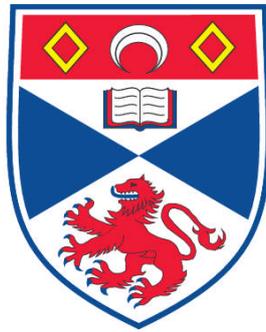


**EXAMINING THE RESPONSE OF TOP MARINE PREDATORS TO
ECOLOGICAL CHANGE USING STABLE ISOTOPE PROXIES**

Nora N. Hanson

**A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews**



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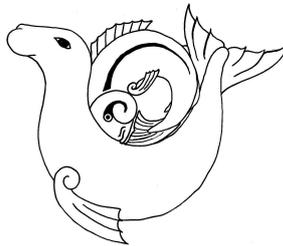
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Examining the response of top marine predators to ecological change using stable isotope proxies

**A thesis submitted in fulfillment of the requirements for the
degree of Doctor of Philosophy**

Nora N. Hanson

June 15th 2011



University of St. Andrews
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Declaration

I, Nora Hanson, hereby certify that this thesis, which is approximately 41,048 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Abstract

Monitoring the response of upper trophic level animals to ecological change is important to understanding the state and stability of ecosystems. Marine predators integrate information over large geographical scales and are relatively long-lived; furthermore, many of these organisms are restricted to terrestrial or freshwater habitats at certain times during their life history and are accessible to researchers. This thesis investigated the response of marine predators to ecological change at a variety of spatial and temporal scales using stable isotope ratio methods with the aims of developing meaningful proxies, or indices, of variability in marine ecosystems.

The first study explored the intrinsic (i.e. ontogenetic) and extrinsic (i.e. environmental) factors important to modulating variation in the stable isotope ratios of C and N in tooth dentin of male Antarctic fur seals (*Arctocephalus gazella*) in the Southern Ocean. In the second study, long-term records of variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of Atlantic salmon (*Salmo salar*) scales and grey seal (*Halichoerus grypus*) tooth dentin provided evidence for large-scale climate forcing across the eastern North Atlantic. In the following study, a more detailed examination of intra- and inter-individual stable isotope variation in Atlantic salmon within a single year was undertaken in an attempt to better understand recent declines in somatic condition of these fish.

The last two studies were concerned with the development of high resolution sampling of fish otoliths using secondary mass spectrometry (SIMS) and the application of this technique to reconstructing the thermal and metabolic histories of individual Atlantic salmon from intra-otolith $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values.

Stable isotope proxies can be used to document shifts in trophic dynamics and animal movement that may be associated with ecological change. Using multiple tissues, elements and species, such studies provide unique monitoring tools at a range of spatial and temporal scales.

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In attempting to write this section of my thesis, I subset my acknowledgments into categories of people I have encountered and had the help of over the last 3 - 4 years (e.g. family, friends, academic, technical, collaborators, etc...). I have quickly come to realise, however, that these distinctions are fairly worthless. In many cases, friends have become family, what began as technical help turned into friendships, academic relationships have come to be near familial. These relationships are some of the most fulfilling and enduring aspects of a PhD thesis and I am extremely grateful to have had the opportunity to develop them.

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1 Introduction

The management and conservation of marine organisms has become increasingly complex in the face of rapidly changing climate conditions (Harley et al. 2006) and ever more ubiquitous human impacts on natural systems (e.g. Halpern et al. 2008). A key challenge is to determine the spatial and temporal extent of the response of biological systems to environmental change, whether they be climatic or anthropogenic in origin. In order to accomplish this, accessible, stable and meaningful measurements are needed that can be used as indices of change. Indices usually are presumed to be proxies that summarise the state of the environment in accessible and highly simplified forms. They are used increasingly by policy-makers and managers to assess the effectiveness of conservation measures (e.g. the development of Ecosystem Quality Objectives for the eastern North Atlantic under the joint policy framework of the International Council for the Exploration of the Seas and the OSPAR Commission). However, there is a need not only to develop more and better indices but also to understand the extent to which they represent key ecological processes and provide an unbiased view of the state of the environment.

Biological indices of predators that that may be geographically wide-ranging, long-lived and that return to coastal and/or freshwater zones integrate variability over large spatial and temporal scales and are a relatively accessible source of information regarding the health of marine ecosystems (Croxall 2006). Additionally, many of these populations interact directly or indirectly with fisheries and so provide an avenue to assess the magnitude and direction of those specific anthropogenic impacts on marine ecosystems.

This thesis investigated the response of marine predators to ecological change at a variety of spatial and temporal scales using stable isotope ratio methods with the overall aim of developing meaningful proxies, or indices, of variability in marine ecosystems.

Stable isotope analysis in animal ecology

Obtaining information about the dynamics of foraging and migration of wild animals, preferably using non-invasive methods, is fundamental to the development of the rationale for approaches to management. Techniques to aid the determination of habitat usage, behaviour and diet have improved considerably in the last century with developments in statistics, telemetry, and chemical analysis. The use of stable isotopes as a chemical proxy to monitor diet and migration is one such development. Understanding the links between climate variation and ecosystem responses is an important concern of 21st century science and stable isotope analysis is a large and rapidly expanding field of enquiry that has much to offer animal ecologists interested in the recent past.

Many elements have naturally occurring stable isotopes but the most commonly used in ecological studies are of C, N, H and O. The natural abundance of the isotopes of these light elements varies greatly and due to advances in mass spectrometry, can now be quantified easily and inexpensively (Griffiths 1991, Gannes et al. 1998). Absolute quantification of isotope abundance is difficult; commonly, isotope ratios are measured relative to a reference standard using instruments that combust, ionize, accelerate and separate isotopes of elements within a gas (referred to as Isotope Ratio Mass Spectrometry or IRMS). The isotope ratio of the sample is given relative to a known standard ratio (Vienna Pee Dee Belemnite for C, atmospheric air for N, and Vienna Standard Mean Ocean Water for O). Ratios are reported in delta notation where:

$$\delta X = 1000 \frac{R_X - R_{std}}{R_{std}} \quad (1.1)$$

X refers to an element and R refers to the ratio of heavy to light isotopes (e.g.: $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). Delta values are reported in parts per thousand, or per mil (‰) (Peterson & Fry 1987, Criss 1995, Sharp 2007). Positive δ values indicate that the ratio of heavy to light isotopes in the sample is greater than that in the standard and negative values mean the opposite is true.

Lighter isotopes (e.g. ^{12}C or ^{14}N) tend to form weaker bonds and react faster (Gannes et al. 1998) than heavier isotopes. Consequently, many natural processes alter the ratio of heavy to light isotopes and this information can be used to study the reaction conditions and the isotopic source of samples (Peterson & Fry 1987,

Griffiths 1991). When stable isotopes are separated during these processes, they are said to fractionate.

Stable isotope analysis in ecological studies can be divided broadly into those focused on natural variation in fractionation of organic matrices (e.g. animal tissues, collagen) and inorganic matrices (e.g. calcium carbonate, hydroxyapatite). In general, stable isotope variation in these compounds can provide information on trophic relationships (process-based information) and migration (source-based information), respectively (Peterson & Fry 1987, Michener & Schell 1994).

Trophic dynamics

Early studies of stable isotope variation in animals reared in the laboratory on known diets showed that the isotope ratio of consumer tissues reflected that of the diet, although there was an enrichment in the heavier isotope for both C and N (DeNiro & Epstein 1978, 1981). The enrichment was related to the prevalence of the lighter isotope in respired carbon and nitrogen in excreted waste. DeNiro & Epstein (1978) noted that that despite tracking diet, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values could vary significantly between individuals raised on the same diet and between tissues within a given individual. They suggested these differences might represent differences in the relative proportions of the major biochemical fractions between individuals and tissues. For example, tissues with a high proportion of lipids could be expected to have low $\delta^{13}\text{C}$ values because of the isotopic discrimination against ^{13}C during lipid synthesis (DeNiro & Epstein 1977, Tieszen et al. 1983). Furthermore, de novo synthesis of biochemicals or their components also might lead to isotopic fractionation that would further alter the difference in isotope ratios between a consumers tissues and those of its diet ($\Delta_{\text{consumer-diet}}$; trophic discrimination factor) (DeNiro & Epstein 1978).

Tieszen et al. (1983) determined $\delta^{13}\text{C}$ trophic discrimination factors for different animal tissues and showed that variation in stable isotope ratios also can arise from differences in tissue metabolism. The rate at which $\delta^{13}\text{C}$ values equilibrated after a switch of diet was heavily dependent on the metabolic turnover rate of that tissue (i.e. the speed at which carbon from a 'new' diet was incorporated into animal tissues). Liver and fat deposits, with high replacement rates, have higher carbon turnover rates and their stable isotope values equilibrate to a change in diet much faster than tissues such as hair which are relatively metabolically inert (Tieszen et al. 1983).

Variation in trophic discrimination factors for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values has been reviewed by McCutchan et al. (2003) and can be shown to depend on differences in diet quality as well as differences in sample preparation procedures. Studies estimating $\delta^{15}\text{N}$ turnover in animals tissues have highlighted the importance of tissue type (e.g. Tieszen et al. 1983, Guelinckx et al. 2007, Buchheister & Latour 2010), animal age/growth rate (e.g. Trueman et al. 2005) and food ration (e.g. Jardine et al. 2004) in determining consumer $\delta^{15}\text{N}$ values. These factors can potentially confound inferences from stable isotope analysis but, if appropriately taken into consideration, they can also provide insight into the trophic dynamics of wild animal populations.

Animal movement

Hobson (1999), and more recently Hobson et al. (2010), provide reviews detailing the abundance and taxonomic breadth of studies using stable isotope analysis to study the movement of animals. Within the marine environment, natural gradients in the proportion of the isotopes of C and N at the base of the food web (now termed isoscapes) can be used to track the movement and foraging of animals (Hobson et al. 2010, Graham et al. 2010). Spatial heterogeneity in marine $\delta^{13}\text{C}$ values exists primarily due to variation in phytoplankton growth rates, cell size and assemblage composition (Goericke & Fry 1994, Bidigare et al. 1997, Popp et al. 1998, Burkhardt et al. 1999); in general, high growth rates and productive areas are associated with higher $\delta^{13}\text{C}$ values. For these reasons, nearshore regions often are enriched in ^{13}C compared to less productive oceanic regions (Hobson 1999, Graham et al. 2010). Large-scale latitudinal gradients with lower $\delta^{13}\text{C}$ values at high latitudes (Kroopnick 1985) are strongly related to differences in the concentrations of CO_2 in the seawater (Hofmann et al. 2000).

Spatial variation in $\delta^{15}\text{N}$ values of primary producers is dependent on the source of inorganic nitrogenous nutrients and biological processes associated with nitrogen cycling (Miyake & Wada 1967, Graham et al. 2010) whereas variation in ocean water $\delta^{18}\text{O}$ values ultimately tracks variations in source water. For example, differences in freshwater $\delta^{18}\text{O}$ inputs and evaporative processes can alter the global distribution of marine water $\delta^{18}\text{O}$ values (Bigg & Rohling 2000).

The dynamic distribution of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ at the base of the food web ultimately is reflected in the isotope ratios of consumer tissues and these gradients can be used to follow the movement of animals between habitats (for a compre-

hensive review, see references in Hobson 1999, Elsdon & Gillanders 2003, Hobson et al. 2010, Graham et al. 2010). In the marine environment, stable isotopes have been used to track geographical movements of animals between foraging habitats (Clementz & Koch 2001, Cherel & Hobson 2007) and ontogenetic shifts in habitat usage (Guelinckx et al. 2006, Shephard et al. 2007, Dufour et al. 2008). While the development of marine isoscapes is still in its infancy (Graham et al. 2010), in the future they may allow detailed spatial reconstructions of migration and habitat residency of marine predators.

Retrospective analyses

Stable isotope analysis offers a major advantage over more traditional techniques for evaluating diet (e.g. stomach or scat content analysis) and animal movement (e.g. telemetry) because of the observer's ability to retrospectively retrieve temporally resolved information from mineralised or keratinised tissues that grow continuously and are metabolically inert after formation (e.g. teeth, fish scales, otoliths, feathers, baleen). This also extends to soft tissues (e.g. muscle, organs) that have varying rates of isotopic incorporation.

Tracking individuals

Sampled chronologically, many 'hard parts' of animals can provide a robust natural tag that records ontogenetic variation in trophic dynamics. In some mammalian species, the dentin and cementum within teeth is deposited in alternating layers of light and dark material, forming annual and sub-annual banding patterns (often called growth layer groups, GLGs) that are used in age estimation (Laws 1953, Hewer 1964). Sub-sampling of consecutive layers of teeth for stable isotope analysis yields life-time records of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ that have been related to dietary shifts (Hobson & Sease 1998, Mendes et al. 2007, Rountrey et al. 2007, Knoff et al. 2008, York et al. 2008, Newsome et al. 2009a) and age of weaning (Newsome et al. 2006, Rountrey et al. 2007, York et al. 2008). Growth in keratinised structures, such as pinniped (seal) vibrissae, whale baleen and reptilian scutes, often is less easily characterised but can be sampled sequentially and also has been used to study ontogenetic and seasonal changes in foraging strategies and individual diet specialisation (Hobson et al. 2004a, Zhao & Schell 2004, Hall-Aspland et al. 2005b, Cherel et al. 2009,

Newsome et al. 2009b, Zanden et al. 2010, Newland et al. 2011).

In fish, the primary structures used for age determination are scales and otoliths (“ear stones”). Due to the nature of scale growth, however, stable isotope analysis is currently limited to a particular life stage (Hutchinson & Trueman 2006) whereas otoliths are sampled across all life stages at high resolution for both stable isotope ratios and elemental concentrations (for reviews, see Campana 1999, Elsdon et al. 2008). Technological advances in the methodology for extracting and analyzing small samples of material for stable isotope analysis have markedly improved the temporal resolution that can be quantified from fish otoliths (Gao 1999, Wurster et al. 1999, Dufour et al. 2008, Weidel et al. 2007). Primarily, the stable isotopes of C and O are used in otolith research to infer individual thermal, metabolic and movement histories (Wurster & Patterson 2001, Gao & Beamish 2003, Wurster & Patterson 2003, Jamieson et al. 2004, Wurster et al. 2005, Zazzo et al. 2006, Dufour et al. 2007, Weidel et al. 2007, Dufour et al. 2008).

Tracking populations

At the population level, otolith stable isotope ratios and elemental concentrations have become important determinants of stock connectivity and natal origins (Thorold et al. 1998, Rooker et al. 2008, Barnett-Johnson et al. 2008) of commercially important fishes. When samples have been collected over many years, the link between otolith $\delta^{18}\text{O}$ values and ambient water temperature can be exploited in order to examine the thermoregulatory response of fish stocks to long-term changes in water temperature (Jones & Campana 2009).

Stable isotope analysis of other commonly archived material, often embracing mammalian teeth and fish scales, offers qualitative indices of trophic systems often achieving temporal scales that are rare in ecological studies (e.g. 40+ years of contiguous data). On these timescales, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ have been related to long-term shifts in trophic level and foraging habitat (Satterfield & Finney 2002, Hobson et al. 2004a, Christensen & Richardson 2008, Aubail et al. 2010), as well as inter-decadal climate forcing (Newsome et al. 2007a, Sinnatamby et al. 2009). The new and rapidly developing field of stable isotope ecology therefore encompasses short-term (i.e. life history) and longer-term (i.e. decadal) processes at a variety of geographical scales; these indices can help researchers to understand the relationships between climate variation, anthropogenic effects and ecosystem responses in the recent past.

Aims and structure

The aim of this thesis was to investigate the response of three selected marine predators to ecological change at a variety of spatial and temporal scales using stable isotope ratio methods. To achieve this, it was necessary to further develop methodology for extracting samples from accretionary biogenic hard-parts of marine predators for isotope analysis and for relating this information to both intrinsic (i.e. ontogenetic) and extrinsic (i.e. environmental) variability.

In Chapter two, both ontogenetic and long-term shifts in the trophic dynamics of male Antarctic fur seals (*Arctocephalus gazella*) are investigated using the $\delta^{13}\text{C}$ values, $\delta^{15}\text{N}$ values and growth increment of individual growth layer groups (GLGs), or annuli, of their canine teeth. These changes are related to environmental variability.

Chapter three explores spatio-temporal scales of ecological forcing in the eastern North Atlantic Ocean across two trophic levels by comparing decadal trends in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Atlantic salmon (*Salmo salar*) and grey seals (*Halichoerus grypus*). Intrinsic and extrinsic factors affecting these data were considered and used to test the spatial and temporal scales of ecological forcing in the eastern North Atlantic.

In Chapter four, the timing and magnitude of trophic effects on Atlantic salmon somatic condition and energy reserves was tested. The hypothesis that variation in body condition indices within a single year is directly related to the foraging conditions experienced at sea was only partially upheld; however, by analysing multiple tissues from the same individual, evidence was provided for an early, marine, onset of nutritional stress related to the body condition of the fish - prior to the start of the return migration to freshwater for spawning.

The next two chapters sought to extend the limits of sampling resolution in the otoliths of wild Atlantic salmon and describe individual-based profiles of thermal and metabolic histories. Chapter five compares a traditional sampling procedure using computerised micro-milling and continuous flow - isotope ratio mass spectrometry (CF-IRMS) techniques with secondary ion mass spectrometry (SIMS), a technique which is relatively new to animal ecologists. Detailed variation in wild Atlantic salmon otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values was described using a novel technique to estimate the chronology and date of aragonite formation for the sample spots.

This thesis was written with chapters prepared in a manuscript format for submission to peer-reviewed journals. Chapters two and five had been published before

submission and it is expected that chapters three, four and six will be submitted to high quality journals after appropriate changes are made to formatting.

Chapter two was published in 2009 the journal Marine Ecology Progress series by the authors N. N. Hanson, C. M. Wurster, M. I. Bird, K. Reid and I. L. Boyd and is entitled “Intrinsic and extrinsic forcing in life histories: patterns of growth and stable isotopes in Antarctic fur seal teeth” Volume 388, pp 263-272.

Chapter five was published in 2010 in the journal Rapid Communications in Mass Spectrometry by the authors N. N. Hanson, C. M. Wurster, Edinburgh Ion Microprobe Facility (EIMF) and C. D. Todd and is entitled “Comparison of secondary ion mass spectrometry and micromilling/continuous flow isotope ratio mass spectrometry techniques used to acquire intra-otolith $\delta^{18}\text{O}$ values of wild Atlantic salmon (*Salmo salar*)” Volume 24, pp 2491-2498.

The final chapter of this thesis summarises the general outcomes and insights gained throughout the previous chapters, and critically evaluates the role and limitations of stable isotope proxies in studies monitoring top predators in marine ecosystems and suggests future avenues for informative research.

2 Intrinsic and extrinsic forcing in life histories: patterns of growth and stable isotopes in male Antarctic fur seal teeth¹

ABSTRACT: Life-time records of the trophic sources of carbon, nitrogen and of growth rate can be generated from biogenic structures that show accretionary growth, including fish scales, whale baleen and the teeth of some animals. Records generated from individual teeth can also be combined to provide longer time series elucidating changes in environmental conditions encountered by a population. Both intrinsic (i.e. ontogenetic) and extrinsic (i.e. environmental) factors are important in modulating variation in growth and the apparent dietary sources of C and N. Canine teeth of a large marine predator, the male Antarctic fur seal (*Arctocephalus gazella*) from South Georgia, were used to investigate both intrinsic and extrinsic sources of variation. Substantial ontogenetic shifts occurred in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in individual teeth, indicating a change in the trophic sources of C and N as individual animals age. Over the 40 yr period from 1964 to 2005, and after statistical reduction of ontogenetic variation, long-term declines were detected in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, indicating that the population has become more dependent on energy from a lower trophic level. A concurrent decline in annular tooth growth may be a consequence of rapid population growth during this period. The time series of $\delta^{13}\text{C}$ values was also inversely correlated with sea surface temperatures in the region, although isolating a causal relationship remains elusive. These analyses suggest that both intrinsic and extrinsic sources of variation, and their interaction, must be considered from such

¹N.N. Hanson, C.M. Wurster, M.I. Bird, K. Reid & I.L. Boyd (2009) Intrinsic and extrinsic forcing in life histories: patterns of growth and stable isotopes in male Antarctic fur seal teeth. Marine Ecology Progress Series 388:263-272. doi: 10.3354/meps08158. Reprinted with kind permission of Inter-Research.

time series data; failure to do so could result in a biased interpretation.

Introduction

Apportioning biological variation between intrinsic (i.e. ontogenetic) and extrinsic (i.e. environmental) variables amongst free-ranging vertebrates is a central challenge in understanding how environmental factors influence populations, with particular relevance for predicting the effects of future environmental change. The present study examines this question in a species of seal that is exposed to considerable interannual and long-term variation in its food supply.

The biological response of top predators may be influenced by direct factors such as changes in habitat availability as well as indirect factors, such as prey abundance and distribution that result in changes to competitive dynamics between species sharing resources (Constable 2006, Croxall 2006). Long-term monitoring of the diet of top predators can provide information concerning trophic dynamics at the ecosystem level as well as population dynamics of both predators and their prey (Croxall 2006, Reid et al. 2006). In some circumstances, such as in the Southern Ocean, characterising and understanding the relationships between top predators, their prey and environmental variability represents an important step towards ecosystem-based management of populations of commercially exploited species (Constable 2006).

While approaches to trophic studies have included stomach and scat analysis, radio-labelling and, more recently, quantitative fatty acid analysis, stable isotopes of carbon and nitrogen are increasingly used to better inform ecologists of trophic relationships. Predators are typically enriched in ^{15}N relative to their prey, with an average increase of $3.3 \pm 0.26\text{‰}$ between trophic levels (McCutchan et al. 2003), and therefore $\delta^{15}\text{N}$ values can be used to resolve trophic positions (Peterson & Fry 1987). In contrast, $\delta^{13}\text{C}$ values vary less ($\sim 1\text{‰}$) between trophic levels but are often used to indicate the geographic source of prey items due to well-documented variation in $\delta^{13}\text{C}$ with different photosynthetic processes (Hobson 1999). In the marine environment, phytoplankton $\delta^{13}\text{C}$ values can vary in relation to cell physiology, morphology, growth and the source of inorganic carbon (Michener & Schell 1994, Burkhardt et al. 1999). Variations in the $\delta^{13}\text{C}$ values of some animals can be linked to benthic versus pelagic or nearshore versus offshore feeding habitats (Hobson et al. 1994, Michener & Schell 1994, Hobson 1999, Cherel & Hobson 2007).

Pinnipeds show incremental growth in their teeth throughout their lives. Thus

the life-history of individual animals may be reflected in the incremental pattern of growth, represented as both the rate of change in tooth growth and changes in the chemical composition of each growth layer (Newsome et al. 2006, 2007a, Boyd & Roberts 1993). Like the teeth of many seal species, the teeth of Antarctic fur seals (*Arctocephalus gazella*) are formed from annular growth layers of dentin. These layers form a series of stacked cones, the outer edges of which are visible on the exterior of the tooth and have been used to determine the age and relative growth rate of individuals (Boyd & Roberts 1993). These layers, called ‘annuli’, are presumed to be metabolically inert after their formation. Thus, the isotopic compositions of annuli are thought to encode a temporal record that can be used to examine changing diet and trophic status throughout the life of an individual animal (Balasse et al. 2001, Newsome et al. 2006, 2007a, Hobson & Sease 1998, Knoff et al. 2008). Additionally, variation in the width of each annulus may be related to variations in the growth of the animal (Boyd & Roberts 1993), serving as a proxy measurement of growth rate that may correlate with the total food available to individuals at different stages of their lives. However, there is a natural and constant decline in annulus width with the age of the animal that must be taken into account before relating this information to food availability or growth (Boyd & Roberts 1993).

The teeth of Antarctic fur seals have the potential to offer an insight into their trophic life-history and potentially into broader environmental variability. When the teeth of seals dying of natural causes are collected over time and in sufficient numbers, it is possible to build a time-series of changes in chemical composition and layer deposition. These patterns can be used in two main ways; first, to examine life histories of individuals and second, to examine changes in relation to the calendar year of deposition. In the present study, contrasting insights from these records are used to examine the hypotheses that dietary variation, reflected in dietary C and N, and annulus width are affected at least as strongly by intrinsic factors such as ontogenetic development or longevity as they are by extrinsic factors such as environmental conditions. Time series of stable isotope variation also are used to investigate broad-scale perturbations and long-term changes in marine ecosystems that may be commonly reflected in the biological responses of top predators (Forcada et al. 2005, Croxall 2006, Murphy et al. 2007). It is reasoned that since fur seals that breed on South Georgia range widely over a region that is characterised by highly dynamic oceanographic conditions (Boyd et al. 1998, 2002), any residual

signal contained within fur seal teeth once intrinsic life-history effects are partitioned out, is likely to reflect general ecological changes at a regional scale.

Materials & Methods

Tooth preparation and stable isotope analyses

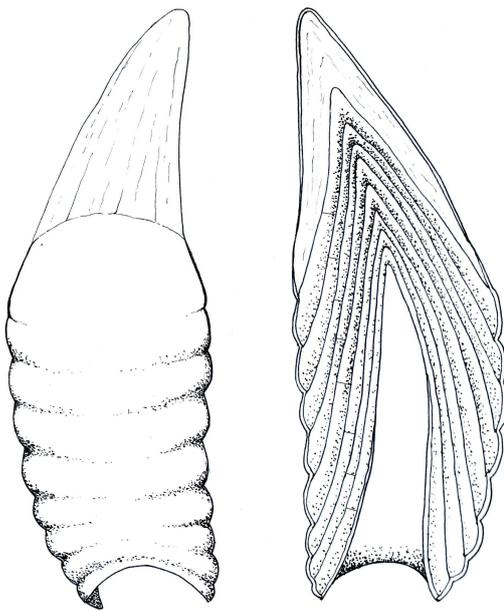


Figure 2.1: Diagram of the outer surface and a longitudinal cut through a 8-yr old male Antarctic fur seal canine tooth. Growth layer groups (GLGs) were visually identified and measured on the etched surface of the tooth. Black horizontal band: area where measurements were taken. Powdered dentin samples were extracted for analysis along consecutive annuli.

Antarctic fur seal teeth were collected from males that died of natural causes at breeding beaches on Bird Island, South Georgia (54°00'S, 38°03'W) during December-January. Left and right upper canines were extracted after the carcass had decayed sufficiently to allow extraction using tooth pliers. We randomly selected a sub-sample of these teeth, archived at the British Antarctic Survey (Cambridge, UK) and the Sea Mammal Research Unit (St. Andrews, UK) for analysis. An average of 10 teeth were selected from a series of collection years for which teeth were available: 1971, '76, '78, '79, '87, '91, '92, '93, '94, '95, '98, '99, '01, '04 and '06 (collection years are referred to by the second year of the season as in Forcada et al. (2005)). Considering that up to 10 dentinal annuli were sampled from each tooth this meant that samples were available for annuli laid down in all years from 1961 to 2005. Each whole tooth

was sawed in half using a diamond-burred circular saw and one half soaked in 10% formic acid overnight to etch the surface of the tooth and facilitate visual discrimination between individual annuli. The age of the animal was estimated by counting individual growth layer groups (GLGs) determined as one depressed, etched section

and one raised, un-etched section. The number of GLGs was counted three times by a single observer. If two of the three estimates were the same, that age became the best estimate for that tooth. If all estimates differed by no more than a year, then the mean of these estimates was used. If estimates differed by more than one year, the section was re-examined. Each layer width was measured where growth layers were parallel in the center of the dentin and in a step-wise manner so as to account for all layers (Boyd & Roberts 1993) using digital callipers (Figure 2.1).

The standard deviation of width measurements was estimated to be 0.09mm based on re-analysis of 20% of all canine teeth. Each annulus was then assigned to a calendar year by back-counting from the calendar year of collection using the age at death. A total of 141 teeth were cut, aged and measured. Approximately 2-3mg of dentin was extracted for isotope analysis from individual growth layers using an electric drill with a 900 μ m diamond tipped dental burr (FG801LS-009 Medium Grit). Material from the outer surfaces was not used in order to prevent any confounding isotopic effect of the formic acid treatment on the external surface of the tooth and dentin was drilled to a depth of no more than 500 μ m to reduce contamination from other layers. Material was only taken from annuli large enough to reliably drill within the band (mainly the outer annuli deposited in the first 5 years of life) and, where possible, consecutive samples were taken from each successive annual growth band. In order to remove inorganic material, approximately 1.5mg (\pm 0.1mg) of powdered dentin was weighed out into silver capsules then washed with 100 μ l 0.5M HCl and left to dry 24 – 36 hours at 20 $^{\circ}$ C. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and carbon and nitrogen weight percent (%C and %N, respectively) were determined using a Costech Elemental Analyzer fitted with a zero-blank auto-sampler coupled via a ConFloIII to a ThermoFinnigan DeltaPlusXL using Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) at the University of St Andrews Facility for Earth and Environmental Analysis. Stable isotope results are reported as per mil (‰) deviations from the (Vienna Pee Dee Belemnite) VPDB and AIR reference standard scale for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Precisions (S.D.) on internal standards were better than \pm 0.1‰ and 0.2‰ for carbon and nitrogen, respectively.

Data analysis

Two main sources of potential variation were present in the dataset and had to be statistically disaggregated. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in teeth have previously been shown to vary throughout an individual seal's life history (Hobson & Sease 1998, Newsome

et al. 2006) and when life history profiles are aggregated, there is also the possibility of multi-decadal and inter-annual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values arising as a function of this aggregation over the 41 year time period for which data were available. In addition, there was the possibility that differences in stable isotope values may be related to the longevity of an individual animal. To partition the variance in the data between these different variables, and to investigate data in relation to each variable individually, a series of multiple linear regressions (mixed effects models) including all combinations of independent variables was performed to model the data. The weighted deviance of all models was tabulated and compared using a chi-squared test for significance (Boyd 1996). Akaike Information Criterion (AIC) score (Akaike 1976) was also used when determining the model of best-fit. The effects of individual variables were investigated using the partial regression coefficients derived from the best-fitting model. This reduced the effect of the other variables and helped to reconstruct the effect of each individual explanatory variable. Simple bivariate linear regressions were then used on data corrected for other effects to investigate trends in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in relation to each variable (annulus number, age-at-death and calendar year) individually. Linear regression was also applied to the annulus width time series with the effect of annulus number removed to highlight variations in tooth growth over time. Yearly averaged stable isotope time series (corrected for age-at-death and annulus number) were de-trended by subtracting the linear trend and correlated with three similarly de-trended climate indices previously correlated with biological indices of marine animals in the southern ocean – including Antarctic fur seals and local krill stocks (Forcada et al. 2005, Murphy et al. 2007). Because Antarctic fur seal diet is highly dependent on the availability of krill in a given year, it was reasoned that proxy measurements of their diet could be related to the same environmental forcing as krill. Therefore, annual de-trended grid averages of sea surface temperature (ERSSTv2, Smith & Reynolds 2003) and sea ice cover (HadISST1, Rayner et al. 2003) from 20°-100°W and 45°-80°S - a large region encompassing both the breeding and potential feeding grounds of male Antarctic fur seals - and an observation-based Southern Annular Mode (SAM) Index (Marshall 2003) was used. Cross correlation analyses was used to explore the relationship between the climate indices and experimental data lagged up to two years. Statistical computations were performed in R version 2.4.1 (R Core Development Team 2010) and time series data was extracted using the KNMI Climate Explorer (climexp.knmi.nl). The use of cross-dating accuracy checks commonly used in dendrochronology was explored

to ensure the most accurate assignment of growth layers to calendar years. Individual uncorrected width time series were correlated to the mean temporal pattern of all other series from individual animals using the computer program COFECHA version 6.06 (Holmes 1983). First order differencing was used during this process to reduce the intrinsic linear patterns present within tooth measurements. The series intercorrelation provides a measure of the strength of a common signal in the time series and the program highlights individual “problem” series that may be misdated, suggesting how series should be shifted in order to improve the overall temporal signal. These series were moved a maximum of two years back in time because the time of death was known and assignment errors were most likely due to inaccurate identification of the neonatal line in order to improve the chronology. A re-evaluated chronology was then related to the same 3 climate indices as the original chronology to test for any differences due to the adjustments made.

Results

Statistical models

Statistical modelling of the effects of the set of independent variables on $\delta^{13}\text{C}$ values (see Table 2.1) provided an AIC score that was lowest for the model including an interaction between all three explanatory variables (model 11): calendar year, annulus number and age-at-death while model 12 (Year + Age*Annulus) provided a marginally lower AIC for $\delta^{15}\text{N}$ values. Further inspection of models 8-14 showed that there were consistent interactions between terms and that calendar year was especially important. These results suggest that the response variables depend on a complex interaction between calendar year, age-at-death and annulus number and that it would be insufficient to consider one variable without consideration of the other two. An attempt was made to do this by accounting for the linear effects of other variables in order to highlight the effect of the variable of interest. The lowest AIC for annulus width included an interaction between calendar year and annulus number although there was a significant interaction between all three variables. The partial regression coefficients used to extract the relationships for each of the independent variables in the regression models are provided in Table 2.2 along with the descriptive statistics for each resulting dataset. The linear relationship between raw (uncorrected) data and each independent variable is shown in Figure 2.2.

Table 2.1: Results of multiple regression analyses relating stable carbon and nitrogen isotope values and annuli width measurements to calendar year, annulus number and age-at-death. Degrees of freedom are given in parentheses. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ and ns = not significant. Model deviances, r^2 and AIC values are also given.

	Model	k	Year (40)	Annulus (6)	Age (6)	Annulus*age	Year*age	Year*Annulus	Year*Annulus*Age	r^2 (%)	Deviance	AIC	
$\delta^{13}\text{C}$	11	9	-	-	-	-	-	-	***	39.4	29.03	756.5	
	13	6	-	-	ns	-	-	***	-	37.8	38.79	760.26	
	14	6	-	ns	-	-	***	-	-	37.1	41.28	762.75	
	12	6	***	-	-	***	-	-	-	36.3	44.46	765.93	
	10	5	-	-	-	-	-	***	-	35.1	29.03	769.64	
	7	5	***	*	ns	-	-	-	-	35.4	48.5	770.84	
	9	5	-	-	-	-	***	-	-	33.2	50.17	777.09	
	4	4	***	*	-	-	-	-	-	32.8	59.3	779.61	
	5	4	**	-	ns	-	-	-	-	31.6	63.7	784	
	1	3	***	-	-	-	-	-	-	28.5	76.1	794.44	
	8	5	-	-	-	***	-	-	-	12.5	124.98	844.45	
	6	4	-	ns	***	-	-	-	-	11.1	129.6	849.95	
	3	3	-	-	***	-	-	-	-	9.7	134.5	852.82	
	2	3	-	ns	-	-	-	-	-	1.8	155.6	873.91	
	$\delta^{15}\text{N}$	12	6	***	-	-	***	-	-	-	52.4	-133.29	588.18
		11	9	-	-	-	-	-	-	***	52.8	-138.49	588.98
		13	6	-	-	ns	-	-	***	-	50.2	-122.06	599.41
7		5	***	***	ns	-	-	-	-	48.8	-114.06	605.41	
14		6	-	***	-	-	***	-	-	48.8	-114.86	606.61	
10		5	-	-	-	-	-	***	-	48.1	-110.45	609.02	
4		4	***	***	-	-	-	-	-	46.7	-103.2	614.31	
5		4	***	-	ns	-	-	-	-	34	-49.68	667.79	
9		5	-	-	-	-	***	-	-	33.9	-50.07	669.4	
1		3	***	-	-	-	-	-	-	33	-44.6	670.83	
8		5	-	-	-	***	-	-	-	31.5	-41.3	678.17	
6		4	-	***	***	-	-	-	-	26.3	-24.31	693.16	
2		3	-	***	-	-	-	-	-	19.1	2.3	717.8	
3		3	-	-	**	-	-	-	-	6.4	38.8	754.27	
Annulus width		10	5	-	-	-	-	-	***	-	27.3	-2932.84	-67.93
		11	9	-	-	-	-	-	-	***	27.6	-2940.68	-67.77
		13	6	-	-	ns	-	-	***	-	27.3	-2932.87	-65.96
	4	4	ns	***	-	-	-	-	-	26.9	-2926.31	-63.41	
	7	5	ns	***	ns	-	-	-	-	26.9	-2926.37	-61.47	
	14	6	-	ns	-	-	***	-	-	26.9	-2928.1	-61.2	
	12	6	ns	-	-	***	-	-	-	26.8	-2926.39	-59.49	
	6	4	-	***	ns	-	-	-	-	23.8	-2884.77	-21.86	
	2	3	-	***	-	-	-	-	-	23.7	-2882.12	-21.22	
	8	5	-	-	-	***	-	-	-	23.8	-2885	-20.1	
	5	4	***	-	ns	-	-	-	-	11.1	-2729.18	133.73	
	9	5	-	-	-	-	***	-	-	11.1	-2730.22	134.69	
	1	3	***	-	-	-	-	-	-	10.1	-2717.35	143.56	
	3	3	-	-	***	-	-	-	-	3.4	-2645.36	215.54	

Table 2.2: Descriptive statistics for the original datasets and each subsequent dataset calculated from the partial regression coefficients (indicated as ‘b’ and provided below) for Year, Annulus and Age.

