explicit quantification of assignment accuracy and precision, essential for conservation and management applications. Isoscape spatial range is commonly constrained by baseline organism distribution or sample collection feasibility, limiting the scope of marine isoscape applications. The research described in this thesis aimed to develop new approaches to modelling spatial isotopic variability and quantify the potential benefits of an isoscape tool for conservation and spatial based management decisions.

Chapter 3 serves as a methods development chapter, predicting North Sea carbon and nitrogen isoscapes using *in situ* samples of lion's mane jellyfish, building on previous work by MacKenzie et al. (2014). While the isotopic composition of jellyfish may be variable among individuals (Fleming et al., 2015), when prepared according to Mackenzie et al. (2017), jellyfish prove useful baseline organisms in shelf sea environments. In chapter 3 techniques to assess assignment accuracy and precision are also established. I demonstrate that assignment precision can be comparable to that achieved from light-based geolocator tags, and that individual assignments are more than 70% assignment accurate when assigning unknown samples to an area representing approximately 30% of the isoscape (Trueman et al., 2017). The potential of assignment as a tool in fisheries research is also highlighted, through the successful assignment of Atlantic herring populations to their known foraging locations.

Chapters 4 and 5 apply North Sea isoscapes to questions in seabird foraging behaviour research. Using the carbon and nitrogen isoscapes produced in chapter 3, coupled with individual data logger information, the most likely foraging locations during moult were refined. Combining two independent geolocation techniques enabled population and individual-level differences in trophic feeding position to be derived. Foraging behaviours were compared between species and between years with varying environmental conditions,

providing important information that could be helpful in designation of protected areas and effective seabird management.

Although isoscapes limited to the North Sea show useful applications, modelling isotopic variability across the shelf seas of the British Isles has a wider scope for use in spatial based management decisions. In chapter 6, the use of Bayesian hierarchical modelling is explored, to address current limitations to isoscape model development caused by the difficulty of obtaining sufficient samples on larger scales. Using a combination of *in situ* isotope measurements of multiple jellyfish species and environmental variables, the INLA approach enabled precise predictions of carbon, nitrogen, and the first marine sulfur isoscapes. Assignment accuracy was approximately 90% when assigning to 40% of the Shelf Sea area and approximately 80% when assigning to ICES subareas, highlighting the potential applications in UK marine management.

7.2 Methodological assessment

Isoscape prediction is dependent on the quality, spatial resolution, distribution and temporal stability of collected samples. I have demonstrated two different isoscape prediction techniques, ordinary kriging of *in situ* samples (chapter 3), and Bayesian hierarchical modelling using R-INLA, including multiple species and additional environmental data (chapter 6). Where sufficient evenly distributed samples are available, ordinary kriging is recommended, and will produce highly accurate isoscape models which may be applicable over different temporal scales. When sample collection is constrained and additional data is required, the INLA approach enables highly precise isoscape predictions, but with reduced applicability over time. Understanding the temporal range of an isoscape is critical to its use in subsequent research and management decisions. North Sea carbon and nitrogen isotopic patterns are reported to be relatively stable (MacKenzie et al., 2014), but temporal variability across wider shelf seas and of sulfur isotopic variability are currently unknown. I demonstrate relatively high assignment accuracy of samples collected both 5 and 15 years prior to isoscape development, supporting shelf sea isoscape assignment applications where samples fall within this range. However, for extended use further work is required to determine isoscape temporal accuracy.

Assignment to isoscapes is also reliant on numerous assumptions. When isoscape and assignment sample organisms differ, calibration offsets including tissue type and trophic differences need to be considered. In this project I demonstrate two different approaches of calibration-offset estimation. Firstly simple adjustment of isotope values to align assignment individual isotope values within isoscape isotope range (chapter 3), and secondly using an alternative geolocation technique (data loggers) to calibrate assignment individual isotope

values to expected isoscape isotope values extracted at broadly known foraging locations (chapters 4 and 5). Both techniques rely on assumptions of known foraging areas but to different spatial scales based on the availability of complementary information. Isoscape assignment provides a useful tool in understanding space use, determining foraging areas and origin, but should be used with caution, explicitly quantifying known uncertainties and if possible in conjunction with alternative techniques.

