ENVIRONMENTAL HISTORY OF NORTHERN COD FROM OTOLITH ISOTOPIC ANALYSIS

By

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ISOTOPIC ANALYSIS OF NORTHERN COD OTOLITHS

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TITLE: Environmental history of Atlantic cod (*Gadus morhua*) from isotopic analysis of otoliths.

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ABSTRACT

This study was concerned with analyzing the stable isotopic compositions ($^{18}O/^{16}O$, $^{13}C/^{12}C$ ratios) of otoliths from Atlantic cod (*Gadus morhua*) of the northern cod stock off the east coast of Newfoundland and Labrador, Canada. The purpose was to examine whether environmental changes in the northwest Atlantic, particularly cold temperatures, in the early 1990s may have influenced the collapse of this stock. A number of separate studies were also conducted to examine some of the factors which may influence the isotope ratios of otoliths to aid our interpretation.

Cod from both inshore and offshore locations in Newfoundland were collected and $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of muscle tissue were analyzed to quantify the effect of ontogenetic diet change on the isotopic composition of metabolic carbon. Parallel isotopic increase of carbon (+2.6‰) and nitrogen (+3.6‰) in muscle tissue confirmed that cod undergo almost continuous trophic level change related to ontogenetic diet change in early life. Otoliths from some of the same fish were then examined to determine if this trophic level change effects otolith $^{13}C/^{12}C$ ratios. A significant correlation was found between muscle isotope ratios of tissue and otolith. Ontogenetic diet change can explain up to 80% of the otolith's variation in $^{13}C/^{12}C$ with age. An additional decrease of 20% in the proportion of metabolic carbon between juvenile and adult cod explains the remainder of the variation. We then

examined otoliths from northern cod to look at their lifetime $^{13}C/^{12}C$ records. These records were remarkably consistent across the study area indicating that similar biological factors are at work in all cod. Stock components could be identified based on their characteristic isotopic signatures and there was some indication that juveniles from different areas were feeding at different trophic levels. Long-term environmental change seemed to be reflected in decreased $^{13}C/^{12}C$ during the mid-1980s. The exact cause of this change, however, remains uncertain.

To aid in the interpretation of the oxygen records, an extensive survey of $^{18}O/^{16}O$ in seawater was carried out over the Newfoundland and Labrador Shelves, examining spatial and temporal variation. There is an inshore to offshore gradient between low salinity and $^{18}O/^{16}O$ near shore and high salinity and isotope ratios offshore and also a strong linear correlation between isotope ratios and salinity. These observations were combined with isotopic records of individual northern cod otoliths to examine migration and environmental change. Most of the individual records displayed increasing ratios with age indicating an important ontogenetic habitat change towards higher salinity waters. Inshore cod form a distinct group with distinctly different lifetime records of $^{18}O/^{16}O$. There is also a temporal trend towards lower mean adult and juvenile values which may indicate changes in recruitment and is consistent with a southward shift in distribution of northern cod.

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There are many people I need to thank, but the first must be my supervisor, Dr. Henry Schwarcz. His enthusiasm and imagination brought me to McMaster and I have learned a great deal during my time working with him. I also would like to thank my committee members, Drs. Mike Risk and Gordon McDonald for their guidance and support.

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Contributions to co-authored papers:

Chapters 3 to 7 have been prepared as separate papers to be submitted for publication. The contribution of the candidate to each of these co-authored papers is outlined below.

CHAPTER THREE

Title: Characterizing the isotopic composition of seawater of NAFO Divs. 2J3KL in the Newfoundland and Labrador offshore region.

Authors: Jamieson, R.E., and Schwarcz, H.P.

Sample collection was carried out by the candidate with aid from the Dr. E. Colbourne of the Department of Fisheries and Oceans. All water analyses were carried out by the candidate. The paper was written by the first author with comments and editorial input from the second author.

CHAPTER FOUR

Title: The effect of ontogenetic changes in trophic level on the $\delta^{13}C$ of Atlantic cod (*Gadus morhua*).

Authors: Jamieson, R.E., and Schwarcz, H.P.

Samples for this study were provided by the Department of Fisheries and Oceans. All analyses were carried out by the candidate. The paper was written by the candidate with comments and editorial input from the second author.

CHAPTER FIVE

Title: The contribution of metabolic carbon to the $\delta^{13}C$ signature of Atlantic cod (*Gadus morhua*) otoliths.

Authors: Jamieson, R.E., and Schwarcz, H.P.

Sample collection was carried out with the assistance of the Department of Fisheries and Oceans. Sample preparation and analysis were carried out by the candidate. The paper was written by the candidate with comments and editorial input from the second author.

CHAPTER SIX

Title: Carbon isotopic records from the otoliths of Atlantic cod (*Gadus morhua*) from the northern cod stock.

Authors Jamieson, R.E., Schwarcz, H.P., and Brattey, J.

Selection of otoliths was carried out by the candidate with assistance from the third author. Otolith analysis was carried out by the candidate. The paper was also written by the candidate with editorial input and comments from the co-authors.

CHAPTER SEVEN

Title: Life history of Atlantic cod (*Gadus morhua*) from the $\delta^{18}O$ records of otoliths.

Authors: Jamieson, R.E., Schwarcz, H.P., and Brattey, J.

Selection of otoliths was carried out by the candidate with assistance from the third author. Otolith analysis was carried out by the candidate. The paper was also written by the candidate with editorial input and comments from the co-authors.

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GLOSSARY

The following terms may be useful for those not familiar with stable isotopic analysis.

delta (δ) notation

A notation used in stable isotope studies denoted by the equation:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) x\ 10^3$$

where R_{sample} and $R_{standard}$ represent the ratio of the heavy to light isotope (e.g. $^{18}O/^{16}O$, $^{13}C/^{12}C$, $^{15}N/^{14}N$) in a sample and standard respectively. The units are per mil (‰) or per thousand. Various international standards have been adopted for different isotope systems and the ones relevant to this study are described below.

VSMOW - Vienna Standard Mean Ocean Water

An international water standard used to compare oxygen and hydrogen ratios in water samples.

VPDB - Vienna Pee Dee Belemnite

An international carbonate standard for comparison of carbonate oxygen and carbon ratios in carbonates.

N_2 Air standard

An international nitrogen standard

CHAPTER 1

Introduction

In July 1992, a moratorium was imposed on the east coast cod fishery. The stocks had experienced a drastic decline in the early 1990s, and since that time they have remained at extremely low levels. Researchers have focussed a great deal of attention on trying to understand the factors which may have influenced this drastic stock collapse. Progress has been made in recent years in the use of the isotopic analysis of otoliths for environmental reconstruction. Isotopic analysis of otoliths from northern cod may provide important information which can help to uncover if environmental factors played a role in the downturn of the northern cod stock. There are still, however, many unanswered questions about how otoliths acquire their isotopic composition.

Otoliths are aragonite structures found in the inner ear of teleost fish and act as part of the fish's auditory system. They have the potential to provide important information on the environmental conditions experienced by fish as well as changes in physiology and behaviour. Their structure, which consists of concentric layers representing seasonal deposition (Figure 1.1), makes it possible to reconstruct an isotopic history for the lifetime of the fish. The purpose of this study was to assess

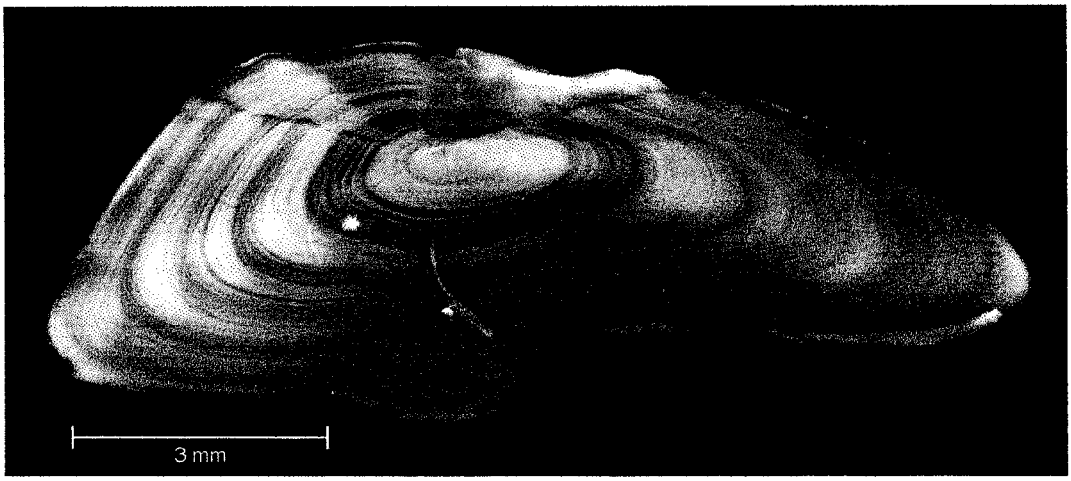

Figure 1.1 Thin section of a cod otolith.

a number of the potential influences on otolith isotopic composition and then to use this information to examine otoliths from the northern cod stock.

1.1 Northern cod

Cod within Northwest Atlantic Fisheries Organization (NAFO) divisions 2J3KL off the east coast of Newfoundland and Labrador constitute what is known as the northern cod stock (Figure 1.2). This stock has historically been the most important to the fishery, providing approximately 70% of the overall catch (Hutchings and Myers 1995). The collapse of this stock has caused a great deal of economic hardship in the Atlantic region.

The Atlantic cod is a member of the Gadidae family which are medium to large size, bottom dwelling, generally marine fish, found in cool waters in the northern hemisphere (Scott and Scott 1988). Atlantic cod can be found in most coastal waters of the North Atlantic, from Iceland and the Norwegian Sea, down to the Bay of Biscay, and from Greenland and southern Baffin Island down to the Gulf of Maine (Lear 1993; Hutchings and Myers 1995). Cod prefer temperatures between -0.5°C to 10°C, but this may vary depending on the time of year, location, and the size of the fish (Scott and Scott 1988). Generally the southern extent of their range corresponds to the 10°C isotherm in April. They can however, be found in waters up to about 20°C (Brander 1994; Brander 1996).

The large shallow banks on the Newfoundland and Labrador Shelves are a

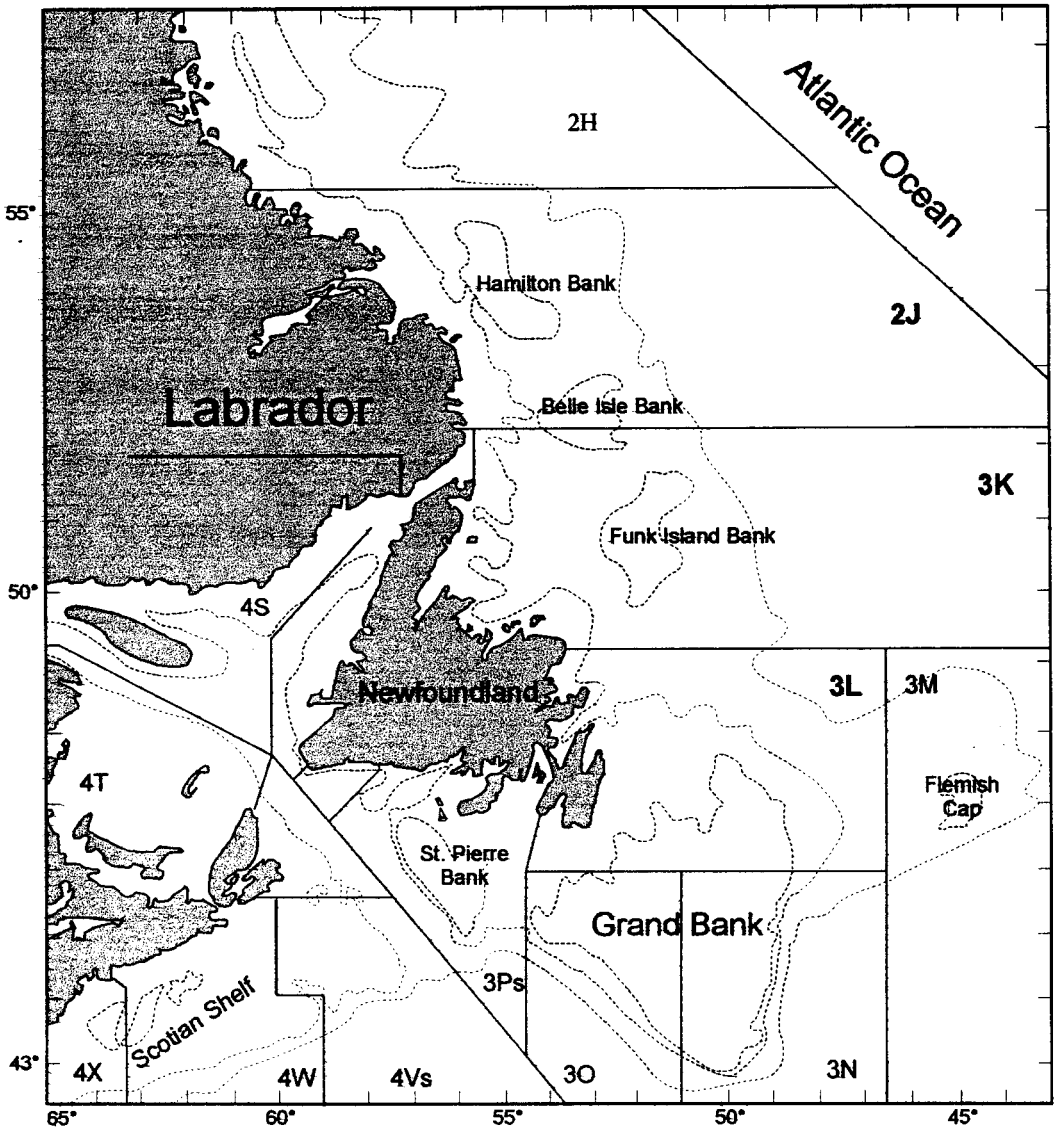

Figure 1.2 Map of NAFO Divisions 2J3KL in offshore Newfoundland and Labrador.

preferred habitat for cod. Traditionally, northern cod migrate between offshore spawning grounds in winter and inshore feeding areas in summer (Rose 1993). During this migration, they tend to move in large schools along trenches in the continental shelf. These trenches are favoured because the water is 2-3°C warmer than the shelf. Cod follow the trenches inshore and spread out to feed during the summer. In autumn they move northward and then back offshore, following a circular route.

Spawning takes place in areas where their eggs are likely to be retained on the shelf. If the eggs are spawned too far south, or at the edge of the shelf, the prevailing currents make it more likely that they will be swept away. When spawning occurs in northern areas and on the shelf, the eggs are swept southward and inshore by currents and are retained. Their spawning behaviour means that cod recruited in a particular area were not necessarily spawned there, and recruitment will depend on where the fish matured (de Young and Rose 1993). Cod in northern areas may therefore play an important role in populating southern areas (Hutchings *et al.* 1993).

While cod migrate between inshore and offshore seasonally, the general distribution of cod on the shelf varies with age. Young cod have been found in inshore waters and in shallow areas of the banks, but they tend to become more widespread over the shelf as they grow. Young cod are recruited into the adult population as they reach maturity at 5 or 6 years of age.

Young cod seem to move from inshore areas where they initially settle and

as they mature they begin the seasonal migration. The concept of discrete inshore populations was not studied in detail until the mid-1990s. An acoustic survey in Trinity Bay, Newfoundland in 1995, revealed a large aggregation of cod in this bay. Since that time, researchers have speculated that this population may represent a combination of resident inshore fish as well as fish which have moved in from offshore areas and remained. This may therefore represent a potential source of recruits to aid the recovery of offshore stocks.

1.2 Stock Collapse

For more than five hundred years, the northern cod have attracted fishermen to Newfoundland. The Vikings first discovered Newfoundland more than 1000 years ago (Kurlansky 1997). They were followed by Basques, British, French, Spanish, Portugese, and Russian fishermen. The cod fishery supported the economies of Europe and spurred the exploration of the northwest Atlantic. Cod provided a relatively cheap source of nutrition which preserved well (Kurlansky 1997). Relatively simple fishing techniques maintained the fishery at a supportable level into the 20th century. It was only in the 1950s, with the advent of the factory freezer trawler, that the cod stocks began to really feel the pressures of overfishing (Hutchings and Myers 1995; Kurlansky 1997). In 1968, catches of northern cod reached a peak of 810 000 tonnes (Garrod and Schumacher 1994), but in the early 1990s the stock collapsed dramatically and by July 1992, the fishery was closed. The

northern cod faces commercial extinction and, at this time, the stock has shown no signs of recovery.

A debate has gone on amongst fisheries researchers as to why this stock collapsed so drastically. Overfishing has obviously been an enormous problem, however, concurrent with the decline in the stocks, below average temperatures were observed in the Northwest Atlantic (Colbourne *et al.* 1994). Some believe that these changes in temperature may have influenced the behaviour of the cod.

Changes in distribution have been evident for the stocks off Newfoundland and Labrador during the past decade (de Young and Rose 1993; Rose *et al.* 1994; Rose *et al.* 2000). There has been a redistribution of the stocks towards the southern Grand Banks. Researchers have attributed this southward migration to temperature changes either as a direct result of the colder temperatures or as an indirect response to other factors such as changes in prey abundance which may be influenced by temperature. Other workers however, blame disparate fishing effort between northern and southern areas (Myers *et al.* 1997). It may therefore simply reflect a greater degree of stock decline in the northern regions. Using otoliths to analyze cod temperature and physiological histories may be able to shed some light on this debate.

1.3 Otoliths as Environmental Monitors

In order to use isotopic analysis of otoliths as a monitor of environmental and physiological conditions it is necessary to have a complete understanding of the

factors which influence this isotopic composition. In the case of oxygen, it has been fairly well established that the oxygen of otolith aragonite is deposited in isotopic equilibrium with the ambient water and temperature (Devereux 1967; Degens *et al.* 1969; Kalish 1991a; Kalish 1991b; Thorrold *et al.* 1997; Radtke *et al.* 1996). Determining temperature from $\delta^{18}O$ measurements though, requires knowledge of the isotopic composition of the water. Seawater $\delta^{18}O$ is related to salinity changes reflecting the relative input of open ocean and freshwater sources (Craig and Gordon, 1965). It is therefore important to assess the potential variability in these variables before attempting to interpret variations in $\delta^{18}O_{oto}$ values as temperature changes.

The story of the carbon isotopic composition of otoliths is much more complicated. Carbon in otoliths is not deposited in equilibrium with environmental dissolved inorganic carbon (DIC) (Degens *et al.* 1969; Radtke *et al.* 1987; Kalish 1991a; Kalish 1991b; Iacumin *et al.* 1992; Radtke *et al.* 1996; Thorrold *et al.* 1997; Schwarcz *et al.* 1998; Weidman and Millner 2000). It appears that it is in fact a mixture of DIC and a very ^{13}C depleted metabolic carbon component. The relative proportion of these two end-members also seems to shift over the life of a fish which is reflected by increasing $\delta^{13}C_{oto}$ values. Schwarcz *et al.* (1998) speculated that cod may undergo an ontogenetic shift in diet to higher trophic levels which would in turn increase the $\delta^{13}C$ value of metabolic carbon. This might explain a large part of the variation in $\delta^{13}C_{oto}$ in the early years of life.

1.4 This Study

A relatively novel aspect of this study is the use of a computer aided micromilling device which makes it possible to look at the lifetime variations in isotopic composition of individual otoliths. Whole otolith or low resolution sampling techniques which have been used in other studies do not allow a true analysis of temporal variation. Micromilling can provide valuable information on the life history of individual fish as well as factors which may influence the entire stock. The sample preparation and milling methods used in this study are outlined in Chapter 2.

In order to use otoliths to examine the potential impact of environmental changes on the northern cod stock, it was necessary to first investigate the factors influencing the isotopic composition of otoliths. Chapter 3 presents the results of a comprehensive study of the isotopic variability of waters on the Newfoundland and Labrador Shelves. The isotope, salinity, and temperature results will provide a framework for interpreting the $\delta^{18}O$ records from otoliths taken from the same area. Chapters 4 and 5 deal with the carbon isotopic composition of cod otoliths are how it may be affected by changes in metabolism and diet. A study was carried out to look at the potential impact of ontogenetic diet shift on the isotopic composition of metabolic carbon in cod. This study was coupled with analysis of otolith $\delta^{13}C$ in the same fish in order to evaluate the relative importance of the metabolic carbon component and the effect of this diet shift on that component.

A clearer understanding of the factors affecting otolith $\delta^{18}O$ and $\delta^{13}C$ allows

us to examine otoliths from northern cod in order to look at how cod may have experienced changes in the environment either as a direct influence on the temperature experienced by the cod, or as an influence on their physiology and behaviour. The results of these analyses are presented in Chapters 6 and 7. While there are still some remaining uncertainties, this research will contribute a great deal to the understanding of the use of otolith isotopic composition to study environmental impacts on fish stocks such as the northern cod.

CHAPTER TWO

Micromilling Method

2.1 Introduction

A relatively novel aspect of this study was the use of a computer-controlled micromilling system. Previous otolith studies have generally analyzed whole otoliths, and used other low-resolution sampling methods (Kalish 1991; Iacumin *et al.* 1992; Radtke *et al.* 1996; Thorrold *et al.* 1997; Stephenson *et al.* 2001). Few have fully utilized the potential of the zoned nature of otoliths to extract life-time histories of the environmental and physiological conditions experienced by fish (Patterson *et al.* 1993; Schwarcz *et al.* 1998; Weidman and Millner 2000; Begg and Weidman 2001; Wurster and Patterson 2001a). For this study, otoliths were milled using a Merchantek MicroMill system (Figure 2.1). This system gives us the capability to analyze the otolith seasonal zonation on a very fine scale (< 20 μm).