Variable	Corrected for:	Mean	se	Minimum	Maximum	Units	n	b(Year)	b(Annulus)	b(Age)
$\delta^{13}\text{C}$	Uncorrected	-18.88	0.09	-22.48	-14.82	‰	250	-0.068	-0.163	-0.158
$\delta^{13}\text{C}$	Year, Age-at-death	-21.52	0.01	-26.05	-21.52	‰	250			
$\delta^{13}\text{C}$	Annulus, Age-at-death	-20.61	0.01	-24.10	-16.74	‰	250			
$\delta^{13}\text{C}$	Year, Annulus	-17.07	0.07	-20.01	-13.37	‰	250			
$\delta^{15}\text{N}$	Uncorrected	9.87	0.07	7.59	14.13	‰	250	-0.052	0.256	-0.115
$\delta^{15}\text{N}$	Year, Age-at-death	7.89	0.01	5.15	12.80	‰	250			
$\delta^{15}\text{N}$	Annulus, Age-at-death	9.76	0.01	7.06	14.76	‰	250			
$\delta^{15}\text{N}$	Year, Annulus	10.17	0.07	8.45	13.36	‰	250			
Annulus width	Uncorrected	0.81	0.01	0.30	2.10	mm	1006	-0.00524	-0.049	
Annulus width	Year	0.95	0.01	0.41	1.08	mm	1006			
Annulus width	Annulus	1.03	0.01	0.50	2.20	mm	1006			

Variation due to annulus number and age-at-death

Model deviances did not detect any significant effect of age-at-death on either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ when $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were adjusted to account for the linear effects of the calendar year in which the dentin annulus was deposited and the annulus number ($\delta^{13}\text{C}$, $\chi^2 = 11.62$, d.f. = 6, $p > 0.05$; $\delta^{15}\text{N}$, $\chi^2 = 11.77$, d.f. = 6, $p > 0.05$). However, the developmental stage of the animal had a significant effect on diet as inferred from both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. This was because stable carbon isotope values decreased linearly with annulus number ($b = -0.165 \pm 0.0411$), although this accounted for only 5% of the variation in the data. But stable nitrogen isotope values increased with annulus number ($b = 0.256 \pm 0.0297$) and accounted for nearly 23% of the variation in the data.

Temporal variation

A simple least squares linear regression model fitted to 41 years of $\delta^{13}\text{C}$ data excluding the effects of tooth annulus number and age-at-death showed there was a significant decline in both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in relation to calendar year ($\delta^{13}\text{C}$, $b = -0.0678 \pm 0.00665$; $\delta^{15}\text{N}$, $b = -0.0521 \pm 0.00623$). Additionally, both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ appeared to oscillate over time (Figure 2.3). After accounting for the effect of decreasing annulus width with age, there was also a significant linear decline in annulus width from 1961-2005 ($b = -0.005 \pm 0.001$). According to this regression, there was an average 0.225mm decrease in annulus width over the 41-year data series. In addition to these long-term declines, $\delta^{13}\text{C}$ values were negatively correlated with SST ($^{\circ}\text{C}$) values in the same year (Pearson product-moment correlation, $p = 0.0128$, d.f. = 39, $r = -0.385$). No other significant patterns appeared from the cross-correlation analysis.

Chronology validation

36.8% of individual annuli width series and 35.6% of isotope series were more closely aligned with the total time series mean pattern once shifted by one to two years. However, these shifts did not significantly improve the standard error of the mean time series except for $\delta^{13}\text{C}$ (two-tailed t-test, d.f. = 37, $p < 0.05$), suggesting that, overall, tooth chronologies were adequately determined in the first instance. The re-evaluated series were not significantly cross-correlated with any environmental

time series, although the relationship between $\delta^{13}\text{C}$ and SST remained strong ($p = 0.065$, d.f. = 39, $r = -0.29$).

Discussion

Intrinsic effects

The present study found evidence for consistent changes in stable isotope ratios over the first seven years of the lives of male Antarctic fur seals once the variation due to the calendar year of deposition and the age-at-death of the animal was removed. Considering that relatively few male seals live more than 10 years (Boyd & Roberts 1993), this suggests that the trophic level at which male Antarctic fur seals forage, and their source of carbon, changes systematically during the period of rapid growth included in this analysis (Payne 1977). Other studies of pinniped teeth (Hobson & Sease 1998, Newsome et al. 2006) have observed high $\delta^{15}\text{N}$ and low $\delta^{13}\text{C}$ values in the first 1-2 years of life presumably due to the metabolic routing of milk protein and lipids during nursing. However, Newsome et al. (2006) found this pattern lacking among male Northern fur seals, presumably due to the short (~4 month) lactation period – a characteristic shared with Antarctic fur seals. Both studies note that there is a large amount of variation in ontogenetic patterns among individuals.

In the present study, larger sample sizes were used and the linear change in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values was described into the seventh year of life, when animals are physically and sexually mature, thus creating a more complete view of ontogenetic variation than previous studies. While not statistically different, when $\delta^{15}\text{N}$ values were averaged for each annulus there was a peak at year two followed by a decrease of nearly 1‰ in the third year. Estimates of isotopic equilibration of dentin after a switch in diet have been suggested to be between one to four months (Balasse et al. 2001). As weaning occurs after approximately four months in these animals, it is plausible that a milk-derived $\delta^{15}\text{N}$ signal could influence the isotopic profile throughout the first ~8months of dentin growth. Little information is available about post-weaning diet; Warren et al. (2006) tagged five male pups after weaning and found that they moved progressively offshore to more oceanic conditions throughout the first winter, potentially following the availability of prey items. This change in foraging location could mask any $\delta^{15}\text{N}$ signal due to the short lactation period and contribute to a decrease in $\delta^{15}\text{N}$ values in the third year if pups begin

to forage predominately on krill at this time. However, it is difficult to draw firm conclusions without more detailed information on juvenile diet.

Because the common prey species of Antarctic fur seals are known to encompass at least three different trophic levels (e.g. krill, krill-eating fish, and piscivorous fish and sea-birds) the results showing an ontogenetic increase in $\delta^{15}\text{N}$ values indicate that male Antarctic fur seals tend to forage on higher trophic level prey as they age. Antarctic krill sampled around the Antarctic Peninsula and the Lasarev Sea had $\delta^{15}\text{N}$ values of 3.6‰ whereas fish species sampled around the Falkland Islands and the Antarctic Peninsula had values ranging from 8.4-11‰ (Dunton 2001, Cherel et al. 2002, Schmidt et al. 2003). Thus the ontogenetic increase in $\delta^{15}\text{N}$ values in Antarctic fur seals dentin indicates that they are including more krill-eating and/or piscivorous fish in their diets rather than directly feeding on krill. The concomitant decline in $\delta^{13}\text{C}$ values could represent this shift to a new diet if fish were relatively depleted in ^{13}C compared to krill, but as the opposite is true (Dunton 2001, Cherel et al. 2002, Schmidt et al. 2003) this explanation seems unlikely. The $\delta^{13}\text{C}$ values of particulate organic carbon decrease with increasing latitude (Goericke & Fry 1994) – a phenomenon also reflected in the isotopic values of Antarctic krill (Schmidt et al. 2003). Therefore, the ontogenetic decline in $\delta^{13}\text{C}$ values may reflect the movement of males to more southern foraging grounds (Boyd et al. 1998). It is likely that these developmental shifts in diet reflect the changing energetic needs and foraging capabilities of male fur seals as they grow.

Extrinsic effects

The computer program COFECHA is a widely used dendrochronological tool that has been useful for cross-dating other biological proxy series such as those obtained from tree rings, mollusc shells and coral skeletons. The series obtained from Antarctic fur seal teeth, however, are much shorter than those commonly used with this program (approx. 7 yrs compared to decades) and this is probably the most limiting factor to its application to tooth time series analysis. Any interpretation of results based on time series that are re-evaluated using COFECHA must consider the limitation of short time series. The use of this tool was explored to improve the dating of the chronology but did not find sufficient evidence to warrant changing it. Such resources from other disciplines could be useful to future studies of this kind, however, and their consideration is recommended.

The apparent changes in stable isotope composition of teeth during the lifetimes of

male Antarctic fur seals discussed in the previous section have taken place against a background of a temporal trend in trophic level and carbon source. Male fur seals at South Georgia have experienced a $\sim 2\%$ decline in dentin $\delta^{15}\text{N}$ values from 1964-2004, representing a substantial drop in trophic level. This result is consistent with Antarctic fur seals becoming more dependent on krill in their diet in the latter part of the time series. Similarly, a decline in $\delta^{13}\text{C}$ values, indicative of a more pelagic/offshore feeding environment (Hobson et al. 1994, Cherel & Hobson 2007) supports this interpretation. It is possible that a proportion of the annual decline in $\delta^{13}\text{C}$ values could be attributed to the Suess effect – the decline in oceanic $\delta^{13}\text{C}$ values of dissolved organic carbon due to anthropogenic input of atmospheric CO_2 . However, this decline has been estimated by (McNeil et al. 2001) to be $0.015\% \pm 0.003 \text{ yr}^{-1}$ in the Sub-Antarctic Zone but a $0.068\% \text{ yr}^{-1}$ decline in $\delta^{13}\text{C}$ values was observed in the present study.

The decline in $\delta^{13}\text{C}$ could also be related to large-scale processes governing primary productivity in the Southern Ocean. It is possible that the trend in $\delta^{13}\text{C}$ values of Antarctic fur seal dentin reflects a global decline in primary productivity (Gregg et al. 2003) - especially pronounced in high latitudes – because of the inverse relationship between algal cell growth rates and $\delta^{13}\text{C}$ (Hofmann et al. 2000, Schell 2000). The decline in both stable isotope time series could be attributed to shifts in diet of fur seals if fur seals are feeding more exclusively on krill swarms than in previous years. (Myers & Worm 2003) reported a substantial decline in worldwide predatory fish stocks since the onset of commercial exploitation, including an analysis of ~ 60 years of trawl survey data from South Georgia that contained information on some fish species found in the diet of Antarctic fur seals. Likewise, in their summary of fisheries activity and policy in the Southern Ocean, (Kock et al. 2007) noted the over-fishing and subsequent collapse of *Notothenia rossi* and other fish stocks in the 1970s. *N. rossi* was reported as being common in the diet during early analyses of Antarctic fur seal stomach contents (Bonner 1968) but was almost absent from the diet in more recent studies (e.g. Reid 1995). This historical loss of predatory fish in the Southern Ocean could provide a partial explanation for the decrease in average trophic level as indicated by $\delta^{15}\text{N}$ values. Indeed the abrupt change in the $\delta^{15}\text{N}$ time series in the early 1970s coincides almost exactly with the period of greatest catches of *N. rossi* around South Georgia (Kock et al. 2007).

These isotopic trends have been accompanied by a significant decline in annulus width, after accounting for the ontogenetic changes, over the course of the time

series. This decline in annulus width could be caused by a density-related response to an increasing fur seal population in the region (Boyd & Roberts 1993). Assuming dentine deposition is indeed an accurate recorder of animal growth, the very high numbers of fur seals at South Georgia may cause competition for food resources between males and a coincident decline in annular growth of individuals. Also, increased competition may mean that a higher proportion of smaller, weaker males have died and been collected at the breeding beach in more recent years.

In addition to these long-term trends, there is evidence that the diet of fur seals has varied on a much shorter time scale. Attempts were made to test for any functional links between fluctuations in physical indices known to effect krill populations and the sub-decadal variation observed in the Antarctic fur seal teeth time series. These indices represent only a small subset of potential sources of extrinsic variation, but their impact on local environmental conditions, and particularly on krill stocks, is well-documented. Relationships between life-history variables in fur seals, some other predators of krill and environmental variability have already been suggested (Forcada et al. 2005, Nicol 2006, Trathan et al. 2006).

A significant proportion of the oscillations in stable carbon isotope values varied inversely with sea surface temperatures in the region. Sea surface temperature could be driving changes in the $\delta^{13}\text{C}$ values measured in the dentin of male Antarctic fur seals by a) causing changes in the annual availability or distribution of food sources – mainly krill, b) causing changes to the baseline $\delta^{13}\text{C}$ signature of phytoplankton or c) causing males to travel to more southerly regions when SST is high and to more northerly regions when SST is low. While climatic forcing of the abundance of Antarctic krill has been suggested (Murphy et al. 2007), the process is not instantaneous and lags behind SST anomalies by two years. Although errors in age estimation could cause improper assignment to calendar years, re-evaluation of the altered time series (as suggested by the COFECHA analysis) did not find a significant correlation at a lag of two years – indicating that changes in krill abundance are not the functional link between the $\delta^{13}\text{C}$ series and SST. Second, if the $\delta^{13}\text{C}$ signal present in the time series was directly related to krill availability one would expect this signal also to be present in the $\delta^{15}\text{N}$ series as presumably males would need to supplement their diets in these years with increased piscivory. Alternatively, SST could be influencing fur seal $\delta^{13}\text{C}$ values by affecting inter-annual variability in $\delta^{13}\text{C}$ values of phytoplankton. (Popp et al. 1998) found that the decline in ^{13}C with latitude in Southern Ocean suspended particulate organic matter was at least partially

driven by changes in algal assemblages and cell growth rates and the availability of dissolved CO₂ in the oceans is considered to be a driver of variation in $\delta^{13}\text{C}$ value of particulate organic carbon (Goericke & Fry 1994, Burkhardt et al. 1999, Popp et al. 1998). Goericke & Fry (1994) showed that CO_{2(aq)} varies inversely with temperature in the global oceans. If these latitudinal differences are partly driven by temperature then inter-annual changes in SST may also change community structure and growth rates of marine algae between years and provide a link between the $\delta^{13}\text{C}$ signal present in Antarctic fur seal dentin time series and SST. However, long-term inter-annual monitoring of $\delta^{13}\text{C}$ values of particulate organic matter in the Southern Ocean does not exist with which to test the above conjecture. Likewise, there is insufficient data on the distribution of male fur seals outside the breeding season to discard the third hypothesis that inter-annual changes in SST could cause males travel to widely different foraging locations (and hence $\delta^{13}\text{C}$ values) – perhaps across the Antarctic Polar Front.

The present study has shown that the annular deposition of dentin layers in the teeth of pinnipeds can document patterns of ontogenetic and temporal shifts in the availability of stable nitrogen and carbon isotopes. A complex set of intrinsic and extrinsic factors affecting diet will mainly account for this variation in stable isotope composition. Attempting to separate these factors is a necessary first step before identification of potentially important links between diet and environmental conditions. The present study has shown that historical patterns of stable isotope deposition in the teeth of male Antarctic fur seals can provide a measure of long- and short-term environmental variability as seen from a top-down ecological perspective.

The author would like to thank colleagues (past and present) at the British Antarctic Survey field station at Bird Island, South Georgia for the collection of fur seal teeth. The author is also grateful to P. Ascough for laboratory assistance and expertise, D. Herd and T. Prave for use of their lab and equipment and to R. Wilson for help and information regarding time series analysis.

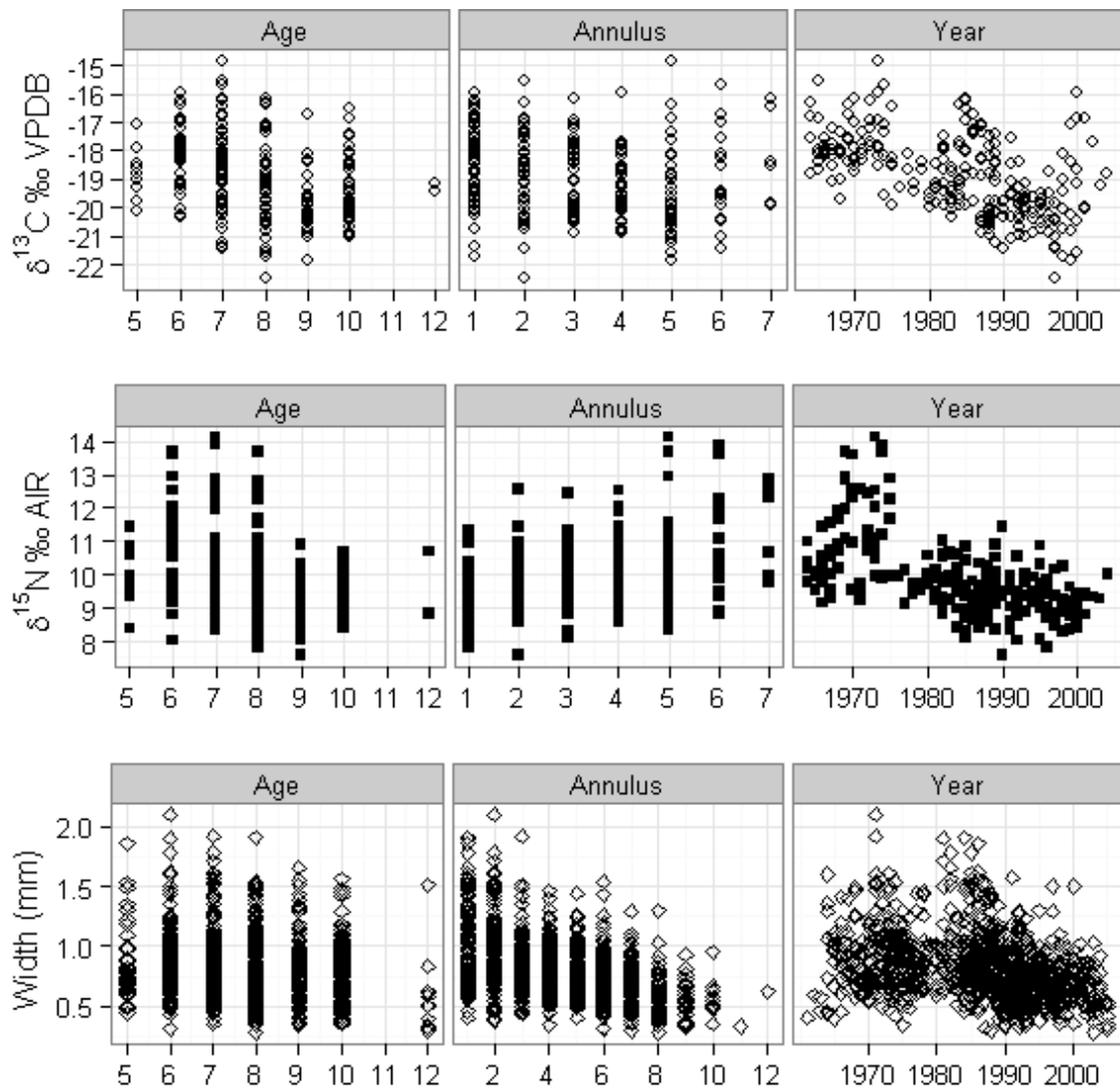


Figure 2.2: Relationships between uncorrected datasets and calendar year, annulus number and age-at-death. Stable isotope values are presented as per mil (‰) deviations from the Vienna Pee Dee Belemnite (VPDB) and atmospheric air reference standard scale for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. ‘Year’ refers to the calendar year of annulus deposition, ‘Annulus’ to the layer number and ‘Age’ to the age-at-death of the animals.

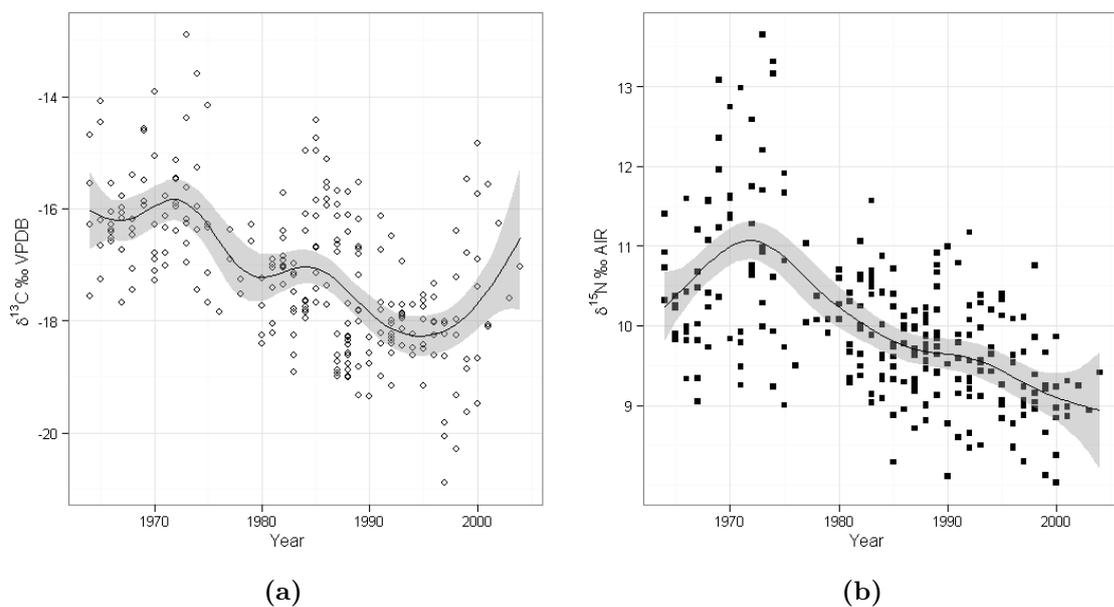


Figure 2.3: The pattern of (a) $\delta^{13}\text{C}$ and (b) $\delta^{15}\text{N}$ isotope values ($\pm 1\text{SD}$) in the organic fraction of tooth dentin from male Antarctic fur seals (*Arctocephalus gazella*), 1964 to 2004. Smooths ($\pm 1\text{SD}$) were generated using locally weighted regression (loess; $\alpha=0.5$).

3 Scales of spatio-temporal ecological forcing across the northeast Atlantic based on predator isotope signatures

ABSTRACT: Indices of trophic change can be used to elucidate a mechanisms of ecological forcing and the response of top predators, but are difficult to compile over many years. Retrospective analyses of variability in stable isotope ratios of metabolically inert tissues provide a means of assessing inter- and intra-annual variation in predator trophic dynamics. Comparison of time series across taxa operating at different trophic levels and/or geographic ranges facilitates inferences about the scale of spatial and temporal connectivity in ecological forcing. Evidence is presented from long-term records of variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of wild Atlantic salmon scales and grey seal teeth for large-scale ecological forcing across the eastern North Atlantic. Atlantic salmon responded strongly to rises in sea surface temperature since the late 1990s, perhaps by shifting towards foraging at upper trophic levels, and this appears to underlie contemporaneous declines in the somatic condition of individual fish and entire annual cohorts. Sustained declines in grey seal $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values since the 1950s probably indicate that grey seal trophic dynamics have responded to regional shelf-zone forcing related to the pronounced effects of human exploitation of fisheries, as well as to historical foodweb re-structuring during the North Sea regime shifts during the 1980s.

Introduction

Major changes in the eastern North Atlantic marine ecosystem have occurred in recent decades in association with global patterns of climate change (Hughes et al.

2010, Pinnegar & Heath 2010, Edwards et al. 2010). Rising air and sea surface temperature (SST) and changes in hydro-climatic forcing have been linked to large-scale biogeographic shifts in the distribution (Beaugrand et al. 2002), phenology (Edwards & Richardson 2004) and abundance (Beaugrand & Reid 2003, Richardson & Schoeman 2004) of plankton species at multiple trophic levels and across wide spatial scales. In the eastern North Atlantic and North Sea, the 1980s has been identified as a period of rapid and marked shifts in ecosystem dynamics as a consequence of climatic forcing (Beaugrand & Reid 2003, Beaugrand et al. 2003, Beaugrand 2004), but the past century also has seen intense human exploitation of marine resources in some areas that can similarly drive changes in ecosystem trophodynamics (Jennings et al. 2002, Pinnegar et al. 2002).

It is difficult to both detect these changes and to identify possible causal mechanisms because of the complexity of marine ecosystems and their underlying variability, even when dedicated surveys (e.g. the Continuous Plankton Recorder) provide annual information. Comparable long term data for large marine predators are rare and other tools are needed to retrospectively investigate how fundamental, large-scale changes (such as rising SST) affect the response(s) of higher level consumers.

Stable isotope analysis can be used to address how marine predators respond to changes in the marine environment. The method centers upon the ratio of heavy to light isotopes in the tissue of consumers and changes therein can be interpreted in relation to the isotope ratios of assimilated prey (DeNiro & Epstein 1978, 1981). Nonetheless, the method also relies upon understanding the pathways of isotopic alteration associated with the assimilation of dietary nutrients. The preferential excretion of the lighter isotope of nitrogen (^{14}N) leads to an increase in consumer tissue $\delta^{15}\text{N}$ values of 2-5‰ (McCutchan et al. 2003) per trophic level. There is a similar, but less pronounced, trophic enrichment in ^{13}C relative to ^{12}C . However, there also are large spatial gradients in $\delta^{13}\text{C}$ at the base of the marine food-web because of variation in the growth, morphology and taxonomy of primary producers and the concentration of aqueous CO_2 (Goericke & Fry 1994, Popp et al. 1998, Gruber et al. 1999). This variation may be reflected in the $\delta^{13}\text{C}$ of consumer tissues (Clementz & Koch 2001, Cherel & Hobson 2007). Thus, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values can provide both process-based (e.g. trophic linkages) and source-based (e.g. foraging distribution) information about consumers (Peterson & Fry 1987).

Retrospective studies of variation in stable isotope ratios is possible for many tissues which remain metabolically inert after their formation; these include teeth

(Koch & Tuross 1994, Newsome et al. 2007a, Hanson et al. 2009), hair (Cerling et al. 2009), balleen (Schell 2000), bone (Bump et al. 2007, Christensen & Richardson 2008) and scales (Satterfield & Finney 2002, Perga & Gerdeaux 2003, Sinnatamby et al. 2009). Comparison between historical isotopic data and modern samples provides a means of making progress towards detecting changes in predator diet or nutrition in response to ecological change over time, and the inclusion of multiple taxa can help to determine temporal trends at different trophic levels and/or geographical ranges. However, factors operative at the level of the individual (such as size, age or gender) can influence isotope values and may obscure or alter long-term patterns. Where possible, these factors should be included as covariates in the statistical analysis of data.

Here, we assess how the trophic dynamics of two ecologically important marine predator species - Atlantic salmon (*Salmo salar* L.) and British grey seal (*Halichoerus grypus* (Fabricius)) - operating at different trophic levels, have been affected over a period of rapid change in their respective marine environments in the eastern North Atlantic. At a large spatial scale these species occupy the same oceanic region and are therefore subject to the same broad-scale climate variability; but they also forage within distinct parts of the North Atlantic system. Grey seals are neritic foragers whereas Atlantic salmon range over oceanic depths. Both species return to sites where they are accessible for sampling and individuals of both have the potential to integrate variability in the shelf and oceanic pelagic ecosystems over broad spatial and temporal scales. The aim of this study was to test the spatio-temporal scale of ecological forcing in the eastern North Atlantic by comparing patterns of long-term trophic change in these two species; if similar, it is assumed that large-scale and long-term ecological forcing related to climate changes in the region drive the trophic responses of these predators.

Materials & Methods

Sample collection and preparation

Atlantic salmon scales Atlantic salmon scales were sub-sampled from the extensive archive maintained by Marine Scotland (Inchbraoch House, South Quay, Ferryden, Montrose, DD10 9SL, UK) for the River North Esk on the east coast of Scotland. Because these data derive from the river-mouth commercial fishery, the

day of capture can be relied upon as being the day of completion of the marine migration. The fork length, weight, sex and date of return for each salmon was recorded. Sea and river ages were determined from scale samples. Body condition factor (W_r ; Blackwell et al. 2000) was calculated as:

$$W_r = W/W_s \quad (3.1)$$

where W is the weight of the fish at capture and W_s is the standard weight predicted from a specific length/weight regression equation for salmon sampled at the fishery (see Todd et al. 2008 and chapter 4 for calculations). Between 20 and 40 one-sea winter (1SW) return adults were sub-sampled from 16 selected years between 1963 and 2008. The years were chosen to embrace periods of pronounced ecological changes in the eastern North Atlantic (Beaugrand & Reid 2003, Beaugrand 2004) and also years over which particularly striking recent declines in the mean somatic condition factor of returning fish have been found (Todd et al. 2008, Bacon et al. 2009). Salmon were allocated to one of four condition factor categories : Category 1 ($0.99 < W_r < 1.09$), Category 2 ($0.90 < W_r < 0.97$), Category 3 ($0.85 < W_r < 0.89$) and Category 4 ($W_r < 0.79$), where Category 1 includes salmon returning in 'normal' to 'good' condition and those in Category 4 were in 'poor' condition. 'Poor' condition factor (low W_r) Atlantic salmon also show a strong tendency to markedly compromise lipid reserves (Todd et al. 2008): in extreme cases, fish that return up to 30% under-weight for their length may have lipid stores reduced by as much as 80% compared to full condition fish.

Between 5 and 10 scales per fish were cleaned in deionised water using a toothbrush to remove tissue material prior to stable isotope analysis. Due to the under-plating of Atlantic salmon scales with collagen layers (Hutchinson & Trueman 2006), only the outermost section of the scale - representing the last season of growth (following the winter annulus) was excised manually for analysis (see Sinnatamby et al. 2008, 2009) using a micro-scalpel blade under a dissecting microscope.

Grey seal canines A sub-sample of 42 archived female grey seal canine teeth (held at the Sea Mammal Research Unit, University of St. Andrews) was randomly selected from those collected during culls in 1972 and 1981 at the Farne Islands for age estimation and stable isotope analysis of dentin collagen. Between 2007 and 2009, upper canine teeth were also collected from 24 grey seals stranded on the east

coast of Scotland. Tissue material was removed from fresh teeth by bringing to a low boil and mechanically cleaning the teeth using a scalpel and toothbrush. All teeth were thoroughly rinsed in 70% ethanol, followed by deionised water and then air dried. Maximum canine length (tip of crown to end of root) and width (mm) were measured using digital calipers. Canine mass (mg) also was recorded. Methods for age estimation in canines of the grey seal were based on Mansfield (1991) and Bernt et al. (1996).

A longitudinal thin section (100-120 μm) was removed from the root section of each tooth using a precision low speed diamond saw (Buehler® IsoMet, University of Warwick, Coventry, CV4 7AL, UK). Sections were stored in deionised water until viewing under a binocular microscope with transmitted light. Polarised light filters were used to improve resolution of growth layers and digital photographs taken. The number of complete growth layer groups (GLGs) in the cementum was read three times by one researcher (NNH) and once by another experienced researcher (S.Murphy, University of St. Andrews). Where age estimates differed, the most commonly reported age or, if all estimates were different, median age was recorded as the best estimate. If estimates all differed by more than one year, the section was re-examined. After thin sectioning, a transverse cut was made to free a segment of the root for stable isotope analysis and which clearly included both the inner dentin material and the outer layers of cementum. The pulp cavity in grey seal canine teeth essentially is occluded by dentinal layers by age 4 or 5 (Hewer 1964) and these layers are not distinguishable without examination of a thin section; therefore, dentin samples were a mixture of these early years. Powdered samples of dentin (~30mg) were extracted from each tooth segment using a hand-held rotary tool (Dremel, P.O. Box 98 Broadwaterpark North Orbital Road Denham, Uxbridge Middlesex UB9 5HJ) fitted with a diamond burr (Komet 801 016, 600 μm tip), and subsequently transferred onto aluminum foil and stored in plastic vials.

Stable isotope analysis

Approximately 0.6mg of excised Atlantic salmon scale material was weighed into tin capsules for isotopic analysis. Scale sub-samples were not demineralised (see Trueman & Moore 2007a, Sinnatamby et al. 2007). Powdered dentin samples were treated with 0.5M HCl for ~24h at room temperature to extract collagen. Samples were rinsed with deionised water until neutrality and lyophilised. Between 1 and 1.6 mg of collagen was weighed into tin boats for analysis. All samples were analysed by

Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) at the University of St Andrews Facility for Earth and Environmental Analysis for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The instrument was a Costech Elemental Analyzer fitted with a zero-blank auto-sampler coupled via a ConFlo III to a ThermoFinnigan DeltaPlusXL. Stable isotope results are reported as per mil (‰) deviations from the (Vienna Pee Dee Belemnite) VPDB and AIR reference standard scale for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Precisions (SD) on internal standards were better than $\pm 0.20\text{‰}$ and $\pm 0.25\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ respectively.

Data analysis

To examine temporal trends in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of grey seal dentin, it was necessary to assign calendar years to those samples. Because the year of death was known, dentin years could be calculated by subtracting the best estimate of age from the year of death; as outlined above, the dentin samples represent an amalgamation of ~ 4 years of juvenile life. Notwithstanding this limitation, because the primary interest here was in linear trends in the seal time series, rather than assigning $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to specific calendar years, the pooling of the early years in juvenile dentin samples did allow testing for significant linear patterns. Salmon scale $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were, however, assigned accurately to calendar years because samples derived from an intensive annual monitoring scheme on the River North Esk, Scotland.

The focus of statistical analyses was to characterise temporal patterns for each species and isotope ratio whilst identifying and quantifying intrinsic (e.g. sex, body condition, age) effects important to variation in those $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Due to constraints in the samples available, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements of both salmon scales and seal dentin represented grouped, unbalanced data (Table 3.1). Because of this, linear mixed effects models fitted by maximum likelihood were used (*lme4* package, Version 2.11.1; Bates & Maechler 2010) to examine the variation in $\delta^{13}\text{C}_{seal}$, $\delta^{15}\text{N}_{seal}$, $\delta^{13}\text{C}_{salmon}$ and $\delta^{15}\text{N}_{salmon}$ values.

For Atlantic salmon, there was the possibility that pre-spawning body condition could be related to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values both within and across years, and that effects might be sex-specific. For grey seals, the age-at-death did not represent true lifespan because most samples were obtained from deliberately culled individuals; however, because there is high mortality in the early years of grey seal life, age-at-death represented an estimate of minimal longevity and was therefore retained in

the analyses. Estimated ages-at-death were binned into four age classes: 3-5, 7-10, 10-20 and >20 years. Due to the variable states of decay of seal carcasses, it was not possible to obtain accurate morphometric data to estimate size or body condition of these animals.

The magnitude and significance of temporal patterns in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were assessed by constructing a maximum linear mixed effects model with a random slope and intercept for the effects of ageclass (for seals) and body condition (for salmon) both within and across calendar years. Sex was included in the models as a fixed effect. Models were simplified by iterative removal of terms and the best predictive model was selected by Akaike Information Criterion (AIC) values (Akaike 1976). Due to small sample sizes and the relatively large number of parameters, bias-corrected AIC values (AICc) were computed for each grey seal model (Hurvich & Tsai 1989; Equation 3.2).

$$AICc = AIC + \frac{2k(k+1)}{n-k-1} \quad (3.2)$$

where k is the number of parameters and n is sample size.

Intervals for the 95% highest posterior density (HPD) were estimated for model coefficients by Markov chain Monte Carlo (MCMC) methods ($n = 10,000$, using the *mcmc* function in R package *lme4*, Bates & Maechler 2010). For random effects, an exact restricted likelihood ratio test based on simulated values was used to test whether the variance was significantly different from zero using *exactRLRT* in the R package *RLRsim* (version 2.0-6; Scheipl 2011).

Inter-annual variation in the slope of the relationship between salmon stable isotope ratios and body condition category was assessed in relation to SST anomalies in the Norwegian Sea (1° gridbox centered on 64.5°N , 4.5°E) averaged from February - August (the period represented by scale growth). All data analysis was performed in R (version 2.11.1; R Core Development Team 2010).

Results

The best predictive models to describe variation in $\delta^{13}\text{C}_{seal}$, $\delta^{15}\text{N}_{seal}$, $\delta^{13}\text{C}_{salmon}$ and $\delta^{15}\text{N}_{salmon}$ values and the coefficients of random effects are presented in Table 3.2 and Table 3.3. There was a significant random effect of year on Atlantic salmon

scale $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($\sim 23\%$ and 10.5% of the variance, respectively) and the coefficients of the effect for each isotope were significantly and positively correlated (Pearson's product moment correlation, $p = 0.04$, $r = 0.51$). Within years, variation in the slope of $\delta^{13}\text{C}$ values in relation to body condition explained a small proportion of the total variation. Male salmon had significantly higher $\delta^{13}\text{C}$ values than did females; however, the magnitude of the effect of sex ($0.003 < b < 0.18$) was below that expected due to analytical error ($\pm 0.20\%$).

Neither $\delta^{13}\text{C}_{\text{salmon}}$ nor $\delta^{15}\text{N}_{\text{salmon}}$ values showed significant linear trends over the years analysed (1963-2008; Figure 3.2) when all values were pooled, but significant trends in $\delta^{15}\text{N}$ values did emerge when partitioned by body condition factor category (Table 3.3). Condition categories 1 and 2 (good condition) had negative effects on the slope of $\delta^{15}\text{N}$ values over the time series whilst condition categories 3 and 4 (poor condition) had positive effects (Figure 3.1 and Table 3.3). There was a significant positive correlation between the slope of the relationship between $\delta^{15}\text{N}$ and W_r category and SST anomalies ($p = 0.02$, $r = 0.56$) and a significant negative relationship between the slope of the relationship between $\delta^{13}\text{C}$ and W_r category and SST anomalies ($p = 0.04$, $r = -0.51$; Figure 3.3).