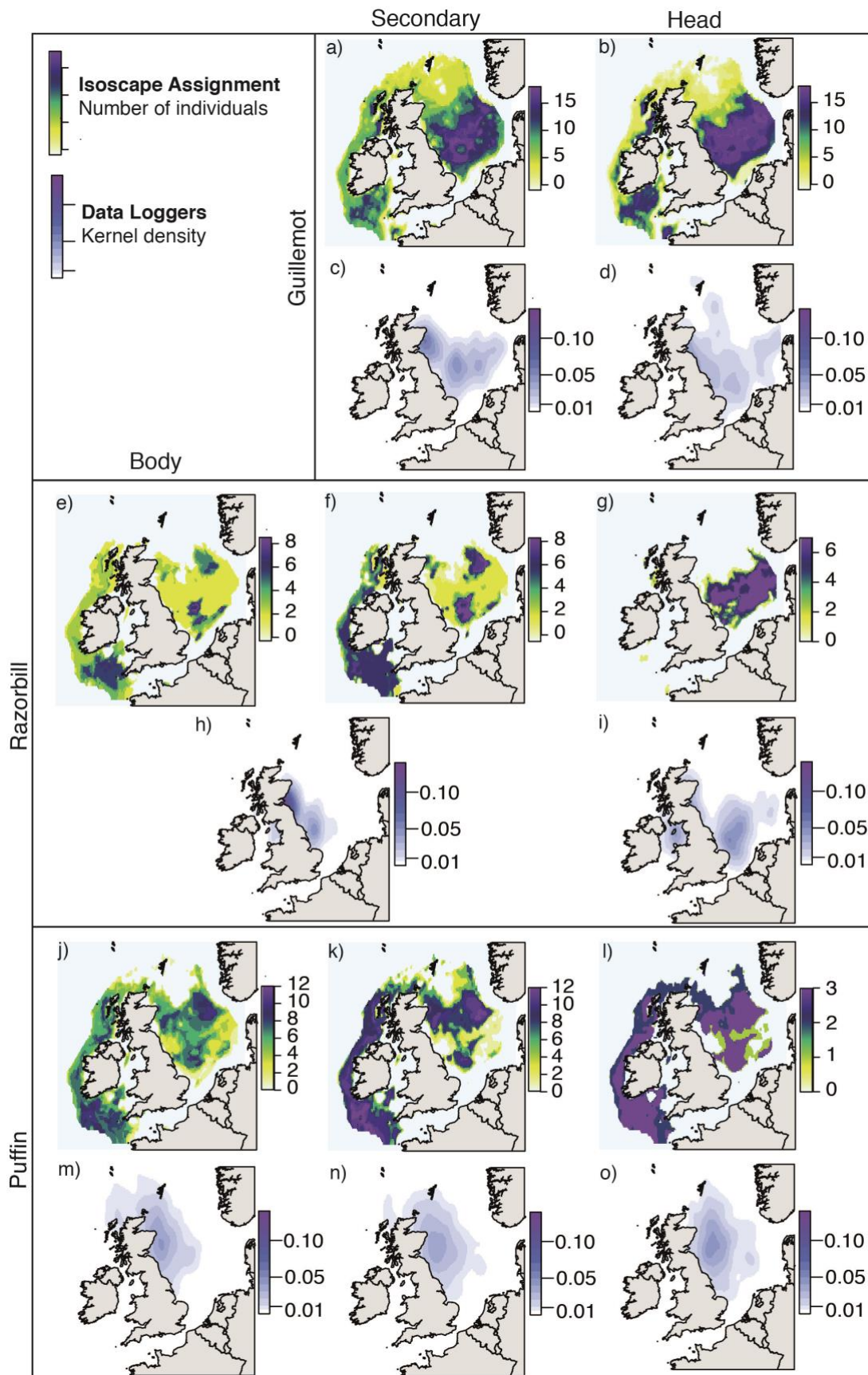


Figure 7.1 Assignment of carbon and nitrogen feather isotopes of UK breeding seabird (guillemots, razorbill and puffin) populations to the UK Shelf Sea carbon and nitrogen isoscapes (upper), and kernel density surfaces based on location coordinates derived from data logger tags for the same populations during the corresponding seasons (lower).

7.3 Management and Conservation - Future applications

Continued production of more robust and spatially explicit marine isoscapes, with quantifiable uncertainty, enables a vast range of management evidence requirements to benefit from this approach (MMO, 2015), as well as providing a tool to investigate academically interesting marine ecology questions which were not necessarily possible before.

7.3.1 Marine Protected Area (MPA) designation

Understanding where animals spend their most vulnerable time periods, where they obtain resources and through which areas they migrate is critical information for spatial based management. Particularly within shelf sea environments, where space use conflicts between marine development, fishing, shipping channels and protected areas are most prevalent. By assigning numerous ecologically important species, across a range of trophic levels, to the shelf sea isoscapes, dominant areas of nutrient origin will be highlighted, providing an alternative approach to traditional methods of MPA designation. Repeated assignments over subsequent years allows temporal change in dominant productive regions to be assessed, to better manage MPA boundaries over time.