While the isotopic composition of whole otoliths represents an integrated value for the life of the fish, it is possible to get much more information about its life history by analyzing on an annual, seasonal, or even finer scale. In small otoliths whole otolith analysis may be sufficient, but for larger otoliths, much more information can be obtained by analyzing at a finer resolution. Improvements in mass

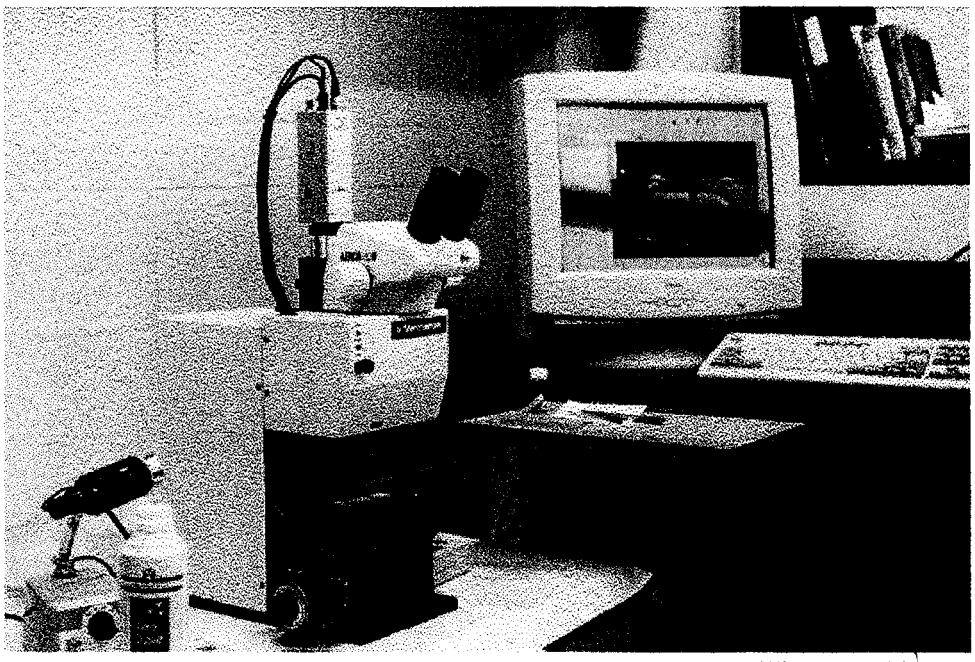

Figure 2.1 Merchantek MicroMill System. The micromilling machine is connected to the computer at the right for real-time viewing and control of the milling process.

spectrometry have also allowed micromilling techniques to be refined. In the past, relative large quantities of carbonate were required for an isotopic analysis. Today it is possible to analyze samples less than 20 μg.

2.2 Sample Preparation

The archive otoliths obtained from the Department of Fisheries and Oceans had been collected during research and commercial trawls over more than fifty years. These were stored dry in envelopes. Other otoliths were extracted directly from fish caught for this study. These otoliths were cleaned with a dilute bleach solution and rinsed well with deionized water.

The otoliths were embedded in an Araldite® resin (Figure 2.2) and then an Isomet low-speed saw was used to cut a 200-300 μm section from each otolith. The section must be cut through the nucleus of the otolith in order to reveal a complete section of all the growth bands. In order to do this with one pass, the saw was mounted with a pair of diamond tipped wafering blades separated by plastic spacers to give the desired thin section thickness. These sections were polished to remove any remaining rough edges from the sectioning process and then mounted on petrographic slides with Araldite® resin. The sections were then polished using different grits (800 grit to 0.3 μm) to render the seasonal zones clearly visible. The resulting thin sections are thin enough to clearly see the growth increments, yet are thick enough to retrieve enough sample for isotopic analysis. The Micromill is

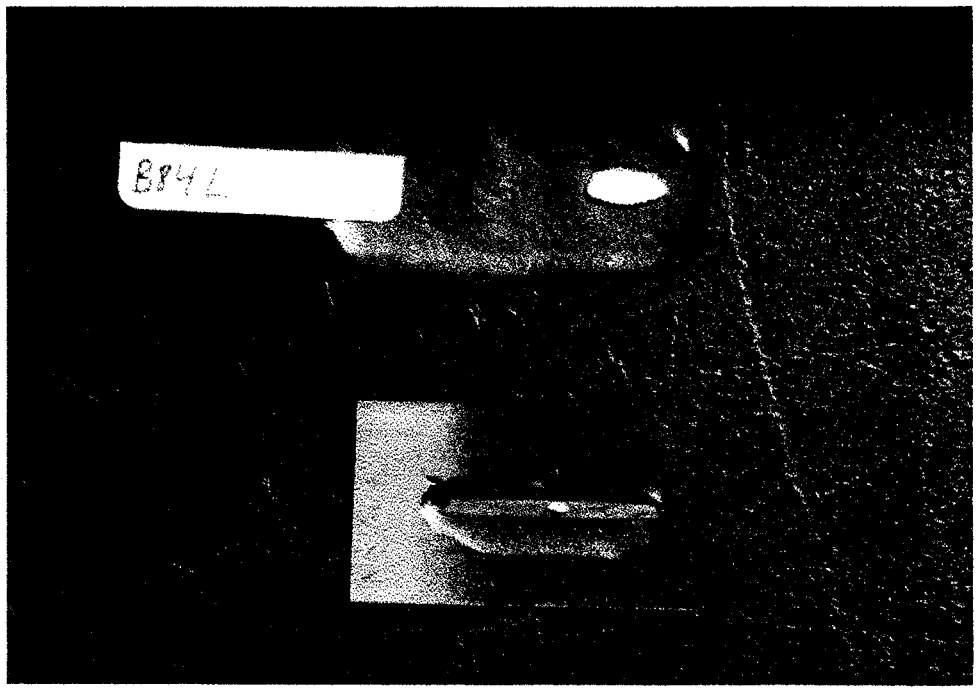

Figure 2.2 An otolith embedded in resin at top, and an otolith thin section at the bottom.

equipped with a removable sample plate and the slides are attached with a heat-softened cement.

2.3 MicroMill System

The Merchantek Micromill system consists of a x-y stage with a drill that can move in the vertical (z) axis, and which is capable of movement with a 1 μm resolution along all axes. The sample can be viewed through a built-in stereo microscope or through a video image shown on the computer screen in real-time. The entire system is computer controlled allowing parameters such as drill speed and light intensity to be controlled. It also allows for interactive control of the milling process. The system can switch between transmitted light and reflected light for maximum visibility of growth structures. The milling tools are carbide bits.

2.3.1 Digitizing sample paths

Milling involves taking samples along set paths defined using software of the milling machine. Using this technique, it is possible to follow individual growth bands. Older milling systems utilized "off-line" methods for defining sample paths which involved taking photographs of the sample. In contrast, the present system allows this procedure to be carried out using a video image of the sample through the computer. This allows the operator to make immediate changes to the milling paths.

In order to mill a sample, x-y coordinates must be inputted into the computer to define the path of the drill. This is done by connecting small linear paths to make curves corresponding to the growth bands of the otolith (Dettman and Lohmann 1995). The paths are graphically overlaid on the video image of the sample. The mouse is used to "trace" the major boundaries of the growth bands. The computer then interpolates a number of intermediate paths between the paths which have been entered manually. The exact number of interpolated paths is determined according to the path width specified by the operator. Alternately, a number of paths can be specified and the program will determine the appropriate width. The milling depth can be specified as well. The drill has the ability to automatically detect the top of the sample and then can mill down to a specified depth. It is also possible to correct automatically for sample tilt by measuring the depth at three reference points. This feature allows for better control of sample size.

Once the paths have been input, it is possible to control the speed of both the drill bit and the movement of the sample stage. It is also possible to mill one path at a time, or choose to multiple paths to be milled in sequence, in order to speed processing.

2.3.2 Collecting samples

Once a sample path is milled, the carbonate power is left behind on the stage. This powder is collected manually using a scalpel. The carbonate adheres to the

scalpel and the sample can be transferred very efficiently to a small metal cup for later analysis. More than 95% of the milled powder is recovered for analysis. After milling, any residual powder is blown off the sample with a jet of clean air.

2.4 Reproducibility

Two otoliths were re-milled to check precision and the results are shown in Figure 2.3. Even very small deviations in the isotopic composition can be reproduced. The average standard deviation between pairs of samples milled from the same zone was ±0.07‰ for both $\delta^{13}C$ and $\delta^{18}O$. This means that very little of the variation seen in the individual isotopic records is due to analytical uncertainty and therefore represents real variations of isotopic composition in the samples.

2.5 Conclusions

These new micromilling techniques allow a great deal more flexibility for sampling strategies than has ever been possible before. The introduction of direct computer control has enhanced the flexibility of these systems allowing for a greater degree of control. With the MicroMill system it is easy to change sample path width and depth for more control of sample size and spatial resolution. Real-time video and microscope viewing allows the operator to monitor the milling process and if necessary to adjust the sample paths for greater accuracy when following complicated growth structures. The only draw-back may be that this degree of sampling

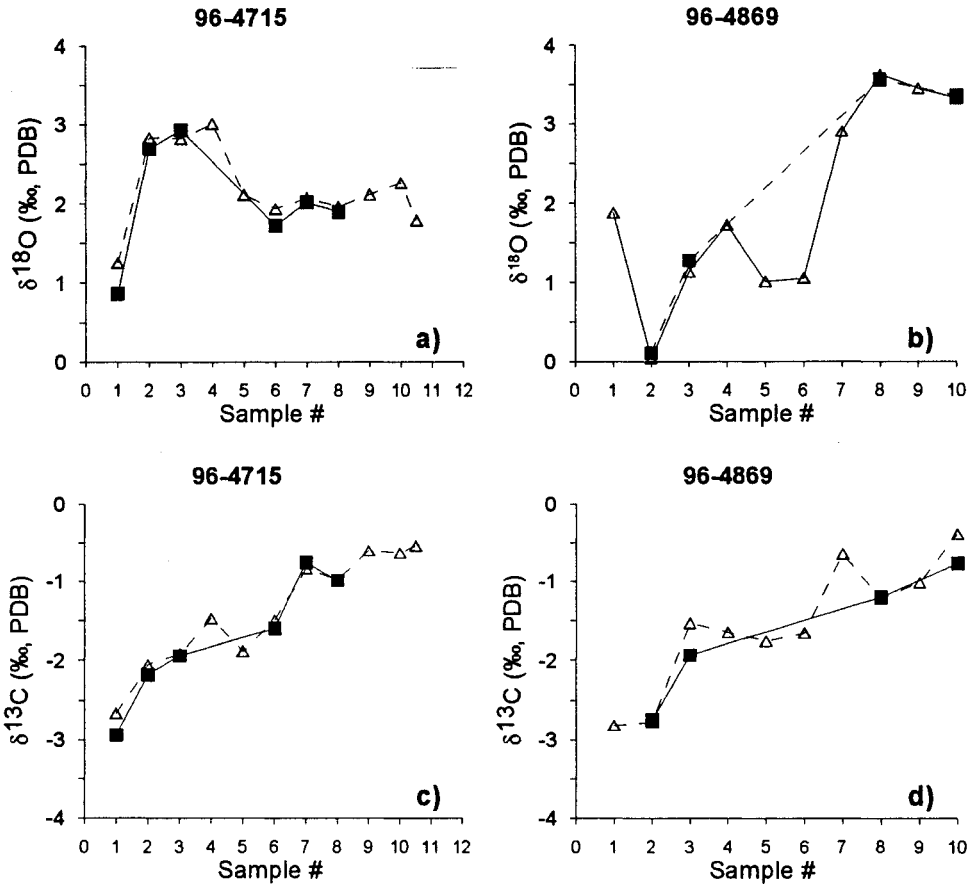

Figure 2.3 Replicate analyses of individual a) and b) $\delta^{18}O_{oto}$ records and c) and d) $\delta^{13}C_{oto}$ records.

resolution increases the time necessary to sample one individual otolith. An average adult otolith may take two or three days to mill at a seasonal resolution.

These micromilling techniques are finding uses not only in otolith research, but also in the study of other biological carbonates (e.g. Weidman *et al.* 1994; Dettman *et al.* 1999; Wurster and Patterson 2001b). Eventually it will be possible not only to measure annual and seasonal variations, but even daily variations. The only limit will be the ability of the mass spectrometers to analyze extremely small samples.

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CHAPTER THREE

Characterizing the isotopic composition of seawater of NAFO Divisions 2J3KL in the Newfoundland and Labrador offshore region.

3.1. Abstract

In order to accurately interpret the $\delta^{18}O$ records of northern cod otoliths, it is necessary to have an understanding of the spatial and temporal variation in $\delta^{18}O$ of seawater in the 2J3KL study area. Water samples were collected along the Newfoundland and Labrador Shelves during April and July 1998. An increase in both $\delta^{18}O$ and salinity across the shelf is a reflection of the relative influence of the Labrador Current and the North Atlantic Current. Generally, north to south differences appear to be minimal except where they reflect changes in depth between areas of the shelf. Seasonal differences are also minimal especially at depth. In the surface water they reflect inputs of sea ice melt water and solar heating.

A clear linear relationship exists between $\delta^{18}O$ and salinity with only a small variation between spring and summer. These relationships are represented by the equations:

$$\delta^{18}O_{SW} = 0.657\ S - 22.61 \qquad (r^2 = 0.894)$$

$$\delta^{18}O_{SW} = 0.579\ S - 20.29 \qquad (r^2 = 0.810)$$

respectively where S represents salinity. At the maximum salinity, there is a

distinctive trend of variable $\delta^{18}O_{SW}$ (±1‰) at almost constant salinity. This trend may represent a mixing between two high salinity water masses.

3.2 Introduction

Determination of paleotemperatures from the isotopic analysis of carbonates requires that the $\delta^{18}O$ composition of the precipitating fluid be known. Often this is not the case. Generally, ocean water has a $\delta^{18}O$ close to 0‰ (VSMOW), but it varies from place to place depending on factors such as the relative amounts of evaporation and condensation, inputs of fresh water from rivers or ice melt, and the mixing of waters from different areas (Craig and Gordon 1965).

NAFO Divisions 2J3KL span an area along the Newfoundland and Labrador coast between approximately 46° and 55°N and are inhabited by the northern cod stock. The purpose of this study is to describe the spatial variation in $\delta^{18}O$ over this area in order to provide the necessary framework for interpreting the $\delta^{18}O$ records of otoliths from this area. The oxygen isotopic composition of otoliths, which are composed of the $CaCO_3$ polymorph aragonite, is related to both the temperature and the oxygen isotopic composition of the ambient water. Therefore, in order to accurately calculate temperature from otolith $\delta^{18}O$ it is necessary to have a full understanding of both the spatial and temporal variations in the $\delta^{18}O$ of seawater.

3.2.1 Oceanographic conditions off Newfoundland and Labrador

The oceanography of the Newfoundland and Labrador Shelves is dominated by the southward flow of the Labrador Current (Petrie and Anderson 1983; Petrie and Isenor 1985). This current brings relatively fresh, cold water from the north. Inshore and offshore branches of this current are recognized. In the area of the Grand Banks, the inshore branch flows along the coast through the Avalon Channel and then west along the south coast to the Gulf of St. Lawrence. Part of the offshore branch splits off and flows east to the north of the Flemish Cap while the rest flows along the edge of the bank (Figure 3.1). Warm, salty water is carried north to the area by the Gulf Stream which becomes the North Atlantic Current as it flows east-northeast around the southern edge of the Grand Bank. This water dominates the deeper areas off the shelf. Exchange can occur between the two currents along the edge of the shelf forming "slope waters" (Petrie and Anderson 1983; Petrie and Isenor 1985). The presence of these two currents with their characteristic water masses leads to a strong inshore - offshore gradient of salinity and temperature.

The freshwater component of the Labrador Current comes mainly from runoff and ice-melt occurring in Hudson and Baffin Bays (Myers *et al.* 1990; Mertz *et al.* 1993). Changes in this input may be important to interannual variations of salinity on the Newfoundland Shelf, but it seems that ice-melt in the Labrador Sea is largely responsible for the seasonal salinity cycle in this area (Myers *et al.* 1990). The largest seasonal variation occurs in the surface waters with a salinity minimum around

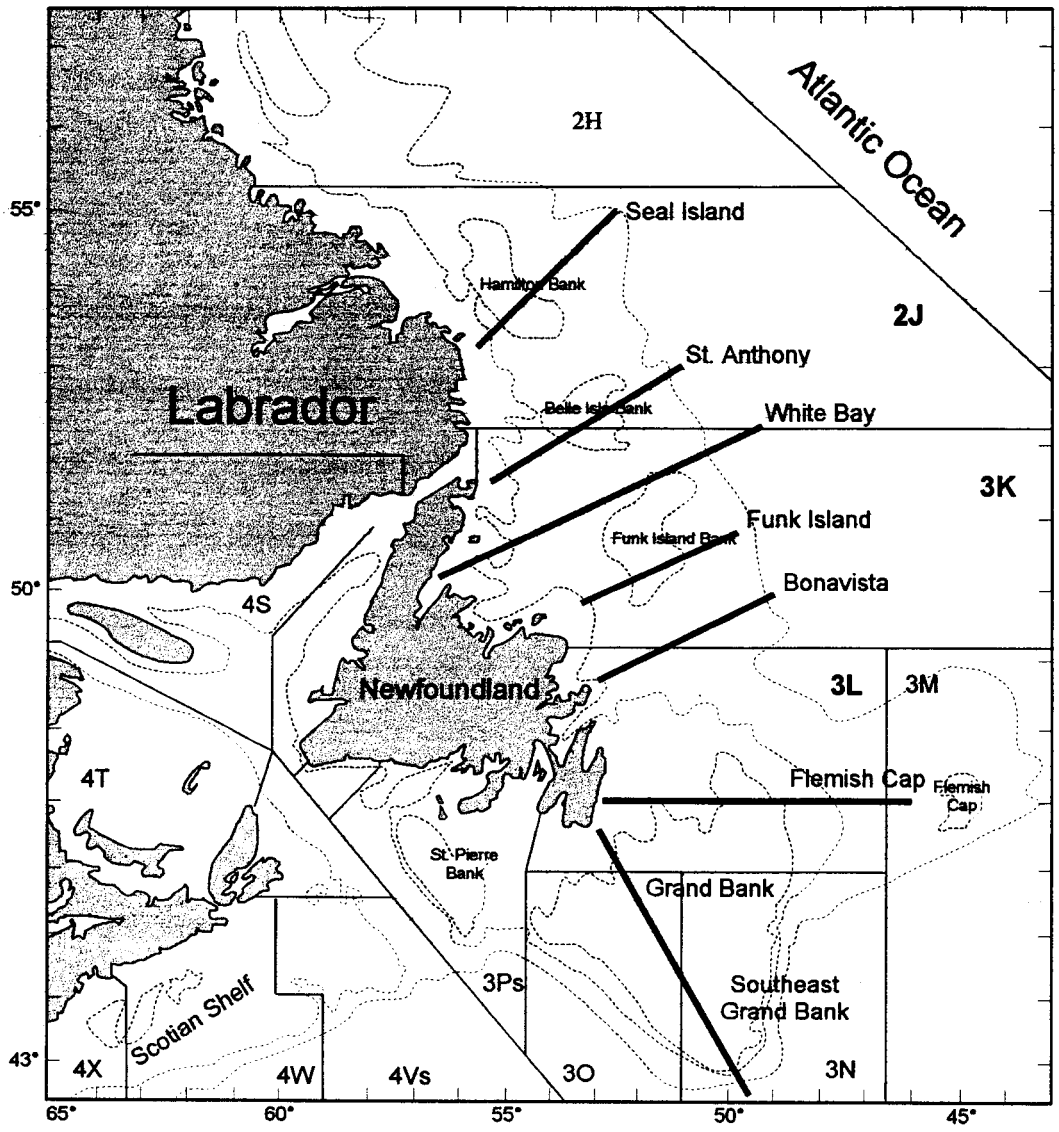

Figure 3.1 Map of offshore Newfoundland and Labrador illustrating the sampling transects for the spring and summer water sampling cruises.

September and a less pronounced minimum occurring at depth around December (Petrie and Anderson 1983; Myers *et al.* 1996). Temperatures also vary across the shelf. The bottom temperature across the northern Grand Bank ranges from less than 0°C inshore and rises to 3°C at the shelf edge.

Seasonal cycles of heating and mixing lead to changes in the vertical structure of the water column. In winter two layers dominate in deeper areas of the shelf. A layer of fresher, cool water sits on a warm, salty bottom layer which originates with the deeper slope waters. During summer (April-May), surface heating leads to the formation of a thin (30 to 40 m) layer of warm water. The cold winter water becomes sandwiched between this thin upper layer and the warm bottom layer forming the cold intermediate layer or CIL (Colbourne *et al.* 1994). This layer is defined by temperatures less than 0°C. It reaches its maximum thickness in winter and averages 200 ± 35 m. Cold years promote the formation of a larger CIL as larger ice extent leads to colder water temperatures (Colbourne *et al.* 1994).

3.2.2 The isotopic composition of seawater

Variations in the isotopic composition of ocean water reflect the interaction of open ocean water with the relatively ^{18}O depleted freshwater inputs such as river drainage and ice-melt. The relative importance of evaporation and precipitation also play a role (Craig and Gordon 1965). When a single ^{18}O depleted freshwater source is mixed with ocean water a linear $\delta^{18}O$ - salinity relationship develops. Fairbanks

(1982) used the $\delta^{18}O$ - salinity relationship to trace the origin of freshwater inputs to the New York Bight and the Gulf of Maine. His model is based on the observation that there is a regular change in the $\delta^{18}O$ of meteoric water with latitude along the east coast of North America. For instance, the St. Lawrence has a $\delta^{18}O$ of -10‰ while subarctic waters can be approximately -22‰ or lower (Gat and Gonfiantini 1981). The value of the freshwater input is reflected in the intercept at zero salinity. Using this information, the region of origin for the freshwater input can be estimated.

Craig and Gordon (1965) determined the $\delta^{18}O$ - salinity variation for the North Atlantic:

$$\delta^{18}O_{sw} = 0.61\ S - 21.2 \qquad (1)$$

where S represents salinity. Other workers have determined similar relationships for specific coastal water masses. For example, Tan *et al.* (1988) showed that for Browns Bank, Nova Scotia:

$$\delta^{18}O_{sw} = 0.523\ S - 18.179 \qquad (2)$$

while Fairbanks (1982) found on the Scotian Shelf:

$$\delta^{18}O_{sw} = 0.442\ S - 15.55 \qquad (3)$$

($r^2 = 0.99$; $n = 4$). Fairbanks (1982) also showed that for slope water in the New York Bight area:

$$\delta^{18}O_{sw} = 0.628\ S - 21.67 \qquad (4)$$

($r^2 = 0.99$; $n = 31$). Gao (1997) reported a correlation of:

$$\delta^{18}O_{sw} = 0.524\ S - 18.38 \qquad (5)$$

($r^2 = 0.915$; $n = 73$) for waters in NAFO Division 4Vs on the northeast Scotian Shelf. All of these relationships have relatively negative freshwater end-members which indicate inputs from northern areas although some are also influenced by the St. Lawrence and other major rivers on the east coast.