Significant declines both in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values (-0.015% year $^{-1}$ and -0.03% year $^{-1}$) were seen for grey seal dentin, even after accounting for the potential random effects of age class (Figure 3.2 and Table 3.2). The model with the lowest AICc value for grey seal dentin $\delta^{13}\text{C}$ values included a significant random effect of age class, and accounted for $\sim 18\%$ of total variation. Coefficients of the random effects showed that dentin $\delta^{13}\text{C}$ values were lower in juveniles (-0.12%) and lowest (-0.23%) in animals that were ages 10-20 y when they died and higher in the 6 - 10 year ($+0.20\%$) and >20 y ($+0.15\%$) categories. The best predictive model for $\delta^{15}\text{N}_{\text{seals}}$ values did not include any random effects and was fitted as a simple linear regression. Models for both $\delta^{13}\text{C}_{\text{seals}}$, $\delta^{15}\text{N}_{\text{seals}}$ highlighted a difference between males and females; however, the low t-values and wide 95% confidence intervals associated with the estimated coefficients indicate that the effect is not well explained. Because there were only five males and 49 female grey seals in the analysis, statistical power is too low to reliably estimate any sex differences in seal dentin isotope ratios.

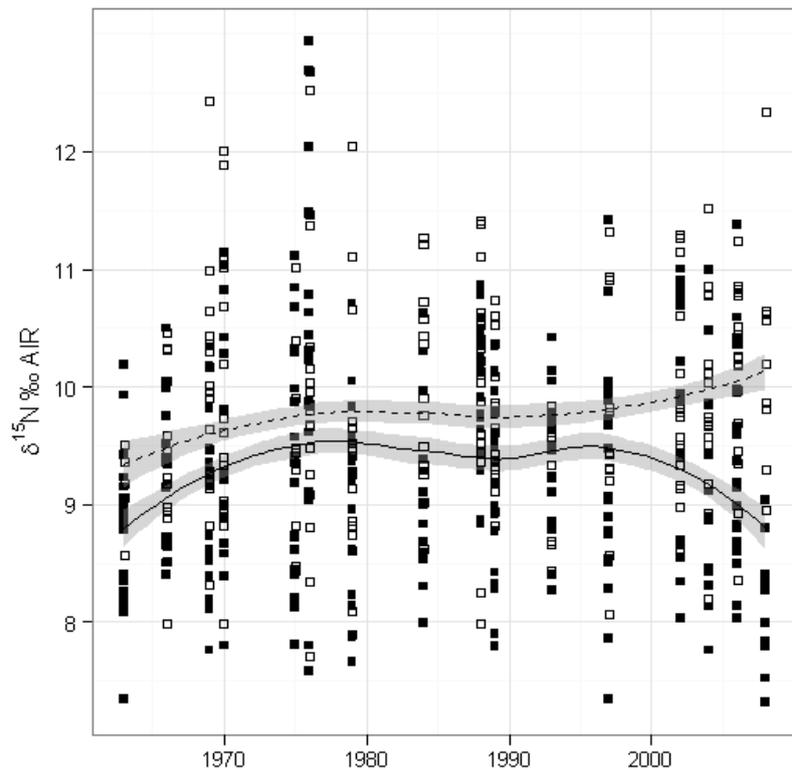


Figure 3.1: The pattern of $\delta^{15}\text{N}$ variation in wild Atlantic salmon scale collagen from the River North Esk one sea-winter stock. Solid squares and line refer to fish that returned to the monitoring station with a body condition factor (W_r) > 0.90 ; open squares and dashed line refer to fish that returned with a body condition factor < 0.85 . Smooths ($\pm 1\text{SD}$) were generated using locally weighted regression (loess; $\alpha=1$).

Discussion

The marine feeding signatures of Atlantic salmon and grey seals followed dissimilar patterns; as a generalisation, linear temporal trends in the probable trophic level were evident for both species and both stable isotopes, but at different temporal scales. Systematic changes in stable isotope ratios were apparent for Atlantic salmon between ~ 1997 and 2008, but only when Atlantic salmon stable isotope values were partitioned according to condition factor. This strongly indicates a link between changes in marine trophic dynamics and pre-spawning somatic condition of returning adult fish. Grey seal trophic dynamics probably responded to regional shelf-zone forcing related to the pronounced effects both of human exploitation of fisheries and the North Sea regime shift in foodweb structure of the early to mid 1980s

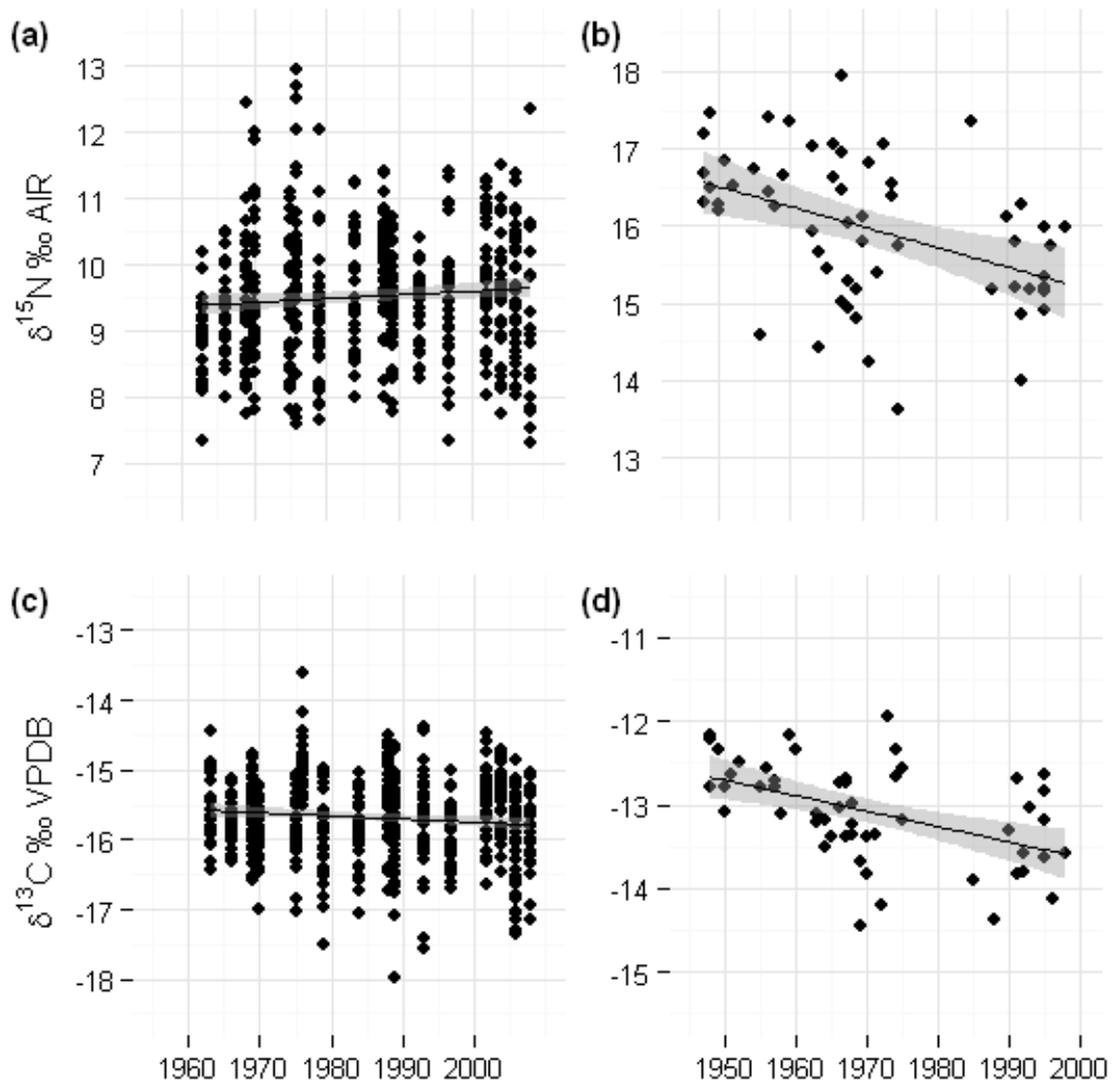


Figure 3.2: Mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values ($\pm\text{SE}$) of wild one sea-winter Atlantic salmon scales (a,c) and grey seal dentin collagen (b,d). Standard errors were estimated by bootstrapping. Single dots represent a sample size of one. Salmon scales were collected from fish returning to the River North Esk, Scotland and grey seal canine samples were collected from animals that were culled or stranded on the east coast of Scotland between 1970-present.

(Beaugrand 2004). Atlantic salmon, operating in a broader and more oceanic sector of the eastern North Atlantic, appear to have responded later in the time period examined and possibly to different processes and/or drivers. Taken overall these results of themselves provide little evidence that the trophic dynamics of grey seals exploiting the neritic waters of the eastern North Atlantic, or salmon exploiting the

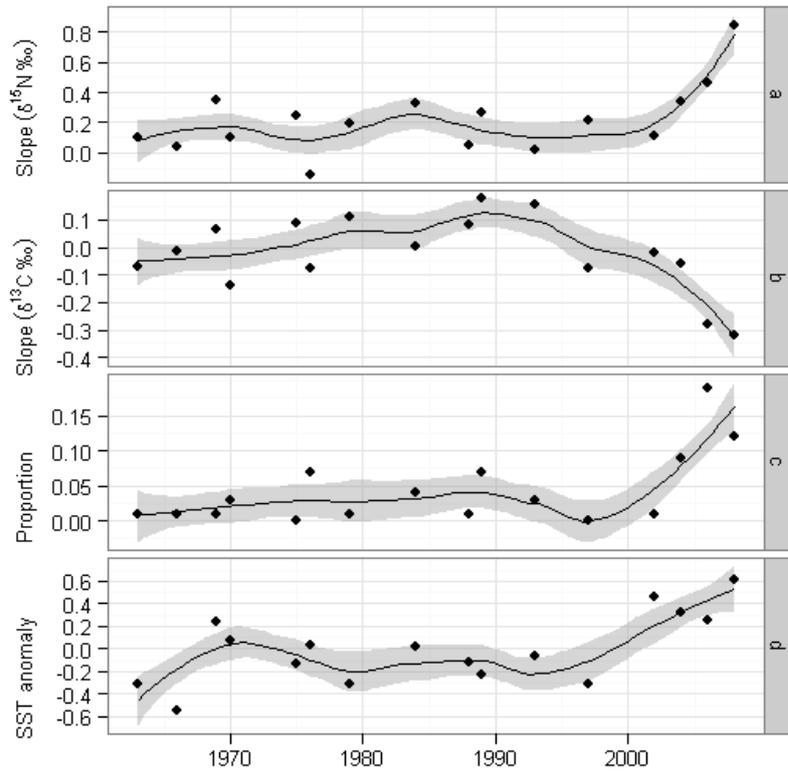


Figure 3.3: Synchronicity in the timing of shifts for wild Atlantic salmon in (a) the slope of the relationship between $\delta^{15}\text{N}$ and W_r category over time, (b) the slope of the relationship between $\delta^{13}\text{C}$ and W_r category over time, (c) the proportion of $W_r < 0.79$ salmon caught in the River North Esk monitoring program and (d) sea surface temperature anomalies in the Norwegian Sea (64.5°N , 4.5°E) averaged from February - August. Smooths ($\pm 1\text{SD}$) were generated using locally weighted regression (loess; $\alpha=0.5$).

open ocean, were being forced at the same temporal scales. Mixed effects models highlighted the complexities of interactions between intrinsic (e.g. body condition, age) factors and temporal elements of stable isotope time series.

Atlantic salmon

Pooled Atlantic salmon scale collagen $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values did not exhibit linear trends over time, although there were significant inter-annual fluctuations in mean values (Figure 3.2), and the two stable isotope ratios were strongly correlated. For example, the strongest annual effect on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values occurred in 1976 when both were anomalously high. Similarly, Sinnatamby et al. (2009) found little evi-

dence for long-term change in the marine trophodynamics of 1SW Atlantic salmon from Canadian and European rivers, but did find significant differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between years; however, they did not consider the effect of body condition in their analyses.

In the present study, it was apparent that body condition was an important factor explaining temporal variation of both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values; for $\delta^{13}\text{C}$, the coefficients of random effects show that the magnitude of the relationship between condition factor and $\delta^{13}\text{C}$ varied between years. In the latter part of the time series, the effects were predominantly negative (i.e. the lowest condition factor category also had the lowest $\delta^{13}\text{C}$ values). It also was apparent that not only did the poorest condition fish (W_r categories 3 and 4) show the highest scale $\delta^{15}\text{N}$ values, but also that these were becoming more enriched in ^{15}N in the later years of the sub-sampled time series, whilst $\delta^{15}\text{N}$ values for the highest condition fish (W_r categories 1 and 2) were in decline (Figure 3.1 and Figure 3.3). When there was a positive slope between stable isotope ratios and condition category, poor condition fish had relatively high stable isotope ratios in that particular year. There was a marked change in the years following ~ 1997 (see also Todd et al. 2008), when the discrepancy in stable isotope ratios between the highest and lowest condition categories began to enlarge.

The synchronicity of these shifts for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is striking and warrants further consideration. Atlantic salmon primarily inhabit the surface 10m of the water column (Holm et al. 2006, Dadswell et al. 2010) and their growth and survivorship is tightly linked to sea surface temperatures in the eastern North Atlantic (e.g. Friedland et al. 2009). In the present study, significant correlations between within-year slopes (with respect to condition factor category) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and Norwegian Sea SST anomalies were found (Figure 3.3). Todd et al. (2008) linked the recent and marked declines in Atlantic salmon mean W_r specifically to a progressive and sustained rise in mid-winter SST anomalies in the Norwegian Sea. Consequently, the proportion of salmon caught in the River North Esk monitoring program that returned in body condition category 4 ($W_r < 0.79$) also showed a marked increase around the late 1990s commensurate with the increase in $\delta^{15}\text{N}$ values and decrease in $\delta^{13}\text{C}$ values of a sub-sample of these fish (Figure 3.3). These relationships strongly suggest that relative to fish returning in good condition, salmon returning in the poorest condition experienced a markedly different marine feeding regime and this divergence between the stock components increased progressively between ~ 1997 and 2008, in conjunction with rising SST anomalies in the Norwegian Sea.

Variation in bulk $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of consumer protein generally can be explained in terms of differences in diet or trophic level and habitat use. Higher values of both isotope ratios are often associated with predation at higher trophic levels, although the average trophic increase in $\delta^{13}\text{C}$ values is less than that of $\delta^{15}\text{N}$ ($\sim 0.5\text{‰}$ compared to $\sim 2.4\text{‰}$ for poikilotherms fed a high-protein diet; McCutchan et al. 2003).

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of prey items also may vary spatially, reflecting physical and biological processes affecting the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of primary producers (Miyake & Wada 1967, Michener & Schell 1994, Gruber et al. 1999, Hofmann et al. 2000). In the eastern North Atlantic, large-scale geographic variation in $\delta^{15}\text{N}$ is of a lower magnitude than that expected from changes in trophic level (e.g. see preliminary isoscapes in Graham et al. 2010) whereas $\delta^{13}\text{C}$ values in dissolved inorganic carbon (DIC) can decrease by $>1\text{‰}$ over the latitudinal range 45°N to 75°N in this same area (Gruber et al. 1999). Such large-scale latitudinal gradients in $\delta^{13}\text{C}_{\text{DIC}}$ are reflected in the tissues of plankton (Graham et al. 2010) and may resonate up the food web. On the regional scale, higher $\delta^{13}\text{C}$ values generally are associated with high algal growth rates (Laws et al. 1995, Bidigare et al. 1997) and more productive regions (e.g. inshore and/or upwelling zones), although considerable spatial heterogeneity in plankton $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values exists in oceanographically complex regions (Schell et al. 1998).

The portion of scale growth analysed in this study was accreted during the 5-7 month period following the end of the last winter annulus and the day of capture. The precise geographical location of maturing 1SW salmon during these particular months is unknown but limited tag data suggest that they are distributed within the Norwegian Sea, and particularly north of the Faroes (Hansen & Jacobsen 2000, Dadswell et al. 2010). The data available in this ocean region suggests that during February - March adult and pre-adult fish predominantly forage on zooplankton crustaceans ($\sim 25\%$ frequency of occurrence; mainly amphipods) and forage fish ($\sim 50\%$ frequency of occurrence; mainly lantern fish, pearlsides and barracudinas for 1SW fish; Jacobsen & Hansen 2000, 2001, Rikardsen & Dempson 2010).

Data for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the different components of salmon diet in the eastern North Atlantic are scarce, but where planktonic crustaceans and forage fish from the same region have been compared (Hobson et al. 1995, Møller 2006, Bode et al. 2008, Petursdottir et al. 2008), fish tissues have higher $\delta^{15}\text{N}$ values commensurate with foraging at higher trophic levels. This trophic segregation in

$\delta^{15}\text{N}$ values of salmon prey might suggest that fish that had returned to the River North Esk in the poorest condition (i.e. markedly underweight for their length) had foraged at progressively higher trophic levels, and probably with a greater proportion of fish than crustaceans in their diets during their final spring months at sea. The concomitant divergence in $\delta^{13}\text{C}$ values between the condition categories in the latter part of the time series could indicate geographic segregation of foraging and more negative values of the scales of poor condition fish may relate to these particular fish foraging in areas of low productivity or at higher latitudes.

However, interpretation of consumer $\delta^{15}\text{N}$ values may be complicated by the effects of nutritional stress; if intake of dietary protein is severely restricted or ceased, the body continues to synthesize necessary metabolites from the existing endogenous nitrogen pool. Similar to the trophic enrichment process, this re-amination of amino acids causes an enrichment of ^{15}N in consumer proteins. High $\delta^{15}\text{N}$ values can, therefore, also be associated with periods of nutritional stress (e.g. Doucett et al. 1999, Jardine et al. 2004, Guelinckx et al. 2007, Gaye-Siessegger et al. 2007). There is evidence for this 'starvation' effect in metabolically active tissues of fish such as muscle and liver (Jardine et al. 2004, Gaye-Siessegger et al. 2007), including return-migrant Atlantic salmon (Doucett et al. 1999), and the feathers of some birds (Hobson et al. 1993, Cherel et al. 2005). However, other studies have reported mixed results (Kempster et al. 2007, Williams et al. 2007, McCue & Pollock 2008) and none have investigated this effect in scale tissue samples.

That low condition factor salmon predated at higher trophic levels may seem counter-intuitive considering that forage fish consumed by Atlantic salmon generally have higher energy densities and lipid content than planktonic crustaceans (Spitz et al. 2010), and that in some regions salmon have been found to prefer fish over crustaceans (Jacobsen & Hansen 2001). However, individual salmon can exhibit a degree of specialisation in their prey consumption (i.e. many salmon tend to predate a single prey type; Hansen & Pethon 1985, Jacobsen & Hansen 2001) and it is conceivable that a component exists within the River North Esk stock that specialises on upper trophic level prey items during the marine life-stage. These salmon may not be able to attain or maintain high W_r factors throughout the marine migration due to the relatively low availability of forage fish compared with crustacean species (Jacobsen & Hansen 2001). Furthermore, whilst it is evident that fish returning in poor condition had severely compromised lipid reserves (low W_r), they continued to grow in length throughout the last spring at sea (as evidenced

by the addition of scale circuli) and did not exhibit any evidence of scale Ca^+ resorption which is associated with periods of extreme food deprivation, such as during the upriver spawning migration (Persson et al. 1998). Thus, the evidence is weak for a prolonged period of severe starvation (on the order of several months) that might be necessary to drive high scale $\delta^{15}\text{N}$ values in Atlantic salmon.

The the patterns in Atlantic salmon scale $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, when partitioned by body condition, are strongly related to rising SST anomalies in the Norwegian Sea. This result is consistent with previous links between salmon recruitment (Beaugrand & Reid 2003, Friedland et al. 2009), growth (Friedland et al. 2009), somatic condition (Todd et al. 2008, Bacon et al. 2009) and SST. However, the present results and analyses provide strong evidence for trophic and/or spatial segregation of ocean feeding of salmon returning in variable condition. Taken together, the increase in the proportion of salmon returning in poor condition and the increase in the slope of the relationship between W_r and scale $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the latter part of the time series suggests that a larger proportion of salmon are feeding at upper trophic levels and in areas of lower productivity or at higher latitudes, and possibly both these factors are acting contemporaneously. These environmental conditions evidently are detrimental to 1SW Atlantic salmon pre-spawning body condition and probably are driven in part by the indirect effect of rising sea surface temperature on the availability and abundance (or quality) of zooplankton and small nekton prey.

Grey seals

The isotopic ratio of carbon in grey seal tooth dentin appeared to be related to age class. Dentin samples are representative of only the first few years of growth (due to the cementum growth and occlusion of the pulp cavity at this time; Hewer 1964) and replacement or turnover of collagen in these layers is extremely unlikely (Koch & Tuross 1994). Thus the changes in dentin $\delta^{13}\text{C}$ values with age class do not reflect ontogenetic changes in foraging, but rather the connection between 'early life' signatures and age-at-death could indicate that different foraging strategies in the early years of life influence survivorship. Seals culled at ages >20 y had slightly and significantly higher $\delta^{13}\text{C}$ signatures in early years which might signal that these particular seals adopted a more benthic foraging strategy during this life stage that was advantageous to survival. However, the result could also be an artefact of the uneven distribution of age classes over the time series. For example, older animals were over-represented in early years (Table 3.1), when $\delta^{13}\text{C}$ values were higher and

clearly more data are needed to fully understand the effects of early life trophic dynamics on longevity.

In contrast to Atlantic salmon, grey seal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values showed clear and steady linear declines over the time series. Scat analysis has shown that the incidence of sandeels (*Ammodytes* spp), haddock (*Melanogrammus aeglefinus*) and whiting (*Merlangius merlangus*) increased in the diet of grey seals between the 1980s and 2002 (Hammond et al. 1994, Hammond & Grellier 2005). This represents a downward shift in trophic level exploited that coincided with the observed decline in grey seal dentin $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values reported here. Christensen & Richardson (2008) also noted a significant decline in bone collagen $\delta^{15}\text{N}$ values for harbour porpoises (*Phocoena phocoena* (L.)) stranded along the Dutch North Sea coast. Their time series, ranging from 1848-2002 was relatively stable until the 1960s after which $\delta^{15}\text{N}$ values declined markedly until ~1978 when values stabilised once more. They suggested that the decline could have been attributable to porpoises feeding on smaller fish and/or feeding at lower trophic levels in recent years, both of which could have been a product of a wider-scale decrease in the abundance and size of upper trophic level species associated with over-exploitation in the 20th century (Jennings et al. 2002, Pinnegar et al. 2002). It is possible that the interaction between competition from fisheries and changes in environmental conditions since the ~1980s, and which have been considered to be poor for the recruitment of some upper trophic level fishes (e.g. cod, plaice, sole; Cook & Heath 2005), have forced both grey seals and harbour porpoises to forage at lower trophic levels in the latter years of the time series.

Neither Christensen & Richardson (2008) nor the present study can discount the possibility that the declines in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of top predators in the North Sea reflect temporal changes to stable isotope dynamics at the base of the food-web rather than trophic shifts (e.g. due to nutrient dynamics, productivity, etc.). Declines in predator $\delta^{13}\text{C}$ values are further complicated by the incursion of isotopically “light” anthropogenic CO_2 in the marine environment (the “Suess effect”; Quay et al. 1992, Newsome et al. 2007a, Christensen & Richardson 2008, Aubail et al. 2010) and/or a reduction in primary productivity (Schell 2000, Hirons et al. 2001), both of which can cause a reduction in ^{13}C . The decline in $\delta^{13}\text{C}$ in the grey seal dataset was approximately -0.015‰ year^{-1} , well within the Suess effect of -0.026‰ year^{-1} estimated for dissolved inorganic carbon (DIC) in the North Atlantic (Körtzinger et al. 2003). Never the less, concurrent declines both in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$

values reduces the likelihood that the Suess effect, and not a trophic effect, is the sole cause of the decline in $\delta^{13}\text{C}$ values. Evidence for a pronounced shift in diet from scat analyses (Hammond et al. 1994, ?) together with a similar response in isotope proxies of another generalist predator in the same region over the same time period (Christensen & Richardson 2008), indicates that those temporal changes in $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values of grey seals do reflect a change in trophic dynamics.

The extent to which large-scale climate forcing is driving these changes is unclear; rising SST has caused major biogeographic shifts in the distribution of several fish species and is associated with poor survival conditions for larval cod (Beaugrand et al. 2003, Pinnegar & Heath 2010) - a species that was abundant in the diet of grey seals during the 1980s (Hammond et al. 1994). However, severe overfishing of species historically important in grey seal diet make it difficult to separate the effects of human over-exploitation and climate forcing; a reduction in fish size (Jennings et al. 2002), abundance or change in trophic level (Pinnegar et al. 2002) due to fisheries all could have forced a downward shift in grey seal trophic level. Moreover, the magnitude and rapidity of change in grey seal $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values compared with the subtle changes in the Atlantic salmon dataset may signal the additive effects of large-scale climate forcing and direct competition with fisheries to which this species is exposed as a shelf-zone neritic forager.

Long-term records of variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of Atlantic salmon and grey seals provide evidence for large-scale climate forcing across the eastern North Atlantic, but there are clear differences in the response of these predators to ecological change. Atlantic salmon appeared to respond strongly to rises in SST after ~1997 by shifting towards foraging at upper trophic levels and this appears to be affecting the condition of return-migrant adult fish. Grey seals, by contrast, appear to be more susceptible to long-term, and perhaps more localised ecological changes in the shelf waters that have altered the availability of upper trophic level prey. Spatio-temporal scales of ecological forcing on predator trophic dynamics can thus range from relatively recent effects across the pelagic eastern North Atlantic to longer-term effects in the shelf seas. This probably reflects the multiple layers of ecological drivers, some of which will be anthropogenic, and that arguably have had the longest influence upon the shelf seas through the impacts of fishing and other human activities. This study also suggests that these local influences on ecological dynamics may become more pervasive because of ocean climate-driven forcing at the larger spatial scale.

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Table 3.1: Sample size, mean stable isotope value and standard deviation for each calendar year grouping. The value of grey seal ages in each year is also given.

Species	n	Year	Mean $\delta^{15}\text{N}$	s.d.	Mean $\delta^{13}\text{C}$	s.d.	Age
Atlantic salmon	30	1963	8.80	0.60	-15.57	0.44	-
	31	1966	9.27	0.67	-15.73	0.33	-
	39	1969	9.42	0.91	-15.57	0.45	-
	29	1970	9.71	1.14	-15.84	0.41	-
	30	1975	9.45	0.89	-15.78	0.51	-
	39	1976	10.27	1.39	-14.94	0.38	-
	35	1979	9.20	0.80	-15.89	0.53	-
	28	1984	9.52	0.87	-15.88	0.51	-
	39	1988	10.07	0.70	-15.33	0.58	-
	40	1989	9.38	0.71	-15.91	0.70	-
	30	1993	9.32	0.54	-15.70	0.66	-
	32	1997	9.41	0.98	-15.93	0.46	-
	32	2002	9.80	0.93	-15.47	0.51	-
	32	2004	9.62	0.94	-15.38	0.47	-
	40	2006	9.75	0.92	-16.07	0.72	-
	21	2008	9.06	1.31	-15.86	0.57	-
	Grey seal	3	1948	16.73	0.44	-12.40	0.33
2		1949	16.98	0.68	-12.35	-	32, 32
2		1950	16.23	0.06	-12.95	0.21	31, 31
1		1951	16.85	-	-12.64	-	30
1		1952	16.51	-	-12.48	-	29
1		1955	16.73	-	-12.79	-	26
1		1956	14.60	-	-12.57	-	16
2		1957	16.93	0.68	-12.76	0.06	24, 24
1		1958	16.25	-	-13.12	-	23
1		1959	16.66	-	-12.16	-	22
1		1960	17.35	-	-12.33	-	21
2		1963	16.49	0.77	-13.17	0.06	18, 18
2		1964	15.05	0.86	-13.34	0.23	17, 17
1		1965	15.46	-	-13.38	-	16
2		1966	16.85	0.29	-12.88	0.21	15, 15
4		1967	16.60	1.21	-12.88	0.33	14, 14, 14, 14
3		1968	15.43	0.56	-13.19	0.19	13, 13, 13
2		1969	15.00	0.26	-14.06	0.54	12, 12
2		1970	15.96	0.22	-13.61	0.33	11, 11
2		1971	15.54	1.82	-13.36	-	10, 10
1		1972	15.40	-	-14.21	-	9
1		1973	17.05	-	-11.95	-	8
2		1974	16.47	0.13	-12.51	0.23	7, 7
2		1975	14.68	1.49	-12.88	0.44	6, 6
1		1983	15.12	-	-	-	15
1		1985	17.35	-	-13.90	-	17
1		1988	15.18	-	-14.37	-	13
1		1990	16.13	-	-13.31	-	20
2		1991	15.51	0.40	-13.27	0.81	5, 16
3		1992	15.05	1.15	-13.69	0.17	4, 12, 16
3		1993	15.84	1.18	-13.04	-	16, 17, 18
5	1995	15.32	0.40	-13.07	0.44	9, 11, 12, 12, 25	
2	1996	15.93	0.27	-14.13	-	15, 23	
1	1997	15.77	-	-	-	3	
1	1998	15.98	-	-13.57	-	7	
1	2003	14.88	-	-	-	15	
1	2004	15.42	-	-	-	12	
1	2005	15.74	-	-	-	4	

Table 3.2: Results of mixed effects models fit by maximum likelihood to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of collagen extracted from recently collected and archived samples of Atlantic salmon scales and Grey seal tooth dentin. 95% confidence intervals are given in parentheses were calculated from the Highest Posterior Density intervals of Markov chain Monte Carlo simulations ($n = 10,000$).

	Isotope	N	d.f.	Random effects	No. groups	Variance	% Variance	S.D.	p-value	Effect	Coefficient	t-value
Atlantic salmon	$\delta^{13}\text{C}$	527	5	year	16	0.08	22.41	0.27 (0.25 - 0.68)	p<0.001	intercept	-15.71 (-15.86 - -15.56)	-186.59
				slope W_r within years	4	0.01	1.96	0.08 (0.07 - 0.27)	p=0.001	sex	0.1 (0.003 - 0.18)	2.12
				residual		0.27	75.96	0.52 (0.49 - 0.55)				
	$\delta^{15}\text{N}$	527	4	year	16	0.09	10.34	0.3 (0.2-0.5)	p<0.001	intercept	9.46 (9.18-9.72)	79.41
				slope W_r across years	4	0.00	0.01	0.009 (0.004-0.04)	p<0.001			
				residual		0.78	89.65	0.88 (0.83-0.94)				
Grey seal	$\delta^{13}\text{C}$	54	6	year	33	0.13	46.43	0.36 (0-0.46)	p=0.005	intercept	-12.69 (-13.2 - -12.27)	-62.64
				ageclass	4	0.05	17.86	0.22 (0-1.3)	p=0.006	year	-0.015 (-0.025 - -0.002)	-2.44
				residual		0.10	35.71	0.31 (0.37-0.57)		sex	-0.34 (-0.74 - 0.27)	-1.45
	$\delta^{15}\text{N}$	58	2							intercept	16.62 (16.42 - 16.82)	80.62
										year	-0.03 (-0.022 - -0.038)	-3.78
										sex	0.56 (0.12 - 1)	1.28

Table 3.3: Coefficients of significant random effects on the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Atlantic salmon scales and grey seal dentin from linear mixed effects models estimated by maximum likelihood. W_r category = body condition factor (see Eqn Equation 4.1 for calculation).

	Group	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
		Year ^a	W_r category ^b	Year ^a	W_r category ^b
Atlantic salmon	1963	-0.51	-	0.09	-0.007
	1966	-0.16	-	-0.03	-0.013
	1969	-0.04	-	-0.01	0.033
	1970	0.18	-	0.00	-0.078
	1975	-0.07	-	-0.15	0.018
	1976	0.56	-	0.63	0.011
	1979	-0.20	-	-0.31	0.029
	1984	0.00	-	-0.18	-0.020
	1988	0.43	-	0.17	0.067
	1989	-0.07	-	-0.39	0.058
	1993	-0.12	-	-0.22	0.071
	1997	-0.01	-	-0.13	-0.052
	2002	0.21	-	0.16	0.005
	2004	0.02	-	0.26	-0.005
	2006	0.16	-	-0.02	-0.173
	2008	-0.37	-	0.11	-0.133
		$W_r = 0.99-1.09$	-	-0.010	-
	$W_r = 0.90-0.97$	-	-0.001	-	-
	$W_r = 0.80-0.85$	-	0.005	-	-
	$W_r < 0.79$	-	0.014	-	-
Grey seal	Age: 3 - 6 yrs	-	-	-0.12	-
	Age: 6 - 9 yrs	-	-	0.20	-
	Age: 10 - 20 yrs	-	-	-0.23	-
	Age: + 20 yrs	-	-	0.14	-

^aintercept

^bslope

4 Multi-tissue stable isotope variation and the ‘skinny’ salmon story

ABSTRACT: Recent declines in the somatic condition of wild one sea-winter Atlantic salmon (*Salmo salar*) returning to southern European rivers may be driven by suboptimal environmental conditions during the marine residency in the eastern North Atlantic. However, linking marine environmental conditions and dietary history of individual fish to their somatic condition upon capture in coastal waters is difficult using traditional tracking techniques. Because isotopic incorporation rates vary in different tissues, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation was assessed in multiple salmon tissues and related to body condition factor in order to assess the timing and magnitude of trophic effects on salmon quality. A significant positive relationship between condition factor and muscle stable isotope values partially upheld the hypothesis that individual variation in somatic condition indices within a single year are directly related to the foraging conditions experienced at sea. A significant negative relationship between somatic condition and $\delta^{15}\text{N}$ values of liver tissue provided evidence for an early, marine, onset of nutritional stress - prior to the start of the freshwater spawning migration. The effects of lipid extraction and among tissue differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are also discussed.

Introduction

Unprecedented declines in the abundance of wild Atlantic salmon across their distributional range are well-documented and have been linked to reductions in marine survival and recruitment (ICES 2010). In the eastern North Atlantic, climatic conditions in the post-smolt nursery area during the first summer at sea are important for growth mediated survival (Friedland et al. 2000, 2009). Recent increases in

sea surface temperature (SST) in the eastern North Atlantic, more pronounced at lower latitudes, and northward shifts in the distribution of warm-water zooplankton concurrent with declines in the abundance of cold-water species (Beaugrand et al. 2002, Beaugrand & Reid 2003, Richardson & Schoeman 2004), have been identified as a critical concern for the future viability of southern European Atlantic salmon stocks (Friedland et al. 1998, 2000, Beaugrand & Reid 2003, Todd et al. 2008, Bacon et al. 2009, Friedland et al. 2009, Todd et al. 2010). Whilst most studies of the interaction between marine climate and Atlantic salmon have focused on indices of abundance (Beaugrand & Reid 2003, Friedland et al. 2009, 2000, Vøllestad et al. 2009, ICES 2010), Todd et al. (2008) and Bacon et al. (2009) have explored a more qualitative assessment of Atlantic salmon stocks by investigating long-term trends in size and somatic condition of returning adults. Bacon et al. (2009) found significant long-term declines in both length and weight of one sea-winter (those fish returning to freshwater after a single winter at sea; 1SW) salmon in the majority of rivers analysed. Both studies confirmed a striking decadal decline in body condition of returning 1SW stocks in multiple Scottish fisheries beginning ~1997, despite using two different indices of condition. Furthermore, body condition factor (W_r ; Equation 4.1) is sigmoidally related to body lipid content (Todd et al. 2008); long-term declines in mean condition factor, and inferred reduction in individual lipid reserves, of returning 1SW stocks to Scottish fisheries are likely to have direct impacts on salmon fecundity and reproductive success (Thorpe et al. 1998, Todd et al. 2008). Increasingly high marine mortality coinciding with decreasing reproductive success are of major concern for the conservation, management and population viability of southern European Atlantic salmon stocks.

A key question to explore in order to address conservation and management concerns is to what extent are long-term declines in Atlantic salmon recruitment, size and body condition a product of changing marine conditions? Because of their diverse and anadromous life histories, climate change has implications for all stages of Atlantic salmon life histories Todd et al. (2010).

Salmon that are en route to their natal streams to spawn and are intercepted in coastal fisheries are, by necessity, approaching sexual maturity. What triggers the onset of sexual maturation (or rather, release from its inhibition) at sea and the subsequent 'return' migration is debatable; however, the interaction of environmental cues (e.g. photoperiod, body condition, growth rate) and genetic cues (e.g. which set a condition 'threshold' or growth rate) appear important (Hutchings &

Jones 1998, Thorpe et al. 1998, Jonsson & Jonsson 2007). In their life history model for Atlantic salmon, Thorpe et al. (1998), suppose that there are two important 'maturation' events in the year preceding reproduction in which the fish must assess their body condition (lipid reserves) and its rate of change. If the criteria are met to surpass some genetically determined threshold of these parameters, then maturation continues but if the body condition is considered insufficient, continued maturation is inhibited. Jonsson & Jonsson (2007) concluded from scale analyses of eight populations of Atlantic salmon returning to Norwegian rivers that fish return earlier when the growth rate during the first year at sea is low and suggested that growth rate itself has an important genetic basis. In contrast, Hutchings & Jones (1998) provided evidence for population specific growth rate thresholds but in their model simulations, increased growth rate was associated with earlier maturation. Clearly, the plasticity in Atlantic salmon life histories makes predicting the links between environmental conditions (e.g. determining growth, lipid reserves) and population dynamics (e.g. maturation, survival) difficult. However, empirical evidence suggests that maturing 1SW fish need to have 'passed' some threshold(s) of growth in length, growth rate or somatic condition in order to have continued their maturation into the final spring and summer months at sea; the extent to which these parameters are determined by genetic heritability or environmental conditions is still unknown.