Within this project, I have demonstrated the power of North Sea isoscape assignments to refine and monitor seabird moult locations (chapters 4 and 5). Assignment to the British Isles shelf sea isoscapes also shows great promise, with most likely foraging locations of all species, broadly reflecting the corresponding data logger geolocation areas (Fig. 7.1). With an expanded isoscape range, this approach is no longer limited to North Sea overwintering populations, but can be used to study any tagged animal foraging within the shelf seas of the British Isles. Isoscape assignment tools can also be extended to any marine animal to understand movement, migration and foraging behaviours. For example, Quaeck-Davies (2017) assigned muscle samples from spiny dogfish (*Squalus acanthias*) pregnant females and their embryos to the North Sea isoscapes (Trueman et al., 2017), to determine which regions of the North Sea are important for assimilation of resources at different life stages (Fig. 7.2).

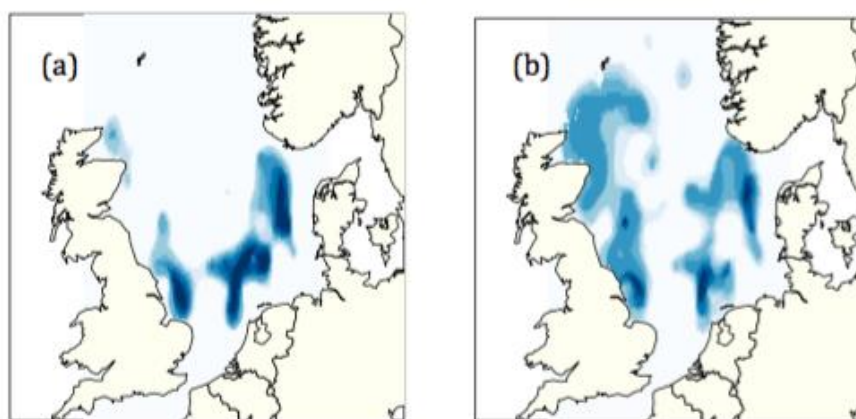


Figure 7.2 Geographic assignment to the North Sea carbon and nitrogen isoscapes (Trueman et al, 2017) of spiny dogfish muscle from pregnant females (a) and their embryos (b). The assignment areas are summed over the number of individuals assigned ($n=19$). (Quaeck-Davies, 2017)

7.3.2 Marine food supply chain traceability and fisheries management

Global illegal, unregulated and unreported (IUU) fishing is estimated to cost up to £20 billion per year and accounts for approximately 18% of total catch (Agnew et al., 2009).

Fisheries substitution, where incorrect provenance is claimed is one method of fisheries fraud. The Common Fisheries Policy (Commission, 2015) states commercial fish products must be labelled with catch location, however reliable, retrospective forensic verification techniques are currently lacking. Isoscape assignment provides a possible means to verify catch locations, offering benefits to sustainable fisheries management and improved consumer trust. In addition, isoscape assignment could (for instance) identify nursery grounds or the spatial origin of migratory animals arriving with exotic pathogens. Isoscapes can provide a greater understanding of population connectivity in highly migratory and highly priced species such as tuna, offering a complimentary approach to expensive tagging and genetic studies (Lorrain et al., 2014, Garza, 2016). These same techniques could also provide a means to track illegal marine organism trafficking and practices, such as shark finning and unethical prawn farming, back to their origin.

To initially assess the potential of the shelf sea isoscape assignment potential in fisheries management and traceability, I calculated assignment accuracy of a collection of ecologically and commercially important species with known catch locations (Fig. 7.3), using isotope data provided by Jennings and Cogan (2015). Samples were grouped by species and assigned to the shelf sea $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isoscapes (chapter 6) to an area representing approximately 30% of the isoscape area. Carbon ($+2\text{‰}$) and nitrogen ($+0.5\text{‰}$) calibration offset values were applied as per herring assignments in chapter 3 (Trueman et al., 2017) and

trophic level estimates for each species were taken from Mackinson and Daskalov (2007). Assignment accuracy was greater than 70% for all ray and skate species, highlighting the potential for such techniques in conservation projects. Commercial fish species assignment accuracy varied from 97% for Atlantic cod to just 35% for European plaice, likely due to differences in migration behaviours and tissue turnover rates. Further work is required to quantify the scope of isoscape assignment tools for fisheries management and traceability applications, but initial results show great promise.