With this study we hoped to 1) determine a $\delta^{18}O$ - salinity relationship and 2) characterize the spatial and seasonal variations in $\delta^{18}O_{sw}$, salinity, and temperature for the waters on the Newfoundland and Labrador Shelves (NAFO 2J3KL). This is the first large scale isotopic study known to have been carried out in this area.

3.3 Methods

The Department of Fisheries and Oceans conducts regular oceanography cruises to collect water samples over the continental shelf off Newfoundland and Labrador. Samples for this study were collected during spring (April 25 - May 1) and summer (July 23 - August 2) of 1998 aboard the CCS Teleost along standard transects which are illustrated in Figure 3.1. Only three transects were conducted during the spring cruise (Teleost cruise 98-062); the Southeast Grand Bank, Flemish Cap, and Bonavista transects. The spring cruise was affected by bad weather and the Flemish Cap and Bonavista Transects were not fully completed. A total of 84 samples were collected. The summer cruise (Teleost 98-068) was much longer and extended further up the coast to Labrador. All the transects shown except the Southeast Grand

Bank were conducted at this time and 238 samples were collected.

Water samples were collected at standard depths of 5, 20, 50, 75, 100, 150 and 200 m as well as at the bottom. Other measurements such as salinity and temperature were made at the same time *in situ* using a conductivity-temperature-depth (CTD) recorder. Samples were not collected at all depths for every station and therefore the data coverage is not complete. In some cases, only bottom measurements were made. Water for isotopic analysis was collected in 50 mL glass vials which were tightly capped and sealed with electrical tape. The samples were kept refrigerated until analysis.

The procedure used for analysis of the $\delta^{18}O$ of the water samples was modified from that used by Epstein and Mayeda (1953). Samples of 0.2 mL were equilibrated with CO_2 in sealed pyrex tubes in a 25°C water bath for a week. The CO_2 was analyzed on a SIRA Series II dual-inlet IRMS. The results are presented as per mil (‰) values relative to the Vienna Standard Mean Ocean Water (VSMOW) international standard. Replicate analysis gave an average standard deviation of ± 0.10‰

3.4 Results

3.4.1 North to South Variability

Sampling transects across the Newfoundland and Labrador shelves allows us to characterize the oxygen isotopic variability in the 2J3KL area and its relationship

with salinity and temperature variations. As expected, $\delta^{18}O$ and salinity can be related by a significant positive linear correlation. This is true for both the April and August data sets which are given by the equations:

$$\delta^{18}O_{sw} = 0.657\ S - 22.61 \qquad (r^2 = 0.894) \qquad (1)$$

$$\delta^{18}O_{sw} = 0.579\ S - 20.29 \qquad (r^2 = 0.810) \qquad (2)$$

respectively, where S is the salinity given in psu (practical salinity units). The April data are much more closely distributed about the regression line (Figure 3.2a) compared with the August data set (Figure 3.2b). This is partly due to the fact that the August data contain approximately three times the number of samples. There does though, seem to be some tendency towards non-linearity in this set. At lower salinities, the $\delta^{18}O_{sw}$ values seem to curve up from the regression, while at the highest salinity $\delta^{18}O_{sw}$ appears to vary almost independent of salinity. There is a small difference between calculated intercepts for the April and August data sets, tending towards a more ^{18}O enriched source in summer. The North Atlantic mixing line has been added to each graph for comparison (Craig and Gordon 1965). The April correlation comes much closer to this line. The August correlation is parallel, but indicates a slight ^{18}O depletion in the water values. The intercept of Craig and Gordon (1965) falls between the two data sets at $\approx$-21‰.

Correlations between $\delta^{18}O$ and salinity can be constructed for each of the individual transects and the regression statistics for this analysis are presented in Table

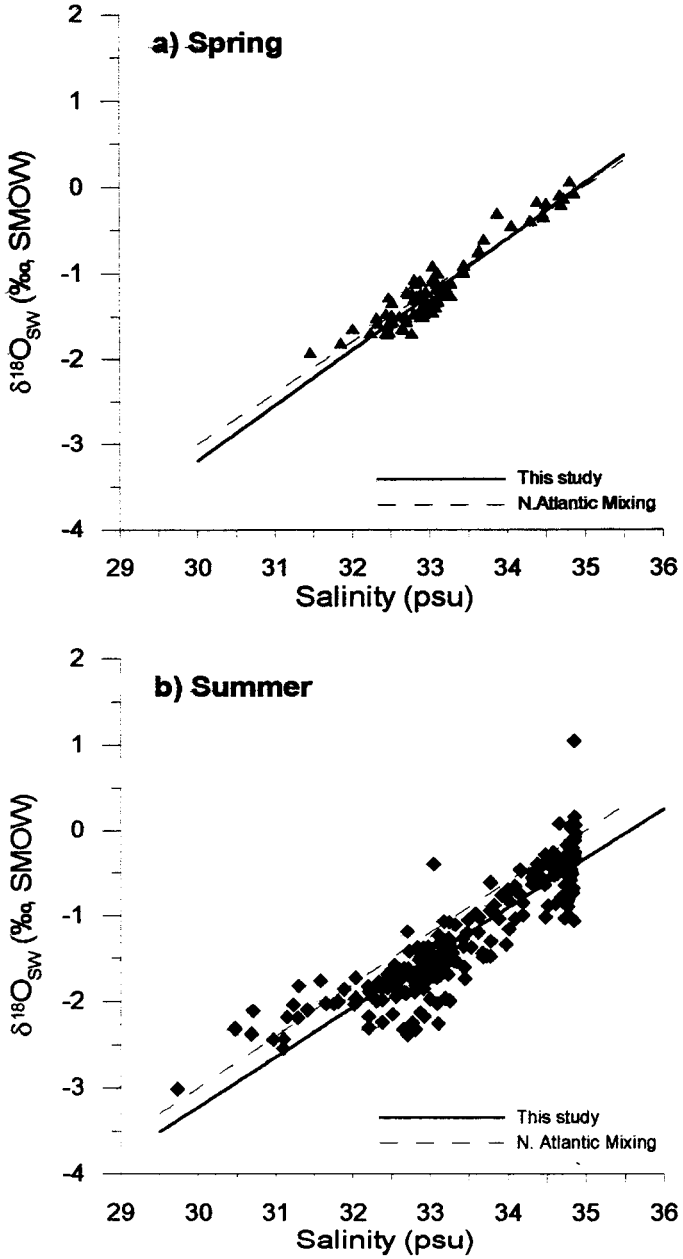

Figure 3.2 $\delta^{18}O_{SW}$ - salinity correlations for a) spring and b) summer 1998. The solid lines represent the regression for each group while the dashed lines represent the North Atlantic mixing line of Craig and Gordon (1965).

3.1. There does not appear to be a great deal of variability between the various transects. The only exception is the Southeast Grand Bank (SEGB) transect which has a weaker correlation ($r^2 = 0.598$). Only 12 samples were collected from this transect with a small range in salinity (approximately 32 to 33 psu). There seems to be a freshening in waters along both the Flemish Cap and Bonavista transects. Estimates of the $\delta^{18}O$ of the freshwater component vary between approximately -19 and -23‰. A more ^{18}O depleted value of -24.4‰ was estimated from the SEGB data.

A summary of the average $\delta^{18}O_{SW}$, salinity, and temperature data for each transect is offered in Table 3.2. The average $\delta^{18}O_{SW}$ for spring (-1.17 ± 0.47‰) is not significantly different from the average summer value (-1.21 ± 0.68‰). The range for the entire study is between -3.01 and +0.16‰. There appears to be very little indication of a north to south trend in $\delta^{18}O_{SW}$. The most southerly transect, SEGB, averages -1.35 ± 0.23‰ while the most northerly, Seal Island, averages -1.27 ± 0.80‰. The Funk Island and St. Anthony transects have slightly higher $\delta^{18}O_{SW}$ values but the standard deviations indicate that this is not a significant difference. Seasonal difference is apparent only in the range of values for each transect. The maximum $\delta^{18}O_{SW}$ values are similar but the summer minimum values are slightly lower than those in the spring for all transects. Salinity also shows very little north to south trend over the study area. The averages are: spring: 33.11 ± 0.69 psu; summer: 33.50 ±

Table 3.1 Least-squares fits to data for $\delta^{18}O$ and salinity (S) of seawater samples from 2J3KL area: $\delta^{18}O = m\ S + b$

	n	m	b	r^2
April 1998	**84**	**0.647**	**-22.6**	**0.894**
Southeast Grand Bank	12	0.702	-24.4	0.598
Flemish Cap	38	0.665	-23.2	0.902
Bonavista	34	0.614	-21.5	0.917
July/August 1998	**238**	**0.579**	**-20.6**	**0.810**
Flemish Cap	64	0.630	-22.2	0.888
Bonavista	40	0.583	-20.6	0.805
White Bay	37	0.535	-19.4	0.884
Seal Island	36	0.601	-21.4	0.835
St. Anthony	25	0.590	-21.0	0.903
Funk Island	25	0.650	-22.9	0.828

Table 3.2 Summary of $\delta^{18}O_{SW}$, salinity, and temperature data for each transect.

	$\delta^{18}O_{SW}$ (‰)		Salinity (psu)		Temperature (°C)	
	mean	**range**	**mean**	**range**	**mean**	**range**
Spring 1998	**-1.17 ± 0.47**	**-1.94 to 0.05**	**33.11 ± 0.69**	**31.46 to 33.86**	**0.55 ± 1.62**	**-1.73 to 4.54**
Flemish Cap	-1.12 ± 0.44	-1.73 to 0.05	33.18 ± 0.63	32.32 to 34.86	0.68 ± 1.16	-1.32 to 3.64
Bonavista	-1.17 ± 0.54	-1.94 to -0.11	33.12 ± 0.84	31.46 to 34.73	-0.15 ± 1.71	-1.73 to 3.31
Southeast Grand Bank	-1.35 ± 0.23	-1.71 to -0.93	32.88 ± 0.25	32.24 to 33.18	2.16 ± 1.36	0.48 to 4.54
Summer 1998	**-1.21 ± 0.68**	**-3.01 to 0.16**	**33.50 ± 1.07**	**29.74 to 34.88**	**2.62 ± 3.79**	**-1.66 to 13.13**
Flemish Cap	-1.34 ± 0.52	-2.03 to 0.07	33.14 ± 0.78	31.30 to 34.87	3.24 ± 4.80	-1.56 to 13.13
Bonavista	-1.20 ± 0.69	-2.19 to 0.06	33.32 ± 1.06	30.71 to 34.86	2.18 ± 4.20	-1.66 to 12.48
Funk Island	-0.81 ± 0.76	-2.44 to 1.05	34.04 ± 1.06	30.98 to 34.86	2.85 ± 2.84	-1.65 to 10.80
White Bay	-1.24 ± 0.68	-2.54 to 0.16	33.90 ± 0.99	31.10 to 34.88	2.84 ± 2.98	-1.46 to 10.99
St. Anthony	-0.94 ± 0.67	-2.38 to 0.08	33.88 ± 1.08	30.69 to 34.86	2.47 ± 2.67	-1.45 to 9.90
Seal Island	-1.27 ± 0.80	-3.01 to -0.27	33.44 ± 1.22	29.74 to 34.87	1.88 ± 2.78	-1.58 to 7.68

1.07 psu; the overall range is 29.74 to 34.88 psu. The average salinity is very consistent between the transects, but again the minimum values in summer tend to be lower. Salinity never reaches 35 psu and the average maximum for each transect is very consistent at 34.85 ± 0.04 psu.

Temperature shows the strongest tendency to vary between the north and south of the study area and also varies between spring and summer. The average temperatures are: spring: 0.55 ± 1.62°C; summer: 2.62 ± 3.79°C. For the three spring transects, there is a tendency towards warmer average values from Bonavista in the north to SEGB in the south although the range of values observed at each transect is similar. In the summer, the trend is very slight and not significant considering the overall amount of variation. The lowest observed temperatures are generally consistent between seasons and all transects with an average of approximately -1.7°C.

3.4.2 Inshore to Offshore variability

The most important variations in the 3KL area seem to occur between the inshore and offshore waters. Figure 3.3(a-b) presents typical profiles of bottom water $\delta^{18}O_{sw}$ along the Flemish Cap and Bonavista transects from near shore to the slope. The spring and summer data are both included and the depth is plotted for comparison. Each transect has a distinct pattern of $\delta^{18}O_{sw}$ variation across the shelf. The Flemish Cap transect passes over the shallow areas of the northern Grand Bank

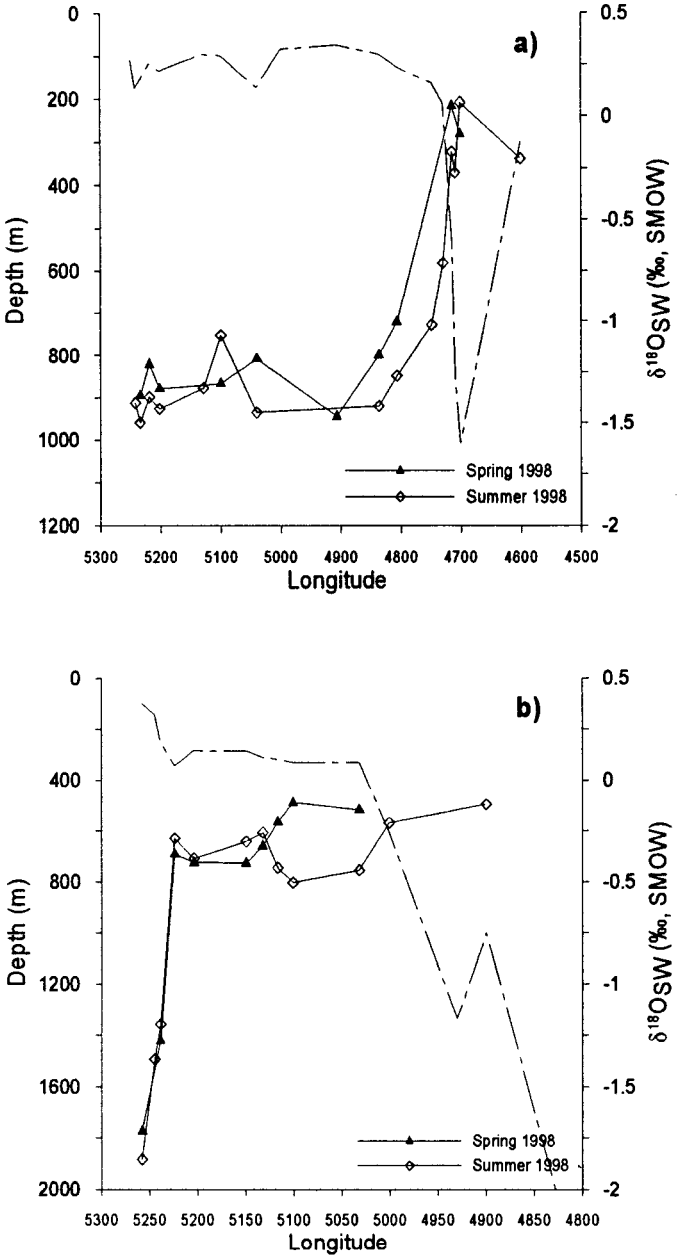

Figure 3.3 Bottom water $\delta^{18}O_{SW}$ profiles from near shore to offshore for a) Flemish Cap and b) Bonavista transects. Both spring and summer data are included. The dashed lines represent depth profiles for each transect.

(80 and 200 m), and deepens to approximately 1000 m over the shelf slope. It then passes over part of the Flemish Cap which is between 200 and 300 m deep (Figure 3.1). Near shore, the $\delta^{18}O_{sw}$ values are relatively lower (≈-1.5‰), but they increase rapidly to values approaching 0‰ at the edge of the shelf and into the deep waters off the slope. The shelf in the Bonavista area is deeper (average of ≈300 m), and the $\delta^{18}O_{sw}$ values, which start out relatively ^{18}O depleted (≈-2‰) near shore, are more ^{18}O enriched over the shelf and into the deeper waters of the slope (-0.5 to 0‰). Seasonal variation of $\delta^{18}O_{sw}$ does not appear to be important in the bottom waters of either transect. Trends for the other transects are generally similar to the Bonavista transect. The only exception is the White Bay transect which has characteristics of both the Bonavista and Flemish Cap transects. There are very ^{18}O depleted values near shore (≈-1.5‰) which then increase to ≈-1‰ over most of the shelf and shoot up to values near 0‰ at the edge of the shelf.

Similar transect profiles can be constructed to illustrate the variations seen in $\delta^{18}O_{sw}$ with depth. Figures 3.4(a-b) and 3.5(a-b) again present the Flemish Cap and Bonavista transects but with the addition of data for each sampling depth. Generally, there is a great deal more overlap between different depths in the spring data for both the Flemish Cap and Bonavista data. The Flemish Cap profile seems to exhibit a well mixed water mass over the shelf with a slight separation at the shelf edge. More differentiation is evident between shallow waters and deeper waters along the

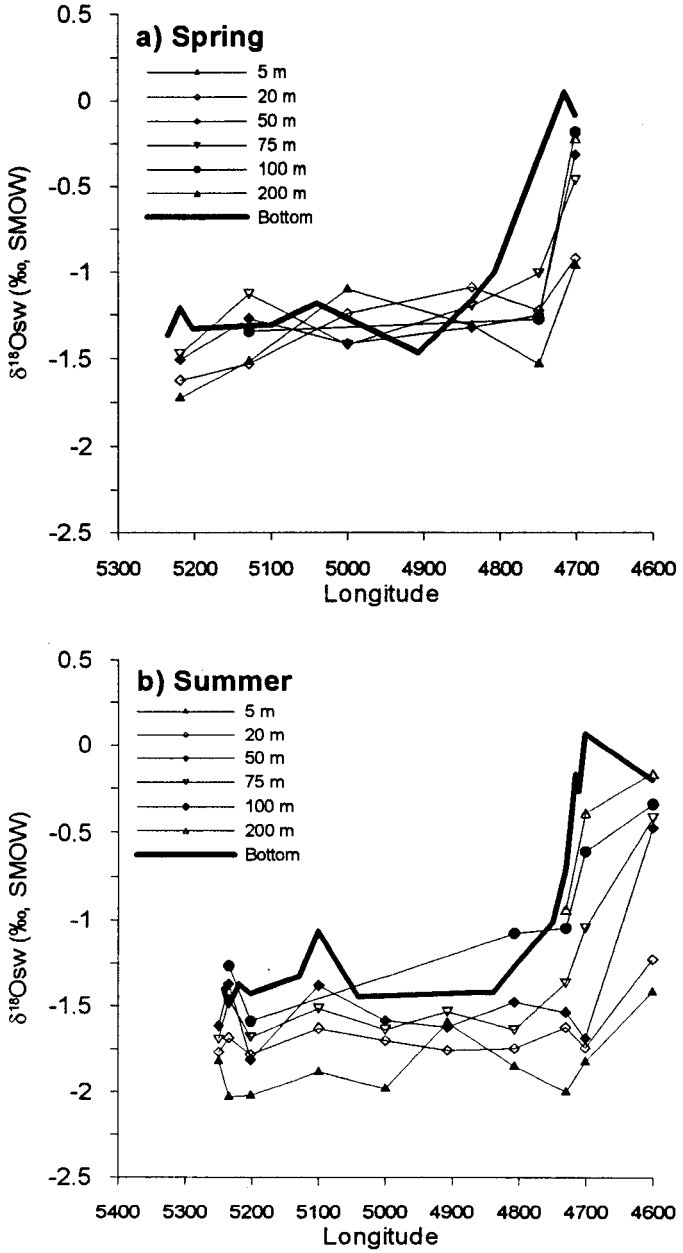

Figure 3.4 Near shore to offshore $\delta^{18}O_{sw}$ profiles for different depths along the Flemish Cap transect in a) spring and b) summer 1998.

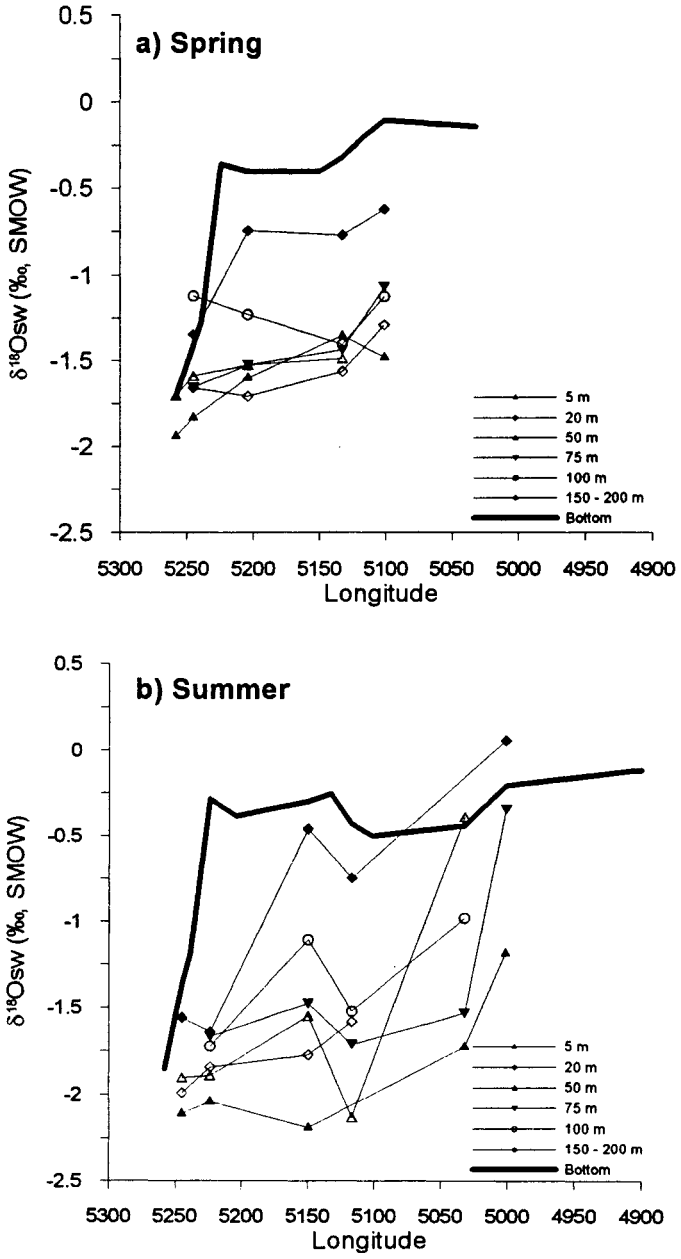

Figure 3.5 Near shore to offshore $\delta^{18}O_{sw}$ profiles for different depths along the Bonavista Transect in a) spring and b) summer 1998.