Assuming that 1SW salmon have 'passed' a maturation threshold and in doing so, are 'committed' to returning to freshwater to spawn the same year, the fact that these stocks are suffering long-term declines in average length and somatic condition has several implications. First, because the declines are only monitored in salmon that have survived the marine migration, it is possible that there is disproportionate mortality of longer and better condition fish at sea, driving down the average indices of those that do make it to freshwater. Second, long-term declines in somatic condition and growth may suggest that the 'genetic threshold' for early maturation is changing (i.e. marine selection may be favoring small, skinny fish); and third, suboptimal environmental conditions during the last few months at sea - after 1SW fish are 'committed' to return - could trigger a dramatic loss of weight and lipid reserves, driving high individual variation in somatic condition of survivors. Because long-term declines in both length and body condition are apparent, it seems likely that they are the result of complex interactions between genetically heritable traits and environmental conditions within years; but one way to assess the magnitude of intra-annual environmental influences on salmon quality is to test for differences in

marine feeding during the last months at sea in relation to eventual pre-spawning body condition. This requires linking knowledge of the dietary history of the fish with indices obtainable from fish that have been captured in coastal waters. Assessing trophic effects on returning salmon quality, even within a single year, is difficult due to the paucity of empirical data on the distribution, diet and body condition of Atlantic salmon at sea.

The oceanic distribution and migrational timing of 1SW Atlantic salmon is poorly understood; salmon tagged in autumn and winter in the Norwegian Sea and north of the Faroe Islands were recaptured the following year in Scotland and other southern European countries (Hansen & Jacobsen 2000, 2003). During their spawning migration, salmon have been estimated to travel at speeds up to 50 - 100km day⁻¹ (Dadswell et al. 2010), meaning that fish captured off the north coast of Scotland in summer could have been located in a large potential region of the eastern North Atlantic during their final months at sea. However, given the predominant flow of the North Atlantic sub-polar gyre (NAspG) and distribution of preferred thermal habitat (4-8°C) in early spring, it is likely that fish of southern European origin will have been foraging within the southern Norwegian Sea during this time (Dadswell et al. 2010). Changes in trophodynamics in this region, such as declines in euphausiids and the subarctic copepod *Calanus finmarchicus* have not been beneficial for Atlantic salmon stocks (Beaugrand & Reid 2003, Friedland et al. 2009). However, at-sea studies of salmon diet and condition during the latter part of the marine migration are presently lacking and providing time resolved estimates of diet necessary to examine trophic effects on eventual pre-spawning condition would be difficult and expensive to obtain for 1SW fish during spring. The stable isotope ratios of carbon and nitrogen in the tissues of adults returning to freshwater provide a means to assess the marine trophic history of Atlantic salmon in relation to their somatic condition and energy reserves.

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of animal tissues reflect the isotopic signatures of dietary sources once isotopic fractionation between diet and consumer (also termed trophic discrimination) has been accounted for (DeNiro & Epstein 1978, 1981). Trophic discrimination estimates vary between taxa and tissues but, in general, there is a tendency for the heavier isotope of nitrogen (^{15}N) and, to a lesser extent, carbon (^{13}C) to accumulate in the tissues of the consumer (Post 2002, McCutchan et al. 2003). Furthermore, the rate of isotopic incorporation depends on the growth rate and metabolism of the tissue. In general, rapidly-growing and more metabolically

active tissues (i.e. faster rate of protein turnover) such as liver and blood plasma will equilibrate with a new diet faster than less metabolically active tissues with slower turnover rates such as muscle and collagen (Tieszen et al. 1983, Perga & Gerdeaux 2005, Trueman et al. 2005, Guelinckx et al. 2007, del Rio et al. 2009, Buchheister & Latour 2010). Stable isotope analysis of multiple tissues from the same individual can therefore provide time-resolved information on dietary sources (Dalerum & Angerbjörn 2005, Phillips & Eldridge 2006, del Rio et al. 2009).

To address the hypothesis that long-term declines in wild 1SW Atlantic salmon growth condition and quality are driven by suboptimal environmental conditions during the last few months at sea, the magnitude and timing of marine trophic effects on indices of individual fish quality was assessed within a single year. Linear relationships between the stable isotope signatures of multiple tissues from the same individual and their condition factor and lipid content were sought from a sample of salmon returning to a mixed-stock fishery on the north coast of Scotland in 2009. The temporal resolution provided by examining multiple tissues with varying isotopic turnover rates (liver, white and red muscle, and scale collagen) provides a more comprehensive insight into marine trophic effects and salmon quality.

Materials & Methods

Sample collection & preparation

27 1SW wild Atlantic salmon were selected from salmon caught in a coastal mixed-stock fishery (Melvich [58.57° N, 3.92° E], North Scotland) during the 2009 netting season (June-August). Fork length (to 0.5 cm) and weight (to 0.01 kg) of each fish were used to calculate a condition factor by means of the relative mass index, W_r (Blackwell et al. 2000):

$$W_r = W/W_s \tag{4.1}$$

where W is the weight of the fish at capture and W_s is the standard weight predicted from a specific length/weight regression equation for salmon sampled at the fishery derived by the regression length percentile (RLP) method, treating multiple year-classes as separate populations and regressing the 75th percentile of $\log(\text{weight})$ at each 1cm grouping against $\log(\text{length})$ (Todd et al. 2008). Subsampling of in-

dividuals for analysis was not random; salmon were selected from the total catch in order to cover a range of condition factors. Scale samples were removed from rows posterior to the dorsal fin and above the lateral line as is standard for Atlantic salmon (Shearer 1992b). Scale impressions were made on acetate strips for confirmation of sea-age (Bryce Whyte, Marine Scotland, Montrose).

Approximately 2 cm³ of red muscle and white muscle was excised from tissue posterior to the dorsal fin and above the lateral line. A similar amount of liver tissue also was removed. Tissues were rinsed with deionized water and stored at -80°C. These three soft tissue types were chosen for analysis because of differences in their isotopic incorporation rates as well as their different functions in salmon physiology. Red muscle is used for aerobic, routine and sustained swimming activity and utilizes lipid stores for rapid mobilisation of free fatty acids (FFA) whilst white muscle primarily utilises glycogen as an energy store and functions in anaerobic locomotion - important during burst swimming activity associated with prey capture, predator avoidance and the upstream spawning migration. The liver is an important site of lipid metabolism in fish and has a faster isotopic turnover rate than muscle tissues (Trueman et al. 2005, Guelinckx et al. 2007). The lipid content of the whole fish was determined from a subsample of homogenate using a modified Bligh & Dyer method (see Todd et al. 2008 for details). Lipid content was determined in triplicate and the mean reported as entire fish (EF) percent lipid content (%Lipid).

Prior to isotope analysis, soft tissue samples were lyophilised and homogenised with a mortar and pestle. The ground tissue sample was divided and lipids were extracted from one half using accelerated solvent extraction (Dionex ASE 200, 4 Albany Court, Camberley, GU 16 7QL, Surrey, UK). Method A from Bodin et al. (2009) was used because this ASE protocol was found to have the least influence on $\delta^{15}\text{N}$ values. Scale samples were visually inspected for signs of scale resorption or regeneration that could confound stable isotope analyses; any such scales were excluded from analysis. Scales were prepared for isotope analyses as outlined by Sinnatamby et al. (2008) by excising scale material deposited after the winter check (Figure 4.1) and therefore representative of post-winter growth. Approximately 1 mg of soft tissue and 0.6 mg of scale tissues was weighed into tin capsules for analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using a Costech Elemental Analyzer fitted with a zero-blank auto-sampler coupled via a ConFlo III to a ThermoFinnigan DeltaPlusXL using Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS) at the University of St Andrews Facility for Earth and Environmental Analysis. Stable isotope results

are reported as per mil (‰) deviations from the Vienna Pee Dee Belemnite (VPDB) and atmospheric air reference standards for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Duplicate analyses were run for ~20% of total samples and precisions ($\pm\text{SD}$) for internal standards were better than $\pm 0.15\text{‰}$ for both carbon and nitrogen. Precisions for within- and between-run replicates of tissue samples were $< \pm 0.22\text{‰}$ and $\pm 0.38\text{‰}$ for carbon and nitrogen analyses, respectively.

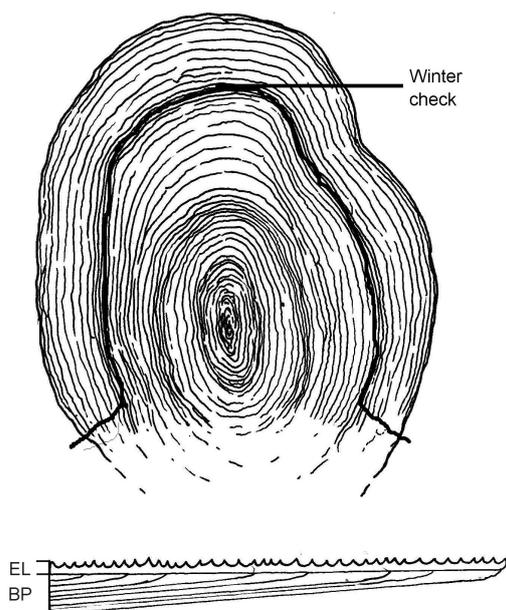


Figure 4.1: Illustration of a typical 1SW wild Atlantic salmon scale showing the post-winter portion of scale growth excised for stable isotope analysis. Only the outer margin is sampled because, due to the under-plating growth of successive collagen layers in the basal plate (BP), material that was deposited earlier (inner portion) will be mixed with collagen layers deposited later in life. Carbon in the the external layer (EL) of apatite was expected to have a negligible effect on scale $\delta^{13}\text{C}$ values.

Data analysis

The effect of lipid extraction on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and variance within each tissue type was assessed using analysis of variance (ANOVA) and Bartlett tests of homogeneity of variance. Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values among tissues was assessed using ANOVA. Multiple linear regression models were specified to examine the relationship between somatic condition (W_r), whole fish lipid content (% lipid) and

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of white muscle, red muscle, liver and scale tissue. Collinearity of the independent variables was assessed by examining variance inflation factors. Due to small sample sizes and relatively large number of parameters, bias-corrected Akaike information criterion values (AICc) were computed for each model (Hurvich & Tsai 1989; Equation 4.2).

$$AICc = AIC + \frac{2k(k+1)}{n-k-1} \quad (4.2)$$

where k is the number of parameters and n is sample size. Regression models were simplified by the iterative exclusion or addition of variables and reductions of AICc values. All data analysis was performed in R (version 2.11.1; R Core Development Team 2010).

Results

Salmon somatic condition factor ranged from 0.75 to 1.04 and whole fish lipid content (%Lipid_{EF}) ranged from 5.81% to 14.58%. $\delta^{13}\text{C}_{\text{bulk}}$ values were lowest in the red muscle ($-23.30 \pm 0.54\text{‰}$) and highest in the scales ($-15.910 \pm 0.35\text{‰}$) whereas $\delta^{15}\text{N}_{\text{bulk}}$ values were lowest in the scales ($9.18 \pm 0.59\text{‰}$) and highest in the red muscle tissue ($11.74 \pm 0.56\text{‰}$).

Lipid extraction significantly increased $\delta^{13}\text{C}$ values for all tissues (one-way ANOVA, $F_{1,52} = 112.75$, $p < 0.001$ for liver; $F_{1,52} = 997.03$, $p < 0.001$ for red muscle; $F_{1,52} = 28.92$, $p < 0.001$ for white muscle) but had no significant effect on $\delta^{15}\text{N}$ values (Figure 4.2). Although $\delta^{13}\text{C}$ values were most variable in bulk liver and white muscle tissue samples (CV = 4.2% and 2.8%), this variation is significantly reduced after lipid extraction. Variation in $\delta^{15}\text{N}$ values was not significantly effected by lipid extraction (Table 4.1) but $\delta^{15}\text{N}_{\text{bulk}}$ values were used in subsequent regression analysis in order to avoid confounding effects of any minor alterations in $\delta^{15}\text{N}$ values that might arise from the removal of labile ^{15}N -depleted compounds (Trueman et al. 2005).

There were significant differences in $\delta^{13}\text{C}_{\text{bulk}}$ ($F_{3,104} = 121.76$, $p < 0.001$) and $\delta^{15}\text{N}_{\text{bulk}}$ values ($F_{3,104} = 650.37$, $p < 0.001$) between the four tissue types. Differences persisted in $\delta^{13}\text{C}$ values after lipid extraction ($F_{3,104} = 545.2$, $p < 0.001$), although the magnitude of discrepancies between the tissues was greatly reduced (Table 4.1).

The results of multiple linear regression models between condition indices and

4.0 Results

Table 4.1: Mean, standard deviation and coefficient of variation (CV) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for each tissue type; p-values are given for Barlett tests for homogeneity of variance between bulk and lipid extracted samples. LE = lipid extracted

Tissue	Mean $\delta^{13}\text{C}$	$\pm\text{SD}$	CV (%)	p-value	Mean $\delta^{13}\text{N}$	$\pm\text{SD}$	CV (%)	p-value
Liver _{bulk}	-21.44	0.96	4.2	<0.001	9.62	0.55	5.8	0.934
Liver _{LE}	-19.39	0.28	1.5		9.83	0.54	5.5	
Red muscle _{bulk}	-23.30	0.54	2.3	0.234	11.74	0.56	4.8	0.696
Red muscle _{LE}	-19.12	0.43	2.2		11.91	0.52	4.4	
White muscle _{bulk}	-19.50	0.54	2.8	0.019	10.87	0.50	4.6	0.518
White muscle _{LE}	-18.84	0.34	1.9		10.84	0.57	5.2	
Scales	-15.91	0.35	2.4		9.18	0.59	6.4	

Table 4.2: Coefficients (and p-value) of stable isotope values of each tissue type regressed against condition indices for $n = 27$ wild 1SW Atlantic salmon; W_r is pre-spawning condition factor and $\%Lipid_{EF}$ is the lipid content of whole homogenised fish. LE = lipid extracted. Models were selected on the basis of reduction in AIC values. Variance inflation factors were < 2.3 . Where no p-values are reported, the null model was preferred.

$\delta^{13}\text{C}$							
	Liver	Red muscle	White muscle	Scale	d.f.	F	r^2 (adj)
W_r	-0.03 (0.01)	-	-	-	1, 24	7.67	0.21
$\%Lipid_{EF}$	-2.05 (< 0.001)	-	-	-	1, 24	38.91	0.60
$\delta^{13}\text{C}_{LE}$							
W_r	-	-	-	-		-	
$\%Lipid_{EF}$	-	-	-	-		-	
$\delta^{15}\text{N}$							
W_r	-0.09 (0.018)	0.07 (0.044)	-	-	2, 23	3.3	0.16
$\%Lipid_{EF}$	-2.77 (0.028)	-	2.49 (0.074)	-	2, 23	2.77	0.12

isotope values within each tissue are shown in Table 4.2. The most striking relationships were found between bulk $\delta^{13}\text{C}$ values in the liver and both the somatic condition factor and whole fish lipid content; fish in good condition with higher lipid reserves had significantly lower liver $\delta^{13}\text{C}$ values. Condition factor and whole fish lipid content were significantly and negatively related to bulk liver $\delta^{15}\text{N}$ values but positively related to red and white muscle $\delta^{15}\text{N}$ values, respectively.

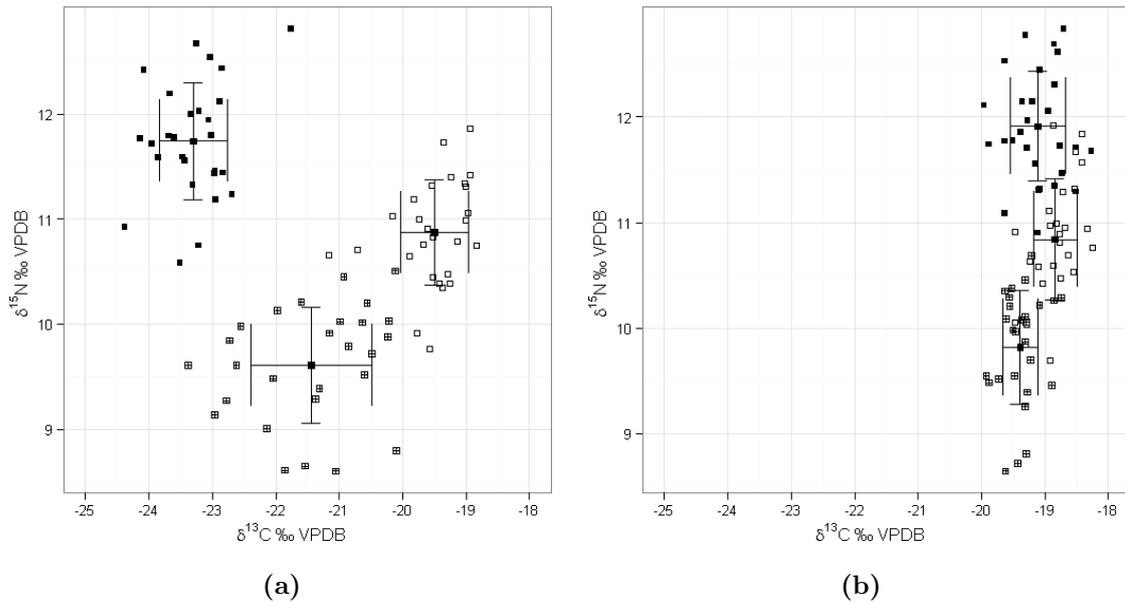


Figure 4.2: Variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the liver (\boxtimes), red muscle (\blacksquare) and white muscle (\square) of 1SW wild Atlantic salmon analysed in bulk (a) and after lipid extraction (b).

Discussion

This study compared the commonly used trophic proxies, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, in the tissues of returning wild 1SW Atlantic salmon to two measures of fish quality: somatic condition factor (W_r) and lipid reserves (% lipid in whole homogenized fish). Uniquely, the study included isotopic measurements of four different tissues from each individual fish, that have different metabolic functions and isotopic turnover rates, thus providing a more detailed analysis of the physiological implications of isotopic discrimination.

Effect of lipid extraction

As expected, the removal of isotopically 'light' lipids during the lipid extraction process significantly increased $\delta^{13}\text{C}$ values of all soft tissues and significantly reduced $\delta^{13}\text{C}$ variation in liver and white muscle. That $\delta^{15}\text{N}$ values were not significantly affected by lipid extraction may be due to the use of semi-polar solvents and high temperatures used in the ASE protocol (Bodin et al. 2009). Other lipid extraction techniques have had wide-ranging effects on fish tissue $\delta^{15}\text{N}$ values (Trueman et al. 2005, Sweeting et al. 2006, Logan & Lutcavage 2008, Elsdon et al. 2010), invalidating

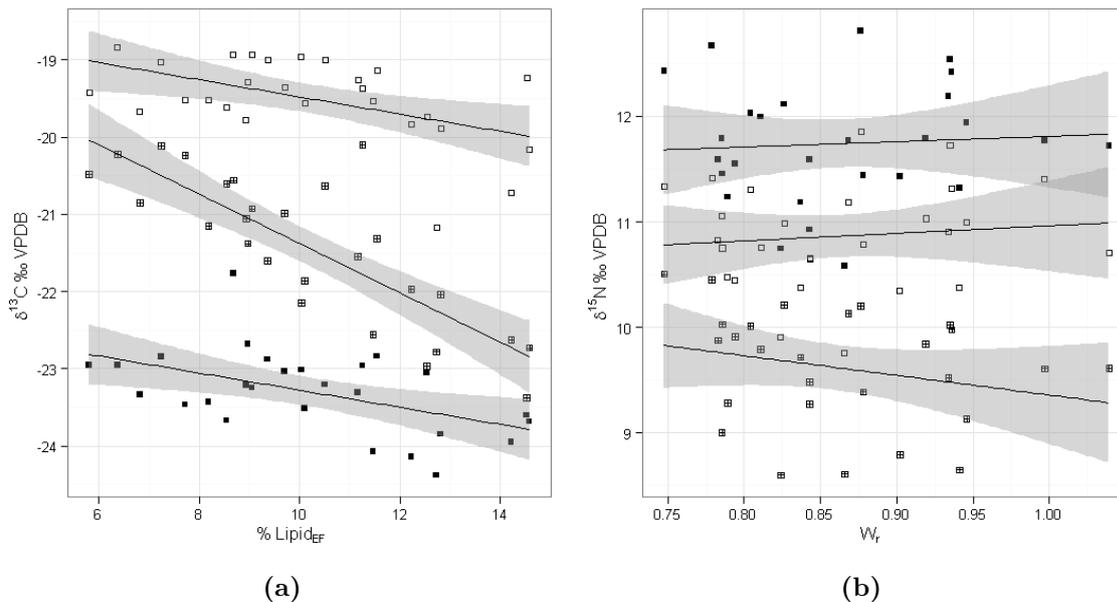


Figure 4.3: The relationship ($\pm 1SD$) between tissue $\delta^{13}C_{\text{bulk}}$ values and the whole Atlantic salmon lipid content (%) determined by the Bligh & Dyer method (a) and the relationship between $\delta^{15}N_{\text{bulk}}$ values and condition factor (b). Liver (\boxplus), red muscle (\blacksquare) and white muscle (\square).

assumptions that a standard technique may be applied to all fish tissues. The use of ASE protocols for lipid extraction of soft fish tissue samples is recommended but, when available, it is most informative to run samples in duplicate for $\delta^{13}C$ and $\delta^{15}N$ of both bulk and lipid-extracted material as in the present study.

$\delta^{13}C$ and $\delta^{15}N$ variation among tissues

Mean $\delta^{13}C$ and $\delta^{15}N$ values of bulk white muscle and liver reported in the present study were similar to those reported for Atlantic salmon in other studies (Trueman et al. 2005, Dempson et al. 2010). Individual Atlantic salmon ($n = 27$) displayed a consistent pattern of isotopic enrichment among tissues. The pattern of ^{13}C -enrichment in Atlantic salmon soft tissues showed that white muscle values $>$ liver values $>$ red muscle values whereas the pattern of ^{15}N -enrichment in Atlantic salmon soft tissues was red muscle $\delta^{15}N$ values $>$ white muscle $>$ liver. The isotopic signature of scale collagen was very different from that of soft tissues.

Variation in the C and N stable isotope ratios of different tissues within an individual generally can be attributed to a) biochemical composition b) isotopic 'routing'

and c) differential protein turnover rates (Pinnegar & Polunin 1999, Jim et al. 2004, Phillips & Eldridge 2006, del Rio et al. 2009, Newsome et al. 2010). Isotopic routing is unlikely to play a major role in determining differences in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values among the soft tissues analysed, because salmon are carnivorous and obtain protein, lipids and carbohydrates from similar dietary sources (del Rio et al. 2009). The separation of stable isotope ratios between the various tissues analysed in the present study can be explained primarily by variation in lipid content and amino acid composition, and the isotopic composition of individual compounds; and by the different temporal scales of isotopic integration.

During the synthesis of lipids, the lighter isotope of carbon, ^{12}C , is preferred as pyruvate is oxidised to form acetyl coA (DeNiro & Epstein 1977) causing the $\delta^{13}\text{C}$ value of lipids to be much lower than that of other molecules. Commonly, tissues with a high lipid content have disproportionately low $\delta^{13}\text{C}$ values (Focken & Becker 1998, Tieszen et al. 1983) reflecting this process. Using $\delta^{13}\text{C}_{bulk}$ values as a proxy for lipid content, the results suggests that lipid concentrations are greatest in red muscle. Although accounting for a relatively small proportion of total fish mass, red muscle tissue of adult Atlantic salmon (including of those individuals analysed in the present study) does contain a high proportion of lipids (Doucett et al. 1999; A. Howe, pers. comm), reflecting the energetic demands on this tissue during the homing migration. Being a major constituent by weight of salmon carcasses, white muscle is an important lipid store but has a lower percent lipid than red muscle or liver tissue (Doucett et al. 1999, Pinnegar & Polunin 1999; A. Howe, pers. comm); accordingly, $\delta^{13}\text{C}$ values were lowest in this tissue. Liver is an important site of lipid metabolism and had the highest concentration of lipids in juvenile rainbow trout (*Onchorhynchus mykiss*) (Pinnegar & Polunin 1999), although in spawning Atlantic salmon, red muscle tissue contained the most lipid (Doucett et al. 1999) followed by liver tissue. This difference most likely reflects a change in the allocation of lipid reserves with age and reproductive stage; pre-spawning adult Atlantic salmon are likely to have high red muscle lipid contents illustrative of the high energetic demands of upstream migration (Jonsson et al. 1997, Doucett et al. 1999). The liver $\delta^{13}\text{C}$ values intermediate between red and white muscle in the present study are in agreement with the results of Doucett et al. (1999).

This suggests that the majority of among-tissue variation in $\delta^{13}\text{C}$ values can be ascribed to differences in biochemical (i.e. lipid) composition; however, significant differences between soft tissues also existed after lipids had been extracted. The gen-

eral pattern after lipid extraction was for high $\delta^{13}\text{C}$ values in white muscle followed by red muscle and then liver. These 'residual' discrepancies may be attributable to variable rates of isotopic incorporation between the tissues, which in turn is closely related to tissue protein turnover rate (del Rio et al. 2009).

Similar to $\delta^{13}\text{C}$, the consistent among-tissue pattern of ^{15}N depletion is likely a product of variation in tissue amino acid composition and protein turnover rates. Fish liver tissue tends to have low $\delta^{15}\text{N}$ values relative to muscle tissues (Pinnegar & Polunin 1999, Jardine et al. 2005, Perga & Gerdeaux 2005, Trueman et al. 2005), although in cases of severe nutritional deprivation, $\delta^{15}\text{N}$ values of liver tissue can exceed those of muscle (Jardine et al. 2004, Doucett et al. 1999). Evidence for this degree of starvation was not found in the present study. The $\delta^{15}\text{N}$ value of essential/indispensable amino acids, which cannot be synthesized by consumers but are derived directly from dietary protein, tend to resemble $\delta^{15}\text{N}$ values of the diet ("source") whereas non-essential/dispensable amino acids, which may be incorporated directly from dietary protein or wholly synthesized *de novo* within the consumer, can exhibit considerable enrichment in ^{15}N (Gaebler et al. 1966, del Rio et al. 2009). Therefore differences in the relative abundance and $\delta^{15}\text{N}$ values of individual amino acids in the tissues of Atlantic salmon may drive bulk tissue $\delta^{15}\text{N}$ value. Without experimentally rearing salmon and measuring amino acid composition and $\delta^{15}\text{N}$ values, it is difficult to draw conclusions from the present data because of the complexity of stable isotope fractionation and nutritional physiology (del Rio et al. 2009). As a generalisation, low $\delta^{15}\text{N}$ values of fish liver likely reflect the greater proportion of indispensable amino acids in this tissue (Trueman et al. 2005, Pinnegar & Polunin 1999) whereas the relative abundance of dispensable amino acids, such as taurine, in muscle tissues may account for ^{15}N enrichment (van der Boon et al. 1989, Pinnegar & Polunin 1999).

Scale stable isotope values were markedly different from soft tissues from the same individual; a $\Delta\delta^{13}\text{C}_{\text{muscle-scale}} \approx 2\text{-}4\text{‰}$ and $\Delta\delta^{15}\text{N}_{\text{muscle-scale}} \leq 1\text{‰}$ is not uncommon in other studies (Satterfield & Finney 2002, Pruett et al. 2003, Estrada et al. 2005, Sinnatamby et al. 2008). Scale tissue is composed primarily of collagen (Zylberberg et al. 1992), with an upper carbonate layer (EL; Figure 4.1), and once material is deposited it is considered metabolically inert so that stable isotope signatures are expected to reflect those of dietary protein at or near the time of deposition (Tieszen et al. 1983, Jim et al. 2004, Trueman & Moore 2007b). The outer scale portion excised for analysis should integrate stable isotope variation deposition over

the last 6 - 8 months of salmon marine migration (i.e. from mid-winter until time of capture) but due to underplating of consecutive collagen layers (Hutchinson & Trueman 2006), it may be biased towards material deposited early in the final spring period and therefore scale stable isotope values represent a different, or longer-term, dietary signal from that of muscle tissues. Additionally, the abundance of the amino acid glycine (~33% of fish scale collagen Ikoma et al. 2003) in fish scales might represent an incidence of isotopic routing of a relatively ^{13}C -enriched amino acid (Fantle et al. 1999, Hare et al. 1991) to a specific tissue (Estrada et al. 2005).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation among individuals

In order to assess the magnitude and timing of environmental (i.e. trophic) effects on the health of returning 1SW Atlantic salmon, linear relationships were characterised between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of four tissue types and two metrics of fish quality, condition factor (W_r) and whole fish lipid content (%). For both stable isotope ratios, significant relationships were found in the liver tissue; as the most 'metabolically active' tissue analysed, this result suggests that trophic effects within the last weeks to month at sea are important determinants of ultimate Atlantic salmon condition and energy reserves. All relationships were weak, however, and there was a large degree of scatter within the data indicating that other processes besides simple shifts in trophic level were at work.

Condition indices and $\delta^{13}\text{C}$ Bulk liver $\delta^{13}\text{C}$ values were negatively related to both condition factor and whole fish lipid content; a ~1‰ decline in $\delta^{13}\text{C}$ values amounted to an increase in W_r of ~0.4 and 2% in lipid content. The liver is a major site of *de novo* fatty acid synthesis and metabolism in salmon (Lin et al. 1977, Arnesen et al. 1993); observed inter-individual variation in the $\delta^{13}\text{C}$ of this tissue could be a consequence of either or both of (a) variation in the $\delta^{13}\text{C}$ values of the lipid compounds ($\delta^{13}\text{C}_{lipid}$) themselves and (b) variation in the concentration of lipids within liver tissue. The distinction between these is important to unravel because if (a) were true, experimental evidence suggests that lower $\delta^{13}\text{C}_{lipid}$ values are indicative of a deficit of lipid in the diet, forcing fish to produce the majority of necessary lipid compounds *de novo* (Gaye-Siessegger et al. 2004a). The results of the present study would thus suggest that fish returning with a high condition factor and greater lipid reserves suffered suboptimal foraging conditions at sea (i.e. a lack of sufficient dietary lipids), which seems counter-intuitive. In contrast, if (b)

were true, the results suggest that fish in good condition and greater total body lipid stores also have higher lipid concentrations in the liver.

Neither $\delta^{13}\text{C}_{\text{lipid}}$ nor lipid content of liver was measured directly in this study, but because tissue stable isotope ratios were determined both before and after lipid extraction, there was a surrogate measure of tissue lipid content that helped to determine the extent to which (b) might be true. C:N ratios are often used as proxies for lipid content under the assumption that because lipids are composed primarily of carbon with little nitrogen, tissues with higher lipid concentrations will have higher C:N ratios (Sweeting et al. 2006, Logan & Lutcavage 2008 but see Fagan et al. 2011). Therefore the change in C:N ratios of liver tissue before and after lipid extraction ($\Delta\text{C:N}_{\text{bulk-LE}}$) provides an indicator of lipid content. The highly significant relationship ($p < 0.001$, $t\text{-value} = -12.90$, $b = -1.02$, $r^2 = 0.88$, Figure 4.4) between this indicator and bulk liver $\delta^{13}\text{C}$ values provides strong evidence for interpretation (b). Furthermore, Focken & Becker (1998) showed that whole fish lipid content is linearly and negatively related to $\delta^{13}\text{C}$ value of whole homogenised fish carcasses and Doucett et al. (1999) provided evidence for a linear relationship between liver $\delta^{13}\text{C}$ values and liver lipid content in adult Atlantic salmon.

Doucett et al. (1999) also showed that as adult Atlantic salmon move upstream during the spawning migration, $\delta^{13}\text{C}$ values of red muscle, white muscle and especially liver increase - concurrent with a decline in the lipid content of these tissues. The authors propose that this process reflects the mobilisation or catabolism of free fatty acids (FFAs) as lipid stores are depleted during sustained swimming activity. In the present study, poor condition fish are analogous to those most advanced in the upstream migration in the study of Doucett et al. (1999); however, the fact that only liver tissue shows a negative relationship with condition factor can be taken as evidence that fish returning in poor condition, albeit low on total body lipid reserves, have not yet begun to mobilise lipids store in red or white muscle.

Perhaps most interesting is that no significant relationships existed with salmon body condition or lipid content once tissue lipids had been extracted. $\delta^{13}\text{C}$ of lipid-extracted tissues can be expected to vary with the geographic source of carbon in the diet and also with trophic level; the results, albeit from a limited sample size, thus suggest a broadly common geographical foraging area for returning 1SW salmon in the last months of their marine migration in 2009.

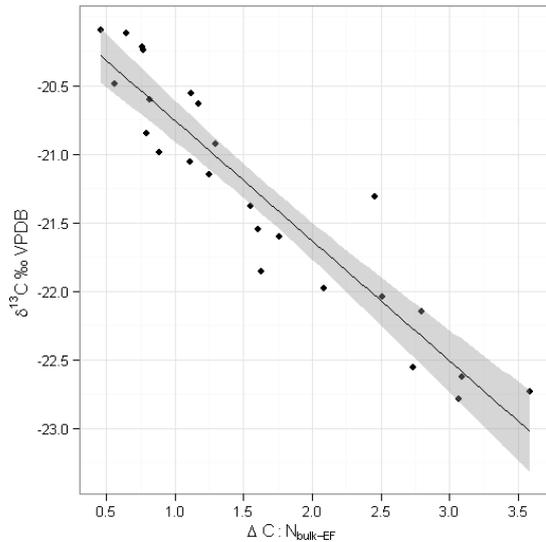


Figure 4.4: The linear relationship ($\pm 1SD$) between $\delta^{13}C$ values of bulk liver tissue and liver $\Delta C:N_{bulk-LE}$ (a proxy for tissue lipid content) in wild 1SW Atlantic salmon.

Condition indices and $\delta^{15}N$ Similar to $\delta^{13}C_{bulk}$ values, both condition factor and whole fish lipid content were negatively related to $\delta^{15}N$ values in liver tissue; however, the trend was reversed in white muscle. $\delta^{15}N$ values are commonly considered a proxy for trophic level (DeNiro & Epstein 1981, Zanden & Rasmussen 2001, McCutchan et al. 2003). However, the magnitude of enrichment in ^{15}N with trophic level is neither consistent across taxa (Vanderklift & Ponsard 2003) nor amongst individuals because processes such as variation in growth rate (Trueman et al. 2005), diet quality (Gaye-Siessegger et al. 2003), and feeding level (Gaye-Siessegger et al. 2004b, Jardine et al. 2004, Guelinckx et al. 2007) can significantly change the magnitude of tissue to diet isotopic spacing ($\Delta\delta^{15}N_{consumer-diet}$) and confound attempts to reconstruct historical diet. In the present study, the aim was not to reconstruct estimates of absolute trophic level or different dietary contributions (e.g. using stable isotope mixing models; Moore & Semmens 2008, Parnell et al. 2010) but simply to test for any trophic effects on salmon quality. Furthermore, because all tissue samples were taken from individual adults at a similar life stage, it is unlikely that variation in growth rate would significantly affect tissue stable isotope ratios.

White muscle tissue $\delta^{15}N$ values incorporate dietary information extended over several months (Jardine et al. 2004, Trueman et al. 2005, Sakano et al. 2005, Guelinckx et al. 2007, Church et al. 2009, Hammond & Savage 2009, Buchheister

& Latour 2010) and may be considered a reflection of longer-term diet whereas the rapid turnover of liver provides information regarding the most recent diet (Trueman et al. 2005, Guelinckx et al. 2007). Given these estimates for fish muscle tissue, the positive relationship between condition factor and red muscle $\delta^{15}\text{N}$, and whole fish lipid content and white muscle $\delta^{15}\text{N}$ values, suggests that feeding at a higher trophic level (i.e. with a greater proportion of forage fish and squid in the diet) during the final months at sea, may ultimately result in better body condition and higher energy reserves. However, the change in the slope of the relationship for liver tissue - although of a similar magnitude - indicates that either salmon shift trophic levels when close to coastal waters (i.e. poor condition fish begin to feed at higher trophic levels), or that catabolic processes begin to have an effect on poor condition fish during this time. A lack of dietary intake can elevate tissue $\delta^{15}\text{N}$ values in a fractionation process similar to trophic fractionation except that the organism begins to re-process old protein rather than new. Given that the guts of all fish analysed in this study were empty and that loss of appetite and cessation of feeding in maturing salmon is likely to begin at sea (Jacobsen & Hansen 2000), the latter hypothesis is preferred. Gaye-Siessegger et al. (2004b) found that decreasing feeding rates were correlated to an increase in $\delta^{15}\text{N}$ values of whole Nile tilapia (*Oreochromis niloticus*) and Guelinckx et al. (2007) reported a significant effect of food deprivation on $\delta^{15}\text{N}$ values of liver tissue in the sand goby (*Pomatoschistus minutus*), but not in white muscle - which was attributed to the more rapid metabolic turnover of liver tissue manifesting the catabolic effects of starvation more rapidly. Liver $\delta^{15}\text{N}$ values also increased by nearly 2‰ in wild Atlantic salmon ascending freshwater rivers to spawn (a time when little to no feeding occurs Doucett et al. 1999). A similar process could be responsible for the results in the present study; a lack of sufficient dietary protein during the final weeks at sea would increase the $\delta^{15}\text{N}$ of the existing N pool available for liver metabolism. Subsequently, liver $\delta^{15}\text{N}$ in those fish experiencing a degree of nutritional stress would be elevated. In addition to evidence provided by liver $\delta^{13}\text{C}$ values, liver $\delta^{15}\text{N}$ values imply that poor condition fish experience earlier onset of the effects of starvation than those returning in good condition and with high lipid reserves.