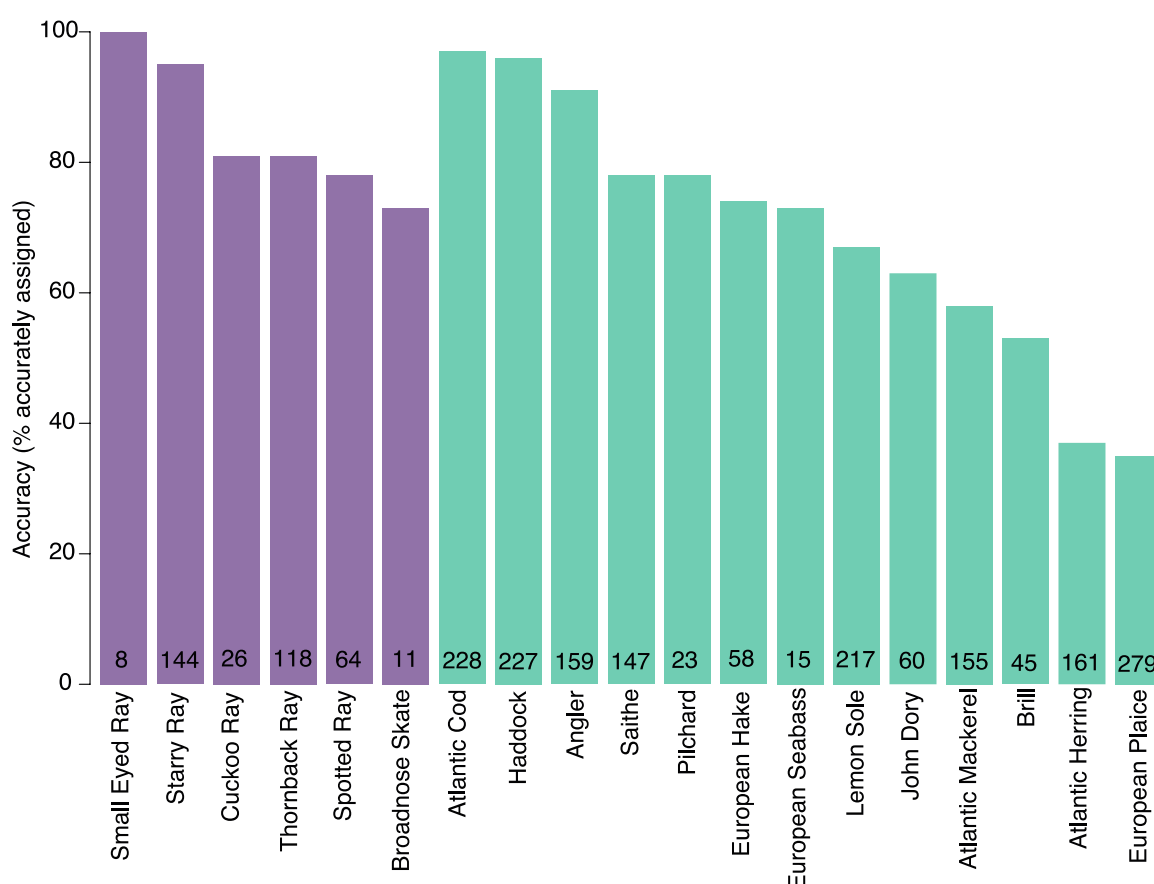


Figure 7.3 Assignment accuracy to the UK Shelf sea carbon and nitrogen isoscapes of known catch location ecologically (purple) and commercially (green) important fish species. Sample carbon and nitrogen isotope values taken from Jennings and Cogan (2015) ranging from 2002 to 2010, and assigned to an area representing approximately 30% of the UK Shelf Sea isoscape. Numbers within bars represent number of samples.

7.4 Conclusion

In a changing economic, political and environmental climate, it seems sensible to develop integrated, inexpensive methods to address spatial issues in marine ecology, conservation and food security and governance. Innovative new approaches providing cost effective and relatively quick solutions are critical to the improved management and conservation of the marine environment, particularly at a time of rapid socio-economic and

legislative change. In addition, whilst it is no longer possible to wait for perfect solutions, decisions must be made and management programmes put in place in the face of uncertainty. Methodologies that are able to explicitly quantify this uncertainty will become more important in making sure the best possible strategies are implemented. Marine isoscapes offer a tool to potentially start addressing some of these key management priorities.

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