Bonavista transect. Waters below 200 m are clearly ^{18}O enriched compared to the shallow waters. Seasonal variation, which was not evident in the waters along the bottom, appears higher in the water column. There is notably more spread in the summer between different sampling depths and as noted earlier, tends towards decreased $\delta^{18}O_{sw}$.

The trend of increased $\delta^{18}O_{sw}$ with depth is accompanied by trends in salinity, and temperature. This is clearly illustrated in the data presented in Table 3.3. Here we have calculated the average values at different depths for each transect. While $\delta^{18}O_{sw}$ increases with depth, salinity slowly increases from values of approximately 32 psu at the surface to almost 35 psu at depth. Variability also seems to decrease with depth indicating that the water is much more homogeneous. Again, there is a slight trend towards lower $\delta^{18}O_{sw}$ values in summer. The variation in temperature is a little more complicated. Temperature is clearly influenced by season; increasing by over 10°C between spring and summer. Between approximately 75 and 150 m, temperatures decrease and are often negative; corresponding with the depth of the CIL. They increase again below this, often to values of 3 or 4°C. As with $\delta^{18}O_{sw}$ and salinity, there is much more consistency in deeper than in shallow waters.

3.4.3 Temperature and $\delta^{18}O_{sw}$

The oxygen isotopic composition of $CaCO_3$ is related to both the $\delta^{18}O$ of

Table 3.3 Summary of the average $\delta^{18}O_{sw}$, salinity, and temperature data for the spring and summer data sets divided according to depth.

Depth (m)	n	$\delta^{18}O_{sw}$ (‰, VSMOW)	Salinity (psu)	Temperature (°C)
Spring				
5	14	-1.52 ± 0.26	32.52 ± 0.46	0.71 ± 1.30
20	12	-1.39 ± 0.24	32.64 ± 0.35	0.60 ± 1.45
50	14	-1.30 ± 0.33	32.90 ± 0.34	0.47 ± 1.73
75	14	-1.26 ± 0.29	33.06 ± 0.31	-0.10 ± 1.40
100	11	-1.09 ± 0.28	33.30 ± 0.27	-0.16 ± 0.62
150	7	-1.09 ± 0.28	33.30 ± 0.27	-0.16 ± 0.62
200	2	-0.48 ± 0.26	34.17 ± 0.53	1.94 ± 1.44
250 - 350	8	-0.41 ± 0.35	34.33 ± 0.46	2.45 ± 1.25
> 500	2	-0.02 ± 0.07	34.83 ± 0.03	3.40 ± 0.24
Summer				
5	28	-1.99 ± 0.39	31.76 ± 0.88	10.48 ± 2.12
20	27	-1.66 ± 0.42	32.72 ± 0.66	4.51 ± 2.94
50	29	-1.51 ± 0.57	33.18 ± 0.65	0.01 ± 2.00
75	28	-1.39 ± 0.53	33.44 ± 0.67	0.04 ± 2.12
100	29	-1.25 ± 0.48	33.52 ± 0.63	0.14 ± 2.00
150	18	-1.41 ± 0.36	33.31 ± 0.36	-0.62 ± 0.85
200	22	-0.68 ± 0.44	34.28 ± 0.43	1.97 ± 1.52
250 - 250	31	-0.57 ± 0.35	34.49 ± 0.36	2.72 ± 1.17
400 - 850	12	-0.52 ± 0.24	34.79 ± 0.04	3.38 ± 0.11
1000	14	-0.15 ± 0.43	34.86 ± 0.01	3.26 ± 0.06

ambient water and temperature. It would therefore be worthwhile to examine the relationship between $\delta^{18}O_{SW}$ and temperature in this area which will aid in the interpretation of carbonate $\delta^{18}O$ values. The presence of distinct water masses with characteristic salinities and temperatures leads to a correlation between $\delta^{18}O_{SW}$ and temperature. Figure 3.6 presents two plots of $\delta^{18}O_{SW}$ versus temperature for the a) spring and b) summer data sets. The data has been separated into ≤ 75 m and > 75 m depths. These plots demonstrate that in deeper water there is a strong linear relationship between $\delta^{18}O_{SW}$ and temperature. In spring, the relationship is more compact, but the deep water relationship ($r^2 = 0.843$; $n = 33$) is still much stronger than the shallow relationship ($r^2 = 0.091$; $n = 51$). The summer data exhibits considerably more scatter, but again the deep water relationship is highly significant ($r^2 = 0.956$; $n = 127$) compared to the shallow water ($r^2 = 0.164$; $n = 111$).

3.5 Discussion

Over 300 water samples with complementary salinity and temperature data were collected over the 2J3KL area. This large collection of data allows us to characterize the waters on the Newfoundland and Labrador shelves which are inhabited by the northern cod stock. Analysis of these data reveals that the most significant variations occur with depth and between near shore and offshore at the shelf edge while north to south variations are much less important.

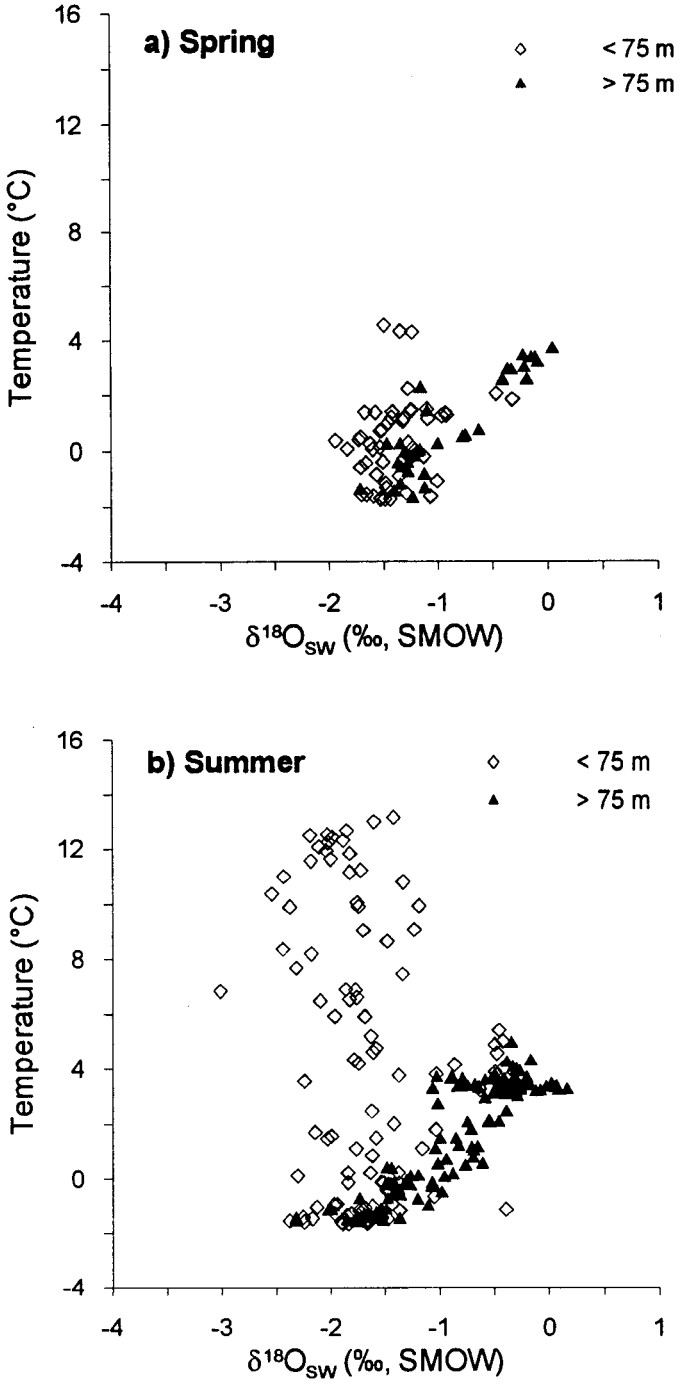

Figure 3.6 Temperature versus $\delta^{18}O_{SW}$ plots for a) spring and b) summer 1998. The solid triangles are samples from > 75 m and the open diamonds are samples from < 75 m.

Generally, the $\delta^{18}O_{SW}$ - salinity correlations for the two data sets, as well as the individual transects, are consistent with the findings of other studies in the North Atlantic. The correlations of Craig and Gordon (1965), Tan *et al.* (1988), and the slope waters studied by Fairbanks (1982) are the most similar. The influence of the Labrador Current and its freshwater component from the waters of Hudson Bay and Baffin Bay on the composition of waters along the North American coast is confirmed by the very negative $\delta^{18}O_{sw}$ intercepts at zero salinity which vary between -23 and -19 ‰. The high salinity component converges on a salinity of approximately 34.8 psu and a $\delta^{18}O_{SW}$ of approximately 0‰ which is consistent with the ocean water value of Craig and Gordon (1965) (S = 34.93‰; $\delta^{18}O_{SW}$ = +0.12‰). There is, however, a very unusual, almost vertical, relationship between $\delta^{18}O_{SW}$ and salinity at these very high salinities. This resembles a trend found in Gao (1997) in Scotian Shelf waters. Tan and Strain (1996) also observed a similar trend at a station in the Hudson Strait. All of these points represent deep waters (> 400 m). At present, we have no explanation for this relationship. If this represented a mixing relationship, the freshwater component would have to be extremely ^{18}O depleted ($\approx$-136‰) which is not reasonable. It is possible that it may represent mixing between two high salinity water masses, but this possibility cannot be assessed at this time. This may indicate that at very high salinities, a separate indicator such as Sr may be required for precise assessment of $\delta^{18}O_{SW}$.

Local ice-melt has been identified as an important source of seasonal salinity variation off the coast of Newfoundland. The shape of the summer curve is consistent with input from ice-melt. When ice forms, it leaves behind a water with a higher salinity. The $\delta^{18}O_{sw}$ however, does not change significantly (O'Neil 1968; Tan and Fraser 1976). Strain and Tan (1993) found that $\delta^{18}O_{sw}$ - salinity curves evolve over the course of freezing and melting of sea ice. During melting, the correlation departs from linearity as fresh, but isotopically similar, water is added. Curving of the correlation leads to an increased estimate of the isotopic composition of the freshwater component which could complicate estimates of source regions. At lower salinities, the summer $\delta^{18}O_{sw}$ - salinity correlation in the 2J3KL area seems to curve away from the linear relationship. The calculated $\delta^{18}O_{sw}$ intercept is also higher in summer than for the spring value. The maximum ice extent off Newfoundland and Labrador generally occurs during March and lasts longer further north (Prinsenberg *et al.* 1997) while a salinity minimum occurs in the fall indicating that melt water could be an important component of the water column in July.

Cod have historically undergone large migrations between inshore and offshore areas, therefore understanding the variations in $\delta^{18}O_{sw}$ across the shelf will aid in the interpretation of cod otolith $\delta^{18}O$ records. It turns out that the largest spatial variations are seen between the near shore and offshore areas. The dominance of the fresh, cold Labrador Current over the shelf and the warm, salty North Atlantic

Current near the slope would lead us to expect that variations in $\delta^{18}O_{sw}$ would also be discovered.

Generally, $\delta^{18}O_{sw}$ is lower near shore than it is offshore. The relative influence of the two major currents can explain this variation. However, the spatial scale of this ^{18}O enrichment differs between transects. Along the Flemish Cap transect, $\delta^{18}O_{sw}$ remains at low values across the banks and only increases as depth increases at the slope. In contrast, the Bonavista transect $\delta^{18}O_{sw}$ increases rapidly near shore to relatively high values over the shelf. The difference between these two situations appears to lie in the relative depth of the shelf in each region. The Flemish Cap transect passes over shallow areas of the Grand Banks while the Bonavista transect passes over the northeast Newfoundland Shelf with water between approximately 300 and 400 m deep. $\delta^{18}O$ increase with depth is clear for both the spring and summer data sets (Table 3.3). The other transects are also consistent with this hypothesis, generally resembling the Bonavista transect in $\delta^{18}O_{sw}$ variation with depth.

There is little seasonal variation in the $\delta^{18}O_{sw}$ of deeper waters (Figures 3.3 to 3.5). There is, however, significant seasonal variation in the water column. During spring, waters are well mixed after the cooling of winter. This is reflected in the overlap between the $\delta^{18}O_{sw}$ profiles at different depths observed in spring (Figures 3.4a and 3.5a). As the water column becomes more stratified in summer, with the

formation of the CIL, $\delta^{18}O_{sw}$ tends to increase more regularly as a function of depth (Figures 3.4b and 3.5b). Again, the lowest values are found at the surface and the highest values at the bottom. The surface waters also become more ^{18}O depleted in summer which is likely related to melt water input.

Temperature correlation with $\delta^{18}O_{sw}$ is linked to both the influence of the Labrador Current and the North Atlantic Current as well as seasonal heating of surface waters. At depth along the slope, the warm waters of the North Atlantic Current are associated with higher salinity and higher $\delta^{18}O_{sw}$ while the Labrador Current is a cold, low salinity water mass with generally lower $\delta^{18}O_{sw}$ values. The range of observed temperatures increases during the summer as a result of solar input to surface waters. These waters also have lower salinities and this leads to a great deal of scatter at low $\delta^{18}O_{sw}$ (Figure 3.6b).

Interannual variation can not be assessed with the current data set. Gao (1997) observed secular variation in Scotian Shelf waters and it is likely that this would be observed in the 2J3KL area on a decadal time scale due to long-term changes in climate. Variations in salinity are observed over the Newfoundland and Labrador Shelves. Changes in atmospheric conditions influence ice formation which in turn can have an effect on salinity, especially in surface waters. On a decadal time scale the salinity may vary by as much as 1 psu. Since $\delta^{18}O$ records of otoliths typically span periods of up to 6 - 8 years, this spatial and seasonal variability may

make it very complicated to assess otolith $\delta^{18}O$ in terms of environmental change.

3.6 Conclusions

Our analysis of water from the Newfoundland and Labrador shelves has demonstrated the importance of the Labrador Current and the North Atlantic Current to the isotopic composition in this area. The two water masses can be distinguished by combining $\delta^{18}O$, salinity and temperature analysis. This influence leads to a distinct inshore to offshore gradient in $\delta^{18}O_{SW}$, salinity, and temperature which is largely associated with changes in depth across the shelf. Near shore we encounter a water mass which is characterized by low $\delta^{18}O_{SW}$ values between -2 to -1‰ and salinities between 31 and 32 psu. These waters are generally cooler, but experience more seasonal fluctuation, especially in shallow waters. Surface waters may also be affected by seasonal inputs of high $\delta^{18}O_{SW}$, low salinity waters from the melting of sea ice. Offshore, $\delta^{18}O_{SW}$ values approach 0‰ and salinity increases to almost 35 psu. A very distinctive trend of variable $\delta^{18}O_{SW}$ with almost constant salinity has been noted in the most saline waters. The cause of this relationship is not presently known.

This study has provided a $\delta^{18}O$ - salinity relationship for the Newfoundland Shelf region of NAFO Divisions 3K and 3L. Only a slight variation was found between the relationships derived for the spring and summer of 1998. Seasonal variation, however, can be important as a cause of variations in the range of observed

temperature, salinity and $\delta^{18}O_{SW}$. These variations may be reflected, in turn, in $\delta^{18}O$ values of otolith aragonite when sampled on a seasonal scale (Jamieson *et al.* 2001). Understanding the spatial and temporal fluctuations in $\delta^{18}O_{SW}$ as well as salinity and temperature in the 2J3KL area will aid in the interpretation of otolith $\delta^{18}O$ for the determination of temperature as well as tracing of cod movements related to particular water masses.

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CHAPTER FOUR

The effect of ontogenetic changes in trophic level on the $\delta^{13}C$ of Atlantic cod (*Gadus morhua*).

4.1 Abstract

Stable isotopes have the potential to reveal information about food webs and the diet of particular species. Muscle tissue of 86 Atlantic cod (*Gadus morhua*) from inshore and offshore waters off Newfoundland, Canada, were analyzed for $\delta^{13}C$ and $\delta^{15}N$. The results have shown that a continuous change in trophic level occurs between the ages of three and five reflecting ontogenetic changes in diet. $\delta^{15}N$ analysis shows that cod muscle $\delta^{15}N$ increases by +3.6‰ corresponding to an increase of 1 trophic level. Parallel increases in $\delta^{13}C$ lead to an increase of 2.6‰. There is a clear relationship between $\delta^{13}C$ and $\delta^{15}N$:

$$\delta^{15}N = 1.3\delta^{13}C + 39.3 \qquad (r^2 = 0.820)$$

The carbon and nitrogen results are consistent with a food web based on phytoplankton. Juvenile cod feed on small zooplankton and eventually become piscivorous as adults moving through continuous trophic levels as they grow. An extreme increase was observed in the $\delta^{13}C$ values of inshore age 1 cod which ranged between -20.1 and -16.9‰. This increase is not seen in age 1 fish from the offshore supporting an inshore specific ^{13}C source. Juvenile cod undergo a shift from pelagic

to benthic prey at approximately 10 cm. This ^{13}C enrichment probably represents a seagrass or macroalgae input to benthic prey. Adult cod values plateau at approximately +15‰ and -18‰ for $\delta^{15}N$ and $\delta^{13}C$ respectively as change in diet becomes less important. The fact that ontogenetic shifts are so clearly reflected in the isotopic composition of cod muscle tissue indicates that isotopic analysis can be used to continuously monitor changes in trophic level and feeding behaviour. This can provide a valuable link between cod and their environment which will contribute to better management strategies for this endangered species.

4.2 Introduction

Conventionally, trophic levels have been used to define the position of an organism within a food web. It is well known, however, that an organism will not feed on the same prey throughout its life. Organisms can undergo ontogenetic changes in diet usually associated with changes in size and habitat. In fish, including Atlantic cod, feeding changes occur between the larval, juvenile, and adult stages. These changes in diet would imply that cod would not maintain a constant trophic level but would instead exhibit a gradual increase in trophic level throughout their lives. Stable isotopic analysis has been used to examine the trophic structure of various ecosystems; these studies have largely assumed that organisms can be confined to one trophic level and have ignored shifts in trophic level which may be associated with ontogenetic changes in behaviour. A single species would thus exhibit

a gradual increase in trophic level as it undergoes a transition from juvenile to adult feeding behaviour. Schwarcz *et al.* (1998) speculated that shifts in cod otolith $\delta^{13}C$ might be related to the effect of ontogenetic shifts in trophic level on tissue $\delta^{13}C$. Isotopic techniques can give insight to the extent and rate of this ontogenetic shift in diet revealing information about life history and interactions with the environment.

The life history of Atlantic cod involves ontogenetic shifts in both habitat and diet. Atlantic cod (*Gadus morhua*) is a demersal species: after hatching, larval cod (<4 cm) live in the upper water column for several weeks after which they settle to a demersal habitat. Age 0 cod (<10 cm) prefer shallow inshore waters, but they begin to move further offshore as they grow and mature. By age 3 to 4, they are found widely distributed in deeper waters on the shelf (Dalley and Anderson 1997; Anderson and Gregory 2000).

While often considered generalists because they will eat a wide variety of prey, cod actually tend to focus on a few principal prey items (Tyler 1972; Lilly 1987). Adult cod are largely piscivorous (Lilly 1987; Pálsson 1994; Casas and Paz 1996). Capelin (*Mallotus villosus*) is one of the main prey items of cod in the Newfoundland and Labrador area, but they are known to feed on many other types of fish including sand lance (*Ammodytes sp.*), redfish (*Sebastes sp.*), flatfish (*F. pleuronectidae*), eelpout (*F. zoarcidae*), and Arctic cod (*Boreogadus saida*) (Lilly 1987; Pálsson 1994; Casas and Paz 1996). They will also eat various crustaceans the most important being crab and shrimp. Many other prey have been identified including gammarid and

hyperiid amphipods, mysiids, euphausiids, polychaetes, cephalopods, and echinoderms (Lilly 1987; Casas and Paz 1996).

It appears that cod can continue to change their feeding habits over many years. Stomach content studies have shown that cod undergo ontogenetic changes in their feeding habits which continue over most of their lives. A comparison of the feeding habits of the various North Atlantic cod stocks (Pálsson 1994) found consistent changes in prey preference with cod size. Crustaceans were an important prey item for small cod whereas the diet of older cod was dominated by fish (>50%). There was a clear trend of increased predation on fish prey with increasing cod size. Prey other than fish or crustaceans were never more than 15% of the total diet and generally decreased in importance for older cod. For the Newfoundland stock (2J3KL), crustaceans made up greater than 50% of the diet for fish under 20 cm in length. The proportion of fish prey increased significantly as the fish grew to 40 cm and then stabilized.

Lilly (1987) reviewed cod feeding habits in Newfoundland and Labrador waters. Regional variations occur in the relationship of diet to cod length. A wide range of prey is however eaten by all fish and dietary change with age is sometimes gradual. In NAFO division 3L, small cod ate mainly euphausiids while medium cod (40 - 69 cm) ate sand lance and capelin. Crab and flatfish seem to become more important for larger fish (>60 cm). In divisions 2J and 3K, flatfish was also found to be important for much larger fish (>75 cm). Generally, only cod >35 cm eat adult

capelin, while juvenile capelin can be eaten by cod as small as 20 cm. Capelin is most important for fish between 40 and 69 cm. Seasonal and annual fluctuations in prey abundance can affect cod diet.