Conclusions

This study was greatly improved by the analysis of multiple tissues. Studies of wild fish populations tend to focus on dorsal white muscle (e.g. Jardine et al.

2008, Dempson et al. 2010, Box et al. 2010) which can provide useful discrimination between groups; however, where the variable of interest occurs between individuals and is likely to have physiological consequences - such as somatic condition - stable isotope ratios from multiple tissues may prove most informative. There is still a great deal of uncertainty surrounding the usage of stable isotope analysis in understanding the performance of wild individuals and populations (see del Rio et al. 2009). Where laboratory experiments are not feasible for the animals of interest, stable isotope analyses in the future may be improved by analysing multiple tissues, pre- and post-lipid extraction and by combination with other analytical methods such as fatty acid analysis and enzymatic activity assays.

The present study provided little evidence for significant environmental effects governing trophic dynamics of returning Atlantic salmon and their ultimate return condition in 2009 - shifts in muscle stable isotope values characteristic of whole trophic levels ($\Delta\delta^{15}\text{N} \approx 2.5\text{‰}$; $\Delta\delta^{13}\text{C} \approx 0.5\text{‰}$; McCutchan et al. 2003) were related to relatively small shifts in W_r or lipid reserves. However, because these results are restricted to a single year and relatively small number of individuals, this result does not directly address the question: what process(es) ultimately are responsible for changes in the quality of returning Atlantic salmon. Longer-term studies combining indices of quality, climate and trophic dynamics are necessary to address this.

By focusing on a single year and analysing multiple tissues from the same individual, the present study does provide evidence for an important finding: that many 1SW Atlantic salmon arriving at coastal locations en route to their natal freshwater rivers are already experiencing nutritional stress. That a greater proportion of these fish are returning to southern European rivers in recent years (Todd et al. 2008, Bacon et al. 2009), and are likely at an enhanced and sustained risk of starvation prior to and during their freshwater spawning migration, should be of major concern to those interested in both the marine and freshwater management of these stocks.

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5 Towards high resolution micro-sampling of intra-otolith $\delta^{18}\text{O}$ values in wild Atlantic salmon (*Salmo salar*)¹

ABSTRACT: The chemical signals in the sequential layers of fish otoliths have the potential to provide fisheries biologists with temporal and spatial details of migration which are difficult to obtain without expensive tracking methods. Signal resolution depends, however, on the extraction technique used. The use of mechanical micromilling and continuous flow isotope ratio mass spectrometry (CF-IRMS) methods were compared with secondary ion mass spectrometry (SIMS) to obtain $\delta^{18}\text{O}$ profiles from otoliths of wild Atlantic salmon (*Salmo salar*) and these used to corroborate the time of freshwater emigration of the juvenile with macroscopic patterns within the otolith. Both techniques showed the transition occurring at the same visible feature on the otolith, allowing future analyses to easily identify the juvenile (freshwater) versus adult (marine) life-stages. However, SIMS showed a rapid and abrupt transition whereas micromilling provided a less distinct signal. The number of samples that could be obtained per unit area sampled using SIMS was 2 to 3 times greater than that when using micromilling/CF-IRMS although the $\delta^{18}\text{O}$ values and analytical precisions ($\sim 0.2\%$) of the two methods were comparable. In addition, SIMS $\delta^{18}\text{O}$ results were used to compare otolith aragonite values with predicted values calculated using various isotope fractionation equations.

¹N.N. Hanson, C.M. Wurster, EIMF & C. D. Todd (2010) Comparison of secondary ion mass spectrometry and micromilling/continuous flow isotope ratio mass spectrometry techniques used to acquire intra-otolith $\delta^{18}\text{O}$ values of wild Atlantic salmon (*Salmo salar*). Rapid Communications in Mass Spectrometry 24:2491-2498. doi: 10.1002/rcm.4646. Reprinted with the kind permission of John Wiley & Sons.

Introduction

Otoliths or “ear stones” are paired inner ear structures of teleost fish that function in hearing and balance (Popper & Coombs 1982). Typically, they are comprised of calcium carbonate (usually of the polymorph aragonite, but may include the polymorph vaterite) and organic matrices, including the protein otolin (Murayama et al. 2002). As a fish grows its otoliths increase in size by the accretion of mineral layers precipitated from the surrounding endolymphatic fluid within the inner ear (Campana 1999). Because otoliths accrete acellularly and are metabolically inert after formation, they can be considered as permanent “natural tags” that provide a time-series of water chemistry encountered by the fish throughout its lifetime (Campana 1999). Stable oxygen isotope ($\delta^{18}\text{O}$) values of otolith aragonite have been shown to precipitate at or near physico-chemical equilibrium with the ambient water composition for many species, with fractionation mediated by temperature (Campana 1999, Thorrold et al. 1997, Høie et al. 2004b, Kalish 1991, Storm-Suke et al. 2007). Thus, provided that the $\delta^{18}\text{O}$ values of ambient waters are known or can be estimated, the water temperature during deposition of otolith aragonite can also be estimated. Several temperature dependent $\delta^{18}\text{O}$ fractionation equations have been developed for otolith aragonite (Thorrold et al. 1997, Høie et al. 2004b, Storm-Suke et al. 2007, Patterson et al. 1993) as well as inorganic aragonite (Kim et al. 2007). Despite a lack of evidence for kinetic or metabolic effects on oxygen isotope fractionation (Thorrold et al. 1997, Høie et al. 2003), the intercepts of these equations differ significantly, suggesting that inter-specific differences in some mechanism, such as metabolic rate (Høie et al. 2004b, Storm-Suke et al. 2007), can influence the equilibrium relationship, making the use of a single universal equation describing temperature-mediated $\delta^{18}\text{O}$ fractionation inappropriate. Nonetheless, the use of species-specific equations or those derived for closely related species can provide accurate information regarding thermal histories (Høie et al. 2004b, Storm-Suke et al. 2007).

That $\delta^{18}\text{O}$ values of otolith aragonite can adequately reflect ambient water conditions is of considerable importance to fisheries biologists interested in gaining a better understanding of the migratory behaviour and thermal requirements of animals which can be notoriously difficult (and expensive) to track using conventional tag or telemetry methods (Elsdon & Gillanders 2003). Tracing ontogenetic changes in marine habitat exploitation and distribution by means of intra-otolith $\delta^{18}\text{O}$ values

has been applied to many commercially and ecologically important species, including haddock (Begg & Weidman 2001), cod (Gao 2002), orange roughy (Shephard et al. 2007), halibut (Gao & Beamish 2003) and sockeye salmon (Gao & Beamish 1999). Extraction of annual or seasonal samples from otolith layers previously has relied on developments in mechanical micromilling (Shephard et al. 2007, Gao 1999, Wurster et al. 1999) but secondary ion mass spectrometry (SIMS) has now greatly increased the potential resolution of otolith profiles (Weidel et al. 2007). The small beam size, and the ability to sample spots as close as $\sim 30\mu\text{m}$, together with increased precision, allows otolith isotope profiles to be obtainable perhaps to a daily resolution, depending on the accretion rate and size of otolith layers. SIMS can eliminate the need for physical separation of carbonate in discrete layers and provide insight into fine-scale differences in individual behaviour and environmental history.

The Atlantic salmon (*Salmo salar*) is an anadromous species. Adults spawn in freshwater and the juveniles grow slowly for 1-5 years prior to smoltification in springtime and downstream migration to sea. Population mean river ages (i.e. age at smoltification) tend to vary with latitude, but most smolts in Scotland are either 2 or 3 years old (at ~ 30 g weight) at migration (Metcalf & Thorpe 1990, Todd et al. 2010). Post-smolts may return to freshwater the following summer as mature adults after only one winter at sea (so-called one sea-winter fish; 1SW; $\sim 1-5$ kg) or may remain at sea for 2-5 winters before returning at a larger size as multi sea-winter (MSW) adults. Scottish 1SW adults typically migrate to the Norwegian Sea in the eastern North Atlantic, whereas a large proportion of Scottish MSW fish range as far as West Greenland during their marine migration (Hansen & Quinn 1998). Although salmon in the marine environment probably migrate to ocean areas of a relatively narrow temperature range (Friedland et al. 1998, 2003, Reddin & Shearer 1987), such wide-ranging migration destinations may be characterized by differences in physico-chemical parameters (e.g. trace elements or isotopes) of surface ocean waters reflected in the organism's tissues, including the otoliths.

Micro-structural layers of the otolith are visible only with magnification but macro-structural bands of opaque and translucent material reflecting seasonal and/or annual patterns of accretion are easily visible on a polished section under low magnification. The reasons for visible differences in the opacity of these layers are not completely understood but thought to result from differences in the relative mineral and organic concentrations of the zones (Høie et al. 2008, Jolivet et al. 2008), which has been linked to variations in temperature (Høie et al. 2008) and feeding regime

(Høie et al. 2009). Macroscopic bands of opaque and translucent material correspond to seasonal growth and can thus often be annually resolved (Høie et al. 2008, Campana & Thorrold 2001). In 1SW Atlantic salmon, deposition of microincrements during the high-growth marine phase has been estimated to be approximately every 8 days based on a study of pen-reared fish (Wells et al. 2003); thus, visualization of both of macro- and micro-structural bands in relation to isotope sampling frequency can be used to estimate temporal resolution. If sampling is possible at a monthly resolution, $\delta^{18}\text{O}$ profiles across the entire otolith of Atlantic salmon should reflect 1) annual seasonality in the freshwater habitat that drives changes in $\delta^{18}\text{O}_{\text{water}}$, 2) the shift in $\delta^{18}\text{O}_{\text{water}}$ at the freshwater:marine migratory transition and 3) differences in $\delta^{18}\text{O}_{\text{water}}$ encountered during the ocean migration.

Here, the spatial (i.e. temporal) resolution and precision of $\delta^{18}\text{O}$ profiles derived from the micromilling/CF-IRMS and SIMS techniques are compared in wild Atlantic salmon otoliths. The aim was to relate the ontogenetic shifts in $\delta^{18}\text{O}$ values (e.g. the freshwater to seawater transition for the smolt stage) to macro-structural banding (i.e. annuli) in the otolith in order to visually determine the area of the otolith representative of the juvenile freshwater and adult marine phases. This visual discrimination can help guide future isotope analyses to either or both phases of the life cycle. These data also permitted a preliminary assessment of spatio-temporal otolith thermometry, with specific reference to its potential for discriminating patterns of individual fish behaviour during their marine migrations.

Materials & Methods

Otolith collection and preparation

The sagittae of Atlantic salmon are the largest of the three pairs of otoliths. These were dissected from the heads of two wild 1SW salmon caught in mixed stock fisheries during the netting seasons (June-August) at Strathy Point (58.58° N, 4.02° E) in 2007 and from two 1SW fish caught 4 miles east at the Melvich fishery (58.57° N, 3.92° E) (North Scotland) in 2008. Samples from two different fisheries were used due to the closure of Strathy Point fishery in 2007. Salmon from the two years do, however, share the same life history/maturation schedule in being 1SW adults. Length and weights were measured and a sample of scales was taken to determine river age and confirm that fish had spent one winter at sea and their age at smolt

emigration. Otoliths were cleaned of any membrane, rinsed with deionised water and stored in sterile Eppendorf tubes until further analysis. Each otolith was set in round epoxy resin mounts (24.5mm), ground to the core (otolith origin) and polished along the sagittal plane (Figure 5.1). Because of the differences in otolith size between individuals, image analysis software (Rasband 1997-2009) was used to estimate the number of samples obtained per unit area of exposed otolith; this facilitated comparison between the two sampling techniques, albeit from different otoliths. Otoliths from Strathy fish ST050702 and ST090803 (caught in 2007) were prepared for destructive micromilling whereas otoliths from Melvich fish M063 and M011 (caught in 2008) were prepared for SIMS analyses.

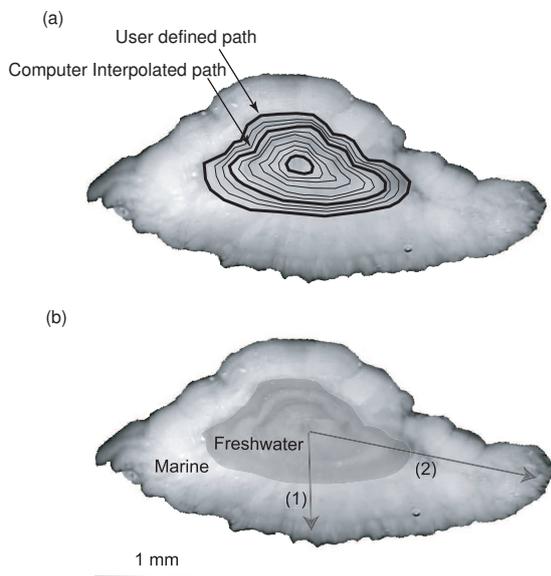


Figure 5.1: Reflected light image of a wild Atlantic salmon (1SW) otolith polished in the sagittal plane showing the alternating macro-structural annuli of opaque and translucent layers. (a) User-defined paths are created by following the outline of annuli and paths were interpolated between these at regular intervals by computer software. (b) The portion of the otolith corresponding to the juvenile, freshwater life stage is shown in grey. The axis of SIMS spot transects (1) and the axis of measurement for micromilling/CF-IRMS samples (2) are also given.

Micromilling/CF-IRMS

The polished otoliths were attached to a New Wave Research™ micro-mill sample plate (Scottish Universities Environmental Research Centre, East Kilbride, Scotland) and sample paths were defined in three dimensions following the contours of the externally visible annual translucent and opaque bands (Figure 5.1). Intermediate paths were interpolated by the computer software between user-defined paths every 80-100µm and milled using the edge of a micro dental drill (0.3mm) from the centre (core) outwards to the edge of the otolith (Wurster et al. 2005). Micromilling followed the banding pattern around the entire otolith and milling depth was set at 40µm. The sample point at which paths crossed the final opaque band was noted – the latter was generally identifiable as the third annual band for fish that had spent two years in freshwater as a juvenile. 50-100µg of sample carbonate was collected using mini-scalpel blades and stored in silver capsules prior to analysis. Consecutive samples were combined when insufficient material was obtained from a single path. Stable oxygen isotope values were determined using an online gas preparation and introduction system (ThermoFinnigan GasBenchIII, Stafford House, Boundary Way, Hemel Hempstead, Hertfordshire HP2 7GE) coupled to a ThermoFinnigan DeltaPlus-XL mass spectrometer (University of St Andrews Facility for Earth and Environmental Analysis). Carbon dioxide was evolved from the sample by reaction with 100% orthophosphoric acid in exetainer vials after atmosphere was replaced with He. Repeat samples of NBS-18 and NBS-19 (international standards) and in-house Carrara marble standards of similar mass to unknown samples provided monitoring of accuracy and precision. Precisions (\pm SD) for internal standards were $<0.15\text{‰}$ for oxygen.

SIMS

Reflected light microscope photographs of the sample mount guided the sampling procedure. Oxygen isotope ratios were measured with a CAMECA-IMS-1270 ion microprobe (#309) (University of Edinburgh) using a ~ 5 nA primary $^{133}\text{Cs}^+$ beam. Secondary ions were extracted at 10 kV, and $^{16}\text{O}^-$ ($\sim 3.0 \times 10^9$ cps) and $^{18}\text{O}^-$ ($\sim 4.0 \times 10^6$ cps) were monitored simultaneously on dual Faraday cups (L'2 and H'2). Each spot analysis involved a pre-sputtering time of 50 s, followed by automatic secondary beam and entrance slit centering and finally data collection in two blocks of five cycles, amounting to a total count time of 40 s. Otolith isotope measurements were

bracketed by 5-10 spot analyses of an internal reference material (UWC-1 calcite) to assess instrument precision.. A beam size of 15 μm was used for $\delta^{18}\text{O}$ measurements, with a step size of 50 μm . These values were chosen as conservative parameters for preliminary analyses. The sampling transect originated near the core of the otolith and terminated at the dorsal edge. Reproducibility of the standard calcite measurements was $< 0.24\%$. For all isotope analyses, $\delta^{18}\text{O}_{\text{otolith}}$ values are reported relative to Vienna Pee Dee Belemnite (VPDB; Coplen 1996).

X-ray diffraction

Otolith material remaining within resin mounts was excavated using micro-scalpel blades, crushed in acetone and scanned using a Philips PW1050/Hiltonbrooks DG2 X-Ray diffractometer (XRD) to determine mineral content, using a zero background sample holder. Analysis was from 23 to 40 2σ lattice plane with a step size of 0.2 degrees for 5 seconds per step. Precisions for this instrument typically are better than 0.01 degrees 2σ and the limit of detection is 0.5-1%.

Otolith thermometry

It was confirmed that all four otoliths used in this study were composed of aragonite and did not contain the calcium carbonate polymorph vaterite, allowing a preliminary assessment of presently available equations for otolith thermometry (Thorrold et al. 1997, Høie et al. 2004b, Storm-Suke et al. 2007, Patterson et al. 1993, Høie et al. 2004a). Only the two high-resolution SIMS profiles (5.2b) were used for this exercise because a) the abrupt shift to marine $\delta^{18}\text{O}$ values allowed determination of a discrete ‘marine zone’ and b) we are interested in discrete ‘snapshots’ of potential geographic distribution that would be obscured by the integrative nature of micromilling the sample. In the absence of a specific fractionation equation for wild Atlantic salmon, several equations were explored to attempt a first assessment of the potential for salmon otolith thermometry; it was expected that the equation for the salmonid genus *Salvelinus* (Storm-Suke et al. 2007) would be most appropriate. The $\delta^{18}\text{O}_{\text{otolith}}$ values, sea surface temperature (SST) months and fractionation equations used are presented in Table 5.2 along with the maximum and minimum latitude inferred from each equation. To back-calculate oxygen isotope values of otolith aragonite assuming equilibrium fractionation, an understanding of the ambient temperature and $\delta^{18}\text{O}$ of the water ($\delta^{18}\text{O}_{\text{water}}$) is required. The Geo-

chemical Oceans Sections Study (GEOSECS) gridded database of $\delta^{18}\text{O}_{\text{seawater}}$ values (LeGrande & Schmidt 2006) (available at: <http://data.giss.nasa.gov/o18data/>) and sea surface temperature (SST) data (National Oceanic & Atmospheric Administration's Optimally Interpolated SSTv2 data provided by the Earth System Research Laboratory Physical Sciences Division, Boulder, Colorado, USA, from their web site at <http://www.esrl.noaa.gov/psd/>) from 45.5° N to 88.5° N and -29.5° E to 29.5° E were used to explore the distribution of $\delta^{18}\text{O}$ values of aragonite ($\delta^{18}\text{O}_{\text{equilibrium}}$) precipitated in equilibrium with ambient water. SST data are considered applicable for this exercise because Atlantic salmon mainly inhabit surface (5-10m) waters with only periodic dives (Holm et al. 2006). The 'expected' $\delta^{18}\text{O}_{\text{equilibrium}}$ values were compared to those measured in the otoliths ($\delta^{18}\text{O}_{\text{otolith}}$) (\pm SD of SIMS standard measurements). Three identifiable values were extracted from each otolith profile: the first 'marine' value after smolt emigration (corresponding to the first positive $\delta^{18}\text{O}_{\text{otolith}}$ value), the highest value sampled in the outer translucent portion of the otolith (corresponding to winter temperature minima because high $\delta^{18}\text{O}$ values indicate lower water temperatures) and the final value obtained for each transect (corresponding to the second summer temperature near the place of capture). Because smolt emigration from freshwater is primarily during the month of May in Scotland (Shearer 1992a), February is the annual thermal minimum and June is the month immediately prior to capture for these fish, these months in 2007 and 2008 were used for estimates of $\delta^{18}\text{O}_{\text{equilibrium}}$ values. It is important to consider these sample values as estimates of the winter and summer signals because the 'true' start, winter and final values could be intermediate to the sampling spots.

Results

Salmon lengths, weights and otolith measurements are presented in Table 5.1. Annual bands were visible in sagittal sections of otoliths under low magnification (16x), appearing as alternating bands of opacity and translucency under reflected light (Figure 5.1b). The validity of inferred macro-structural annual banding visible in the otolith was independently corroborated by fish scale readings to confirm the river and sea age of each fish. Age estimation of Atlantic salmon by scale readings is a well established practice (Shearer 1992b) and was considered an appropriate validation of annual banding in the otolith. It became apparent that the outer, wide opaque otolith zone corresponded to the period of rapid growth associated with the

first summer of the marine phase, based on knowledge of the river and sea ages of the fish. For example, ST050702 spent two years as a juvenile in freshwater and the otolith showed three opaque bands corresponding to the two years of the freshwater phase and one year spent at sea.

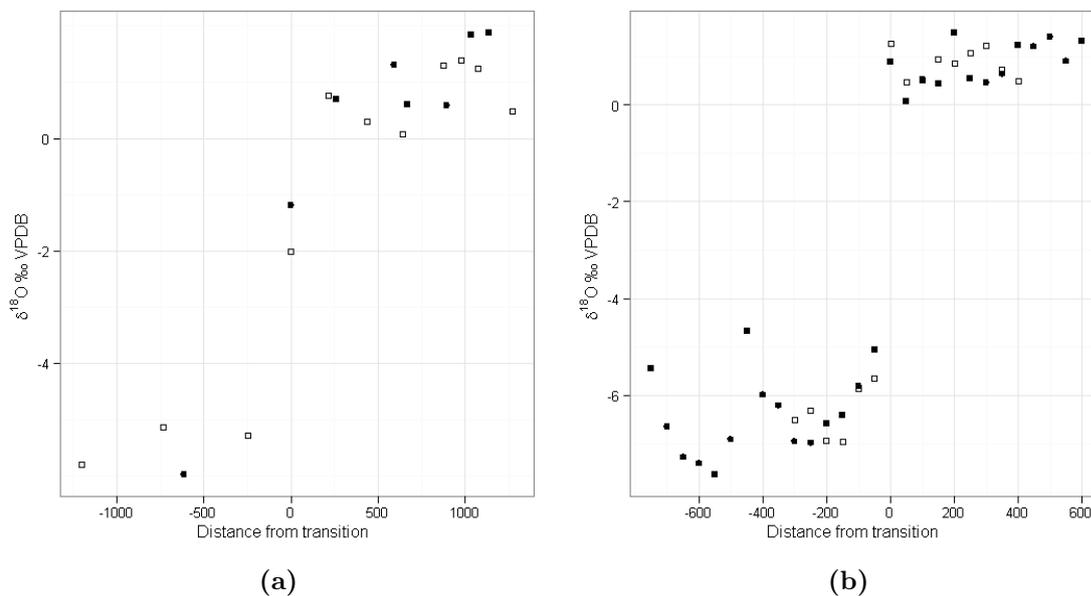


Figure 5.2: The pattern of $\delta^{18}\text{O}$ otolith values across wild Atlantic salmon (1SW) otoliths generated from (a) the micro-milling technique and (b) secondary ion mass spectrometry (SIMS). Profiles in (b) are standardised along the abscissa so that 0 mm corresponds to measurements at the start of the marine phase and error bars represent analytical precision. The grey area in both plots represents oxygen isotope values indicative of freshwater residency. Error bars represent \pm analytical error.

$\delta^{18}\text{O}$ values of otoliths measured using micromilling/CF-IRMS and SIMS

Eight and eleven $\delta^{18}\text{O}_{\text{otolith}}$ samples were obtained by micromilling ST050702 and ST090803, respectively. This corresponded to 1.19 and 1.20 samples per mm^2 of otolith surface. Between $76\mu\text{m}$ and $489\mu\text{m}$ of micromilling paths (measured from the core to the rostrum) were necessary to obtain sufficient material ($\sim 80\mu\text{g}$) for isotope analysis using CF-IRMS. $\delta^{18}\text{O}$ values varied from a minimum of -6.0‰ to a maximum of 1.9‰ in ST050702 and from -5.8‰ to 1.4‰ in ST090803, reflecting the anadromous (freshwater to marine) transition in the fish life-cycle. Negative

Table 5.1: Measurements of 1SW Atlantic salmon (*Salmo salar*) and their otoliths used in the present study.

ID	Data caught	Location	Sex	Length (cm)	Weight (kg)	Otolith sagittal area (mm ²)	n	Samples/mm ²
ST050702	05/07/2007	Strathy Point	F	54.0	1.81	6.73	8	1.19
ST090803	09/08/2007	Strathy Point	M	62.0	2.50	9.20	11	1.20
M011-08	14/07/2008	Melvich	F	53.0	1.88	7.82	28	3.58
M063-08	02/07/2008	Melvich	M	60.0	1.84	6.44	15	2.33

Table 5.2: Summary of the otolith profile $\delta^{18}\text{O}$ values used to predict the latitudinal range distribution of 1SW Atlantic salmon M011-08 and M063-08, with the SST months and temperature fractionation equation used to derive each estimated range.

ID	Life stage	$\delta^{18}\text{O}$ (‰) VPDB	$\delta^{18}\text{O}$ (‰) VSMOW	Criterion	Month	Latitude range				
						Kim et al. (2007)	Storm-Suke et al. (2007)	Høie et al. (2004)	Thorrold et al. (1997)	Patterson et al. (1993)
M011-08	Emigration	0.89±0.24	31.83±0.24	Starting value	May 2007	na ¹	45.5 - 57.5	na ¹	na ¹	na ¹
	Winter	1.39±0.24	32.36±0.24	Highest value	February 2008	45.5 - 52.5	50.5 - 63.5	45.5 - 49.5	na ¹	45.5 - 50.5
	Final	1.31±0.24	32.27±0.24	Final value	June 2008	45.5 - 59.5	52.5 - 66.5	45.5 - 58.5	58.5 - 58.5	45.5 - 58.5
M063-08	Emigration	1.26±0.24	32.21±0.24	Starting value	May 2007	45.5 - 55.5	49.5 - 61.5	na ¹	na ¹	45.5 - 55.5
	Winter	1.22±0.24	32.18±0.24	Highest value	February 2008	45.5 - 48.5	46.5 - 61.5	45.5 - 47.5	na ¹	45.5 - 48.5
	Final	0.48±0.24	31.42±0.24	Final value	June 2008	53.5 - 58.5	45.5 - 58.5	54.5 - 58.5	na ¹	53.5 - 58.5

¹Inferred latitudes were south of the study area (45.5°)

$\delta^{18}\text{O}$ values ($-5.6\pm 0.4\text{‰}$) were obtained near the core of the otoliths and shifted to positive $\delta^{18}\text{O}$ values ($1.0\pm 0.6\text{‰}$) beyond the translucent-opaque transition zone. Because the adult fish were caught in the marine environment, at the conclusion of their marine migration but prior to beginning their freshwater spawning re-entry, the outermost $\delta^{18}\text{O}_{\text{otolith}}$ values are indicative of oceanic/coastal water chemistry. However, because micromilled samples cross many layers within the otolith, this signal cannot provide detailed information on migratory routes such as short-term estuarine incursions. The two micromilling profiles (5.2a) showed similar patterns of variation in $\delta^{18}\text{O}_{\text{otolith}}$ values, with intermediate $\delta^{18}\text{O}_{\text{otolith}}$ values obtained across the freshwater:marine transition zone over a lateral distance of $\sim 400\mu\text{m}$. Because this distance was measured along the longest otolith growth axis (core to rostrum), it is not spatially comparable to other studies, so we have expressed each sample location as a percentage of the total distance as in Zazzo et al. (2006).

During SIMS analyses, twenty-eight $\delta^{18}\text{O}_{\text{otolith}}$ measurements (3.58 samples/ mm^2) were made on a single transect of otolith M011 and fifteen on otolith M063 (2.33 samples/ mm^2), starting from the core (freshwater) area and sampling outwards across the marine phase. This provided two to three times as many samples/ mm^2 as could be obtained by micromilling owing to the ability to sample discrete spots every $50\mu\text{m}$. $\delta^{18}\text{O}$ profiles varied from a minimum of -7.6‰ to a maximum of 1.5‰ (M011) and from -7.0‰ to 1.3‰ (M063) otoliths, respectively, with negative $\delta^{18}\text{O}$ values (average = $-6.4\pm 0.8\text{‰}$) obtained within the inner freshwater zone and positive $\delta^{18}\text{O}$ values (average = $0.8\pm 0.4\text{‰}$) occurring at the outer opaque region. Seasonal variation in $\delta^{18}\text{O}_{\text{otolith}}$ values is apparent for the freshwater region of the M011 otolith, corresponding to the smolt age of two years. This seasonality was not expected to be reflected in the marine portion of the isotope profile because a) the marine environment is more temporally homogenous than freshwater environments with respect to both temperature and $\delta^{18}\text{O}_{\text{water}}$ and b) there is considerable fishery/research vessel evidence that post-smolt salmon and adult salmon occupy a relatively narrow temperature preference in the open ocean (Friedland et al. 1998, 2003, Jonsson & Jonsson 2004a, Hansen & Jacobsen 2000). Hence one would not expect, a priori, to see a clear seasonal periodicity in $\delta^{18}\text{O}_{\text{otolith}}$ values during the marine migration. Both profiles showed a clear and marked increase in $\delta^{18}\text{O}_{\text{otolith}}$ values (5.9‰ for M011 and 6.9‰ for M063) during the smolt migration phase and the transition occurred abruptly; within $\sim 50\mu\text{m}$. In contrast to micromilled otolith profiles, the SIMS $\delta^{18}\text{O}$ values indicated an abrupt shift with no intermediate val-

ues between the freshwater and marine phases (Figure 5.2). The transition from freshwater to saltwater indicated by this shift in $\delta^{18}\text{O}$ values measured by SIMS corresponded to the start of the final macro-structural opaque zone.

Otolith thermometry

The $\delta^{18}\text{O}_{\text{otolith}}$ values, SST months and fractionation equations used are presented in Table 5.2 along with the maximum and minimum latitudes recorded from each equation. The match between measured $\delta^{18}\text{O}_{\text{otolith}}$ values and the distribution of $\delta^{18}\text{O}_{\text{equilibrium}}$ values predicted for the eastern North Atlantic following the Storm-Suke et al. (2007) equation are presented in Figure 5.3.

Discussion

Both techniques confirmed the expectation that the change from freshwater to marine residency is conserved within otolith $\delta^{18}\text{O}$ profiles, and that this occurs at the final transition from translucent to opaque zones (i.e. at the start of the last annual band). This visible macro-structural marker may therefore be used with confidence to guide future analyses focusing specifically either on the freshwater and/or marine phases in this species. Kennedy et al. (2002), using $^{87}\text{Sr}/^{86}\text{Sr}$ ratios, also recorded the smolt emigration to marine residency co-occurring with the start of the final opaque zone; the transition in the strontium isotope ratio profile was smoothed over a distance of $\sim 400\mu\text{m}$, rather than stepped. The abrupt change (6-7‰) found in the present study over a small spatial scale ($50\mu\text{m}$) in the otoliths of fish has not been found in the literature.

Precision of the CF-IRMS analyses was better than SIMS, but required larger sample sizes that reduced spatial, and hence temporal, resolution. Higher resolutions and precisions can be obtained with micromilling using a Dual Inlet mode (Gao & Beamish 1999, Høie et al. 2004b, Wurster et al. 2005, Zazzo et al. 2006, Shephard et al. 2007, Dufour et al. 2008) and may reveal detailed fish migratory behaviour, but even considering that other analytical systems may require only 15-30 μg of sample material to routinely provide precisions of 0.1‰, the temporal resolution of daily (Weidel et al. 2007) or near-weekly analyses that can potentially be obtained by SIMS may outweigh the decline in analytical precision for many ecological questions – especially where the species or age group of interest has relatively small otoliths

and researchers are interested in transitions that may occur rapidly. Dufour et al. (2008) showed that conventional micromilling techniques using a dual inlet system could resolve oxygen and carbon isotope profiles to 23 ± 1 days in the outer portion of otoliths from 0 year alewife (*Alosa pseudoharengus*). However, the paths extracted with micromilling techniques tend to integrate isotopic signals over a larger area (up to $400\mu\text{m}$ in this study, $\sim 150\mu\text{m}$ in Dufour et al. 2008, $\sim 120\mu\text{m}$ in Zazzo et al. 2006) and to deeper depths ($\sim 40\mu\text{m}$) than SIMS, where spots can be $\sim 15\mu\text{m}$ in diameter and extend only 2- $3\mu\text{m}$ into an otolith. This surface analysis assures a more discrete sample that is less likely to obscure signals that could be lost during sample powder integration. In the early post-smolt summer marine phase, a single SIMS spot measuring $\sim 15\mu\text{m}$ in diameter corresponded to approximately one micro-structural increment (Figure 5.4); if this is the case, milling to $40\mu\text{m}$ depth at this point on the otolith would mix more recently formed layers with that sampled because of the three dimensional accretion of otoliths. Moreover, SIMS spot analysis assures evenly-spaced samples regardless of physical plane of the otolith sampled so that the number of samples it is possible to obtain is not constrained by the area of the otolith being sampled. For example, micromilled samples from inner otolith regions will, by the nature of the growth of otoliths (i.e. radiation in crystal growth from a single or multiple ‘primordia’ outwards), need to incorporate larger distances for a single sample because of the shorter perimeter of the sample path compared to the longer perimeters (and hence more sample material) obtained from paths in the outer regions. During periods of rapid growth and high metabolic rates, otolith increments are wider (Wright et al. 2001); samples taken during these time periods will have better temporal resolution than samples taken during periods of slow otolith accretion. In this instance, the temporal resolution both of micromilling and SIMS techniques will depend on the area of otolith sampled, but because isotope values obtained using SIMS can be representative of $15\mu\text{m}$ of the surface of a sample, the amount of time incorporated into that value will necessarily be less than that obtained by micromilling. Given that Wells et al. (2003) estimated micro-increment deposition to be every 8 days in pen-reared Atlantic salmon otoliths, our SIMS spots during the summer marine phase (Figure 5.4) may represent sub-monthly samples but this deposition estimate has not been validated in wild Atlantic salmon.

Regardless of the differences in precision and resolution between the two techniques, it is encouraging that similar patterns and values of the oxygen isotope composition were found across all fish for both techniques. Other micromilled otoliths

also revealed similar patterns of variation (N. Hanson, data not presented). The pattern of $\delta^{18}\text{O}$ values achieved by either technique records the migratory shift between freshwater and marine habitats, although micromilling/CF-IRMS provided a smoothed signal rather than the abrupt shift recorded by SIMS. The concordance between the two techniques supports the use of micromilling techniques to examine life history events likely to cause major shifts in $\delta^{18}\text{O}_{\text{otolith}}$ values.