Cod undergo the largest changes in diet before they are three or four years old. A number of recent studies have looked specifically at changes in feeding in juvenile cod. In their study of juvenile cod in Trinity Bay, Newfoundland, Grant and Brown (1998b) found that small crustacean zooplankton (<4mm), especially calanoid copepods, were the preferred diet among the age 0 cod (4.2 to 7.4 cm) while larger invertebrates were preferred by age 1 cod (7.9 to 14.3 cm). This shift in feeding preference from planktonic to benthic seemed to occur at approximately 8 to 10 cm in size. Lomond *et al.* (1998) also found a very rapid shift in diet from pelagic to benthic at 60 to 100 mm in juvenile cod from Trinity Bay. They believed that this shift occurred as the cod became large enough to consume the larger benthic prey. Keats *et al.* (1987) observed a shift in feeding related to size class in cod from Conception Bay, Newfoundland. A transition was observed from small cod (<96 mm) which fed mainly on planktonic organisms such as copepods (<1mm), to larger cod (96 to 125 mm) which ate planktonic as well as benthic organisms, to cod 166 to 235mm which fed almost entirely on benthic organisms. This study also noted an increase in the size of prey with increasing size of cod. Demersal juvenile cod from Georges Bank have been shown to undergo feeding transitions as well (Auditore *et al.* 1994). Older demersal cod (>80mm) gradually eat more benthic prey and tend to

eat larger prey as they grow. Of course while the median prey size may tend to increase with cod size, this does not preclude large cod from eating smaller prey. Abundance can also be an important factor in prey choice and cod may eat large numbers of a small abundant prey (Lilly 1987).

Researchers can observe shifts in feeding using stomach content analysis and observation, but this is a tedious and costly method (Michener and Schell 1994). Stable isotopes can offer an easier method of analyzing trophic level changes. They have the advantage that they generally represent the long-term assimilated diet rather than the short-term diet reflected in stomach contents. Using isotopic methods to study the ontogenetic variations in the feeding habits of cod can provide information which is important for understanding cod life history. The question now is whether ontogenetic shifts in cod diet will produce detectable changes in isotopic composition.

Isotopic analysis of food web structure is made possible because the isotopes of carbon and nitrogen exhibit regular increases with increasing trophic level (e.g. Fry 1988). The fact that these increases occur indicates that there must be either a preferential assimilation of the heavy isotope or a preferential excretion of the light isotope (DeNiro and Epstein 1978; Rau *et al.* 1983). In either case, the isotopic composition of an organism increases relative to its food. This effect (the isotopic trophic level effect) is seen in each step up the food web so that top predators have the highest isotope values. The trophic level effect for carbon is generally observed to be small compared to that for nitrogen. Field and laboratory studies have shown

that carbon becomes ^{13}C enriched by approximately 1‰ for each increase in trophic level with estimates ranging from approximately 0.5 to 2‰. (DeNiro and Epstein 1978; Rau *et al.* 1983; Thayer *et al.* 1983; Fry 1988; Perry *et al.* 1999. France and Peters (1997) compiled values from different studies and found that the average increase was ecosystem dependent with a value of 1.1‰ proposed for open ocean systems. Since tissue carbon values are much higher than diet, normally $\delta^{13}C$ more closely reflects the primary carbon source at the base of a food web. Larger increases of 3 to 4‰ per trophic level generally make nitrogen a better tracer for trophic level changes. Minagawa and Wada (1984) found an average increase of 3.4 ± 1.1‰ for nitrogen while other estimates have ranged from 3 to 3.8‰ (Wada *et al.* 1987; Fry 1988; Hobson and Welch 1992; Hesslein *et al.* 1993). Combining carbon and nitrogen analyses can provide a powerful tool for unravelling complex food webs.

As ecologists have discovered the utility of stable isotopes, studies of the behaviour of fish and other animals using stable isotopes have become more prevalent. A number of studies have previously documented correlations between isotopic composition and length or age for fish (Thayer *et al.* 1983; Hobson and Welch 1992; Hobson and Welch 1995; Vander Zanden *et al.* 1998; Kline *et al.* 1998; Beaudoin *et al.* 1999; Griffin and Valiela 2001) and other animals such as seals (Lesage *et al.* 2001) and polychaetes (Hentschel 1998) which have been linked to changes such as feeding and migration. We set out to establish that lifetime feeding changes could be an important source of variation in the carbon and nitrogen isotopic compositions of

Atlantic cod, demonstrating that cod continuously change their trophic level as they age. To show this we collected cod of various lengths from locations in both inshore and offshore Newfoundland, Canada.

We assume in this study that there is a relationship between prey-size and prey trophic level, and also a relation between the size (and therefore the age) of a cod and the average size of its prey. This should lead to an increase in the isotope value of the prey with size of the cod, necessary for detecting changes in feeding. It is possible that a fish may feed on different prey items within the same trophic level or that the relationships between prey size and prey trophic level or between predator size and prey size are not strong (Vander Zanden *et al.* 2000; Griffin and Valiela 2001). In these cases, changes in feeding behaviour would not be seen as a change in the isotopic composition. The base of the marine food web is phytoplankton for which $\delta^{13}C$ ranges between -18 and -24‰ (Fry and Sherr 1984). Most marine food web studies have shown that there is a regular increase in isotopic values from the phytoplankton base for most or all organisms in these systems (Rau *et al.* 1983; Fry 1988). The system that we are studying is a typical marine environment and we expect that this condition should be met.

In order to accurately interpret the results of this type of study we must also understand what part of the diet we are assessing when we look at tissue isotopic compositions. While isotopic analysis can reveal information about the long-term diet, it must be recognized that growth and metabolism will control the time span reflected

in an organism's tissues. A number of studies have concluded that, for fish, the body's isotopic value is much more influenced by growth than by metabolic turnover (Hesslein *et al.* 1993; Herzka and Holt 2000; MacAvoy *et al.* 2001). Smaller fish, which are growing rapidly, will respond quickly to changes in diet (Herzka and Holt 2000), while the growth of older fish is slower and their isotopic value will be slow to respond to changes in diet. The isotopic value of these older fish will more likely represent a long-term average diet while that of juvenile fish will be more representative of recent feeding (Hesslein *et al.* 1993). Herzka and Holt (2000) determined that tissues of larval red drum turned over within 10 days of a shift in diet. While Hesslein *et al.* (1993) and MacAvoy *et al.* (2001) found that older fish responded much more slowly, taking more than a year to completely turnover. The lesser importance of metabolic turnover is generally explained by the slow metabolism of poikilothermic fish. Metabolic turnover may still be an important factor in some longer-lived species however, as found by Overman and Parrish (2001) for walleye in Lake Champlain. They inferred that the walleye were preferentially retaining ^{15}N, causing the fish $\delta^{15}N$ to increase over their lives. These changes in $\delta^{15}N$ were very slow, taking up to 10 years to reach a maximum value. While the isotopic turnover of cod tissues has not been previously determined, we would expect it to be similar to that of other fish. Cod exhibit a decreasing metabolism with age (Edwards and Finlayson 1972), we thus expect that younger fish will respond more quickly to changes in diet.

Other factors may obscure relationships between isotope values and diet change including spatial or temporal changes in the base of the food web (Hobson and Welch 1995; Vander Zanden *et al.* 1998; Lesage *et al.* 2001; Overman and Parrish 2001). Individual specialization (Gu *et al.* 1997; Beaudoin *et al.* 1999), opportunism, and omnivory (Vander Zanden *et al.* 2000) can obscure these connections as well. Beaudoin *et al.* (1999) found that competition from other fish species could cause interpopulation differences in trophic structure.

The Newfoundland cod stocks have experienced drastic declines over the last decade and therefore an increased understanding of their behaviour is important. Stable isotopic analysis can provide a fast and effective means of examining trophic structure in the marine environment without having to resort to more labour intensive methods such as stomach content analysis. The potential effect of lifetime changes in feeding habits on the stable isotope values of cod must be understood before further isotopic studies can address questions of cod behaviour and the effect of changes in the environment.

4.3 Methods

The constraints of the cod moratorium made it difficult to obtain large numbers of samples, especially from offshore areas, therefore the initial samples came from inshore locations. All cod were collected as part of research sampling conducted by the Department of Fisheries and Oceans. In November 1997, twenty-two cod

were collected from inshore Bonavista Bay (Figure 4.1) and a further thirty-two cod were obtained in September 1998. Fish from the first group (Bonavista B) ranged in size from 18 to 75 cm while the second group (Bonavista A) included cod from younger age groups (age 0 and age 1) which ranged in size from 4.9 to 9.8 cm and 13.4 to 17.4 cm respectively. In June 2000, it became possible to obtain a set of samples from an offshore location, adding another 32 fish for a total of 86 specimens. This third set (Offshore) were collected during trawls at a number of offshore locations between approximately 46° to 48°59'N and 47°34' to 50°59'W (Figure 4.1). These cod ranged in size from 16 to 47 cm. A small set (n = 7) of adult cod samples were obtained from St. Mary's Bay on the south coast of Newfoundland in June 1997. Unfortunately, length data was not obtained for these fish although they are known to be adult.

The cod were kept frozen until they could be shipped by courier to McMaster University where dorsal muscle samples were removed and freeze-dried for analysis of $\delta^{13}C$ and $\delta^{15}N$. Samples of 3 mg for carbon and 10 mg for nitrogen were placed in sealed pyrex tubes with an excess of CuO and combusted at 550°C for two and a half hours. The CO_2 and N_2 were cryogenically separated and analyzed on a SIRA Series II dual-inlet IRMS. Replicate analyses of the muscle samples gave average standard deviations of ±0.07 for $\delta^{13}C$ and ±0.13 for $\delta^{15}N$. The carbon and nitrogen isotopic values are reported in per mil (‰) values with-respect-to Vienna Pee Dee Belemnite (VPDB) and air N_2 standards respectively.

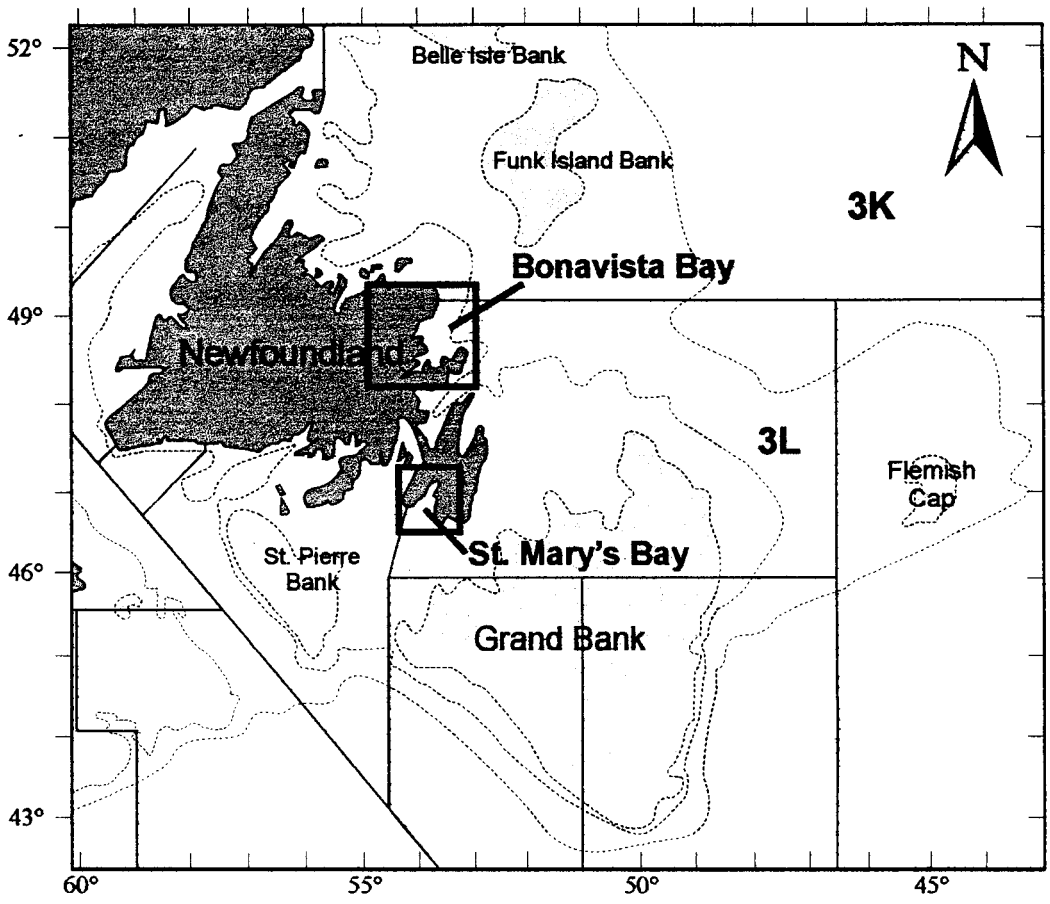

Figure 4.1 Map of offshore Newfoundland indicating the sampling areas in Bonavista Bay and offshore NAFO Div. 3L.

Lipids were not removed from the samples prior to isotopic analysis. Often lipids are removed because they tend to be more depleted in ^{13}C than other biochemical fractions and can therefore skew comparisons between organisms with different lipid contents (DeNiro and Epstein 1977). This is usually of greatest concern when comparing different species, because only one species was examined in this study it was not considered that this would make a significant difference. However, a small experiment was conducted to test this assumption. Lipids were removed from a small number (n = 3) of the samples by treating them with chloroform and methanol and no significant or consistent change was observed in the $\delta^{13}C$ values. C/N ratios were also measured for a subset of the muscle samples over the range of sizes and there was little variation (3.86 ± 0.1) indicating that there was not a significant variation in lipid content.

4.4 Results

4.4.1 Nitrogen

Our analysis of cod muscle tissue reveals a great deal of variation in $\delta^{15}N$ values (+10.6 to +16.8‰) (Table 4.1) indicating that these cod feed on a range of prey items from different trophic levels. It is clear however that most of this variation occurs in fish less than 35 cm in length. Length and $\delta^{15}N$ are significantly correlated for the entire data set ($r^2 = 0.618$) (Figure 4.2). A stronger correlation, however, is found in fish less than 35 cm ($r^2 = 0.703$) while for fish greater than 35 cm there is no

Table 4.1 Average $\delta^{13}C$ and $\delta^{15}N$ (± 1 SD) and range for each size class divided according to location as well as averaged for the total data set. Enrichment values are relative to the average for the smallest size class.

Size class (cm)	n	$\delta^{13}C$ (‰)	Range (‰)	$\delta^{15}N$ (‰)	Range (‰)
Bonavista A					
4.9 - 9.8	15	-20.9 ± 0.1	-21.3 to -20.7	11.7 ± 0.4	10.6 to 12.2
13.4 - 17.4	17	-18.6 ± 0.8	-20.1 to -16.9	12.9 ± 0.4	12.4 to 13.6
Bonavista B					
18	2	-18.9 ± 1.0	-19.9 to -17.9	13.0 ± 0.5	12.5 to 13.5
24 - 26	4	-19.1 ± 0.2	-19.2 to -18.8	14.5 ± 0.5	13.9 to 15.0
30 - 38	4	-18.8 ± 0.2	-19.1 to -18.6	14.0 ± 0.3	13.7 to 14.5
40	2	-18.5 ± 0.2	-18.7 to -18.3	15.5 ± 1.3	14.2 to 16.8
50 - 59	4	-18.4 ± 0.1	-18.5 to -18.3	14.9 ± 0.4	14.4 to 15.5
60 - 68	4	-18.4 ± 0.1	-18.5 to -18.2	14.9 ± 0.1	14.8 to 15.0
70 - 75	2	-18.3 ± 0.1	-18.3 to -18.2	15.3 ± 0.3	15.0 to 15.5
Offshore					
16 - 19	10	-20.0 ± 0.4	-20.8 to -19.5	13.9 ± 0.4	13.6 to 14.8
24 - 27	3	-18.7 ± 0.1	-18.8 to -18.5	15.3 ± 0.2	15.0 to 15.6
31 - 39	6	-18.8 ± 0.3	-19.2 to -18.5	14.7 ± 0.3	14.4 to 15.2
40 - 47	13	-18.6 ± 0.3	-19.2 to -18.1	15.0 ± 0.4	14.5 to 15.6
St. Mary's	7	-19.0 ± 0.2	-19.3 to -18.6	15.2 ± 0.3	14.8 to 15.8
Total Data Set			**Enrichment**		**Enrichment**
0-9	15	-20.9 ± 0.2		11.7 ± 0.4	
10-19	29	-19.1 ± 1.0	+1.8	13.3 ± 0.6	+1.6
20-29	7	-18.9 ± 0.2	+2.0	14.8 ± 0.6	+3.1
30-39	12	-18.8 ± 0.3	+2.1	14.5 ± 0.5	+2.8
40-49	13	-18.6 ± 0.2	+2.3	15.1 ± 0.7	+3.4
50-59	4	-18.4 ± 0.1	+2.5	14.9 ± 0.4	+3.2
60-69	4	-18.4 ± 0.1	+2.5	14.9 ± 0.1	+3.2
70-79	2	-18.3 ± 0.1	+2.6	15.3 ± 0.3	+3.6

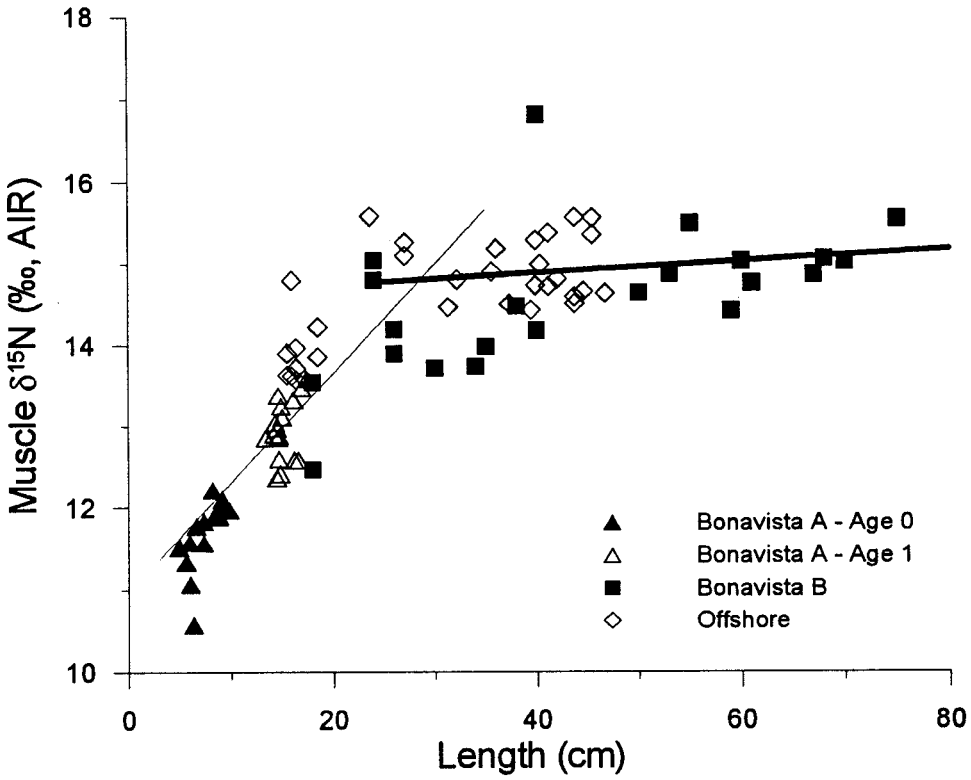

Figure 4.2 Muscle $\delta^{15}N$ versus length with regressions for <35 cm and > 35 cm.

significant $\delta^{15}N$ - length relationship ($r^2 = 0.022$) indicating that the fish reach a plateau in $\delta^{15}N$ at this size range. As a result there is very little variability in $\delta^{15}N$ for fish of this size: ± 0.5‰ which is much less than the overall variation of ≈6‰.

Interpopulation variation seems to be small. The size overlap between the Bonavista B and offshore groups is not perfect, but is adequate for a rough comparison. The same $\delta^{15}N$ - length trend is evident in each group as was present in the entire data set, reaching a plateau at approximately 30 to 40 cm in length. The offshore cod seem to have slightly higher values in the smaller size ranges (10 to 40 cm) than the Bonavista cod (Table 4.1) indicating that perhaps the shift in diet occurs slightly earlier in the offshore, however both reach almost identical plateau values for cod greater than 35 cm, with averages of 14.9 ± 0.7‰ and 14.9 ± 0.4‰ respectively. There were fewer fish in the less than 35 cm size range for Bonavista B (n = 8) than the offshore group (n = 15), and the overall size ranges did not completely overlap which may account for some of the differences. The nitrogen results for St. Mary's Bay (Table 4.1) appear to be slightly higher than the Bonavista and offshore adults (15.2 ± 0.3‰) but the difference is not significant. The overall average for all three adult groups (n = 38) is 15.0 ± 0.5‰.

4.4.2 Carbon

The results for carbon are generally similar to those of nitrogen. Again, a very large range of values was observed for the $\delta^{13}C$ of all cod sampled (-21.3 to -16.9‰)

for an overall span of 3.4‰ (Table 4.1). Assessment of the $\delta^{13}C$ - length relationship is complicated by an extreme ^{13}C enrichment in the 10 - 20 cm length group. There was a significant, although weak, relationship between $\delta^{13}C$ and length for the entire data set ($r^2 = 0.354$) (Figure 4.3). Consistent with $\delta^{15}N$, most of the change seems to occur in the small fish, increasing by 3‰ between fish of 5 cm and 25 cm average length. The 10 - 20 cm cod stand out from the general trend, however. Over this very small range in size, $\delta^{13}C$ varies between -20.1‰ to -16.9‰ ($\Delta = 3.2$‰). Most of these fish are from the Bonavista A group, although two fish from Bonavista B are also in this size range (both 18 cm) and appear to have a similar span (-19.9‰ and -17.9‰). Increases are not evident at this size range for the offshore group which has an average of -20.0 ± 0.4‰. Overall, for fish less than 35 cm the $\delta^{13}C$ - length relationship is significant but weak ($r^2 = 0.357$). If we exclude all of the Bonavista (A and B) age 1 cod, the correlation becomes much stronger ($r^2 = 0.784$). For fish greater than 35 cm there was a weak correlation ($r^2 = 0.257$), and little variation in the average $\delta^{13}C$ (-18.6 ± 0.3‰), confirming that adult cod plateau in $\delta^{13}C$.

The variation between populations was small for $\delta^{13}C$ as it was for $\delta^{15}N$. Comparisons between the Bonavista B, offshore, and St. Mary's adults shows that they have very similar average values (-18.4 ± 0.2‰, -18.7 ± 0.3‰, and -19.0 ± 0.2‰ respectively) giving an overall average of -18.6 ± 0.3‰ for all fish over 35 cm. Again, $\delta^{13}C$ - length trends are evident in both the Bonavista B and offshore groups.

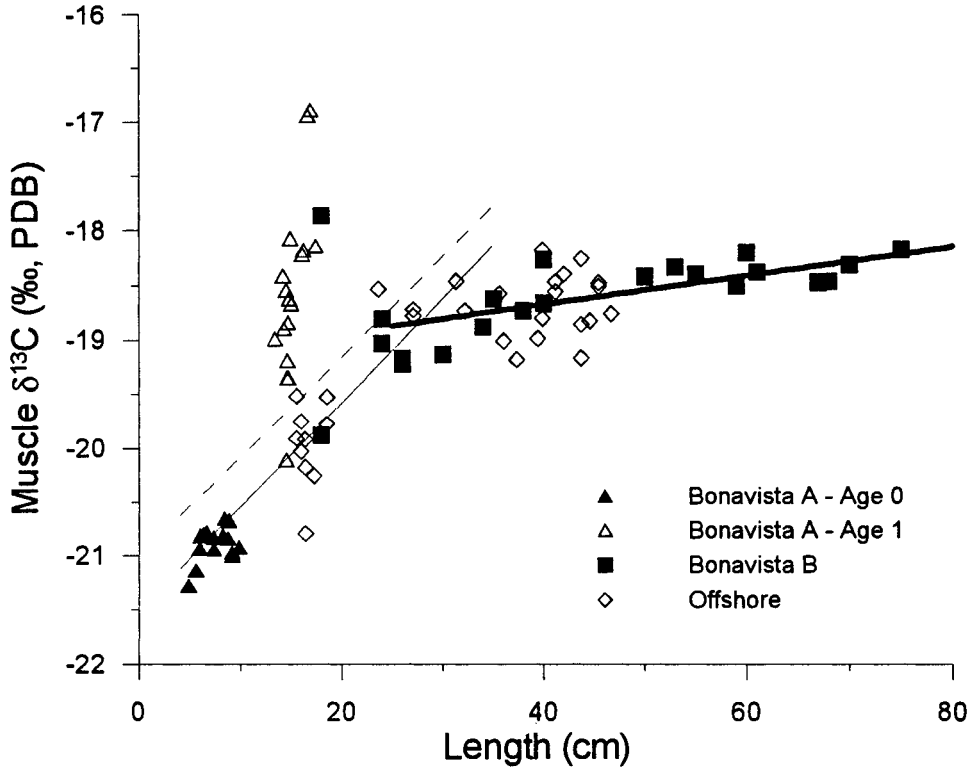

Figure 4.3 Muscle $\delta^{13}C$ versus length with regressions for <35 cm with the age 1 fish (thin solid line), <35 cm without the age 1 fish (dotted line), and for >35 cm.

4.4.3 Correlation of $\delta^{15}N$ and $\delta^{13}C$

Nitrogen and carbon exhibit similar trends with cod length and there is also a positive correlation for $\delta^{15}N$ - $\delta^{13}C$ ($r^2 = 0.397$) (Figure 4.4).

$$\delta^{15}N = 0.86\delta^{13}C + 30.3$$

If we again remove the age 1 fish the correlation improves greatly ($r^2 = 0.820$).

$$\delta^{15}N = 1.3\delta^{13}C + 39.3$$

This is a much clearer relationship than has been found in other studies and this strongly suggests that both $\delta^{15}N$ and $\delta^{13}C$ values are responding to trophic level changes in diet. If we assume that $\delta^{15}N$ is a better indicator of trophic level than $\delta^{13}C$, we can determine the average trophic increase. The average increase in $\delta^{15}N$ values between the smallest and largest size classes is 3.6‰ (Table 4.1). Using an increase of 3.4‰ per trophic level, it appears that cod move up approximately one trophic level over the size range examined in this study. At the same time, the average increase for $\delta^{13}C$ is 2.6‰. This is a rather surprising result as this is quite a large shift compared with shifts of 1-1.5‰ seen in most other studies.

4.5 Discussion

The simultaneous correlations which we have observed between $\delta^{13}C$ and $\delta^{15}N$ of cod muscle and fish length strongly indicates that these changes are related to ontogenetic changes in the diet of cod. The largest part of this change occurs within

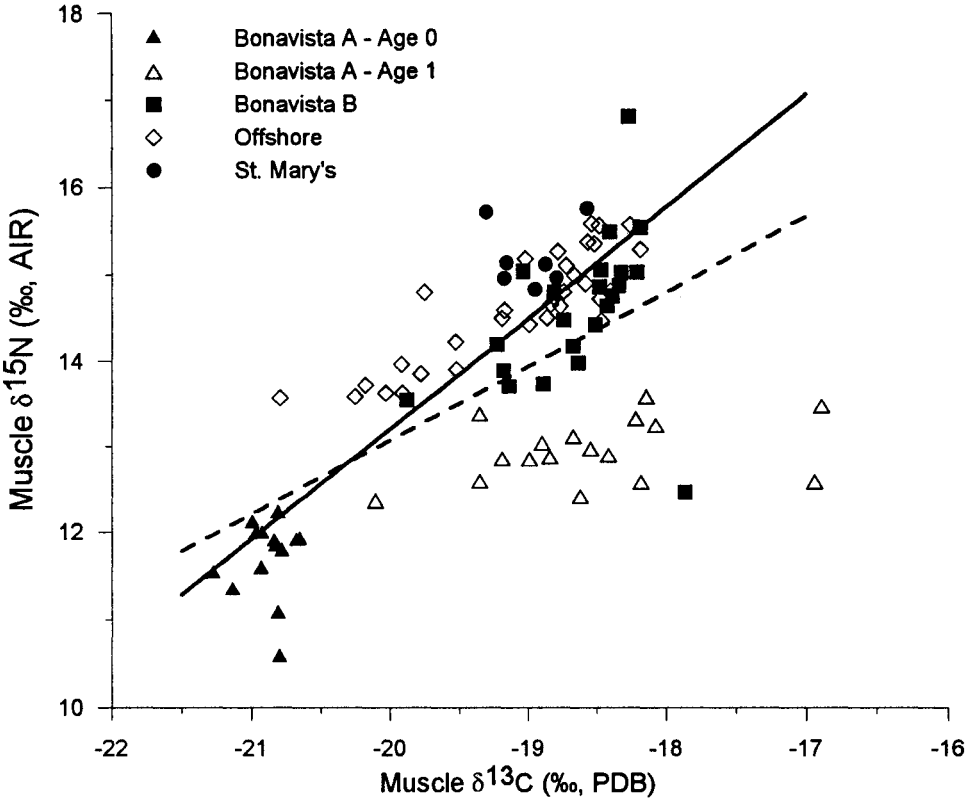

Figure 4.4 Muscle $\delta^{15}N$ versus muscle $\delta^{13}C$. The dotted line represents the regression for the total data set while the solid line represents the regression excluding age 1 cod.

the first 3 or 4 years which is consistent with shifts in feeding observed in other studies (Lilly 1987; Pálsson 1994; Casas and Paz 1996). Over this time it appears that there can be a gradual increase of almost one trophic level. Unlike other isotopic studies of fish, we see a much clearer link between the behaviour of carbon and nitrogen. The only similar study comparing different size classes of Atlantic cod was carried out by Lesage *et al.* (2001) on fish from the St. Lawrence Estuary and the Gulf of St. Lawrence (Table 4.2). They found that $\delta^{15}N$ increased by 1.6‰ between two size classes at both study locations, but curiously they found no increase in $\delta^{13}C$. The fish ranged between 14.5 and 54.5 cm a slightly smaller range than studied here. This may partly explain the discrepancies, but we observe a marked increase in $\delta^{13}C$ even in fish <50 cm in length.

4.5.1 Age 0 cod

The results for the youngest cod sampled (age 0; <10 cm) compare very well with the known characteristics of these fish. They exhibit the lowest values for both $\delta^{13}C$ (-20.9 ± 0.1‰) and $\delta^{15}N$ (+11.7 ± 0.4‰). These values indicate that these fish are two trophic levels above phytoplankton, likely feeding on a diet of zooplankton. While we did not attempt to measure the potential food sources in this area, a study on the south coast of Newfoundland showed that POM in two different bays ranged from approximately -24 to -25‰ for $\delta^{13}C$ and +4.6‰ to +5.2‰ for $\delta^{15}N$ (Dickson

Table 4.2 Comparison of $\delta^{13}C$ and $\delta^{15}N$ results from other studies with the present study.

Location	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Newfoundland nearshore[a]			
(27.8 ± 10.8 cm)	10	-19.0 ± 0.44	13.9 ± 0.57
(52 - 57 cm)	2	-	15.1 ± 0.1
St. Lawrence Estuary[b]			
(230mm)	1	-18.4	14.0
(330 - 545mm)	6	-18.3 ± 0.5	15.6 ± 0.7
Gulf of St. Lawrence[b]			
(145 - 320mm)	11	-19.5 ± 0.5	14.6 ± 0.8
(330 - 466mm)	9	-19.2 ± 0.4	15.8 ± 0.7
Georges Bank[c]	1	-16.4	12.6
This Study			
(49 - 340mm)	48	-19.5 ± 1.1	13.2 ± 1.3
(350 - 750mm)	38	-18.6 ± 0.3	15.0 ± 0.5

[a] data from Lawson and Hobson (2000) (lipids removed) and Hobson and Montevecchi (1991)
[b] data from Lesage *et al.* (2001) - lipids removed
[c] data from Fry (1988)

1986). This agrees with the expected range for marine phytoplankton. If we assume a value of -24‰ for phytoplankton and an enrichment factor of 1.5‰ for zooplankton, then cod feeding on them would be expected to have a $\delta^{13}C$ value of approximately -21‰ which agrees nicely with our findings. Nitrogen isotopic data also support this conclusion. Assuming an enrichment factor of +3.4‰ for both zooplankton and cod, age 0 cod should be approximately +11.8‰ which also matched our results nicely.

The limited variation in $\delta^{13}C$ and $\delta^{15}N$ of age 0 cod indicates that they are all eating a very similar range of prey. There is no overlap in either $\delta^{13}C$ or $\delta^{15}N$ between age 0 and age 1 cod, indicating that these groups have distinct feeding habits. This is again consistent with known transitions in the feeding behaviour of juvenile cod. Physical segregation occurs between age 0 and age 1 cod and they do not follow the same foraging patterns. Age 1+ cod are known to follow a diurnal migration pattern, moving between deeper water during the day and shallow water at night where they feed (Clark and Green 1990; Keats 1990; Grant and Brown 1998a; Grant and Brown 1998b). Age 0 cod will avoid these potential predators (Fraser *et al.* 1996) and will attempt to limit their interaction by following an opposite pattern of behaviour; feeding during the day and dispersing at night when age 1+ cod return (Grant and Brown 1998a; Grant and Brown 1998b). Different age groups of juvenile cod are expected to have very different lifestyles, as is confirmed by our isotopic analyses.

4.5.2 ^{13}C Enrichment in Age 1 Cod

Fish from the Bonavista (A and B) age 1 size class (10 - 20 cm) do not fit into the $\delta^{13}C$ - length trend as defined by data for smaller and larger fish. The overall range for these fish is very large (-20.9 to -16.9‰) and not only do they show much greater increases than the offshore group of the same size (-20.8 to -19.5‰), but they also reach $\delta^{13}C$ values greater than those seen in adult cod. The difference in this group is also evident in the variance which is $\approx \pm 1$‰ compared to ≤ 0.4‰ for $\delta^{13}C$ in all the other groups. The nitrogen values for this group are only slightly higher relative to age 0 cod, and fall within the expected range, therefore this increase in $\delta^{13}C$ cannot represent a large jump in trophic level. The wide range of $\delta^{13}C$ values leads us to believe that another, more ^{13}C enriched source of primary carbon, in addition to phytoplankton, is important in the diet of this group of cod. The relatively even distribution over the entire range of $\delta^{13}C$ also seems to indicate that the diet of these cod is a mixture of two distinct carbon sources.

The more ^{13}C enriched diet component must have a $\delta^{13}C$ value of at least -19 to -20‰. It could, however, be even higher than this since the $\delta^{13}C$ value of the fish represents a mixture of two sources and we don't necessarily see the most ^{13}C enriched end-member. When considering possible sources, we must keep in mind that although the source is ^{13}C enriched, it is not enriched in ^{15}N. This will generally preclude introducing new intermediate trophic steps as this would cause large

increases in $\delta^{15}N$. The source of $\delta^{13}C$ increase must also be specific to the inshore, age 1 fish, as none of the other size groups exhibit large increases, and they are also not seen in the age 1 cod from the offshore group. Even the age 0 cod from the Bonavista A group which were caught at the same time as the age 1 fish are not ^{13}C enriched. The increase may be related to some kind of diet specialization or perhaps opportunistic feeding.

One possible explanation is cannibalism, which has been observed in cod (e.g. Grant and Brown 1998b; Anderson and Gregory 2000) and led to large isotopic increases in the $\delta^{13}C$ values of populations of other fish such as Arctic char (Hobson and Welch 1995). However it would cause correlated increases in $\delta^{15}N$ which are not observed.

The presence of a second population of phytoplankton with different $\delta^{13}C$ values could also possibly explain the higher $\delta^{13}C$ values. Phytoplankton from different water masses have been shown to vary in $\delta^{13}C$ related to factors such as species, temperature, and concentration of CO_2. It is possible that selective feeding may lead to age 1 fish eating prey which feed on these ^{13}C enriched phytoplankton. Fry (1988) observed a decrease in $\delta^{13}C$ of shrimp with depth which he related to changes in $\delta^{13}C$ of phytoplankton. Although this seems like a possibility in this case, age 1 fish feed in shallow water at night and therefore it seems unlikely that they would encounter different phytoplankton sources than the age 0 cod which are

feeding in the same areas during the day (Grant and Brown 1998b). It is also not clear why this would not be seen in age 2 to 3 cod which also spend time inshore.

While phytoplankton usually dominate the base of marine food webs, in coastal areas benthic algae and seagrasses have been found to be important contributors to the food web (Fry *et al.* 1983; Fry and Parker 1979). These plants are generally more enriched in ^{13}C than marine phytoplankton ($\approx$ -21‰) or terrestrial carbon (-31 to -26‰) (Fry and Sherr 1984; Coffin *et al.* 1994). Fry and Sherr (1984) report a range of -5 to -15‰ for benthic macroalgae and -3 to -15‰ for seagrasses from a review of the literature. LeBlanc (1989) measured the isotopic values of seagrasses and macrophytes from an estuary site in Nova Scotia. $\delta^{13}C$ values generally ranged between -12 and -15‰. This wide separation in isotope values between marine phytoplankton and seagrasses has allowed researchers to examine the relative importance of seagrasses and marine phytoplankton to the base of estuarine food webs (e.g. Fry and Parker, 1979; Fry *et al.*,1983). Fry *et al.* (1983) found that there were two distinct groups of plants at the base of a tropical marine food web. Benthic seagrasses, macroalgae and epiphytic algae were all more ^{13}C enriched than other marine plants and this increase was reflected in organisms living in the seagrass meadow environment. Fry and Parker (1979) compared organisms from a seagrass meadow in Texas with organisms from offshore in the Gulf of Mexico. The seagrass animals were 3 to 5‰ higher relative to those offshore, including fish. From this they concluded that the seagrass was an important food source for animals living there.

The total range for age 1 fish from Bonavista Bay (-20.1 to -16.9‰) is intermediate between expected phytoplankton and seagrasses indicating that it is possible that these fish may be ingesting prey which feed on a combination of these sources. Macroalgae and seagrass are also plausible candidates because while they can provide a ^{13}C enriched source, they would not necessarily increase $\delta^{15}N$ values. The nitrogen signature of these types of plants ranges between +5 and +10‰ which is similar to marine phytoplankton (+3 to +12‰) (Minagawa and Wada 1984; Owens 1987; LeBlanc 1989).

There is some evidence from field studies of cod behaviour to support the idea that benthic algae and seagrasses may provide a food source for juvenile cod. A shift in diet is often seen between pelagic and benthic prey at around 10 cm (Keats *et al.* 1987; Grant and Brown 1998b; Lomond *et al.* 1998). This corresponds closely with the timing of shift in $\delta^{13}C$ values found in cod from Bonavista and would explain why no increase is observed in age 0 cod. Observations of young cod have also shown that they will use eelgrass as cover to hide from predators (Gotceitas *et al.* 1997; Keats *et al.* 1987; Keats 1990; Grant and Brown 1998a), and a close association of age 1+ cod and bottom substrates of rock and macroalgae have also been reported (Gotceitas *et al.* 1997). It is possible cod may be preying on benthic invertebrates or fish that feed on the macroalgae. It is also not seen in age 1 fish from offshore where the seagrasses would be absent.

A question that must be asked is whether the increases seen in the age 1 cod

are only an isolated situation related to some type of opportunistic feeding that only occurred during that one year. This question will require further sampling to answer, however the one ^{13}C enriched sample from Bonavista B taken the year before may hint at the possibility that this is not unusual and may represent the normal structure of this food web.

4.5.3 Adult Cod

Cod between 30 and 40 cm reach a plateau in both $\delta^{13}C$ and $\delta^{15}N$ values. In the larger fish (>35 cm), there is very little variation between fish indicating that they are eating a relatively similar diet. These results are comparable with measurements made of cod from nearshore Newfoundland by Lawson and Hobson (2000) and Hobson and Montevecchi (1991) and also with cod from other areas (Lesage *et al.* 2001) although $\delta^{15}N$ seems to show more variation than $\delta^{13}C$ between locations (Table 4.2). δ-values reported by Fry (1988) are also quite low relative to the other studies. Overall, the cod from this study are approximately 3 trophic levels above phytoplankton. The smaller fish eaten by these adult cod would likely have average isotopic values similar to the age 0 cod. Capelin from the St. Lawrence Estuary and the Gulf of St. Lawrence analyzed by Lesage *et al.* (2001) ranged from approximately -21.0 to -19.5‰ for $\delta^{13}C$ and from approximately 13 to 14‰ for $\delta^{15}N$. Two studies have analyzed capelin in the Newfoundland region. Dickson (1986) found a $\delta^{13}C$ of

-22.5 ± 1.4‰ and a $\delta^{15}N$ of 11.8 ± 0.2‰ while Hobson and Montevecchi (1991) measured a $\delta^{15}N$ of 13.0 ± 0.6‰. The results of Dickson (1986) come closest to what we would expect if the diet of adult cod were composed entirely of capelin. We know however, that omnivory will complicate the picture and may in fact suppress the full potential for ^{13}C enrichment in adult cod by introducing more ^{13}C depleted prey. The weak but positive $\delta^{13}C$- and $\delta^{15}N$ - length relationships for fish over 35 cm seems to indicate that $\delta^{13}C$ and $\delta^{15}N$ continue to increase. If fish are selecting the largest possible prey then this may be expected. Also, it has been shown that very large fish (>75 cm) prefer more crabs and flatfish, which indicates that changing diet is a lifelong phenomenon (Lilly 1987).

It is also possible that the full extent of trophic increases in isotope values in adult cod may be suppressed by what may be called "metabolic smoothing". As fish get older, they grow and add new tissue at a slower rate. New tissue will represent an increasingly small fraction of the total mass of the fish. If metabolic turnover is slow, as in older fish, the overall isotopic composition will be slow to change in response to shifts in diet. In some cases, metabolic processes may dominate (Overman and Parrish 2001) and it may be that stomach content analysis will give a clearer picture of diet than isotopic analysis if fish are particularly slow growing.

4.5.4 Inter- and Intrapopulation Variations

Spatial and temporal variations in the base of the food web or variations in the importance of various prey may obscure the overall picture of trophic structure. The sampling for this study took place over a span of four years between 1997 and 2000, and at a number of different locations. As a result, some of the observed variation may represent secular shifts in $\delta^{15}N$ and $\delta^{13}C$ at the base of the food web perhaps driven by changes in the environment. The size overlap between successive years is not large enough to allow a true comparison, but adult cod collected in different years and locations show very little variation. This consistency seems to indicate that spatial and temporal fluctuations in this region are generally not large. Therefore if we do observe changes in carbon and nitrogen isotope values of adult cod, these may indicate important changes in environmental factors such as productivity (Fry and Parker 1979).

Intrapopulation variation is also very small. Within each size class, the standard range in deviation is not usually greater than 0.3‰ for carbon and 0.7‰ for nitrogen except in the age 1 cod. At any particular life stage, it appears that cod eat a very consistent range of prey. This of course does not absolutely identify what the fish are actually eating. It is possible that the fish are either all eating a wide variety of prey or all specializing on a few items (Vander Zanden *et al.* 2000). It is not possible to distinguish between these possibilities with our present data set.

4.6 Conclusions

Our study has shown that it is possible, through the use of stable carbon and nitrogen isotope values, to recognize ontogenetic changes in diet experienced by Atlantic cod in the Newfoundland region. Over a time span of seven or eight years, cod undergo a continuous increase of approximately one trophic level as determined by changes in $\delta^{15}N$. Most of this increase is found in the first three or four years. These findings are consistent with previous conclusions drawn from stomach content analysis. The data are also generally consistent with a phytoplankton base for this food web. Young cod feed on zooplankton and as they grow they move from a crustacean dominated diet to become more piscivorous, incorporating fish prey which are more enriched in both ^{13}C and ^{15}N, and moving through progressive trophic levels.

Parallel increases in $\delta^{13}C$ over this period suggest a trophic level increase in carbon values of about 2.6 ‰, which is larger than have been observed in other marine or fresh-water populations. We do not yet have a model to account for this large trophic increase in carbon isotope values, but this may relate to the preferential metabolic utilization of lipids as an energy source by the cod, resulting in a larger proportional excretion of ^{13}C depleted bicarbonate. Inshore (but not offshore) age 1 cod appear to incorporate carbon from at least two separate sources, one of which is highly enriched in ^{13}C. ^{13}C enriched benthic macroalgae or seagrass may be an important part of the inshore food web. More data are needed on the isotopic composition of potential prey items in inshore waters to confirm this model.

Recognizing ontogenetic behavioural changes in cod is important to increase our understanding of the interaction between cod and their environment as well as for better future management of cod stocks. This study has shown how stable isotopic analysis of tissues can continuously monitor changes in trophic level in a single species. We have shown that ontogenetic changes in feeding behaviour are clearly seen in shifts of isotopic composition. Future studies will expand on this, undertaking broader sampling to get a clearer picture of the various sources of diet variation.

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CHAPTER FIVE

The contribution of metabolic carbon to the $\delta^{13}C$ signature of Atlantic cod (*Gadus morhua*) otoliths.