Comparison of observed $\delta^{18}\text{O}_{\text{otolith}}$ values with the ‘isoscapes’ of expected ($\delta^{18}\text{O}_{\text{equilibrium}}$) given information on $\delta^{18}\text{O}_{\text{water}}$ and SST proved instructive and merits further development. As expected, the results that most closely approximate to empirical observations of 1SW Atlantic salmon distribution were found when using the equation derived for wild Arctic charr. The majority of the derived winter latitude ranges are further south than what is currently known of the ocean distribution of 1SW Atlantic salmon (Holm et al. 2006, Jonsson & Jonsson 2004a, Hansen & Jacobsen 2000). Salmon of Scottish origin have been caught during winter and autumn months north of the Faroe Islands ($\sim 64^\circ \text{N}$), and some are known to travel beyond 70°N . Based on historical catch records and limited tag data, it is thought that salmon of southern European origin travel north to feeding grounds in the Norwegian sea prior to sexual maturation (Todd et al. 2010). Predicted $\delta^{18}\text{O}_{\text{equilibrium}}$ values for most equations at these more northerly latitudes in the eastern North Atlantic are 1-2‰ higher than the estimated ‘winter’ $\delta^{18}\text{O}_{\text{otolith}}$ values, suggesting that wild Atlantic salmon otolith aragonite is not precipitated in equilibrium with ambient seawater conditions according to these equations. The Storm-Suke et al. (2007) equation does, however, return the most plausible result: during the ‘winter’ month both fish were predicted to have been located to the south and east of the Faroes, with M011 at slightly higher latitudes than M063. In June, immediately prior to capture, the results suggest that the fish could have been occupying waters along the Norwegian continental shelf, the north coast of Scotland and to the southeast of the Faroes and Iceland. Importantly, be-

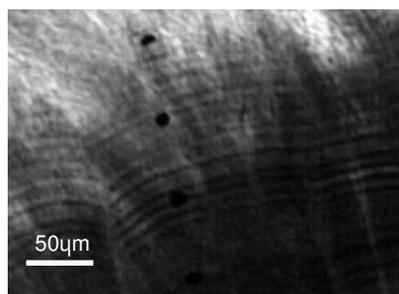


Figure 5.4: Transmitted light image of otolith M063 at 40x magnification. Dark spots represent a single SIMS analysis on the otolith transect spanning approximately one microincrement.

cause the distribution $\delta^{18}\text{O}_{\text{equilibrium}}$ values is a function of gradations in sea surface temperature and $\delta^{18}\text{O}_{\text{seawater}}$, which is itself linearly related to salinity (LeGrande & Schmidt 2006), this technique will be limited to discriminating between latitudes of residence in the eastern North Atlantic and provides only poor longitudinal resolution. However, matching the observed to expected $\delta^{18}\text{O}$ values shows a distinction between the relative latitudes to which each fish migrated (M063 to lower latitudes than M011) in winter 2008, suggesting that the technique may be useful for identifying individual variation in latitudinal migrations of fish in the eastern North Atlantic. Whilst the relative differences in high $\delta^{18}\text{O}_{\text{otolith}}$ between the two fish were consistent across the profiles, it is still possible that a true maximum value for M063 was not sampled. Until smaller step sizes or continuous sampling methods can be developed, inferences must therefore be made with caution.

SIMS sampling of $\delta^{18}\text{O}_{\text{otolith}}$ isotope profiles is a potentially powerful new technique that will become increasingly available to fisheries biologists. It can now achieve higher-resolution sampling at similar precisions to micromilling/CF-IRMS and analysis is confined to the surface of the otolith and provides more discrete $\delta^{18}\text{O}$ values. However, micromills are more accessible to most researchers and have the potential to reveal migratory behaviour that covaries with major changes in isotopic signatures, such as the freshwater:marine transition. The present study has shown that the time at which juveniles migrate to sea co-occurs with the start of the last annual band in the otoliths of 1SW Atlantic salmon and this observation can guide future sampling to focus either on the marine or freshwater life-stage. By exploring the “isoscape” of aragonite $\delta^{18}\text{O}_{\text{equilibrium}}$ values in the eastern North Atlantic in relation to measured $\delta^{18}\text{O}_{\text{otolith}}$ values, it has been shown that only an equation derived for a closely related genus returns plausible (but still inadequate) results for predicting the winter distribution of wild 1SW Atlantic salmon. However, exploration of the spatio-temporal distribution of theoretical equilibrium isotope values utilising ocean databases has the potential to further inform our understanding of the distribution and possible migration routes of other species for which otolith aragonite fractionation equations do exist and where sufficient information regarding water masses of interest is available. For Atlantic salmon, it may be used to discriminate between fish which travel to different latitudes but to be geographically explicit, a specific thermometry equation for this species is necessary.

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6 Reconstructing thermal and metabolic histories of wild Atlantic salmon from otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$

ABSTRACT: Long term declines in wild Atlantic salmon abundance have been linked to reductions in marine survivorship and, in particular, reductions in growth condition (a measure of fish quality) have been correlated to increased mid-winter sea surface temperature anomalies in the eastern North Atlantic. Establishing a causal link between marine climate conditions and salmon somatic condition is difficult without at-sea measurements of environmental and biological parameters, but electronically tagging these animals to obtain this information is difficult and costly. Stable isotopes of oxygen and carbon in the sequential layers of salmon otoliths can act as natural ‘tags’, providing the ability to retrospectively study the thermal and metabolic histories of individual fish during the elusive marine life stage. High resolution intra-otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ profiles obtained using secondary ion mass spectrometry (SIMS) were used to compare the marine thermal and metabolic behaviour of one sea-winter (1SW) return migrant adult salmon in relation to their pre-spawning somatic condition. The results indicate no significant relationship between marine water temperatures experienced by fish and their return body condition. General trends in both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values were similar between fish but individual deviations demonstrate the potential for large differences in individual migration routes and open ocean destinations of wild 1SW Atlantic salmon. It is proposed that intra-otolith $\delta^{13}\text{C}$ values can be informative of the metabolic history

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of individual salmon and record two distinct periods of elevated metabolism during the early post-smolt phase and in the months prior to capture in coastal waters.

Introduction

The wild Atlantic salmon (*Salmo salar*) is an important commercial and cultural species throughout its geographic distribution. Whereas most high seas fisheries have been curtailed or closed over the last three decades, coastal and freshwater fisheries still are in operation in many countries. Despite the reduction in exploitation at sea and in freshwater (2009 showed the lowest catch of wild Atlantic salmon in the North Atlantic since 1960), many stocks still are at reduced reproductive capacity (ICES 2010). The ICES working group on North Atlantic salmon define Southern European stocks as those originating from Ireland, the UK (Northern Ireland, Scotland, England & Wales), France and southwest Iceland. Estimated pre-fishery abundance and spawning escapement of these stocks in the eastern North Atlantic are approaching conservation limits (ICES 2010). There is growing recognition within the scientific community that a major driver of these declines is a reduction in marine survival (Anonymous 2008, Friedland et al. 2009, Todd et al. 2010, ICES 2010).

Atlantic salmon are anadromous fish, emerging from gravel as alevins in freshwater rivers in early spring; the precise timing of this event depends upon the temperature and the rate of development of the eggs. They then spend typically 1-5 years (exceptionally up to 8 years in northern Canada, Klemetsen et al. 2003) feeding on macroinvertebrates before undergoing smoltification and migrating to sea. At sea, they may spend one (one seawinter, 1SW fish) or multiple (multi-seawinter, MSW fish) years feeding on crustaceans, squid and fish in the waters of the North Atlantic before returning to their natal rivers to spawn (Haugland et al. 2006, Jacobsen & Hansen 2000, Rikardsen & Dempson 2010). The decreased survival during the marine phase may result from increased high-seas exploitation but, because of the reduction in marine fishing pressure, other mechanisms must also be invoked. Large-scale recent changes in the distribution and phenology of plankton species in the eastern North Atlantic have been connected with ocean climate change (Beaugrand et al. 2002, Beaugrand & Reid 2003, Richardson & Schoeman 2004); such shifts could be indicators of reduced marine survival of salmon migrating within the Norwegian Sea (Beaugrand & Reid 2003).

Todd et al. (2008) have monitored the lengths, weights and sea ages of wild Atlantic salmon returning to mixed-stock coastal fisheries near Strathy Point in northern Scotland from 1993-2006. Using stock-specific length/weight regression equations to estimate the relative mass index (W_r ; see Blackwell et al. 2000) of individual fish, and by linking this estimate to percent lipid within the tissues of the fish, they showed a significant reduction in mean body condition - and hence fat reserves - of returning 1SW salmon over the time series. This qualitative assessment of the health of mixed stocks immediately prior to freshwater re-entry for spawning may be more of a concern for salmon biologists than the reductions in pre-fishery abundance and spawning escapement, because of its implications for the ability of salmon to successfully complete the freshwater migration and spawn. Whilst Todd et al. (2008) and others (Bacon et al. 2009, Friedland et al. 2009) have recognized the likely contribution of marine climate changes to the reduction in abundance and growth condition of 1SW stocks of wild Atlantic salmon, there is a dearth of empirical data regarding the specific marine migration trajectories of wild stocks. The inability to retrospectively study the environmental conditions experienced by salmon in the North Atlantic has made it difficult to identify times and regions of particular concern. Tracking marked or individual salmon at sea is very expensive and challenging; catch rates and tag returns often are low (Holm et al. 2006, ICES 2010) and, until comprehensive genetic databases are created, stock identification is constrained to large regions of the North Atlantic (e.g. North American stocks versus European stocks; Dempson et al. 2010). Monitoring individuals that return to homewaters has four main advantages; first, it is relatively inexpensive. Second, regional provenance may be inferred depending on the catch location. Third, accurate sea-ages can be ascribed to individuals from scale readings and lastly, annual re-sampling is more achievable. By collecting and analysing the tissues of returning adult salmon, it is possible to make inferences about the environmental changes at sea during their migration by using 'proxy' methods such as stable isotope analysis. Examining chemical signals incorporated into accretionary mineralised structures such as otoliths is one way to gain insight into the details of thermal and metabolic histories of these elusive and long-distance migrants.

A monitoring program established on the north coast of Scotland (Todd et al. 2008) utilises commercially caught wild salmon from mixed stock coastal interceptory nets. The benefits to this research program include a mixed stock catch which is presumed to be representative of several southern European stocks rather than a

single river stock (Jenkins & Shearer 1985), the ability to collect tissue samples from dead fish, and a reliable source of specimens on an annual basis. Importantly, the pattern of variation and decadal decline in somatic condition of this mixed-stock is nearly identical to that of a single, in-river stock on the northeast coast of Scotland, demonstrating the ubiquity of the problem and affirming that the causal factor(s) is(are) attributable to the shared environment of multiple stocks. Thus, whilst river catchment-specific variations in ecological conditions undoubtedly pertain, and will influence the success of particular river stocks, the primary impact is clearly manifest in the shared marine environment and is sufficiently intense as to override any freshwater influences.

Otoliths as natural data storage tags Otoliths are biomineralised structures located within the head of teleost fish that function in balance and hearing (Popper & Lu 2000, Popper & Coombs 1982). They are composed primarily of aragonite, a polymorph of calcium carbonate (CaCO_3), that is deposited on a non-collagenous protein matrix that comprises 0.2-10% of the total otolith material (Murayama et al. 2002, Degens et al. 1969). There are three pairs of otoliths within the inner ear of a fish - the lapilli, sagitta and astersci. Among most non-ostariophysyeans (cyprioid) fishes, the largest of the three is the sagitta and is the pair used throughout this study. The sagittae are oriented in the ventral sacs of the vestibular apparatus (Secor et al. 1991), ventrally and slightly posterior to the brain in Atlantic salmon; the otoliths can be removed by either bisecting the head of the fish from anterior to posterior or using the 'flip-top' method to access the braincase horizontally (Secor et al. 1991). The otoliths are bathed in an endolymphatic fluid which is saturated with calcium and bicarbonate ions (Murayama et al. 2002). Precipitation on the otolith surface is dependent on the composition and pH of the surrounding endolymph fluid (Romanek & Gauldie 1996). The primary source of inorganic elements in the otolith is the water that bathes the gills (in freshwater fishes) or that which is ingested during drinking (in marine fishes). These elements are assimilated into blood plasma, become part of the endolymph surrounding the otoliths and are eventually incorporated into the crystallized structure (Campana 1999). Otolith growth is continuous, often displaying yearly (macroscopic) and daily (microscopic) banding, and the structure is metabolically inert after formation. Unlike many other biogenic carbonates such as those in scales and teeth, otoliths are not susceptible to resorption (Campana & Thorrold 2001) and so provide an environmental and

physiological record of fish life history. Because of these features, otoliths can be sub-sampled at high resolution to provide ontogenetic patterns of stable isotope variation ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) over relatively short time periods of days to weeks.

That the oxygen and carbon isotope ratios of fish otoliths could provide useful information on the life history, metabolism and diet of fishes was recognized as early as 1969 (Degens et al. 1969). McCrea (1950) demonstrated that the fractionation of $^{18}\text{O}/^{16}\text{O}$ between inorganically precipitated calcium carbonate and water was mediated by ambient temperature; using marine shells, Epstein et al. (1951, 1953) later showed that the relationship was consistent within biogenic carbonates (see also Kim & O'Neil 1997). Epstein et al. (1953) reported that if the $\delta^{18}\text{O}$ value of the ambient water is known, the temperature of that water at the time of carbonate formation could be estimated to a precision of $\pm 0.6^\circ\text{C}$. This thermometry equation subsequently was exploited, modified and updated in order to derive estimates of the thermal conditions during carbonate precipitation for numerous marine organisms. Kalish (1991) provided an equation describing the fractionation of $^{18}\text{O}/^{16}\text{O}$ in the otoliths of several species of marine teleosts from the Southern Ocean, and Patterson et al. (1993) described a similar equation for several freshwater species; however, more recent laboratory experiments conducted under controlled conditions suggest that the equation parameters are not consistent across species (Thorrold et al. 1997, Høie et al. 2004b, Storm-Suke et al. 2007) and no equation has yet been estimated for Atlantic salmon. Nevertheless, relative - if not absolute - differences in thermal history as recorded by $\delta^{18}\text{O}$ values should be preserved in the otoliths of Atlantic salmon and can be compared with the somatic condition of the fish prior to river re-entry and the spawning migration. By this means, connections and correlations may be made between a measure of ultimate success of the individual (e.g. size, condition factor) during the marine phase and environmental conditions experienced over the course of that migration.

In contrast to oxygen isotope ratios, carbon isotope ratios in biogenic carbonate are not deposited in isotopic equilibrium with surrounding water. When studying the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of a range of species, Kalish (1991) found that fish with low metabolic rates and/or inhabiting colder waters had otolith $\delta^{13}\text{C}$ values that were closer to those of the ambient dissolved inorganic carbon (DIC) than the otolith $\delta^{13}\text{C}$ values of fish with higher metabolic rates and/or temperature preferences. He suggested that metabolic, rather than kinetic, effects were responsible for driving the relationship between $\delta^{13}\text{C}$ and temperature. In a controlled laboratory experi-

ment, Thorrold et al. (1997) found isotopic disequilibrium of carbon isotope ratios of Atlantic croaker (*Microgonias undulatus*) otoliths and a lack of evidence to support kinetic effects; rather, they reported a significant positive relationship between $\Delta\delta^{13}\text{C}_{DIC-otolith}$ and fish growth rate and a similar, but non-significant, relationship with otolith precipitation rate, suggesting that metabolic rate affects the disequilibrium of carbon isotope ratios in otoliths. More recently, Solomon et al. (2006) estimated the proportional contribution of metabolic carbon (M) to the otoliths of juvenile rainbow trout (*Oncorhynchus mykiss*) to be $\sim 17\%$ by manipulating the $\delta^{13}\text{C}$ value of ambient DIC and diet. Other studies have found values of M between 0 and $\sim 40\%$ depending on the parameters measured and the age of the fish (Høie et al. 2003, Solomon et al. 2006, Dufour et al. 2008, Elsdon et al. 2010). Assuming that at least a proportion of $\delta^{13}\text{C}_{otolith}$ is reflective of metabolic activity, it might be expected that M would change over the lifetime of an individual in concert with changes in size and ambient temperature (Gillooly et al. 2001). Wurster & Patterson (2003) found both seasonal and ontogenetic shifts in $\delta^{13}\text{C}_{otolith}$ in the freshwater drum (*Aplodinotus grunniens*) that they largely attributed to changes in metabolic rate. Thus, $\delta^{13}\text{C}_{otolith}$ values can be considered as proxies for metabolic activity if $\delta^{13}\text{C}_{DIC}$ and $\delta^{13}\text{C}_{diet}$ can be constrained.

Comparing thermal and metabolic histories between and within individual salmon returning with different growth condition factors may provide insights into the oceanic conditions they experienced and clues as to how salmon may respond to future changes in their environment, but detailed profiles for individual fish are necessary to examine variation at small temporal scales. The sagittal otoliths of 1SW Atlantic salmon are small ($\sim 5\text{mm}$, longest axis; rostrum to antirostrum) and the marine zone is perhaps only $\sim 1\text{mm}$ in extent. Here, secondary ion mass spectrometry (SIMS) was applied and this technique was shown to provide, for Atlantic salmon otoliths, higher resolution profiles and more discrete samples (i.e. less mixing) than are attainable by traditional micromilling techniques (Hanson et al. 2010).

The aims of this study were a) to characterise the general pattern of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variation in the otoliths of 1SW wild Atlantic salmon during their marine migration, b) to relate this variation to the sex and relative pre-spawning growth condition of the individual, and c) to compare individual thermal and metabolic histories derived from the respective stable isotope proxies.

Materials & Methods

Sample collection and preparation

Otoliths were dissected from the heads of 27 wild 1SW salmon caught in a coastal mixed-stock fishery (Melvich [58.57° N, 3.92° E], North Scotland) during the 2009 netting season (June-August). Fork length (to 0.5 cm) and weight (to 0.01 kg) of each fish was measured and a sample of scales was collected for aging and growth analysis. Scales were collected from the standard region for Atlantic salmon, 3-6 rows above the lateral line and posterior to the dorsal fin (Shearer 1992b). Otoliths were removed, cleaned of any membrane, rinsed with deionised water and stored dry in sterile Eppendorf tubes. Otoliths from 10 fish were selected for SIMS analysis on the basis of a) the absence of vaterite determined both by visual inspection and bulk X-ray diffractometer scan (see chapter 5) and b) the somatic growth condition factor, W_r , of the fish. Five otoliths were set in each of two round epoxy resin mounts (24.5mm), ground to the core (otolith origin) using P1200, P2500 and P4000 grit SiC paper and polished along the transverse plane. Samples of standard material (UWC-calcite) were also included in each mount in order to monitor precision throughout the analyses. After $\delta^{18}\text{O}$ analyses were completed, and prior to $\delta^{13}\text{C}$ analyses, the blocks were lightly polished to remove SIMS ablation spots.

The pattern of scale circuli deposition was assessed by Bryce Whyte (Marine Scotland, Montrose) in order to confirm that all specimens were 1SW fish and to determine the number of years spent in freshwater before migrating to sea (river age). A chronology of fish length was estimated from the circuli spacing by measuring the linear distance from the scale focus to a) the transition at smolt emigration to sea, b) the middle of the marine winter growth check (contraction of spacing of scale circuli associated with decreased growth during winter) and c) to the scale edge. Using a simple proportional relationship between circuli spacing and fish length (Shearer 1992b) - where S_f is distance from the focus to the feature of interest (i.e. smolt emigration, winter check), S_t is the distance from the focus to the scale edge and L_t is the length of the fish (cm) when caught - the length of the fish at any feature of interest (L_f) can be estimated.

$$L_f = L_t \cdot S_f/S_t \tag{6.1}$$

The length of the fish at smolt emigration and the mid-winter check was estimated (Equation 6.1); for the purpose of establishing a chronology, the timings for these events were assumed to be May 7th (median date of 50% smolt emigration between 2006-2010 on the River North Esk, Scotland, C.D. Todd & J. MacLean pers.comm.) and December 21st. The winter solstice was used as the mid-winter date because salmon are visual predators (Wankowski 1977, Haugland et al. 2006) and can range northwards into high arctic latitudes; at such latitudes, it is intuitive that the lack of available light during mid-winter would be the main constraint on prey capture and hence somatic growth. Evidence from research trawls and longline fisheries indicates that Atlantic salmon are distributed to the north of the Faroes (~62°N) and move further northward and into the Norwegian Sea, perhaps beyond the Arctic Circle during winter (Hansen & Jacobsen 2000, 2003, Dadswell et al. 2010, Jakupsstovu 1988). Few, if any, hours of daylight are available for foraging during the period November - January.

SIMS analysis

Reflected light microscope photographs of the sample mount guided the sampling procedure. Oxygen and carbon isotope ratios were measured with a CAMECA-IMS-1270 ion microprobe (#309) (University of Edinburgh) using a ~5 nA primary $^{133}\text{Cs}^+$ beam. For $\delta^{18}\text{O}$ analyses, secondary ions were extracted at 10 kV, and ^{16}O (~3.0 x 10⁹cps) and ^{18}O (~4.0 x 10⁶ cps) were monitored simultaneously on dual Faraday cups (L'2 and H'2). Each spot analysis involved a pre-sputtering time of 50 s, followed by automatic secondary beam and entrance slit centering and finally data collection in two blocks of five cycles, amounting to a total count time of 40 s. For the $\delta^{13}\text{C}$ analyses, ^{13}C (~3e5 cps) and ^{12}C (~3e7 cps) were monitored simultaneously using an electron multiplier for ^{13}C and a Faraday cup (L'2) for ^{12}C . Each analysis involved a pre-sputtering time of 60 s followed by automatic secondary beam and entrance slit centering and finally data collection in two blocks of twenty cycles, amounting to a total count time of 200s. Otolith isotope measurements were bracketed every 15 analyses by 5 or 10 spot analyses of an internal reference material (UWC-1 calcite, $\delta^{18}\text{O} = 23.28 \pm 0.06$ (VSMOW), $\delta^{13}\text{C} = -2.14 \pm 0.08$ (PDB) J. Valley, pers.comm.) to assess instrument precision and to correct for degradation in the electron multiplier efficiency. The sampling transect originated at the outer margin of the otolith and was terminated once a freshwater isotope signal was recorded (typically ~1mm from the otolith edge). A duplicate $\delta^{18}\text{O}$ transect,

offset by approximately 25 μm from the original, was completed for fish M007-09 (6.3a). Reproducibility of the standard calcite measurements was $<0.21\text{‰}$ for $\delta^{18}\text{O}$ and $<0.43\text{‰}$ for $\delta^{13}\text{C}$. For all isotope analyses, the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values are reported relative to Vienna Pee Dee Belemnite (VPDB) (Coplen 1996).

Data analysis

The relationship between otolith $\delta^{18}\text{O}$ values and ambient temperature has not been measured empirically for wild Atlantic salmon; to date, the most closely related species for which there is an otolith thermometry equation is Arctic charr (*Salvenius salvenius*) (Storm-Suke et al. 2007). Whilst relative differences in thermal profiles rather than absolute temperature estimates were the primary concern of this study, an attempt was made to provide a more appropriate equation for this species by estimating the intercept from the present data, and assuming a slope equal to that for inorganic aragonite equilibrium fractionation of 17.88 (Kim et al. 2007). Average sea surface $\delta^{18}\text{O}$ values (LeGrande & Schmidt 2006) and average otolith $\delta^{18}\text{O}$ values for all individuals for the months of May (2008) and July (2009) were used to calculate $1000\ln\alpha$, and 10^3TK^{-1} was calculated for each month from monthly mean SST temperature estimates from the oiSSTv2 (Reynolds et al. 2002) dataset for 3.5-4.5 $^{\circ}\text{E}$, 58.5-59.5 $^{\circ}\text{N}$ - the 1 $^{\circ}$ gridbox containing the area of salmon capture in Scottish coastal waters. This allowed the estimation of the intercept (Equation 6.2). For comparison, temperature estimates were calculated from both this derived equation, and from that of Storm-Suke et al. (2007) (Equation 6.3, below).

$$1000\ln\alpha = 17.88(10^3TK^{-1}) - 32.563 \quad (6.2)$$

$$1000\ln\alpha = 20.69(10^3TK^{-1}) - 41.69 \quad (6.3)$$

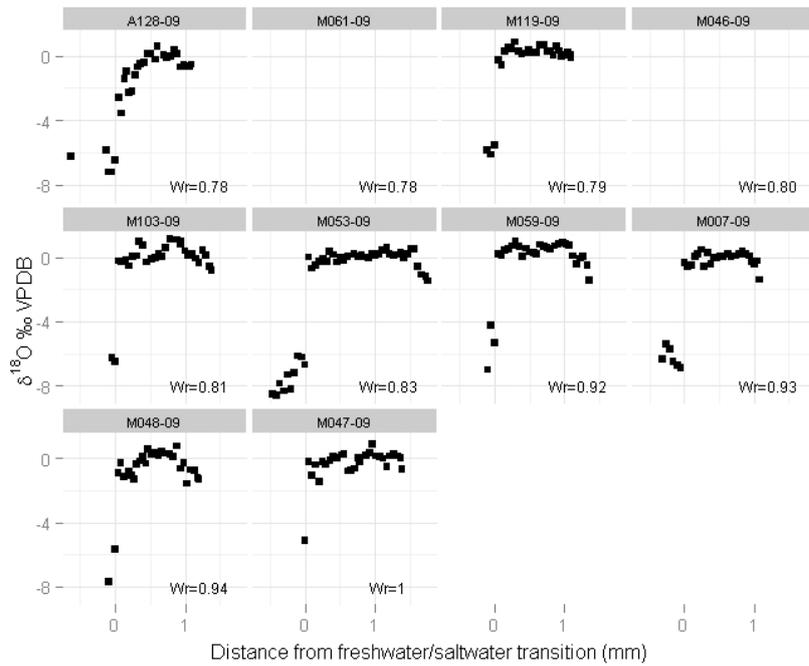
Because sampling transects progressed inwards from the otolith margin to the core of the otolith, the point at which isotope profiles began to reflect freshwater rather than marine residency was recognizable as a substantial decrease in $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values (Figure 6.1). It is important to note, however, that for some fish the transition was more gradual than in others (e.g. A128-09). To compare isotope profiles between

fish, it was necessary to demarcate a value for the start of the marine migration, excluding those values thought to be representative of a freshwater environment, and to re-scale the abscissa to a proportional distance from the freshwater/marine transition. Consequently, all profiles were truncated near the transition where $\delta^{18}\text{O}$ values were $> -4\text{‰}$ and $\delta^{13}\text{C}$ values $> -9\text{‰}$; distances were re-scaled as proportional distances along the otolith, with the first value of the 'marine zone' equal to zero and the final value at the outer margin of the otolith equal to one.

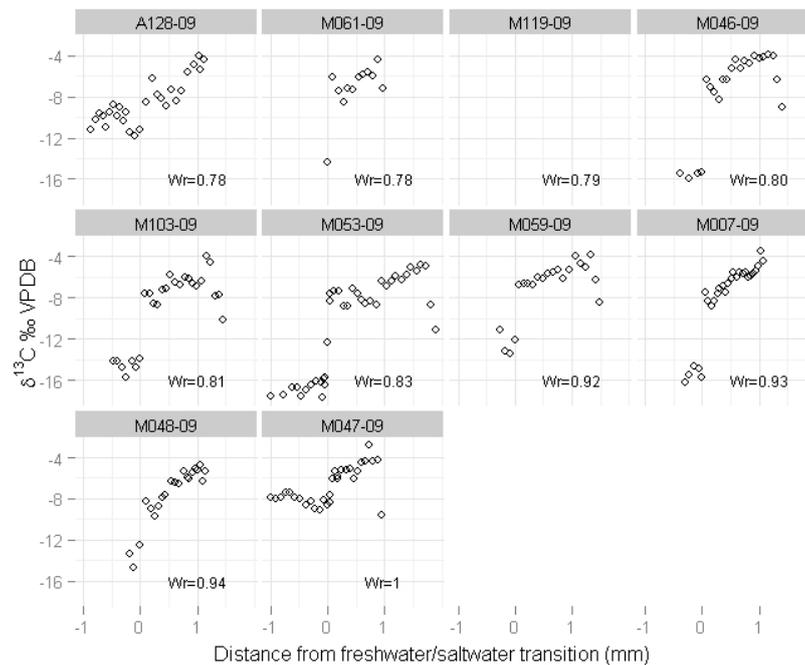
The primary interest was in exploring the chronology of the stable isotope samples; however, using the spacing of micro-increments in the otoliths themselves poses two problems. First, it was difficult to obtain an appropriate thin section for all otolith sections following SIMS analyses; second, the timing and validation of otolith increment formation in adult Atlantic salmon is not well understood. Whilst increment formation has been characterised for embryonic and juvenile salmon (Geffen 1983, Wright et al. 1991), there is only the one study that has attempted to estimate the timing of micro-increment formation in adult Atlantic salmon (Wells et al. 2003). From the otoliths of pen-reared adult salmon, Wells et al. (2003) estimated average micro-increment deposition-time to be just over one week (8 days) in 1SW salmon and noted correlations between scale circuli widths and otolith increment widths in wild adult salmon presumed to be related to seasonal growth patterns. Unlike scale growth, otolith precipitation is not driven solely by somatic growth but, due to the importance of organic molecules (e.g. otolin; Degens et al. 1969, Murayama et al. 2002) in providing nucleation sites for otolith precipitation, otolith growth can still be correlated with somatic - and hence scale - growth (Campana & Thorrold 2001, Wells et al. 2003) .

From correlation with scale growth, the length of the fish and associated date were estimated from scale readings to map a chronology of otolith isotope profiles. To compare the stable isotope profiles with the length of the fish, and the calendar day derived from scale circulus counts, it was necessary to adjust the length and date estimates to proportional distances along the fish scale. Accordingly, the circulus measurement on the scale corresponding to smolt emigration was set to a distance of zero and the distance to the edge of the scale was set to one. As above, smolt emigration date was set to 7 May 2008 and the midwinter check date was set to 21 December 2008. The date of capture provides the third fixed point in deriving the empirical relationship between circulus and date of deposition.

Atlantic salmon do not increase the number of scales as they grow; rather, the



(a)



(b)

Figure 6.1: Profiles of otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values for each individual Atlantic salmon centered at the emigration of the smolt from freshwater (low isotope values) to marine (high isotope values) residency. Analytical precision was $< 0.21\text{‰}$ for $\delta^{18}\text{O}$ and $< 0.43\text{‰}$ for $\delta^{13}\text{C}$. Distances are absolute millimeters from the freshwater/marine transition. Individual plots are arranged in ascending order of growth condition factor (W_r).

scales increase in size as the fish grows in length. Scale radius is related to somatic growth in salmonids (Fukuwaka 1998) and while it is generally accepted that the relationship is unlikely to be precisely linear, back-calculated length estimates based on linear relationships are commonly used for Atlantic salmon (Shearer 1992b). It is apparent from visual inspection that scale growth and circulus deposition is not linearly related to time. For example, two fish may experience at the same time of year environmental conditions that cause a hiatus or “check” formation (= tighter circulus spacing), but the relative position of that check on the scale radial axis will not necessarily be the same between them. This discrepancy may be attributed to variable growth rates; assuming a general von Bertalanffy growth curve for Atlantic salmon, smaller or younger fish could be expected to increase in length more rapidly than a longer or older fish so the deposition of circuli check formations will not be time invariant. Therefore, linear regressions were used to back-calculate fish lengths for each isotope sample along the otolith, but because the timing of scale circulus formation is not ontogenetically constant, polynomial regressions were used to back-calculate calendar date for each isotope sample (Figure 6.2).

There were three main objectives in the statistical analyses: (1) to assess individual variation in isotopic profiles (and hence inferred migratory histories), (2) to compare isotopic data between individuals at specified intervals in relation to their final pre-spawning somatic condition prior to capture and (3) to explore patterns and trends in oxygen and carbon isotope variation for all fish combined. Data points were treated as independent because the physical process of stable isotope incorporation during otolith precipitation, driven primarily by the chemical composition of endolymphatic fluid, should not vary between individuals. The importance of variation in stable isotope profiles at the level of the individual was assessed by comparing a fitted Generalised Additive Model (GAM; *mgcv* package, Wood 2010) that smoothed stable isotope data by calendar date for each individual to a general model that pooled stable isotope data from all individuals together. The model of best fit was determined by minimising the Akaike information criterion (AIC) value (Akaike 1976). To compare isotope data from individuals at specific times during their migration (objective 2), $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values were predicted from individual GAM smooths. For $\delta^{18}\text{O}$, the primary interest was in differences in winter temperatures experienced in relation to pre-spawning temperatures so values were predicted for the winter solstice of 2008 and water temperatures calculated using Equation 6.2 and Equation 6.3. To address objective (3) patterns and trends in $\delta^{18}\text{O}$

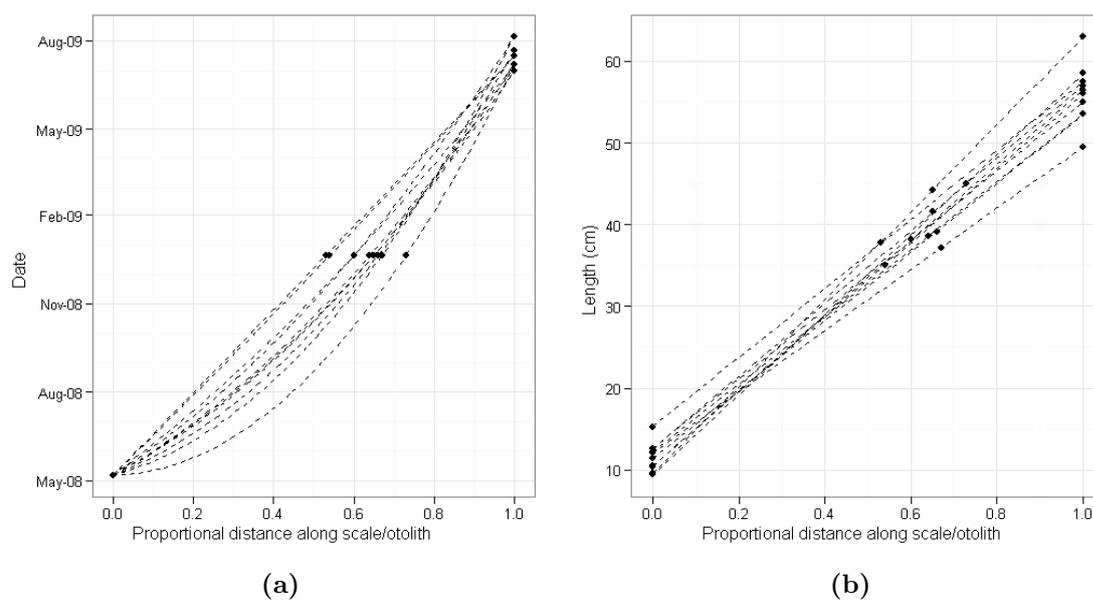


Figure 6.2: Relationship between the proportional distance along the marine zone of scale growth, calendar date (a) and back-calculated fish length (b) using Eqn Equation 6.1 for each individual. Smolt emigration was assumed to occur on May 7th and December 21st was set as the midwinter check. The date of capture provides the final fixed point of each regression. Linear (length) and polynomial (date) regressions between these were used to interpolate values of length and date for each SIMS ablation spot along the otolith.

and $\delta^{13}\text{C}$ variation (response variable) were modelled in relation to the information obtainable from the individual salmon upon capture (sex, condition factor, river age, back-calculated lengths and dates) using semiparametric GAM regression. This approach allowed the estimation of regression models comprised of both factorial predictors (i.e. sex, W_r , river age) and smooth functions of nonlinear predictors (i.e. length and date). Models were assessed on the basis of AIC value (Akaike 1976) and generalised cross validation (GCV) score reduction. All GAMs used an identity link function and gaussian distribution. Visual inspection of model residuals allowed the determination of outliers. All statistical analyses were performed in R (version 2.11.1; R Core Development Team 2010).

Results

SIMS sample spots were approximately $15\mu\text{m}$ in diameter and $\sim 3\text{-}4\mu\text{m}$ in depth (N. Allison, pers.comm.). Between 24 and 34 analyses were obtained per otolith for $\delta^{18}\text{O}$ ($n = 8$ individuals) and between 11 and 24 analyses per otolith for $\delta^{13}\text{C}$ ($n = 9$ individuals). Table 6.1 provides the summary descriptive statistics of isotope values obtained within the marine zone. For the duplicate $\delta^{18}\text{O}$ transect, SEM images guided the matching of points to particular growth zones and once this was completed, the matched profiles were found to be highly correlated (6.3b; $r^2 = 0.89$; Pearson's product-moment test), demonstrating that the pattern of stable isotope variation is repeatable across the transverse section irrespective of the precise alignment of the transect.

Although the pattern of isotope variation was repeatable within an individual, model comparison shows that a GAM conditioned by 'individual' (i.e. a separate smooth function for each individual) was a better fit to the data than one which pooled stable isotope values (AIC = 180.76 vs 363.74). Individual otolith profiles of the marine zone smoothed by back-calculated date are presented in Figure 6.4. There was no significant correlation between the water temperature estimates at the winter solstice (calculated using Equation 6.2 or Equation 6.3) and pre-spawning condition factor.

Smooths generated for all individuals pooled showed that $\delta^{18}\text{O}$ values increased $\sim 1\text{‰}$ from -0.7‰ to a maximum of 0.4‰ at ~ 0.6 of the proportional otolith distance, corresponding to a temperature decrease of $\sim 4 - 5^\circ\text{C}$ (Equation 6.2 and Equation 6.3) to a minimum around January. $\delta^{13}\text{C}$ values increased from -7.4‰

Table 6.1: Summary of the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values obtained within the marine zone of one seawinter (1SW) Atlantic salmon otoliths by secondary ion mass spectrometry (SIMS) analyses. All isotope ratios are reported relative to Vienna Pee Dee Belemnite (VPDB). $\delta^{18}\text{O}$ results were not obtained for two otoliths due to time constraints and an instrumental error; $\delta^{13}\text{C}$ data were not obtained for fish M119-09 because this otolith was accidentally destroyed during sampling. Nd = no data.