5.1 Abstract

Otolith $\delta^{13}C$ values represent a mixture of environmental dissolved inorganic carbon (DIC) and ^{13}C depleted metabolic carbon. The contribution of metabolic carbon to otolith aragonite has not been adequately quantified in the past. Fish were collected from an inshore location in Bonavista Bay on the east coast of Newfoundland, Canada. Both muscle $\delta^{13}C$ ($\delta^{13}C_M$) and otolith $\delta^{13}C$ ($\delta^{13}C_{oto}$) were measured for these fish in order to assess the importance of metabolic carbon to $\delta^{13}C_{oto}$ and whether variations in $\delta^{13}C_{oto}$ could be related to ontogenetic changes in diet. Analyzing an inshore population allows us to minimize the impact of changes in $\delta^{13}C_{DIC}$.

Muscle $\delta^{13}C$ and $\delta^{13}C_{oto}$ were found to be significantly correlated:

$$\delta^{13}C_{oto} = 0.8\delta^{13}C_M + 12.8 \qquad (r^2 = 0.721)$$

This relation indicates that almost all of the size-related variation in otolith $\delta^{13}C$ can be attributed to the ontogenetic changes in diet. The remainder can be explained by a decrease of ≈20% in the relative contribution of metabolic carbon (M) with size. Relatively large individual variability can likely be explained by variations in

metabolism resulting from differences in individual physiology. Seasonal variability may also be a result of metabolic fluctuation with temperature, but it is also possible that seasonal changes in diet or the $\delta^{13}C$ of the food web may influence variation in $\delta^{13}C_{oto}$ values as well. It has also been shown that using measured $\delta^{13}C_{oto}$ and $\delta^{13}C_{M}$ it is possible to track changes in metabolism within groups of fish.

5.2 Introduction

Isotopic analysis of otoliths has found increasing interest for both palaeontological and environmental applications. This method can be used to reveal the environmental and physiological history of fish species, however it has not been used to its full potential. While otolith oxygen is known to be in isotopic equilibrium with the ambient water (e.g. Devereux 1967; Degens *et al.* 1969; Kalish 1991a; Kalish 1991b; Iacumin *et al.* 1992; Thorrold *et al.* 1997), the information recorded in the carbon isotopic record has been more difficult to interpret. Measurements of $\delta^{13}C_{oto}$ in a number of fish species has indicated that although a large proportion of otolith carbon is derived from ambient DIC it is not in equilibrium with this system (Degens *et al.* 1969; Radtke *et al.* 1987; Kalish 1991a; Kalish 1991b; Iacumin *et al.* 1992; Radtke *et al.* 1996; Thorrold *et al.* 1997; Schwarcz *et al.* 1998; Weidman and Millner 2000; Begg and Weidman 2001). $\delta^{13}C_{oto}$ is ^{13}C depleted with respect to expected fractionation of inorganic aragonite precipitation.

Non-equilibrium $\delta^{13}C_{oto}$ values could be either the result of kinetic fractionation effects or of mixing of CO_2 from metabolism of organic matter (nutrient). Metabolic fractionation results from the production of CO_2 from organic matter during metabolism. Kinetic fractionation may occur during the hydration or hydroxylation of CO_2 to form HCO_3^- (McConnaughey *et al.* 1997). Kinetic fractionation, however, should lead to the fractionation of oxygen isotopes as well, but otoliths are formed in oxygen isotopic equilibrium with ambient water. Oxygen equilibrium has been verified for the fish used in this study and will be discussed in a separate paper (Jamieson *et al.* 2001). It seems therefore that apparent $\delta^{13}C$ disequilibrium with marine DIC must result from the addition of metabolic carbon.

Biological carbonates of other organisms have been found to incorporate metabolic carbon. Studies using ^{14}C have shown that metabolic carbon is incorporated into corals (Pearse 1970), sea urchin embryos (Sikes *et al.* 1981), molluscs and barnacles (Tanaka *et al.* 1986). The proportion of metabolic carbon utilized by different organisms varies however. It has also been found that while metabolic carbon is a very important component of the carbonates of terrestrial organisms (up to 90%), aquatic invertebrates seem to incorporate much less, while fish otoliths have intermediate values (McConnaughey *et al.* 1997). Kalish (1991a) estimated that approximately 30 to 35% of the carbon in the otoliths of Australian salmon was from metabolic sources. Schwarcz et al (1998) derived a maximum estimate of 43% for Atlantic cod which would decrease with age. Other studies have

noted the importance of metabolic carbon to otolith $\delta^{13}C$, but they have not attempted to quantify this component (e.g. Iacumin *et al.* 1992; Radtke *et al.* 1996; Thorrold *et al.* 1997).

Variations in $\delta^{13}C_{oto}$ are also found over the life of an individual fish. Precipitation of otoliths occurs from a fluid known as endolymph. Concentric layers of carbonate are deposited around a nucleus and this growth continues throughout the life of the fish, continuously recording its environmental and physiological conditions. Otoliths do not undergo remodelling like other biological tissues and therefore they faithfully record past conditions (Campana and Neilson 1985). Translucent growth zones are formed in winter, while opaque zones form in the summer. By analyzing these individual growth zones, a lifetime history of isotopic composition can be reconstructed.

Studies carried out on otoliths of Atlantic cod (*Gadus morhua*) have shown that the $\delta^{13}C$ signal for an individual cod is not constant but rather increases during the first few years of life and generally reaches a plateau after maturity (Schwarcz *et al.* 1998; Weidman and Millner 2000). Schwarcz *et al.* (1998) showed that the $\delta^{13}C$ values for Nova Scotian cod increased from approximately -5‰ to -2.5‰ during the first 4 or 5 years of life, reaching a maximum of approximately 0‰ at maturity and remaining constant or decreasing thereafter. The fact that these changes are consistent for all fish, independent of year, indicate that they are related to

physiological and behavioural changes as the fish matures and not to secular environmental changes. Patterns in $\delta^{13}C_{oto}$ must be interpreted in terms of changes in DIC, diet, and metabolism. In order to interpret $\delta^{13}C_{oto}$, the relative contributions of each of these components must be determined. The overall picture of how $\delta^{13}C_{oto}$ patterns arise, however, is then complicated by the fact that each of these components is subject to variations caused by seasonal fluctuations as well as changes in physiology, behaviour, and location of the fish.

5.2.1 Dissolved Inorganic Carbon

$\delta^{13}C$ values of oceanic DIC varies spatially as well as seasonally. This is mainly a function of the primary production of organic matter and its degradation as it descends through the water column (Deuser and Hunt 1969; Kroopnick 1980; Kroopnick 1985). Variation in the rate of photosynthesis, mixing of water masses, and differences in the rate of degradation can all contribute to isotopic variability. Generally $\delta^{13}C$ values lie between 1 and 2‰ in surface water. In deeper waters there is a close association between $\delta^{13}C$ and dissolved oxygen concentration which reflects the importance of organic matter oxidation. Organic matter is ^{13}C depleted and its oxidation therefore leads to a decrease in values $\delta^{13}C_{DIC}$ values. As depth increases, $\delta^{13}C_{DIC}$ values decrease, reaching a minimum which corresponds closely with the minimum in dissolved oxygen. Most of the variation occurs in the upper 500 m. At

depth, $\delta^{13}C_{DIC}$ values level off at around +0.5‰. To the extent that DIC of seawater contributes to DIC of the endolymph, these variations in $\delta^{13}C_{DIC}$ may be reflected in $\delta^{13}C_{oto}$ if cod migrate between different depths. Seasonal variations due to fluctuations in photosynthetic activity may also be reflected in $\delta^{13}C_{oto}$.

5.2.2 Metabolic Carbon

The isotope value of metabolic carbon ($\delta^{13}C_M$) is directly related to the $\delta^{13}C$ value of the diet with a slight increase of 1-2‰ (DeNiro and Epstein 1978). Schwarcz *et al.* (1998) speculated that much of the variation seen in otolith $\delta^{13}C$ values over the lifetime of a cod might be attributed to ontogenetic changes in diet. Radtke *et al.* (1996), in a study of cod otoliths, showed that there was a distinct change in otolith $\delta^{13}C$ values between two groups of cod raised on isotopically distinct diets. The importance of ontogenetic changes in diet to cod muscle tissue was confirmed by Jamieson and Schwarcz (2001) showing that the $\delta^{13}C$ value of metabolic carbon varied almost continuously with length as a result of changes in trophic level as the fish ate larger and different prey items. This resulted in an average increase of $\delta^{13}C_M$ values of approximately 2.5‰. If it is the case that DIC of endolymph (and therefore otoliths) contains a large proportion of metabolic carbon, then it is indeed possible that the variation in $\delta^{13}C_{oto}$ is a result of these trophic level changes.

5.2.3 Metabolism

Metabolic rate may vary with age and also temperature (Jobling, 1996). As a fish grows, its metabolism decreases. Metabolism is often measured as oxygen consumption (Q_{O2}). For cod Edwards and Finlayson (1972) found that:

$$Q_{O2} = 0.245W^{0.82} \text{ (mg/h)} \qquad (1)$$

where W is the weight of the fish in grams. As the fish increases in size, oxygen consumption goes down per unit weight. These changes in metabolism would result in changes in the production of metabolic CO_2 and its incorporation into otolith carbonate. The effect of temperature on metabolic rate may also result in seasonal variations in the metabolic contribution.

5.2.4 Calculating the metabolic contribution to DIC of endolymph

It is possible to combine these three components into one equation representing their relative contributions to the HCO_3^- found in the endolymph from which the otolith aragonite will precipitate:

$$\delta^{13}C_{HCO3} = M\,\delta^{13}C_M + (1 - M)\delta^{13}C_{DIC} \qquad (2)$$

where M is the mol fraction of metabolic bicarbonate in DIC of the endolymph. (Schwarcz *et al.* 1998; McConnaughey *et al.* 1997). The three variables M, $\delta^{13}C_M$, and $\delta^{13}C_{DIC}$ may all contribute to the fluctuations seen in $\delta^{13}C_{oto}$ over the lifetime of an individual cod. By measuring $\delta^{13}C_M$ and $\delta^{13}C_{oto}$ from the same fish, we can

calculate the value of M. Expected changes in $\delta^{13}C_M$ and M with age are likely to cause increase in $\delta^{13}C_{oto}$ while the influence in $\delta^{13}C_{DIC}$ is less predictable. If fish are moving to deeper, offshore water with age it is possible that this may result in a decrease in $\delta^{13}C_{oto}$. Fluctuations in older fish are more likely to represent changes in $\delta^{13}C_{DIC}$ as changes in M and $\delta^{13}C_M$ are generally more important early in life.

While these are the main factors, there are also other possible sources of fractionation which may influence the final composition of the otolith. Although HCO_3^- is the dominant carbonate species in seawater, fish take in DIC directly from seawater by diffusion through the gills in the form of CO_2. This CO_2 is then converted to HCO_3^- in the blood. Offsetting fractionations during this process ensure that the resulting HCO_3^- will maintain its seawater isotope value (Schwarcz *et al.* 1998). Fractionation of respired CO_2 and conversion to HCO_3^- should also result in little fractionation and can probably be ignored (McConnaughey *et al.* 1997). A further source of fractionation is that associated with the formation of aragonite from HCO_3^-. The exact magnitude and sign of this fractionation is uncertain and depends to a small degree on temperature. Grossman and Ku (1986) presented data for molluscs:

$$\Delta^{13}C_{Arg\text{-}DIC} = -0.131T(°C) + 2.66 \quad (3)$$

and for the foraminifera *Hoeglundina elegans*:

$$\Delta^{13}C_{Arg\text{-}DIC} = -0.108T(°C) + 2.40 \quad (4)$$

Romanek *et al.* (1992) measured an increase of 2.7‰ for inorganic precipitation of

aragonite independent of temperature at 10 - 40°C. Other factors such as otolith or somatic growth rate seem to have little or no effect on otolith isotopic composition (Kalish 1991a; Thorrold *et al.* 1997).

Taking into account the aragonite-HCO_3^- fractionation, equation (2) may be revised to give an equation for $\delta^{13}C_{oto}$. We can assume an average temperature of 5°C which is the approximate mean for the study area (Narayanan *et al.* 1996).

$$\delta^{13}C_{oto} = M\,\delta^{13}C_M + (1 - M)\delta^{13}C_{DIC} + 2 \qquad (5)$$

A number of otolith studies have postulated that changes in $\delta^{13}C_M$ could be responsible for shifts in $\delta^{13}C_{oto}$ (Kalish 1991a; Radtke *et al.* 1996; Schwarcz *et al.* 1998; Begg and Weidman 2001). Only Radtke *et al.* (1996) attempted to measure otolith $\delta^{13}C$ and metabolic $\delta^{13}C$ in the same fish. They did not however, try to determine the relative proportions of metabolic carbon and they did not relate this to variations seen in the otoliths of wild populations. McConnaughey *et al.* (1997) made a similar comparison for mollusc tissue and shell and used the technique to show that 10% of the shell carbonate was metabolic. We wanted to determine the relative importance of metabolic carbon to the isotopic signature of Atlantic cod otoliths, and at the same time, determine if ontogenetic changes in diet were responsible for the ^{13}C enrichments seen during the first years of otolith growth. In order to study this question we have obtained cod from Bonavista Bay, Newfoundland, an inshore population, and analyzed them for both muscle tissue $\delta^{13}C$ ($\delta^{13}C_M$) and otolith $\delta^{13}C$

($\delta^{13}C_{oto}$). We can furthermore assume that $\delta^{13}C$ of muscle tissue is equal to $\delta^{13}C_M$, since there is no isotopic fractionation during metabolic oxidation (Schoeller *et al.* 1980). Taking fish from the same inshore population should ensure that the fish are experiencing the same environmental conditions and minimize the effect of variables such as $\delta^{13}C_{DIC}$. This allows us to examine the effect of changing diet on $\delta^{13}C_{oto}$.

Determining the relative contribution of metabolic and environmental carbon to otolith $\delta^{13}C$ is critical for the interpretation of otolith $\delta^{13}C$ trends. Otolith carbon has the potential to reveal important information about physiological and environmental changes experienced by the fish. This in turn may aid in studies of fish behaviour as well as fluctuations in productivity.

5.3 Methods

Cod were collected from inshore waters in Bonavista Bay, on the east coast of Newfoundland (Figure 5.1). Two sets of samples were collected for this study in November 1997 and September 1998. Twenty-two cod were collected in the first set and thirty-two cod in the second. The first group (Bonavista B) were mainly larger, older fish ranging in size from 18 to 75cm. The second group (Bonavista A) consisted of younger cod from the age 0 (4.9 to 9.8cm) and age 1 (13.4 to 17.4cm) groups. Cod muscle tissue was analyzed for $\delta^{13}C$ as described in Jamieson and Schwarcz (2001). Otoliths were extracted from these fish and 41 of the 54 were

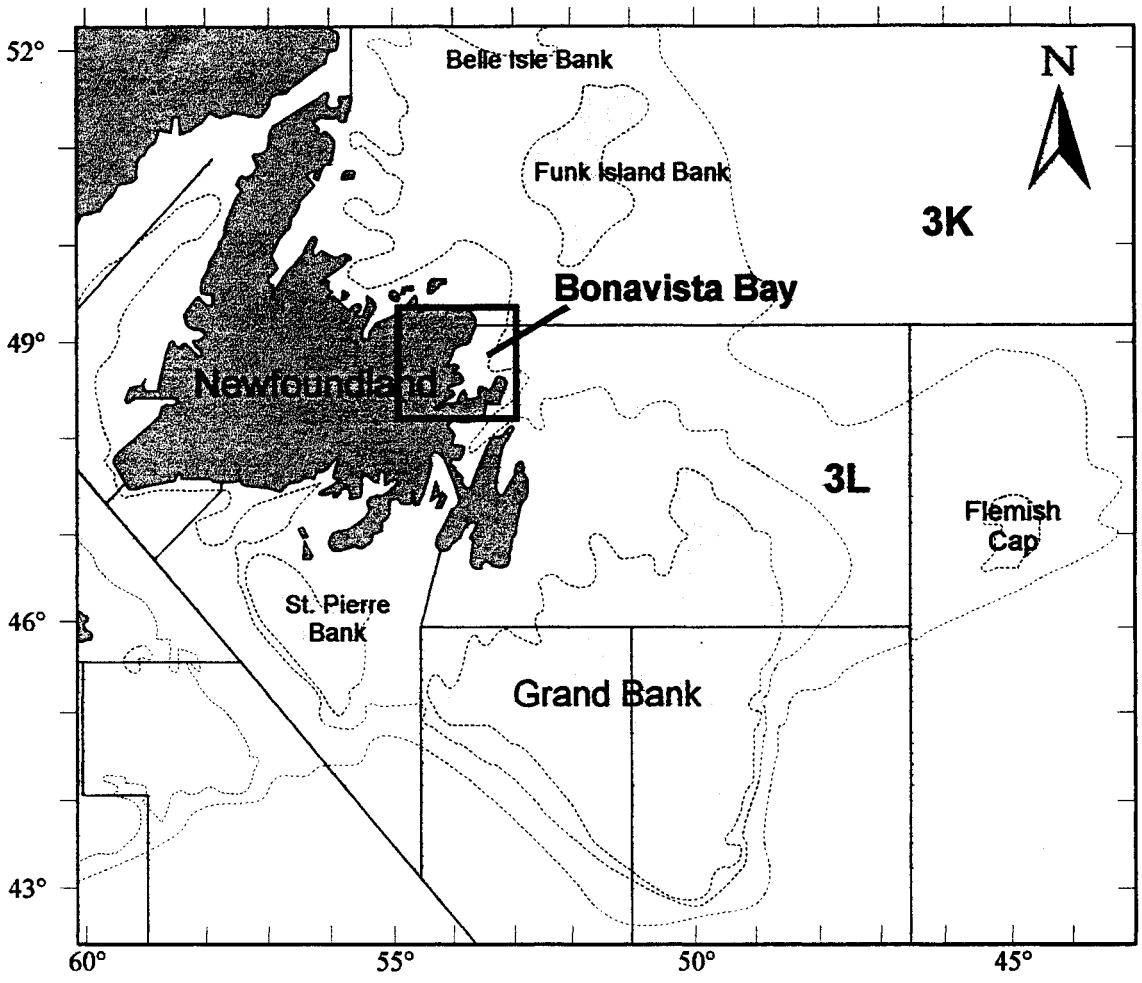

Figure 5.1 Map of offshore Newfoundland indicating the sampling areas in Bonavista Bay and offshore NAFO Div. 3L.

analyzed for $\delta^{13}C_{oto}$. The otoliths were cleaned with a dilute bleach solution and deionized water after extraction to remove any organic material. Larger otoliths from the Bonavista B group were embedded in Araldite® resin and sectioned using a slow speed diamond blade saw. These sections were then mounted on microscope slides and samples were milled from individual opaque and translucent zones using a Merchantek MicroMill system. The isotopic values from the last annual zone representing the newest growth, were used for comparison with $\delta^{13}C_M$. The procedures are more fully described in Jamieson (2001). Otoliths from the younger age 0 and age 1 cod were ground whole for isotopic analysis. The $\delta^{13}C$ values from these fish therefore represent an integrated lifetime value. Since these fish are age 1 and younger, this is equivalent to the last annual band of the older fish, representing a year of growth. Four of the larger age 1 otoliths, however, were milled for a higher resolution examination of seasonal variation during the first year of growth. The thickness of the growth zones dictated the number of samples that could be collected. Four to six samples were taken from the opaque core and one to two were taken from the outer translucent zone.

Aragonite samples were all analyzed on an Optima dual-inlet IRMS fitted with an Isocarb carbonate analyzer. $\delta^{13}C$ values are reported with respect to the VPDB international standard. Reproducibility is ±0.05‰.

5.4 Results

5.4.1 Otolith and muscle tissue comparison

Cod muscle tissue data are described in detail in Jamieson and Schwarcz (2001). The observed range of $\delta^{13}C_{oto}$ is -4.9 to -0.1‰ (Table 5.1) which is significantly correlated with fish length ($r^2 = 0.604$). If the average increase between age groups is examined, there is an overall average increase of +2.9‰ between the average age 0 cod value of -3.9 ± 0.5‰ and the adult cod (>35cm) average of -1.0 ± 0.6‰. A length of 35cm is used as this corresponds with the length where $\delta^{13}C_M$ values apparently plateau (Jamieson and Schwarcz 2001). This increase is very similar to the overall average increase seen in $\delta^{13}C_M$ of +2.6‰. Within each age group, there is a significant amount of variation in $\delta^{13}C_{oto}$ (Δ = 1.5 to 2‰) despite the fact that each size class has a narrow range of $\delta^{13}C_M$ values.

In order to determine the importance of diet change ($\Delta^{13}C_M$) to $\delta^{13}C_{oto}$, we compare $\delta^{13}C_M$ and $\delta^{13}C_{oto}$ from individual fish. They are significantly correlated (Figure 5.2; $r^2 = 0.721$) and can be defined by the equation:

$$\delta^{13}C_{oto} = 0.8\delta^{13}C_M + 12.8 \qquad (6)$$

Most of the variation in $\delta^{13}C_{oto}$ can therefore be explained by the magnitude of $\Delta^{13}C_M$ resulting from ontogenetic changes in diet. The slope of 0.8 means that 80% of the variation can be explained by diet change and only 20% is accounted for by changes in metabolic rate and/or DIC.

Table 5.1 Results of $\delta^{13}C$ analysis of muscle tissue, otolith aragonite samples and calculated M values from Bonavista Atlantic cod. $\delta^{13}C$ values are in ‰ and length is in cm..