ID	Catch date	Sex	Length (cm)	Weight (kg)	W_r	$\delta^{18}\text{O}$				$\delta^{13}\text{C}$			
						n	Max	Min	σ	n	Max	Min	σ
A128-09	22 July 2009	M	62.40	2.29	0.78	24	0.59	-3.57	1.02	13	-3.99	-8.85	1.68
M007-09	30 June 2009	F	54.00	1.76	0.93	24	0.46	-1.40	0.41	22	-3.45	-8.77	1.34
M046-09	07 July 2009	F	55.20	1.62	0.80	nd	nd	nd	nd	18	-3.94	-9.03	1.57
M047-09	07 July 2009	M	58.80	2.44	1.00	28	0.88	-1.44	0.49	17	-2.77	-9.62	1.65
M048-09	07 July 2009	F	57.50	2.15	0.94	27	0.77	-1.57	0.67	18	-4.74	-9.65	1.48
M053-09	07 July 2009	M	56.00	1.74	0.83	34	0.57	-1.49	0.48	24	-4.79	-11.08	1.54
M059-09	16 July 2009	M	57.50	2.10	0.92	27	1.02	-1.42	0.53	17	-3.90	-8.42	1.12
M061-09	16 July 2009	F	53.60	1.44	0.78	nd	nd	nd	nd	11	-4.46	-8.50	1.12
M103-09	05 August 2009	M	49.40	1.16	0.81	28	1.11	-0.81	0.55	19	-3.91	-10.14	1.43
M119-09	05 August 2009	M	56.30	1.70	0.79	24	0.87	-0.58	0.31	nd	nd	nd	nd
All						216	1.11	-3.57	0.66	216	-2.77	-11.08	1.53

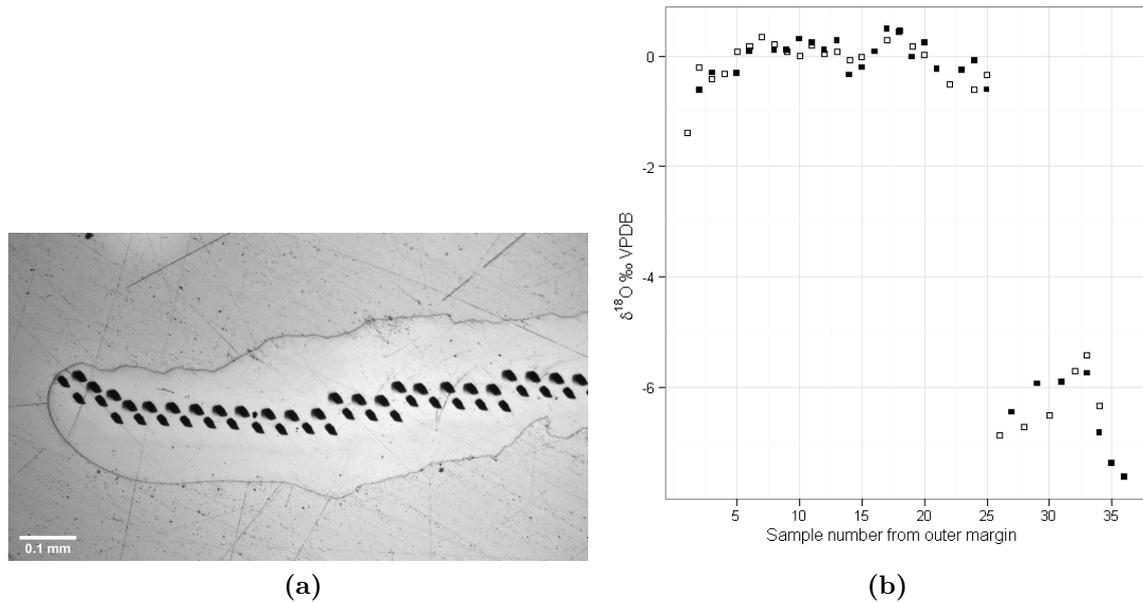


Figure 6.3: Duplicate transects of SIMS spots on otolith M007-09 (a) and $\delta^{18}\text{O}$ (± 0.21) values after SEM images guided matching to growth layers (b).

to a maximum of -4.8‰ at ~ 0.8 of the proportional otolith distance, around April. After visual identification from model residuals, seven outlier $\delta^{18}\text{O}$ data points representing the early marine phase of fish A128-09 were removed and models were re-specified. Pooled $\delta^{18}\text{O}$ values were best predicted by GAM models smoothed by back-calculated length and date (Table 6.2). Surprisingly, the model also included sex (t-value = 4.759, p-value < 0.001, GCV = 0.188); $\delta^{18}\text{O}$ values were on average 0.33‰ higher for the otoliths of male fish (6 individuals, 163 isotope samples) compared to female fish (2 individuals, 50 isotope samples), but the test is weak due to the small number of individuals. For pooled $\delta^{13}\text{C}$ values, the lowest AIC model included length, date and condition factor although the effect of growth condition was not significant.

Discussion

Using high resolution sampling, the pattern of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ across the otoliths of wild Atlantic salmon throughout the marine migration can be effectively described. A chronology relating these values to calendar date and fish growth in length (cm) could be established by matching distance along the marine phase of otolith accretion

Table 6.2: Summary of the generalised additive models fit to $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values obtained within the marine zone of one seawinter (1SW) Atlantic salmon otoliths by secondary ion mass spectrometry (SIMS) analyses. Models were selected by iteratively removing the least significant parameter and comparing AIC and GCV scores.

Model	Response	Covariates	Approximate p-value	AIC	GCV	r^2
1	$\delta^{18}\text{O}$	s(length)	0.045	246.89	0.191	0.383
		s(date)	0.002			
		sex	< 0.001			
		W_r	0.825			
		river age	0.591			
2	$\delta^{18}\text{O}$	s(length)	0.038	244.93	0.189	0.386
		s(date)	0.001			
		sex	< 0.001			
		river age	0.595			
3	$\delta^{18}\text{O}$	s(length)	0.031	243.14	0.188	0.388
		s(date)	<0.001			
		sex	<0.001			
		river age	0.786			
4	$\delta^{13}\text{C}$	s(length)	< 0.001	497.38	1.38	0.444
		s(date)	< 0.001			
		sex	0.544			
		W_r	0.143			
		river age	0.786			
5	$\delta^{13}\text{C}$	s(length)	< 0.001	495.49	1.363	0.447
		s(date)	< 0.001			
		sex	0.575			
		W_r	0.131			
6	$\delta^{13}\text{C}$	s(length)	< 0.001	493.82	1.347	0.45
		s(date)	< 0.001			
		W_r	0.121			
7	$\delta^{13}\text{C}$	s(length)	< 0.001	494.18	1.349	0.445
		s(date)	< 0.001			

to scale circulus (and hence fish growth) increments. This allowed exploration of thermal and metabolic histories within a temporal and growth history framework. The effects of fixed covariates, such as sex, age and condition factor, generally were negligible amongst the restricted number of individuals that were investigated.

Thermal histories

Because there is temperature-dependent oxygen isotope fractionation between aragonite and water, otolith $\delta^{18}\text{O}$ values should be driven primarily by two variables: $\delta^{18}\text{O}$ value of the ambient water ($\delta^{18}\text{O}_{\text{water}}$) and water temperature. Therefore, variation in salmon $\delta^{18}\text{O}_{\text{otolith}}$ profiles could be attributable to their migration between areas of ocean surface waters characterised by different source water $\delta^{18}\text{O}$ signatures and/or by movement between areas of differing temperatures. To estimate thermal histories, it is necessary to constrain the value of $\delta^{18}\text{O}_{\text{water}}$. Ultimately, the $\delta^{18}\text{O}$ values of oceanic waters are reflective of their source; $\delta^{18}\text{O}$ values have been empirically measured for many water masses (see Schmidt et al. 1999 & Frew et al. 2000) and globally interpolated by LeGrande & Schmidt (2006). It is evident from the geographic distribution of $\delta^{18}\text{O}$ values that both freshwater inputs and the presence of sea ice can have large effects on oceanic $\delta^{18}\text{O}$ values. However, $\delta^{18}\text{O}$ values in surface waters (0-10m depth) remain relatively constant throughout much of the migratory range of Atlantic salmon (LeGrande & Schmidt 2006); postsmolts have been found most commonly over ocean depths > 1000m but typically remain mostly within the top 10m of the water column (Holm et al. 2006, Dadswell et al. 2010, Holm et al. 2000) and at salinities >35 psu (Dadswell et al. 2010). It is therefore reasonable to assume a constant $\delta^{18}\text{O}$ value of oceanic water near to 0‰ as a starting point in predicting thermal histories from Atlantic salmon otolith oxygen isotopes.

However, the derivation of an appropriate thermometry equation for wild Atlantic salmon otoliths remains problematic (see discussion in Storm-Suke et al. 2007). Using a regression intercept estimated from the results of the present study (Equation 6.2), >88% of the temperature estimates fall within the range expected from catches of *S. salar* at sea (2 - 14°C; Reddin & Shearer 1987, Holm et al. 2000). This compares with 57% using the field-derived equation of Storm-Suke et al. (2007) and the proportion is negligible (temperature estimates are too warm) using other published fish otolith thermometry equations (Thorrold et al. 1997, Patterson et al. 1993, Kim et al. 2007, Høie et al. 2004b). Nonetheless, once the potential influences of variation in $\delta^{18}\text{O}_{\text{water}}$ and parameter estimates of thermometry equations are

taken into consideration, relative differences in the thermal histories both between and within individuals may become tractable.

That intra-otolith variation in $\delta^{18}\text{O}$ values was much greater than inter-otolith variation suggests that 1SW Atlantic salmon thermal migratory behaviour is relatively constrained and that individuals experience similar physical conditions at sea, perhaps to the extent that they actively choose to remain within surface waters of a relatively narrow thermal range. Comparisons between individuals show that the majority of fish followed similar thermal trajectories; however, away from major surface currents and polar fronts, SST changes in the Norwegian Sea occur over large geographical distances so two fish with a similar thermal trajectory could have migrated to markedly different geographical areas (Figure 6.6). Figure 6.5a shows the general curvilinear pattern of temperature history for all salmon combined, smoothed by date; fish moved from warm coastal waters following freshwater emigration into a range $\sim 8\text{-}10^\circ\text{C}$ by late summer/early autumn, and reached the lowest temperature waters around November - February. They then gradually re-entered warmer waters the following spring, presumably during their return migration into more southerly and warmer coastal waters off Scotland.

The precise oceanic migratory path of 1SW Atlantic salmon still is debated (Dadswell et al. 2010) but the general consensus is that postsmolts from southern Europe (British Isles, France, Spain) migrate north within the continental Slope Current and Norwegian Coastal Current to reach the Norwegian Sea by July - August (Holst et al. 2000, Dadswell et al. 2010, Hansen & Jacobsen 2000). 1SW salmon of southern European origin have been tagged both in the Norwegian Sea and north of the Faroe Islands in autumn and winter, suggesting these areas are important feeding grounds (Hansen & Jacobsen 2000). $8\text{-}10^\circ\text{C}$ water is available in the Norwegian Sea throughout late summer/early autumn of 2008, but these isotherms move south towards the Faroes and into the eastern Norwegian Sea in the late autumn and winter months (Figure 6.6); it is plausible that the average thermal profile generated from 1SW salmon otoliths (6.5a) is representative of their movement within this temperature range and within the North Atlantic Subpolar Gyre (NASpG) (Dadswell et al. 2010).

A few individuals exhibited more variable $\delta^{18}\text{O}$ values (Figure 6.4) than the remainder (e.g. M103-09 and M047-09), suggesting that these particular fish periodically diverged from the common oceanic conditions. Such conditions may have been encountered during excursions into colder regions of frontal or upwelling zones,

where $\delta^{18}\text{O}_{\text{water}}$ values are expected to be higher. Dadswell et al. (2010) suggested that Atlantic salmon may make significant use of the NASpG during their marine migration; if so, the deviations from a narrow range of temperatures shown by M103-09 and M047-09 may represent excursions away from the thermal habitat typical of the gyre. Individual A128-09 was particularly different in that the transition from freshwater to marine oxygen isotope values occurred gradually rather than stepped in this otolith; this may be indicative of a prolonged estuarine residence where $\delta^{18}\text{O}_{\text{water}}$ values tend to be lower in association with freshwater inputs.

The consequences, if any, of these individual 'deviations' for the general success and feeding/growth history of the fish remain unknown. Major changes in the availability, composition and distribution of eastern North Atlantic phytoplankton and zooplankton related to recent ocean climate change have been well documented (Beaugrand et al. 2002, Beaugrand & Reid 2003, Richardson & Schoeman 2004) in the eastern North Atlantic. Particularly pertinent are the long-term declines in euphausiids and copepods - which are major constituents of Atlantic salmon diet (Jacobsen & Hansen 2001, Haugland et al. 2006) - and northward shifts of cold-water copepod species evidenced in these studies. Whilst no significant relationship between $\delta^{18}\text{O}_{\text{otolith}}$ values or estimated winter temperatures and pre-spawning condition factor were recorded in the present study, the distribution of nekton (e.g. squid, forage fish) and zooplankton within the marine environment is patchy and constantly changing. Thus, whilst salmon might successfully locate areas of an optimal temperature it may be that chance effects of plankton and nekton patchiness render those areas suboptimal. Conversely, slight shifts in SST may be reflected by marked changes in prey availability and may mean the difference between salmon finding a productive feeding location, or essentially starving for a period until higher quality areas are encountered. By the same token, however, it may be that these changes in thermal habitat occur on the scale of detailed oceanographic features (such as frontal structures or current meanders) rather than simple but large-scale latitudinal effects. Notwithstanding these qualifications, it is apparent that differences in eventual pre-spawning somatic condition of mature adult 1SW fish at the conclusion of their marine migration do not appear to be driven by large or systematic differences in thermal habitat selection.

Metabolic histories

The general pattern of $\delta^{13}\text{C}$ variation for all otoliths pooled is presented in 6.5b. There is an ontogenetic pattern of $\delta^{13}\text{C}_{otolith}$ values evident in the profiles that corresponds to similar curves found in other studies (Wurster et al. 2005, Gauldie 1996a, Dufour et al. 2007, Gauldie 1996b, Høie et al. 2003, Schwarcz et al. 1998). $\delta^{13}\text{C}$ values in aragonitic otoliths arise from the mixing of bicarbonate of the dissolved inorganic carbon (DIC) component of ambient water and metabolically-derived bicarbonate in the blood (which is itself reflective of dietary carbon), and can be described using a mass-balance model (Schwarcz et al. 1998, Wurster & Patterson 2003, Dufour et al. 2007):

$$\delta^{13}\text{C}_{otolith} = M \cdot \delta^{13}\text{C}_{diet} + (1 - M) \cdot \delta^{13}\text{C}_{DIC} + \varepsilon_{total} \quad (6.4)$$

where M describes the proportion of metabolic carbon in the otolith and ε_{total} is the total fractionation between carbon sources and otolith aragonite, estimated to be between -1.8‰ and -2.7‰ (Solomon et al. 2006 but see also Radtke et al. 1996). Therefore, both ontogenetic and inter-individual differences in $\delta^{13}\text{C}_{otolith}$ could be driven by (i) differences in the proportion of metabolically derived carbon (M), (ii) differences in $\delta^{13}\text{C}_{diet}$ values and/or (iii) differences in $\delta^{13}\text{C}_{DIC}$ values. Possible sources of variability in each of these parameters are discussed below.

$\delta^{13}\text{C}_{diet}$ Atlantic salmon are considered generalist predators, but there is some evidence for ontogenetic changes in diet based on stomach content analyses. Once feeding in oceanic areas, the most common prey items found in the stomachs of post-smolts are hyperiid amphipods, euphausiids and larval fish. Pre-adults in the northeast Atlantic are found to include larger fish such as sandeel, lanternfish and pearlsides in their diets in addition to amphipods, euphausiids and other crustaceans (Jacobsen & Hansen 2000, 2001, Haugland et al. 2006, Hansen & Pethon 1985). $\delta^{13}\text{C}_{diet}$ values for the potential prey of Atlantic salmon are poorly represented in the literature. However, average $\delta^{13}\text{C}_{diet}$ values (after lipid extraction) from the available literature (Sato et al. 2002, Søreide et al. 2006, Møller 2006) of the important crustacean species (euphausiid, amphipods and copepods, mean $\delta^{13}\text{C} = -21.3\text{‰} \pm 1.7$) are approximately 3‰ lower than those of higher trophic level prey such as forage fish (sandeel, capelin, cod, haddock, mean $\delta^{13}\text{C} = -18.2\text{‰} \pm 0.8$).

These differences probably are a result of the trophic enrichment in ^{13}C , which is close to 1‰ in aquatic systems (Zanden & Rasmussen 2001). Thus, diet switching to larger, higher trophic level prey (such as fish) associated with rapid marine growth may account for some of the ontogenetic rise in $\delta^{13}\text{C}$ values within salmon otoliths. It is worth noting, however, that in studies of $\delta^{13}\text{C}_{\text{otolith}}$ where $\delta^{13}\text{C}_{\text{diet}}$ was measured (Dufour et al. 2007, Høie et al. 2003) changes in diet were not sufficient to explain variation in $\delta^{13}\text{C}_{\text{otolith}}$ values.

$\delta^{13}\text{C}_{\text{DIC}}$ The carbon stable isotope value of dissolved inorganic carbonate in marine waters is governed by a complex balance between biological and thermodynamic processes and few studies have measured $\delta^{13}\text{C}_{\text{DIC}}$ directly (Gruber et al. 1999, Kroopnick 1985). Briefly, $\delta^{13}\text{C}$ values in ocean surface waters may vary spatially due to photosynthesis - during which the lighter isotope ^{12}C is preferred, lowering the $\delta^{13}\text{C}$ of phytoplankton relative to surrounding waters - whereas upwelling of remineralised organic material lowers surface $\delta^{13}\text{C}$. Thermodynamic processes include kinetic and equilibrium fractionation between atmospheric $\delta^{13}\text{C}$ and surface water $\delta^{13}\text{C}$ and bulk CO_2 transfer across the atmosphere-ocean interface (Gruber et al. 1999). While variation in surface $\delta^{13}\text{C}_{\text{DIC}}$ remains slight at mid-latitudes, high latitude areas can show large spatial and temporal fluctuations. In the northern North Atlantic, $\delta^{13}\text{C}_{\text{DIC}}$ values ranged from 2.2‰ during summer (September) to 1.1‰ during winter (March) at latitudes beyond 60°N (Gruber et al. 1999); this difference was attributed largely to biological processes connected to the stratification of surface waters during summer months. If this seasonal shift $\delta^{13}\text{C}_{\text{DIC}}$ were to account for variation in $\delta^{13}\text{C}_{\text{otolith}}$ values, one would expect $\delta^{13}\text{C}_{\text{otolith}}$ values to decrease as fish migrate during summer and into winter months. However, the trend in Atlantic salmon otoliths was for an increase in $\delta^{13}\text{C}_{\text{otolith}}$ values over this time from ca. -7‰ to -5‰. Therefore, it is unlikely that changes in $\delta^{13}\text{C}_{\text{DIC}}$ can account for the pattern of $\delta^{13}\text{C}_{\text{otolith}}$ values.

Metabolic carbon In the absence of metabolic effects ($M = 0$) and assuming $\delta^{13}\text{C}_{\text{DIC}} = 1.65\text{‰}$ (intermediate between summer and winter values) and $\varepsilon_{\text{total}} = -2.7\text{‰}$ (Solomon et al. 2006), $\delta^{13}\text{C}_{\text{otolith}}$ values in equilibrium with surrounding DIC should be $\sim -1.15\text{‰}$ whereas measured $\delta^{13}\text{C}_{\text{otolith}}$ values were generally between -6‰ and -8‰. Clearly, there is a metabolic contribution to the carbon pool from which $\delta^{13}\text{C}_{\text{otolith}}$ values are derived. Estimates of M vary widely across taxa and life stages

(for summary see Solomon et al. 2006); however, where both $\delta^{13}\text{C}_{\text{diet}}$ and $\delta^{13}\text{C}_{\text{DIC}}$ have been measured (for juvenile fish only) M ranges from 17% to 32%. These estimates are specific to particular life stages, but in reality metabolic rate will fluctuate over the lifetime of an individual Atlantic salmon in response to changes in non-stationary parameters such as temperature, growth and activity (Smith et al. 2009). Using estimates of respiration calculated from bioenergetic models and temperatures estimated from $\delta^{18}\text{O}_{\text{otolith}}$ values in Lake Annecy whitefish, Dufour et al. (2008) showed a strong correlation between ontogenetic patterns of specific respiration rate (SRR) and $\delta^{13}\text{C}_{\text{otolith}}$ values. Furthermore, in a controlled laboratory setting using Atlantic cod, Høie et al. (2003) found a strong negative relationship between $\delta^{13}\text{C}_{\text{otolith}}$ values and somatic growth rate, which they interpreted as evidence for the negative effect of increased respiration rate on $\delta^{13}\text{C}_{\text{otolith}}$ values. Thus, variation in metabolic activities over the period of rapid growth measured here in 1SW Atlantic salmon is likely to be the major factor contributing to the ontogenetic and inter-individual changes in $\delta^{13}\text{C}_{\text{otolith}}$.

$\delta^{13}\text{C}_{\text{otolith}}$ Assuming that $\delta^{13}\text{C}_{\text{otolith}}$ values can be used as a proxy for metabolic rate due to the incorporation of isotopically light carbon from cellular respiration relative to more positive $\delta^{13}\text{C}_{\text{DIC}}$, the findings of the present study indicate that fish experienced elevated metabolic rate(s) primarily during the early phase of their marine migration and also within the last few months prior to coastal return (6.5b). There was no evidence to support the hypothesis that fish returning in poor growth condition have experienced significantly different metabolic histories from those returning in normal condition, although there are obvious inter-individual differences in $\delta^{13}\text{C}_{\text{otolith}}$ values. In a study of some of the physiological changes experienced by young Atlantic salmon as they move from rivers through estuaries and into the early marine phase, Stefansson et al. (2003) found evidence that postsmolts mobilize energy reserves while conserving protein for somatic growth. This endogenous carbon contribution will be derived from a relatively ^{13}C -depleted energy pool and could help explain the low $\delta^{13}\text{C}_{\text{otolith}}$ values in early in the marine life stage. Additionally, the dietary C of younger and smaller salmon (e.g. the smaller constituents of zooplankton) is likely to be ^{13}C -depleted, and would augment low $\delta^{13}\text{C}_{\text{otolith}}$ values during this stage.

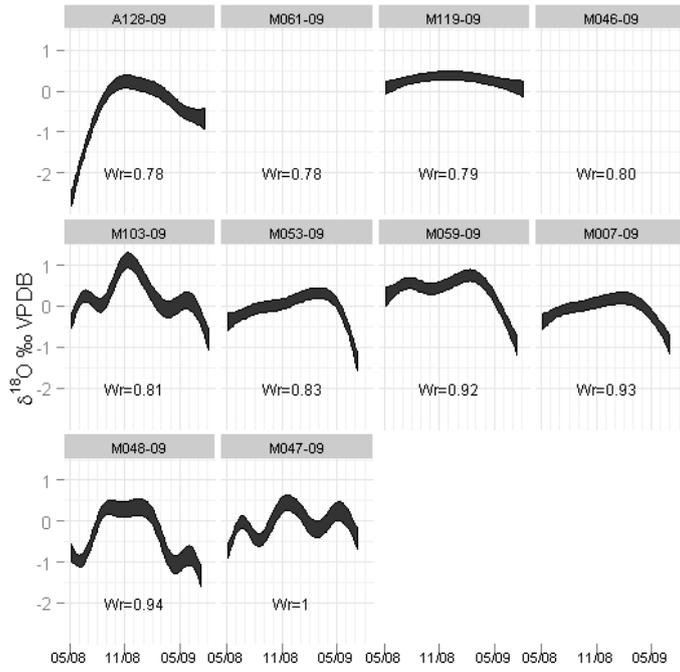
However, the marked decline in $\delta^{13}\text{C}_{\text{otolith}}$ over the last few months of the marine migration cannot be attributed to a rapid diet switch because homing salmon are

known to cease or markedly reduce feeding during this time (Jacobsen & Hansen 2000). All fish used in this study had empty guts upon capture in coastal waters (C. Todd, pers. comm.). Also, changes in $\delta^{13}\text{C}_{DIC}$ values in the coastal region track steep salinity gradients; if declines in $\delta^{13}\text{C}_{DIC}$ values associated with freshwater input are responsible for the decrease in ^{13}C at the edge of the otolith, then salmon would have to have been occupying estuarine environments for several months prior to capture. This is most unlikely given the geographic situation of the coastal interceptory nets where they were caught; the north coast of Scotland is a likely area of first landfall for homing southern European stocks and tag returns have shown that salmon captured on the north coast are destined for various rivers along the east and west coasts of Scotland and even Ireland (Jenkins & Shearer 1985). Moreover, the evidence is that homing salmon move rapidly from open oceanic and neritic waters to estuarine environments (Holm et al. 2006). Rather, it is proposed that the pronounced decline in $\delta^{13}\text{C}_{otolith}$ values in the last few months of their migration represents the incorporation of dietary carbon derived not from newly-assimilated dietary items but from catabolic processes. With the cessation in feeding by salmon, there would be an increase in blood bicarbonate derived from the catabolism of tissue and any increase in metabolism associated with increased swimming activity or nutritional stress would serve only to further decrease $\delta^{13}\text{C}_{otolith}$ values. Therefore, it is concluded that intra-otolith $\delta^{13}\text{C}_{otolith}$ values of 1SW Atlantic salmon are largely reflective of ontogenetic changes in metabolic rate. The relationship between carbon isotopes and condition factor suggests that these fish underwent periods of elevated metabolism, especially during early and late migratory phases, that could be explained by the mobilisation of energy reserves during these periods.

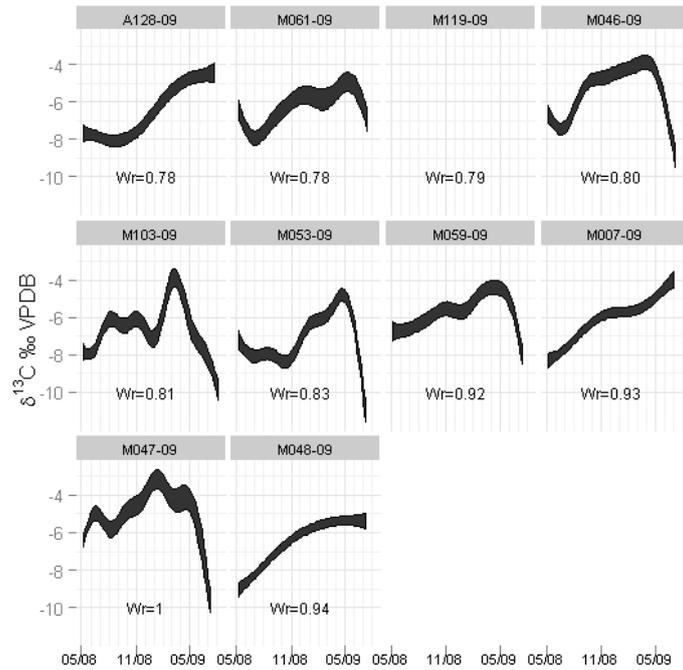
Summary The high resolution profiles obtained here have allowed detailed comparisons of thermal and metabolic histories between and within individuals during comparable periods of their marine migration. The ability to retrospectively study entire marine life histories of fish that survive the marine migration is currently limited to stable isotope analyses of otoliths. Most contemporary biotelemetry technologies do not allow for long-term deployment or retrieval of tags throughout the marine phase and additionally, traditional tags have an extremely low probability of return for Atlantic salmon (e.g. 5 retrievals out of 413 fish tagged; Holm et al. 2006). The results of this study support the hypothesis that Atlantic salmon occupy relatively narrow thermal habitats but also highlight individual differences in thermal

choice. Furthermore, it is proposed that intra-otolith $\delta^{13}\text{C}$ values can be informative of the metabolic history of individual fish. High-resolution sampling is useful for exploring detail at the individual-level but inferences regarding effects of sex, age and condition factor are limited due to the small sample size. Owing to the small size of Atlantic salmon otoliths and need for discrete sampling, the present study was greatly aided by the use of the high resolution SIMS technique and comparable data would not have been forthcoming from traditional micro-milling techniques. Never the less, the relatively high cost and inaccessibility associated with SIMS will always limit the ability to obtain the large sample sizes necessary in some ecological studies.

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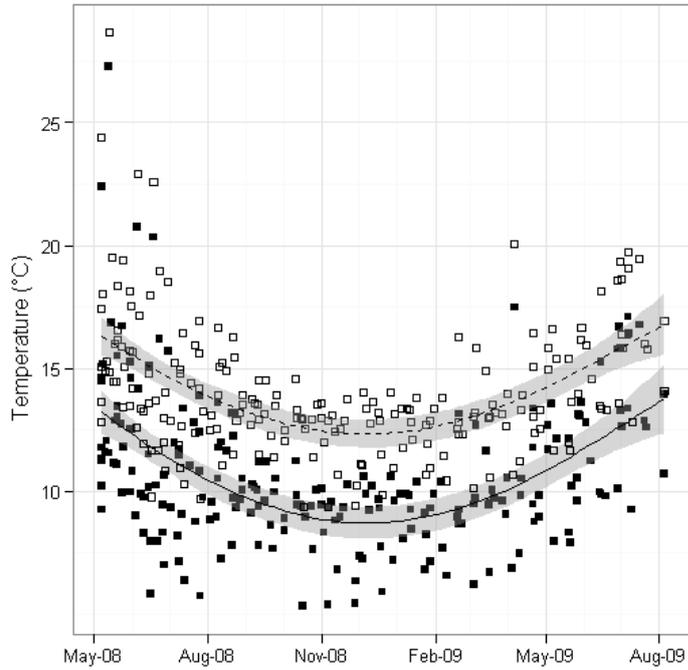


(a)

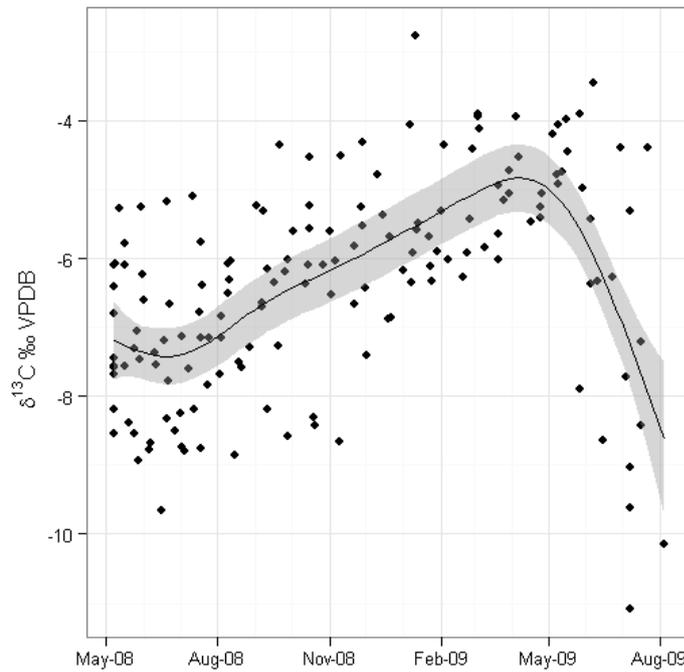


(b)

Figure 6.4: Penalised regression spline smooths of individual $\delta^{18}\text{O}$ (a) and $\delta^{13}\text{C}$ (b) profiles within the marine zone scaled to back-calculated date from May 2008 to August 2009. The black ribbon indicates the smooth \pm standard error. Individual plots are arranged in ascending order of growth condition factor (W_r).



(a)



(b)

Figure 6.5: Temperature estimates ($^{\circ}\text{C}$) calculated from pooled 1SW Atlantic salmon $\delta^{18}\text{O}_{otolith}$ values (a) using Eqn Equation 6.2 (solid line and points) and Eqn Equation 6.3 (dashed line and open points); $\delta^{13}\text{C}_{otolith}$ values (b) by calendar date. Smooths and 95% confidence intervals were generated using generalised additive models.

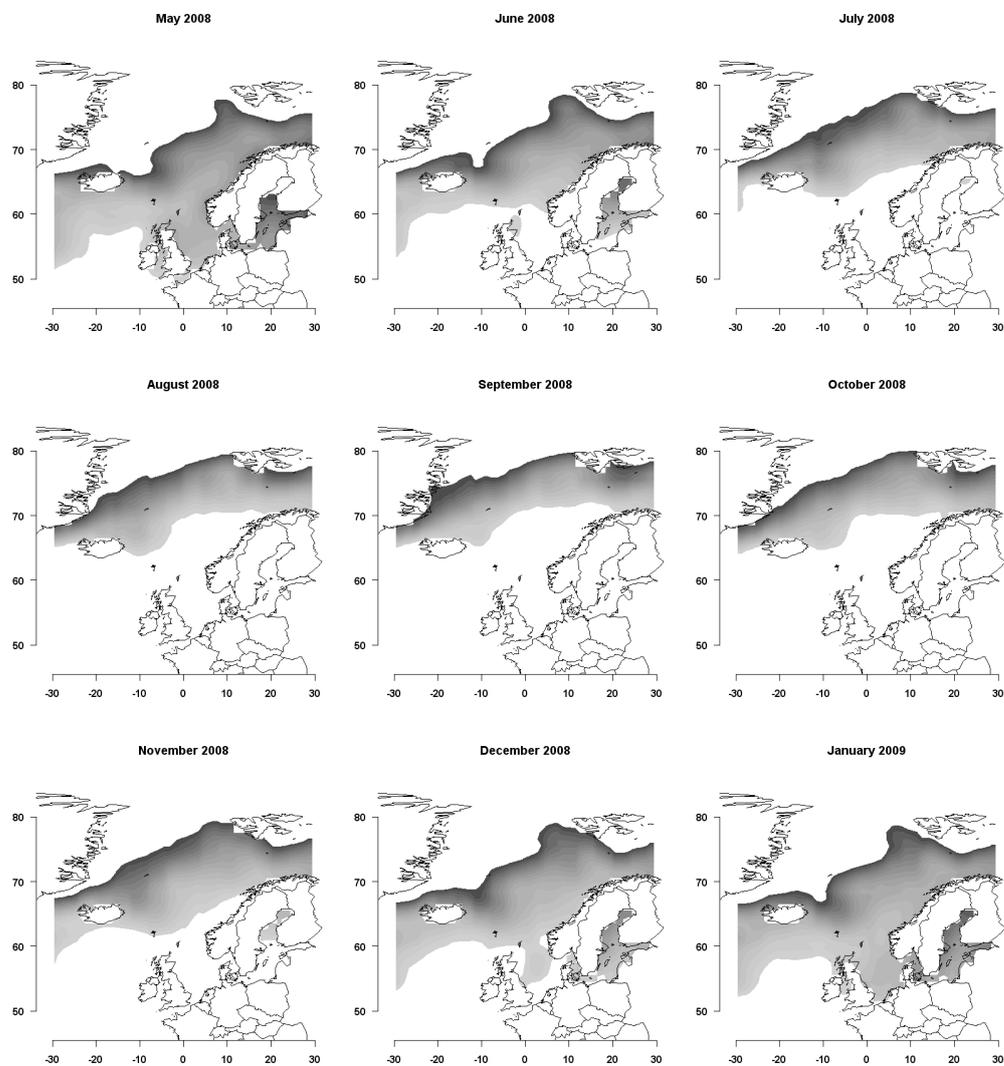


Figure 6.6: The distribution of ocean surface water within the 2 - 10°C isotherm in the eastern North Atlantic. Data compiled from the NOAA optimally interpolated SST dataset (version 2) available from (<http://www.esrl.noaa.gov/psd/data/gridded/data.noaa.oisst.v2.html>) (Reynolds et al. 2002)

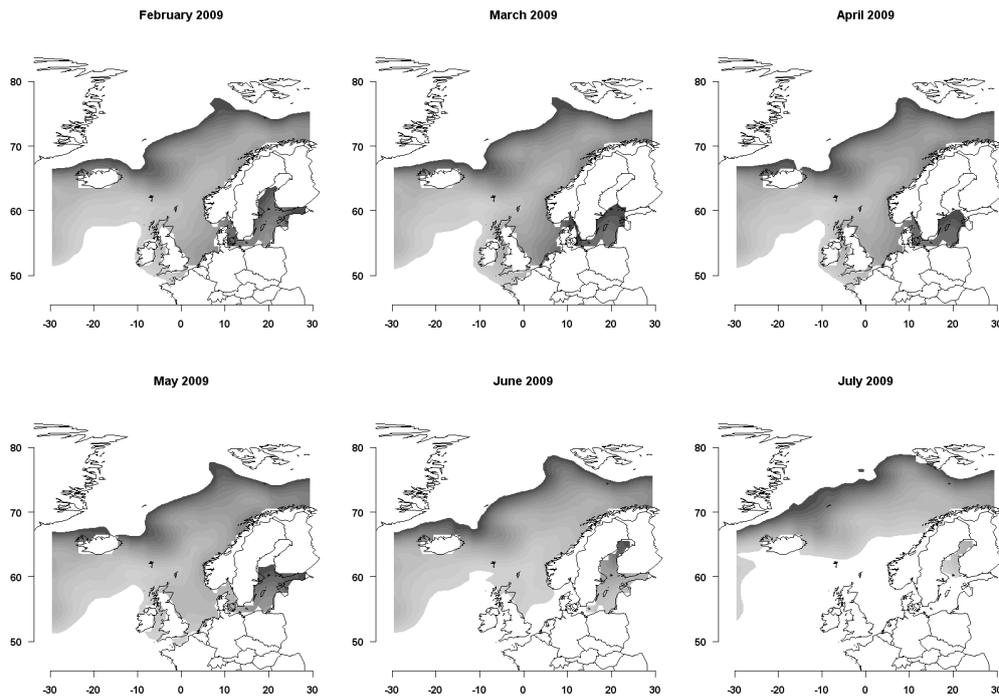


Figure 6.6: The distribution of ocean surface water within the 2 - 10°C isotherm in the eastern North Atlantic. Data compiled from the NOAA optimally interpolated SST dataset (version 2) available from (<http://www.esrl.noaa.gov/psd/data/gridded/data.noaa.oisst.v2.html>) (Reynolds et al. 2002)

7 General Discussion

This thesis examined the response of top marine predators to ecological change using stable isotope proxies; a multi-tissue, multi-isotope and multi-taxa approach allowed the results of these studies to be placed in a range of spatial and temporal contexts. Below, I describe these contexts and evaluate the use of stable isotope proxies as bio-monitoring tools in marine ecology. I review the insights gained during the present research into the response of three marine predators to recent ecological change and discuss directions for future research in stable isotope ecology.

Temporal scales

Ontogenetic Detailed individual-based data on ontogenetic change are extremely difficult to obtain for free-ranging marine predators. Technological and statistical advances, including electronic tagging, has begun to alleviate some of these difficulties; archival tags may last >4 years for some fish and routinely collect information about the physiology of the animal and its abiotic environment (Block 2005). Boyd et al. (2004) provided one definition of this 'biologging science' as "investigation of phenomena in or around free-ranging organisms that are beyond the boundary of our visibility or experience". While isotope ecologists might not consider themselves bi-loggers, by this definition, there are reasons to argue that this definition does apply to them. Stable isotope analysis of accretionary hardparts or multiple soft tissues of marine organisms allows researchers to cross physical and temporal boundaries to study diet, movement, thermal preferences and metabolic histories of individuals. Unlike most electronic tagging techniques, however, chemical proxies often can be obtained at temporal scales from entire life histories to sub-annual resolution.

In chapter 2, individual lifetime profiles of male Antarctic fur seal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were obtained. The general ontogenetic pattern amongst aggregated individuals was one consistent with males foraging at higher trophic levels and at more southerly latitudes later in life. This conclusion was concordant with the foraging demands

and energetic capabilities of these animals as they age. Coupled with age-at-death data and indices of resource availability, these lifetime records can also be used to test relationships between early life foraging habits and longevity in wild populations (so-called 'silver spoon' effects). Preliminary investigations did not find evidence for these effects in male Antarctic fur seals or British grey seals (in chapter 3), but the detailed ontogenetic information obtainable from stable isotope analysis of biogenic hardparts could be useful to investigating this phenomenon in other taxa.

Important parameters during the critical, but not well understood, marine life stage of wild Atlantic salmon, representing a temporal scale of 13 - 15 months, were characterised in chapter 5 and chapter 6. The study was initially motivated by the simple question: "Have individuals returning in poor somatic condition experienced warmer oceanic conditions and metabolic 'stress' compared to those returning in normal body condition?" Retrospective profiling of otolith $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values from the marine growth zone provided an avenue to address this question. While the study did not find any evidence for thermal or metabolic segregation on the basis of return body condition, it was apparent that the marine profiles of individuals were characterized both by a common pattern and individual-based deviations from the common pattern. The common pattern amongst $\delta^{18}\text{O}$ profiles showed a gradual decline in water temperature experienced by the fish to a minimum around mid-winter after which there was a gradual increase in water temperatures. $\delta^{13}\text{C}$ profiles generally followed an ontogenetic pattern of curvilinear increase consistent with a decline in the metabolic proportion of carbon delivered to the otolith as fish age; in the last few months, however, there was a rapid decline in $\delta^{13}\text{C}$ values in the last few months which might be indicative of an increase in metabolic activity associated with the return migration. This study only began to explore the use of otolith 'biologging' to document annual movement patterns (between freshwater and marine residency, and within thermal gradients) coupled with physiology (metabolic rates, dietary shifts).

A temporal resolution down to months or weeks was obtained from intra-otolith isotope profiles once an appropriate dating technique was established. Sub-annual resolution of wild Atlantic salmon trophic history was also obtained (chapter 4) by analysing multiple tissues from the same individual with different rates of isotopic incorporation. The analysis was restricted to the most recent (~6-8) months of the marine migration but different tissues provided different 'windows' from which trophic effects on the body condition of individuals could be assessed. As a general-

isation, there was considerable variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values both between and within individual fish but a few important patterns emerged - the 'tissue timing' of which was critical to the interpretation. For example, a negative relationship between $\delta^{15}\text{N}$ values in the liver and fish somatic condition, but a positive relationship in muscle tissue, shows that there is a temporally shifting trophic dynamic in the last months at sea that has an impact on the somatic condition of returning fish. Because liver tissue is known to turnover rapidly, and salmon are known to dramatically reduce or cease feeding prior to the spawning migration, it was possible to relate $\delta^{15}\text{N}$ value to nutritional stress in the last few weeks at sea. The proportion of "skinny salmon" returning to Scottish freshwaters in poor somatic condition has increased over recent years; the results from the tissue analyses thus suggest that one response of salmon to the ecological changes driving this decline is earlier and perhaps more severe starvation than seen in previous years. However, validation of this effect in more than one year is warranted.

Long-term The results of chapter 4 should be placed in the historical context of declining salmon stocks to appreciate their significance to the study of salmon marine ecology; but lethally sampling multiple tissues from wild Atlantic salmon in many consecutive years is not feasible because of the cost and the effects upon the salmon population. In many research programs, there will be a trade-off between analytical resolution and sample size; one can focus on obtaining high-resolution (e.g. sub-annual) analytical data from few individuals - which may require lethal sampling as in chapter 4 and chapter 6 - or obtaining low resolution (e.g. annual to multi-annual) data from many individuals in a population. In the case of Atlantic salmon, as for many commercially important fish species, retrospective long-term data from many individuals can be obtained from archived collections of fish scales - which were initially collected for purposes of age determination and growth back-calculation. Similarly, the teeth of marine mammal species of conservation and/or subsistence concern (e.g. northern fur seals, Stellar sea lions, Antarctic fur seals) often are collected for monitoring purposes. Archives from such regular monitoring provide an invaluable source of material for retrospective stable isotope analysis because (a) sample collection and preparation is often standardised, (b) samples are collected on a regular basis and (c) other forms of useful information such as morphometric, abundance and dietary data accompany these databases.

Combination of the foresight of British Antarctic Survey staff to collect annual

samples and the relative ease of sampling individual growth layer groups in male Antarctic fur seal canine teeth made it possible to reconstruct ~40 years of trophic variability for the South Georgia population in chapter 2. Because these data were annually resolved, both long-term trends and inter-annual fluctuations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values could be statistically determined and correlated with relevant climatic indices using time series analyses. This process highlighted a relationship between sea surface temperature (SST) and $\delta^{13}\text{C}$ which was postulated to be due to the dynamics of local krill abundance and its association with SST; in warm years, male fur seals may be forced to travel further south to reach their prey. While this explanation is plausible, a major concern of any study investigating long-term stable isotope data is a lack of adequate knowledge of changes in the isotopic baseline through time (e.g. because of changes caused by shifts in primary productivity). This problem is discussed in greater detail on page 139 and possible solutions are proposed.

In chapter 3, two factors prevented the acquisition of stable isotope information for Atlantic salmon and seals on an annual basis. First, it was thought that, given budgetary constraints, a sampling strategy maximising the number of individual salmon analysed in each somatic condition factor category per year was better suited to addressing the question of trophic segregation between these fish than a smaller number of individuals in consecutive years. Indeed, it was because particular years were selected from the scale archive and fish were chosen on the basis of their somatic condition factor that a significant relationship between SST and trophic segregation of these fish was revealed. Second, British grey seal teeth were available only from non-consecutive historical collections and modern samples had to be collected on an opportunistic basis by the author. Because the ages of these individuals were unknown prior to sampling, it was not possible to stratify the sampling to embrace as many calendar years as possible.

Despite these constraints, the archival records of salmon and seal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values provide useful long-term cross-taxa comparisons. The fact that linear trends were evident in grey seal stable isotope values, and that temporal changes in salmon $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were only revealed once data were partitioned by somatic condition factor suggests that the temporal response of these predators to broad-scale ecological change, such as rising SST anomalies in the North Sea and Norwegian Sea (Hughes et al. 2010), has not been synchronous. This may be due to the fact that, over the last century, seals have had to contend both with climate change and

increasing interactions with trophic changes forced by fisheries.

In summary:

- The temporal scales of stable isotope investigations range from weeks or months in tissues with high protein turnover rates or in biomineralised structures that can be sub-sampled at a high resolution by micromilling or SIMS;
- Some of these structures, such as fish otoliths or mammalian teeth, can be sub-sampled across an entire life history profile, providing an ontogenetic track record of isotope change for an individual animal;
- Collected over time, stable isotope analysis of animal tissues can provide long-term records of stable isotope variability.

The temporal scale of investigation largely depends on the tissue or structure being analysed (e.g. turnover rate, mineral deposition time, mechanical wear), the sub-sampling method (e.g. micro-milling vs bulk analysis) and analytical procedure (e.g. CF/IRMS vs SIMS). These structures may also reflect variability at different spatial scales, although the particular life history of the organisms themselves is also important.

Spatial scales

Stable isotope variation also occurs at a variety of spatial scales; the extent to which this variation can be related to the response of top predators to changes in their environment depends upon the structure or tissue being analyzed and the natural history of the organism being studied.

Local scale Because they are acellular and precipitated from an intermediate medium the composition of which is dependent on that of the water ingested by the fish, otolith isotopes primarily reflect local ambient abiotic conditions at the time of deposition (e.g. water temperature, dissolved inorganic carbon, watershed geology; Degens et al. 1969, Campana 1999). These conditions are spatially heterogeneous - mainly varying by latitude - within the distributional range of 1SW southern European Atlantic Salmon (LeGrande & Schmidt 2006, Gruber et al. 1999) and this variation is reflected in the individual patterns of intra-otolith $\delta^{18}\text{O}$ and

$\delta^{13}\text{C}$ values of wild Atlantic salmon (Figure 6.4). While the spatial integration of isotopic information provided by such wide-ranging animals is useful in many instances (e.g. for determining long-term patterns of trophic change; Sinnatamby et al. 2009), the fine-scale detail of local conditions experienced by individual fish can be related to patterns of movement both among and within individual life histories.

Regional scale Other structures and tissues, which are not separated from the metabolism of the organism in this way and from which organic matrices are often used for isotope analysis (e.g. tooth collagen, muscle), are constructed from amino acids ultimately derived from the diet. Because the foraging of marine predators is spatially heterogeneous, as is that of their prey, these tissues integrate the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values over spatial scales that are at least as broad as their foraging range. Thus, the tissue stable isotope ratio of British grey seals can also be a proxy for processes affecting the shelf-zone whereas salmon tissue values will be more closely coupled to processes affecting the open ocean pelagic zone.

Global scale Whilst not explicitly addressed in this thesis, long-term stable isotope analyses of modern populations are sufficiently widespread so as to offer the possibility of investigating global patterns of trophic dynamics. For example, a preliminary literature survey showed that there are several long-term stable isotope studies of marine organisms in the Atlantic Ocean (Jennings et al. 2002, Pruell et al. 2003, Christensen & Richardson 2008, Sinnatamby et al. 2009, Aubail et al. 2010; plus two more contained in the present thesis), the Pacific Ocean (Schell et al. 1998, Hirons et al. 2001, Schell 2001, Satterfield & Finney 2002, Smith et al. 2002, Hobson et al. 2004b, Newsome et al. 2007a, Norris et al. 2007), the Southern Ocean (Hilton et al. 2006, Hanson et al. 2009), and the southern Indian Ocean (Jaeger & Cherel 2011). The organisms featured in these studies also occupy a range of trophic levels; a meta-analysis of such data may reveal global patterns of trophic change.

Within the present thesis, it was possible to compare the declines in tooth collagen $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values both in male Antarctic fur seals in the Southern Ocean and female grey seals in the North Sea. The direction of change was the same for both taxa, implicating a long-term decline in average trophic level for these species, but the magnitude of decline was greater for Antarctic fur seals than for grey seals (-0.068 yr^{-1} vs -0.015yr^{-1} , respectively, for $\delta^{13}\text{C}$ values; -0.052yr^{-1} vs -0.030yr^{-1} for $\delta^{15}\text{N}$ values). This difference in magnitude might result from the difference in

foraging ecology between these two species; grey seals forage on a range of benthic and pelagic fish whereas the Antarctic fur seal diet can encompass a greater range of trophic levels (e.g. krill, krill-eating fish, piscivorous fish). However, the long-term declines in these and other marine predator isotopic records (Hirons et al. 2001, Newsome et al. 2007a, Christensen & Richardson 2008, Aubail et al. 2010) may be related to global patterns of ecological forcing.

In summary:

- Stable isotope analyses can thus be used to investigate local environmental conditions experienced by individuals (chapter 6);
- regional environmental conditions experienced by a population or group of individuals (chapter 2 and chapter 3);
- and to compare global conditions experienced by different taxa (Figure 7.1)

Stable isotope analysis as a bio-monitoring tool in wild populations

A great deal of controlled laboratory research is necessary in order to understand the processes driving variation in the natural abundance of stable isotopes (del Rio et al. 2009). While considerable progress has been made in the physiological understanding of animal stable isotope signals over the past 10 years, there still are many complex factors that contribute to their variability in natural populations. These include differing tissue turnover rates, isotopic routing, lipid content of the tissue, experimental preservation methods, individual growth rates, diet quality and also quantity. Constraining or quantifying all unknowns for wild populations is not feasible; despite this, stable isotope analyses can still fulfill a bio-monitoring need. In chapter 1, a need for accessible, stable and meaningful indices for monitoring marine ecosystems was highlighted. This thesis has demonstrated that, using stable isotopes, information about the response of top marine predators to ecological change can be accessed at multiple trophic levels, at various spatial and temporal scales and for individuals as well as populations. Such information about marine organisms and environments is most easily accessible from organisms that return to coastal or freshwater habitats on a regular basis (e.g. pinnipeds, seabirds, anadromous fish). Tissues that are biomineralised or keratinised at formation are consid-

ered metabolically inert. Data for such tissues and structures therefore represent a reliable and consistent source of information (i.e. it does not change with sample age or cellular contact) and which can be assessed retrospectively. Furthermore, the analytical costs of stable isotope analyses, as with many developing technologies, is no longer prohibitive to their use in routine sampling and long-term monitoring schemes. How well stable isotope data meet the final criterion - that a monitoring index be meaningful - depends on the research context and the availability and quality of additional information. There are two main questions to address in order to maximise the details obtainable from stable isotope proxies in wild populations.

What do changes in stable isotope ratios mean?

Stable isotope ratios clearly are not constant across time or space and it is the information contained within this variation that the observer wishes to interpret. When using these chemical proxies to study the habits of wild animals, it is necessary to acknowledge the numerous possible causes for observed variation because only some of the causes may be of interest.

In the present studies, the meaning of stable isotope variation (as with any biological index) is contextual and depends both upon the spatio-temporal scale of the questions being asked (e.g. at the local level of the individual, or the regional population level; over life histories or multiple decades) and the supplementary information that was available to guide hypothesis testing. As a generalisation, variation was proposed to result from two main processes: changes in animal trophic dynamics resulting from behavioural or life-history changes in the organisms themselves, and changes in the environmental conditions experienced by the organisms related to its location within an isoscape. This could be caused by animal movement or by changes in the geographical distribution of the isotopes.

In chapter 2, stable isotope proxies were deduced to be related both to trophic effects and to animal movement. It was argued that ontogenetic increases in male Antarctic fur seal $\delta^{15}\text{N}$ tooth values probably were related to an increased proportion of upper trophic level prey such as piscivorous fish in the diet. Over the long-term, variation in the average $\delta^{13}\text{C}$ for the population was interpreted to be a result of males moving between oceanic zones near to South Georgia and the Antarctic Front and regions further south nearer to the sea ice edge where $\delta^{13}\text{C}$ values are relatively more negative (Kroopnick 1985, Cherel & Hobson 2007).

Whilst undoubtedly capable of traveling long distances, grey seals spend the majority of their time at sea within relatively close proximity to coastal haul-out sites (McConnell et al. 1999, Matthiopoulos et al. 2004); it was thus unlikely that inter-annual variation in $\delta^{13}\text{C}$ values for these animals (Chapter chapter 3) could be related to their movement within the eastern North Atlantic. Given the simultaneous decline in $\delta^{15}\text{N}$ values, the historical context of human activities in the North Sea, and additional evidence from historical scat analysis (Hammond et al. 1994, Hammond & Grellier 2005), it was reasoned that stable isotope proxies reflected trophic effects in these animals and, in particular, a long-term decline in their average trophic level. The additional empirical data from scat analysis of east coast grey seals, and separate information about the state of the North Sea ecosystem in which they operated, allowed this inference to be made.

In the case of Atlantic salmon, an additional complication to interpreting stable isotope values was the little-studied effect of nutritional deprivation on body tissue $\delta^{15}\text{N}$ values. A statistical relationship between scale $\delta^{15}\text{N}$ value and condition factor may have been a result of poor condition fish feeding at higher trophic levels or essentially starving. While some evidence exists for the effect in fish muscle and liver tissues (Doucett et al. 1999, Jardine et al. 2004, Gaye-Siessegger et al. 2007), to my knowledge, there are no published studies which have investigated the effect of fasting on fish scale stable isotope values. It is important to consider the background the present studies

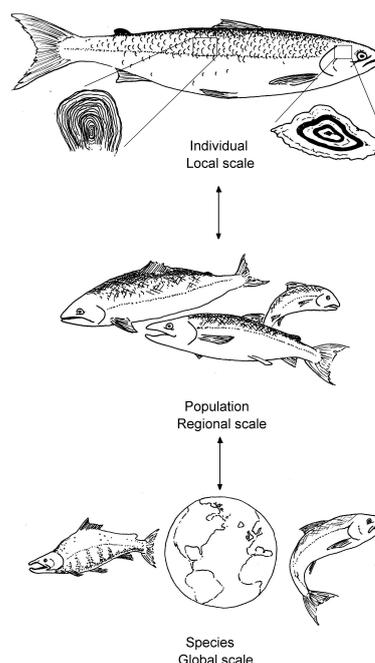


Figure 7.1: Schematic illustration of different scales of monitoring with stable isotope analysis. Detailed information about local environmental conditions experienced by individuals is obtainable from biomineralised structures such as fish scales and otoliths; when samples from many individuals are combined or collected over time, stable isotope analyses can provide information about the regional environmental conditions experienced by that population; comparing stable isotope variation across taxa and regions can also provide a global perspective environmental conditions.

concerning Atlantic salmon somatic condition; Todd et al. (2008) reported that January SST anomalies in the Norwegian Sea were implicated in determining the final somatic condition of return adults some months later. The authors suggested that post-winter marine feeding experience, modulated by SST, may therefore be a key factor driving somatic condition variation. This interpretation suggests that some salmon experienced prolonged periods of food restriction; this period could have occurred post-winter (i.e. fish were in good condition up until winter months and then catabolised energy reserves throughout the following spring/summer) or the period of food restriction may have been sustained over much of the life of the salmon (i.e. 'skinny' fish were skinny throughout their marine life). In either case, it seems probable that poor condition fish experienced sub-optimal feeding conditions at some point during their marine migration; the question remains, however as to whether high $\delta^{15}\text{N}$ values of scale tissue reflects the fact that salmon are essentially catabolising their own tissues during this time ('eating themselves') or that they are foraging at upper trophic levels, which are less abundant and perhaps more difficult to locate and ingest. Scale collagen is likely synthesized from both dietary and endogenous amino acids pools (Hammond & Savage 2009). If foraging conditions at sea were suboptimal for some fish and dietary protein was restricted during this time, it is possible that during the last spring at sea dietary amino acids were preferentially routed towards muscle and skeletal development (i.e. maintaining growth in length) whilst nonessential tissues - such as scale collagen - were more reliant on ^{15}N -enriched endogenous amino acid pools.

In chapter 3, the divergent trends in scale $\delta^{15}\text{N}$ isotope values between fish returning in good and poor condition were interpreted as evidence for trophic segregation at sea rather than a long-term starvation signal in skinny salmon primarily because it was reasoned that starvation must have been unusually prolonged for it to significantly effect the bulk scale $\delta^{15}\text{N}$ isotope value and furthermore, fish scales showed no signs of resorption which is common during periods of extreme fasting. However, until the effects of nutritional deprivation on scale stable isotope values can be determined experimentally (either by food restriction experiments or compound-specific stable isotope analyses), any inferences regarding the mechanisms for ^{15}N enrichment associated with poor condition salmon must be considered with caution.

The results of chapter 4 at first appear to conflict with the Atlantic salmon scale archive results of chapter 3 in that there was no association between somatic condition factor and salmon scale $\delta^{15}\text{N}$ values and a weak, but significant and positive,

association with muscle $\delta^{15}\text{N}$ values. However, those results were restricted to a small number of salmon, spanned a limited range in somatic condition factor values and were obtained for a single year only, whereas the results from chapter 3 were supported by data from over 500 fish and 16 year classes. In this specific context, that liver $\delta^{15}\text{N}$ values were enriched in poor condition fish was encouraging considering that such a highly metabolically active tissue should be the first to reflect any effects of a cessation in feeding prior to capture..

Animal movement, but also physiology were the concerns of chapter 6. Otolith $\delta^{18}\text{O}$ was used as a proxy for ambient water temperature and $\delta^{13}\text{C}$ as a proxy for individual metabolic rate. Although a number of empirical studies have validated the use of otolith thermometry for particular species (Patterson et al. 1993, Thorrold et al. 1997, Høie et al. 2004b, Storm-Suke et al. 2007), as Høie et al. (2004b) and Storm-Suke et al. (2007) noted, the search for a common equation for fish species probably is unrealistic. Laboratory determination of an equation for free-ranging wild species of commercial and/or conservation concern (such as Atlantic salmon) presents a considerable challenge and for some species laboratory-rearing is not feasible. A further complication which receives little attention in the literature is the potential replacement of otolith aragonite with vaterite (a polymorph of calcium carbonate) and the effects this may have for temperature reconstructions from isotope values. Kim & O'Neil (1997) suggested that the $\delta^{18}\text{O}$ of vaterite may be up to 0.6‰ enriched over that of calcite and that it may fractionate independently of water temperature. Clearly, vateritic otoliths or inclusions should be avoided in any otolith thermometry study (e.g. Jessop et al. 2008). Simple, X-ray diffraction techniques combined with visual inspection were used in chapter 5 and chapter 6 to exclude vateritic otoliths from analysis. Despite this, water temperatures estimated from wild Atlantic salmon intra-otolith $\delta^{18}\text{O}$ values using all available otolith thermometry equations usually were warmer than the known thermal preference (Reddin & Shearer 1987, Holm et al. 2000) for this species. An equation using an intercept derived from the empirical data, and assuming that thermal residence matched SST in a particular area, did however provide plausible temperature estimates. Never the less, these results must be interpreted with caution because in the absence of a species-specific thermometry equation, it is not possible to predict absolute thermal habitat - only relative differences (e.g. between individuals or sample spots across the otolith). Despite this limitation, the patterns and relative differences in otolith $\delta^{18}\text{O}$ values do provide comparative data; relative increases and decreases in otolith

$\delta^{18}\text{O}$ values will be linked to water temperatures and so provides a unique way to study the migratory histories of anadromous fish.

Otolith $\delta^{13}\text{C}$ values, in contrast, are directly regulated, to a degree, by fish physiology. The ontogenetic rise in $\delta^{13}\text{C}$ values found in salmon otoliths in chapter 6 are consistent with a metabolic explanatory model. Most intriguing is the indication that there is a pronounced increase in metabolic rate for some individual fish during the final month(s) at sea. Despite the promise of otolith $\delta^{13}\text{C}$ values to describe the detailed history of fish metabolism and diet (Wurster et al. 2005, Elsdon et al. 2010), advances in modeling and more experimental evidence are needed before these parameters can be quantitatively estimated and temporally resolved. However, the results of Chapter chapter 6 point to an association between metabolic rate and fish condition which, if true, would be consistent with the hypothesis that fish returning to Scottish coastal waters in poor condition are nutritionally stressed and suffering early onset of catabolic processes.

Interpreting the meaning of observed changes in stable isotope ratios of wild free-ranging organisms is often complicated by a lack of sufficient experimental evidence but can be aided greatly by the inclusion of extra sources of information such as stomach or scat analysis, indices of animal size or health and the regional context; but at the heart of stable isotope ecology is the final question:

What is(are) the mechanism(s) driving stable isotope variability?

Stable isotope analysis of C, N and O of marine predators does not present a detailed, well-resolved picture of trophic dynamics or animal movement; this is especially true for large marine predators where appropriate data from captive animals could help constrain the mechanisms of isotopic change, but such studies are few (e.g. Hobson et al. 1996, Zhao et al. 2006). Instead, the somewhat 'pixelated' picture presented by stable isotope analysis can be considered a trade-off between providing taxonomic or location-specific detail and the relative ease of sample acquisition, the potentially high through-put of analyses and the wide range of ecologically relevant information derivable.

Importantly, stable isotope proxies of predators do not - of themselves - provide the specific taxonomic information about diet composition yielded by such techniques as scat and stomach analysis (e.g. Hammond & Grellier 2005, Haugland et al. 2006). With recent developments in statistical methods, it has become possible to model the relative contribution of different prey sources to a consumer diet from stable

isotope information (Moore & Semmens 2008, Semmens et al. 2009, Parnell et al. 2010). However, such modeling relies upon temporally accurate and exhaustive characterisation of the stable isotope signatures of all potential prey species or groups of prey (e.g. fish spp, seal spp, penguin spp in the diet of leopard seals; Hall-Aspland et al. 2005a), as well as knowledge of trophic discrimination factors. Furthermore, without some detailed assessment or modeling of changes in the stable isotope baseline signature (Solomon et al. 2011), it is not possible to make reliable predictions of proportionate contribution of prey sources to consumer diets over longer time periods.

The problem of reliable and exhaustive baseline isotope data is a major challenge for future studies of predator dynamics and of ecological change using stable isotope proxies (Solomon et al. 2011). In the marine environment, it may not be possible to retrospectively measure baseline stable isotope variation due to a lack of sufficient samples and/or access to them; therefore, alternative methods to at least constrain this variation must be sought. In chapter 3 a multispecies approach helped to delimit the mechanism(s) responsible for driving long-term change. For example, if large-scale processes affecting the entire marine ecosystem (such as declining primary productivity) were responsible for the observed decline in grey seal stable isotope values one might expect a decline also in the Atlantic salmon time-series. However, the Suess effect as a mechanism responsible for the shift in $\delta^{13}\text{C}$ values cannot be excluded in this case despite the lack of linear trends in average salmon $\delta^{13}\text{C}$ values. In a set of experimental and observation studies, Bump et al. (2007) found that the anthropogenically-induced decline in $\delta^{13}\text{C}$ values is recorded with greater fidelity at upper trophic levels. They reasoned that apex predators integrate variability in stable isotope signatures over broad spatial and temporal scales, leading to a higher signal-to-noise ratio in upper level consumers compared to primary producers. It is possible that a similar mechanism means that grey seals, as top consumers, may incorporate the Suess effect signal more reliably than Atlantic salmon. In the Southern Ocean, however, the Suess effect cannot be invoked to explain entirely the observed decline in fur seal $\delta^{13}\text{C}$ values because the magnitude was greater than the estimated Suess effect (Quay et al. 1992, McNeil et al. 2001; chapter 2). Because of the link between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, it is likely that a shift in foraging location accompanied by a shift in trophic level took place.

Isotopic baselines from primary consumers also may be predicted on the basis of a relatively small number of abiotic factors. Barnes et al. (2009) found that bottom

water temperature was an accurate predictor of spatial variability of $\delta^{13}\text{C}$ values in the queen scallop (*Aequipecten opercularis*) in the North Sea. Such relationships may also be useful for back-calculating isotopic baselines in the study of long-term trophic dynamics.

Isoscapes can be interpolated from empirical measurements and also are useful in delimiting the mechanism(s) of stable isotope change. For example, the isoscape models presented by Graham et al. (2010) show that plausible spatial variation of $\delta^{15}\text{N}$ values in zooplankton in the migratory range of Atlantic salmon and British grey seals is small compared to that expected during trophic enrichment processes. This would support the interpretations outlined in Chapter chapter 3 that changes in consumer $\delta^{15}\text{N}$ values are driven primarily by changes in their trophic position.

The use of multiple species sharing a regional foraging location, prediction of isotopic baselines and consultation of available isoscapes all may help alleviate the challenge of separating baseline isotope effects (i.e. changes in the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of primary producers) from trophic or movement effects (i.e. changes in consumer $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values due to shifts in diet and/or foraging location). Providing plausible mechanisms for observed stable isotope variation in natural systems requires an approach utilising multiple types of data, different observations and analytical approaches.

Response of top marine predators to ecological change

This thesis has demonstrated that stable isotope proxies may be useful bio-monitoring tools at a variety of temporal and spatial scales; in the context of monitoring top predators as indicators of ecological change, stable isotopes are primarily tools to determine trophic and migratory change. Monitoring and describing such changes in predator indices is in itself a valid way to study the ecosystem state, but additional challenges for ecologists are to determine the magnitude and direction of change, the mechanism(s) driving it and the functional links between the mechanisms and observed responses.

All three predator species studied here have exhibited significant changes in stable isotope proxies, both ontogenetically and over multidecadal periods. The decline of $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values in the teeth both of Antarctic fur seals in the Southern Ocean and grey seals in the North Sea raises the question of whether, and to what degree,

top predators may be 'fishing down the foodweb' in response to the removal of upper trophic level prey by commercial fisheries. In the Southern Ocean, it was possible to link long-term $\delta^{13}\text{C}$ values with SST data, thereby providing evidence for climatic forcing on these animals but, as described, the pronounced decline in $\delta^{15}\text{N}$ values in the 1970s was coincident with the collapse of an important predatory fish stock in the region (Kock et al. 2007). Separating climatic and anthropogenic drivers of the response of grey seals was more problematic because of the lack of annual stable isotope data and also because the changes in the climate and human marine resource exploitation are likely both to be implicated in the decline of many fish stocks in the North Sea. In reality, predators are likely responding to a combination of numerous interacting factors.

As planktivores and piscivores, Atlantic salmon are not susceptible to direct competition with fisheries as are upper trophic level predators such as grey seals. Therefore, any changes in their trophic dynamics are likely to result from climatic effects changing the availability or distribution of prey. An interesting and complex picture of effects of a changing marine climate for Atlantic salmon is emerging (Jonsson & Jonsson 2004a, Todd et al. 2008, Bacon et al. 2009, Friedland et al. 2009, Todd et al. 2010). The present thesis has contributed to this important debate by:

- providing a trophic link between rising marine SST anomalies and declining average body condition of returning 1SW Atlantic salmon (chapter 3);
- showing preliminary evidence that 'skinny' Atlantic salmon experienced cessation of feeding and onset of catabolic processes prior to reaching coastal waters (chapter 4);
- rejecting the hypothesis that fish returning in good condition had migrated to colder, more northerly waters and demonstrating the unique marine migratory trajectories for individual fish (chapter 6).

Furthermore, the novel application of SIMS technology to the problem of deriving discrete, high-resolution stable isotope data from small, complex biogenic carbonates (chapter 5) should be an encouragement to stable isotope ecologists to use these instruments and this particular technique in the future. The next phase of research on 1SW Atlantic salmon should expand upon the present datasets; stable isotope proxies have been shown to yield useful and novel data which further improve our understanding of the elusive marine life stage of Atlantic salmon.

Future directions

There are several avenues for productive future research that have emerged from the current thesis. Technical and statistical developments in the field of stable isotope ecology are emerging quickly (e.g. Weidel et al. 2007, Moore & Semmens 2008, Semmens et al. 2009, Parnell et al. 2010, Jackson et al. 2011); keeping abreast only of those which have arisen between 2008 and 2011 has been a considerable challenge. In the medium-term, however, there are two primary lines of inquiry that would help to further the determination of animal trophic ecology and movement history from stable isotope proxies.

Meta-analysis of long-term marine SIA

A review and meta-analysis of currently available data on changes in marine and coastal ecosystem trophic dynamics from long-term proxies of stable isotope variation would be timely. A growing number of research articles document multi-decadal and inter-annual variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of organism tissues and span multiple trophic levels and oceanic regions, but are all limited by a the lack of knowledge of variability in the isotopic baseline (Solomon et al. 2011). Comparison of annual variation across taxa foraging at different trophic levels but within similar regions (e.g. Hobson & Schell 1998, Schell et al. 1998, Hirons et al. 2001, Satterfield & Finney 2002, Newsome et al. 2007a, Norris et al. 2007) would allow for assessment of changes in isotopic baseline and de-coupling of proximate trophic effects and climatic effects at the base of the foodweb. If the mechanism driving isotopic change was related simply to baseline effects, it might be expected that the signal would be present in time-series at multiple trophic levels within a region. Trophic effects in consumers can be partitioned into climatically-induced shifts (e.g. the North Sea regime shift) and anthropogenically-induced shifts in trophic structure (e.g. decline of North Atlantic cod stocks). A synthesis of stable isotope data, in conjunction with quantitative indices of fishing effort and climate variation, could be used to quantify the human impact on marine foodwebs by, for example, comparing patterns of long-term trophic dynamics between similar taxa from regions that are highly impacted by human disturbance (e.g. marine mammals in the North Sea; Christensen & Richardson 2008) and those where human impact is relatively low (e.g. marine mammals in the Southern Ocean; Halpern et al. 2008, Hanson et al. 2009). Such analyses could help to partition effects and incorporate uncertainty into model pre-

dictions and retrospective explanation of long-term marine foodweb change where adequate baseline data are lacking.

Emerging Bayesian techniques for estimating isotopic trophic niche width (i.e. delta space, an estimate of consumer resource use; Newsome et al. 2007b, Jackson et al. 2011), and which are implementable in accessible statistical programs such as R (R Core Development Team 2010), have created a valuable and informative new means to better characterize the response of predators to ecological change. If stable isotope baselines can be adequately constrained, these metrics could be used to investigate the expansion and contraction of population-level resource use through time. These types of data are especially intriguing in the context of human impacts on marine ecosystems; for example, are upper trophic level organisms 'forced' into becoming specialists or generalists in highly exploited areas where marked removal of one or more species is common? Similarly, as marine climate change alters availability of prey due to changes in phenology (Edwards & Richardson 2004) and distribution (Beaugrand et al. 2002), must consumers expand their dietary repertoire in response?

Spatially explicit models of individual migration

Another field of enquiry for which the combination of advances in technology (e.g. the analytical precision of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values using secondary ion mass spectrometry) and statistics (e.g. the development of stable isotope mixing models in a Bayesian framework; Moore & Semmens 2008, Semmens et al. 2009, Parnell et al. 2010) can improve the quality of inferences drawn from stable isotope proxies is the analysis of spatially explicit models of individual migration.

For example, longitudinal transects of high-resolution intra-otolith $\delta^{18}\text{O}$ values represent a means of determining the potential marine migratory routes of wild Atlantic salmon. In chapter 5, publically available isoscapes of seawater $\delta^{18}\text{O}$ values and sea surface temperatures were used to predict spatially and temporally-resolved maps of aragonite equilibrium $\delta^{18}\text{O}$ values. Simple matching of longitudinal transects of intra-otolith $\delta^{18}\text{O}$ values and predicted equilibrium values helped to constrain the latitudinal migration range of individual fish, but these data have the potential to provide more detailed migratory, and perhaps also physiological, data than previously assumed. The application of stable isotope mixing models could help better characterize the metabolic contribution to otolith $\delta^{13}\text{C}$ values while the development of state-space models of intra-otolith $\delta^{18}\text{O}$ values may prove useful to

constrain time-resolved potential migratory paths of anadromous fish. Equally important, such data could permit the observer to eliminate certain proposed migratory trajectories depending on environmental temperatures.

Summary

This thesis demonstrated that stable isotope proxies can be used to document, analyze and interpret the response of wild marine predators to ecological change. Organisms were investigated at a range of timescales using different tissues (fish otoliths and soft tissues; mammalian teeth) suited to informing distinct components of individual life histories (migration, resource exploitation, metabolism) and to tracking changes in trophic dynamics and movement of populations over time. A variety of spatial scales of ecological change could be considered by the nature of the tissue analyzed and the particular biology and life history strategies of the species of interest. Often stable isotope proxies provided otherwise unobtainable measures of the trophic and movement history of marine predators; however, further developments in the quantification or estimation of isotopic baselines for marine systems are necessary for future applications of stable isotope proxies as bio-monitoring tools. Despite this, the body of knowledge of the abundance, behaviour and distribution of stable isotopes in nature is increasing quickly. Future research will potentially allow not only the determination of isotopic niche widths over time but the quantitative estimation of prey contributions to consumer diets. The isotopic ratios of some structures or components of structures (e.g. fish otolith aragonite or tooth hydroxyapatite) could prove to be robust natural tags of considerable use to the reconstruction of detailed migratory history.

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