	Length	$\delta^{13}C_M$	$\delta^{13}C_{oto}$	M (%)		Length	$\delta^{13}C_M$	$\delta^{13}C_{oto}$	M(%)
Bonavista A - age 0					**Bonavista A - age 1**				
NS1	4.9	-21.3	-4.7	35	NS16	14.3	-18.9	-2.3	26
NS2	5.6	-21.1	-4.7	35	NS18	14.5	-20.1	-3.5	31
NS3	6.0	-20.8	-3.5	30	NS19	14.6	-19.2	-2.5	27
NS4	5.9	-20.9	-4.9	36	NS20	14.7	-19.3	-2.9	29
NS5	6.6	-20.8	-3.5	30	NS22	14.6	-19.3	-2.8	29
NS6	6.3	-20.8	-3.8	31	NS23	15.0	-18.7	-2.2	26
NS7	7.3	-20.9	-4.0	32	NS24	14.7	-18.8	-2.6	28
NS8	7.3	-20.8	-4.1	33	NS26	14.5	-18.5	-2.3	27
NS9	8.2	-20.8	-3.2	29	NS27	14.8	-18.6	-1.9	25
NS10	8.4	-20.7	-3.8	31	NS28	16.2	-18.2	-2.3	28
NS11	8.8	-20.7	-3.6	31	NS29	16.6	-16.9	-1.8	27
NS12	8.7	-20.8	-4.0	32	NS30	16.1	-18.2	-1.5	24
NS13	9.1	-21.0	-3.6	30	NS31	16.9	-16.9	-1.8	27
NS14	9.1	-21.0	-4.1	32	NS32	17.4	-18.1	-2.2	27
NS15	9.8	-20.9	-3.2	28					
Bonavista B									
B76	18	-17.9	-2.9	26					
B77	67	-18.5	-0.8	20					
B81	26	-19.2	-1.0	20					
B82	75	-18.2	-0.1	16					
B83	35	-18.6	-1.9	25					
B84	34	-18.9	-2.3	26					
B88	40	-18.3	-0.8	20					
B93	18	-19.9	-2.2	25					
B95	55	-18.4	-1.7	24					
B98	53	-18.3	-0.9	20					

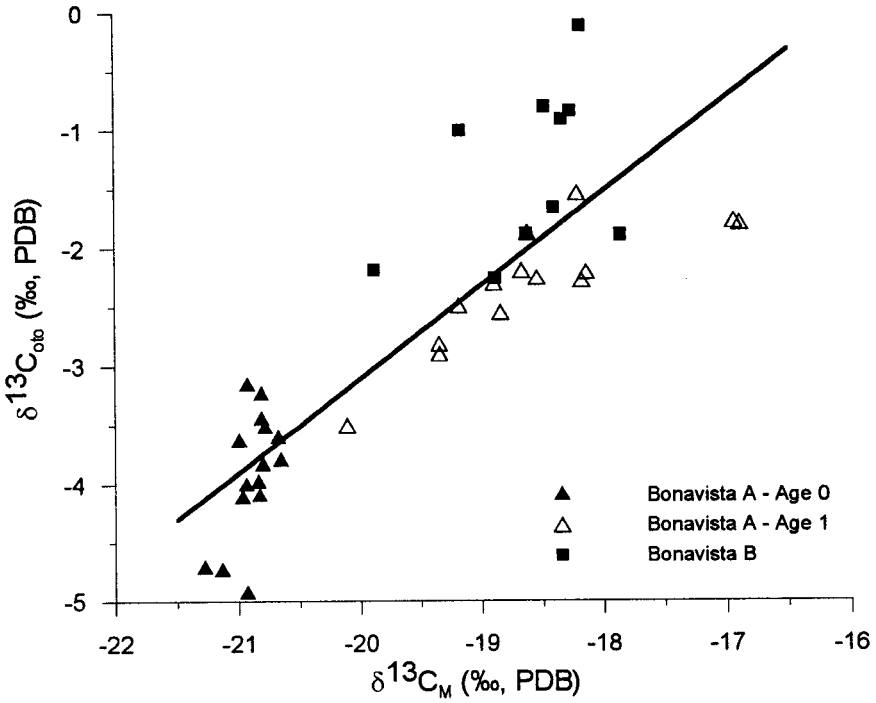

Figure 5.2 Otolith $\delta^{13}C$ vs muscle $\delta^{13}C$ for all Bonavista cod. The line represents the regression for the data set.

The Bonavista A group of age 1 cod provided an opportunity to test the metabolic contribution of $\delta^{13}C_{oto}$ independent of size and age. This group had a relatively small range in size (14.3 to 17.4 cm), but they exhibited a very large range of $\delta^{13}C_M$ values (-20.1‰ to -16.9‰; Δ = 3.2‰). This group also exhibits a wide range of $\delta^{13}C_{oto}$ values (-3.5 to -1.5‰; Δ = 2‰). Most of the data, however seems to lie on the overall correlation between $\delta^{13}C_{oto}$ and $\delta^{13}C_M$.

Using the measured values of $\delta^{13}C_M$ and estimating $\delta^{13}C_{DIC}$, it is possible to calculate the contribution of metabolic carbon (M) at different times in the life of the fish by restating equation (5) as:

$$M = (\delta^{13}C_{oto} - (\delta^{13}C_{DIC} + 2)) / (\delta^{13}C_M - \delta^{13}C_{DIC}) \qquad (7)$$

These results are reported in Table 5.1 assuming a $\delta^{13}C_{DIC}$ value of +1‰. The effect of a change in the assumed value of $\delta^{13}C_{DIC}$ is minimal. Figure 5.3 presents the data as a plot of M versus length. There is a good correlation between M and the Log L represented by the equation:

$$M = -13.0 \text{ Log } L - 42.7 \qquad (r^2 = 0.804) \qquad (8)$$

It is evident that M decreases as the fish get larger; a high of 36% in one of the smallest fish to a low of 16% in one of the oldest and largest cod. The average M is 28 ± 4%. Within each group there is a large variation. The ranges are: age 0: 28 to 36%; age 1: 24 to 31% and adult (>35 cm): 16 to 25%. Therefore within each age class there could be 7 to 10% variation indicating that there can be significant

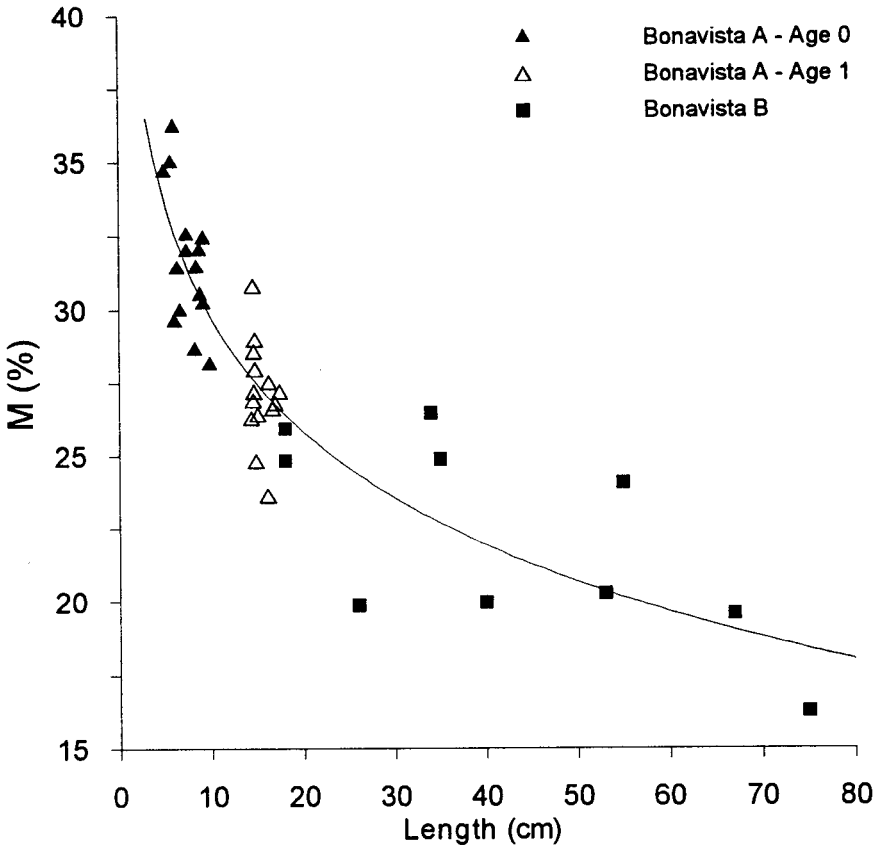

Figure 5.3 Plot illustrating the variation of M with length. The line represents the regression given by the equation: $M = -13.0 \text{ Log } L + 42.7$ ($r^2 = 0.804$).

variation in M within a small size range. There is a considerable degree of scatter in cod > 35cm. There is a range of M values even for cod of a similar size. For example, three cod ranging in size between 34 and 40 cm have a range of M between 20 and 26%.

5.4.2 Individual life trends

It was possible to mill the seasonal zones and reconstruct lifetime records of $\delta^{13}C_{oto}$ for some of the older fish. This would allow us to determine if these cod exhibited the same increase with age observed in other studies and that would be expected if ontogenetic change is important. Figure 5.4 illustrates the trends of $\delta^{13}C_{oto}$ for three of the older Bonavista B cod (6 to 7 years old). It is clear that the fish undergo similar ontogenetic changes in $\delta^{13}C_{oto}$ as seen in other areas. Initial values range between -4 and -2‰ and they increase to adult values of approximately -1.5 to 0‰. There is significant variation in the patterns of ontogenetic changes for individual fish. Cod B82 and B95 exhibit overall lifetime change in $\delta^{13}C_{oto}$ of approximately 2.5‰ while B98 only becomes ^{13}C enriched by approximately 1 to 1.5‰. If we examine the values after the fish appear to have reached maturity (>5 years), the values become more consistent for each individual fish, but there is still variation between the three cod. The average adult $\delta^{13}C_{oto}$ value is -1.0 ± 0.5‰ and the range is approximately 1‰.

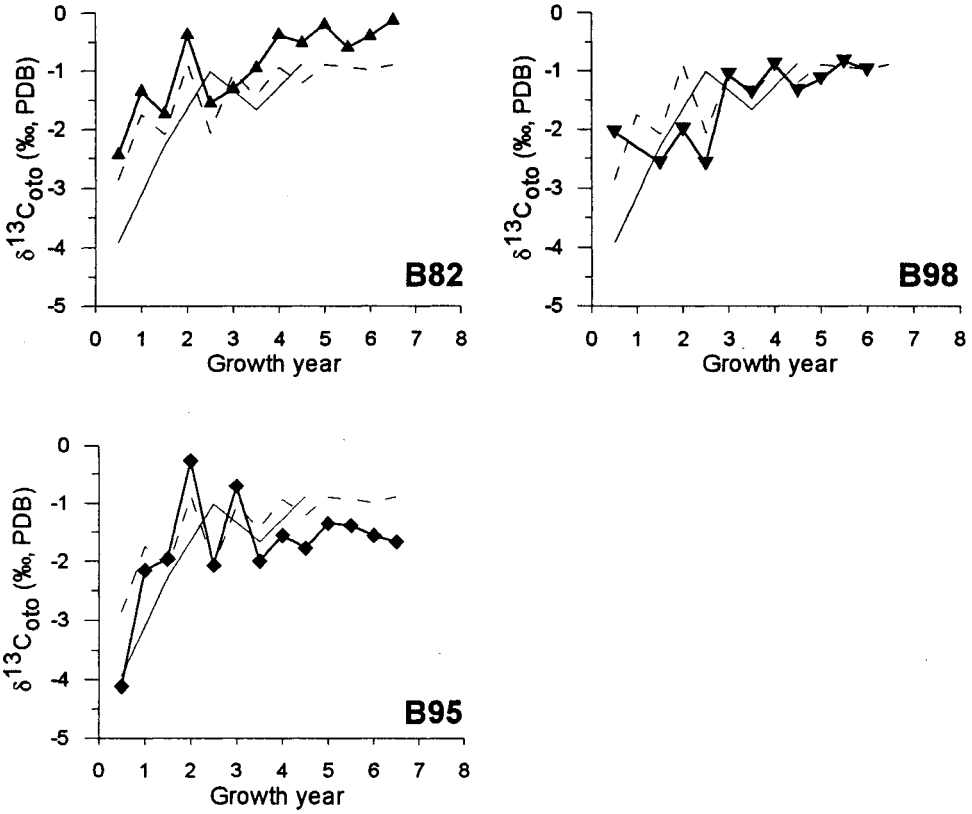

Figure 5.4 Individual lifetime $\delta^{13}C_{oto}$ trends for three of the Bonavista B cod. The dashed line represents the average of the three otoliths, and the thin line represents the average $\delta^{13}C_{oto}$ calculated for length classes from the individual otolith analyses.

These individual records also allow a comparison between $\delta^{13}C_{oto}$ for fish of different sizes and the overall lifetime variation for individual cod. The average $\delta^{13}C_{oto}$ for 10 cm length classes is plotted along with each of the three individual records (Figure 5.4). This average trend agrees quite well with the individual records, but does illustrate the variability of these individual cod.

5.4.3 Seasonal Variation

Seasonal variation is quite pronounced in early life but seems to become less pronounced with age. Figure 5.5 illustrates the average $\delta^{13}C_{oto}$ for adjacent opaque and translucent zones of the three older Bonavista B cod. The translucent (winter) zones are consistently ^{13}C enriched relative to the opaque (summer) zones and the difference can be as high as 2‰. The figure shows however, that the seasonal signal is stronger in younger cod and declines with age. Ontogenetic change in $\delta^{13}C_{oto}$ is superimposed on this pattern so that while an opaque zone will be ^{13}C depleted compared with the adjacent translucent zone, it is possible that the following opaque zone will be more ^{13}C enriched.

A consistent seasonal signal can also be seen in the records for individual cod over the first year of growth (Figure 5.6). These curves have been matched using the $\delta^{18}O_{oto}$ data as a proxy for temperature. The translucent zone is generally more ^{13}C enriched than the opaque zone, agreeing with the records of the older cod. Variation

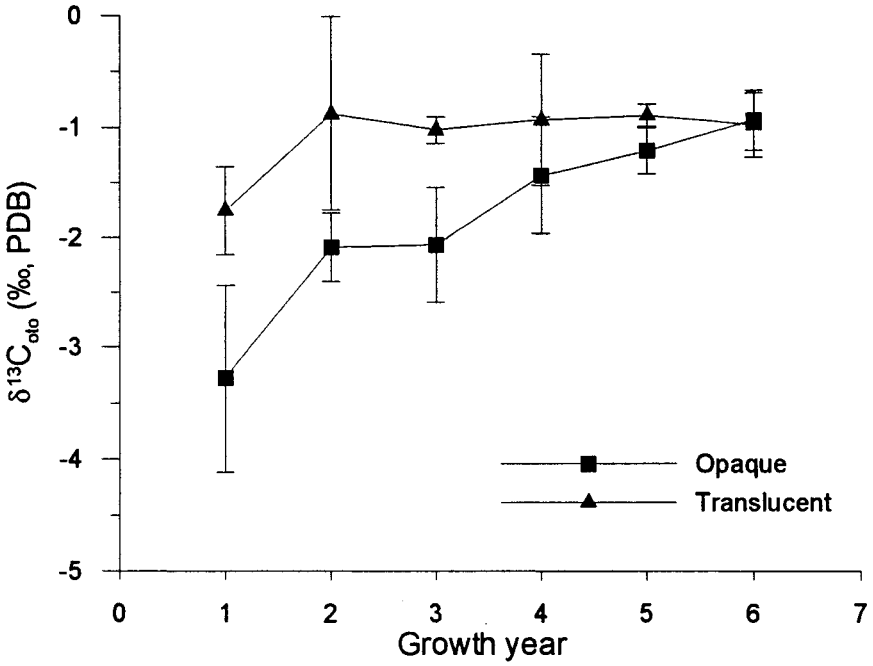

Figure 5.5 Average $\delta^{13}C_{oto}$ (± SD) for adjacent opaque and translucent growth zones for the older Bonavista B cod.

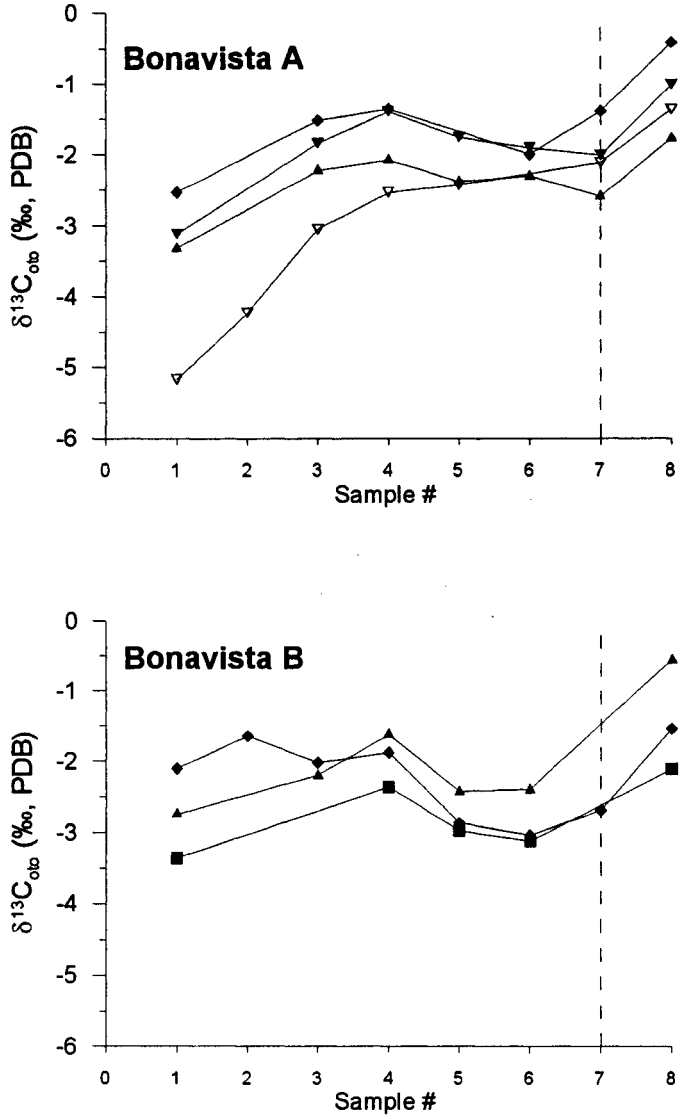

Figure 5.6 Seasonal variation over the first year of growth for individual cod from the Bonavista A and Bonavista B groups. The dashed line represents the boundary between the opaque and translucent zones.

does exist, however, between individual cod. Initial values range between -5.2 and -2.1‰ and are always the lowest. Each otolith initially increases in $\delta^{13}C_{oto}$ and then levels off or declines slightly before increasing again in the translucent zone. Maximum values range between -2.1 and -0.4‰ and are always in the translucent zone. Using the $\delta^{18}O_{oto}$ data to determine the $\delta^{13}C_{oto}$ values associated with the warmest and coldest temperatures, there appears to be no relationship between $\delta^{13}C_{oto}$ and temperature.

5.5 Discussion

5.5.1 Ontogenetic Diet Change

Otolith carbon represents a mixture of two sources: metabolic and seawater DIC. The values of $\delta^{13}C_{oto}$ are clearly below what would be expected for inorganic precipitation from ambient DIC. By comparing $\delta^{13}C_{oto}$ and $\delta^{13}C_{M}$, this study clearly demonstrates that changes in the metabolic component are important to the variability seen in cod otoliths. About 80% of the variability seen in this group of cod can be explained by changes in $\delta^{13}C_{M}$ related to ontogenetic changes in diet. Lifetime enrichment in ^{13}C in otoliths has also been seen in other species, which would lead us to suspect that ontogenetic changes in the contribution and isotopic composition of metabolic DIC are important in these species as well (Begg and Weidman 2001; Stephenson *et al.* 2001; Mulcahy *et al.* 2001). Understanding the importance of diet

to $\delta^{13}C_{oto}$ makes it possible to relate changes in $\delta^{13}C_{oto}$ to changes in trophic level either temporally or between ecosystems.

5.5.2 Metabolic Change

Despite the fact that most of the change in $\delta^{13}C_{oto}$ with age can be related to ontogenetic changes in $\delta^{13}C_M$, it is still necessary to vary the proportion of metabolic carbon incorporated into the otolith to explain the full variation of $\delta^{13}C_{oto}$ with size. This is consistent with the idea that metabolic rate slows as a fish grows. The actual proportion of metabolic carbon being contributed to endolymph HCO_3^- may decrease as the fish ages. Using equation (5), M was calculated using the values of $\delta^{13}C_M$ and $\delta^{13}C_{oto}$ measured for this group of cod. Figure 5.3 demonstrates that M does indeed decrease with fish size. M decreases very rapidly in younger cod. These fish are growing rapidly and M proportionally decreases with weight. This may account for the overall variability seen in these age groups. Previous studies were not able to directly assess the effect of ontogenetic diet variation which will especially affect the percentage calculated for younger fish. If we had assumed that $\delta^{13}C_M$ was constant at the average adult value of approximately -18.5‰ for all fish, the proportion of metabolic carbon would be overestimated by almost in the youngest fish 7 - 8%.

Older cod incorporate approximately 16% to 25% metabolic carbon. These values are slightly lower than those calculated by Kalish (1991a) (~32 to 35%) for

Australian salmon but are similar to the estimate of 20% made by Weidman and Millner (2000) and within the range estimated by Schwarcz *et al.* (1988) (12 to 43%) for Atlantic cod. Individual variability still seems to be relatively large as M can vary by up to 5 or 6% in fish only differing in length by less than 5 cm. An important implication of this relationship between otolith $\delta^{13}C$ and $\delta^{13}C_M$ is that it gives us the ability to track changes in M with age.

5.5.3 Individual Variation

Apart from differences in $\delta^{13}C_{oto}$ related to size, variation between individual fish is also evident. Examining the average $\delta^{13}C_{oto}$ associated with each age group, it is apparent these values are consistent with both the absolute values and the overall trend observed over the life of an individual cod (Figure 5.4). Age 0 cod are consistently lower (average -3.9 ± 0.5‰) and the average $\delta^{13}C_{oto}$ increases to an average adult value of -1.0 ± 0.6‰. It is again evident, though, that after accounting for this size-related increase, there is still variation within age groups. There is an average range in $\delta^{13}C_{oto}$ of approximately 1.5 to 2‰ for each group. These fluctuations may be associated with differences related to the physiology or behaviour of individual fish. Changes in individual metabolism, individual preferences in diet, or migration between waters of different temperature or water composition (migration/ depth change) may all affect $\delta^{13}C_{oto}$.

All of the cod used in this study were caught in Bonavista Bay at approximately the same time of year (September and November). The age 0 and age 1 cod were all caught in shallow water (<10 m). It could be assumed therefore that, within each age group, the fish would be experiencing similar environmental conditions. They should also exhibit similar behaviour and favour similar environments. The behaviour of juvenile fish has been studied in the inshore bays of Newfoundland. It is known that age 0 juvenile cod tend to stay in localized areas of the inshore; mainly driven by predator avoidance (Grant and Brown 1998a; Grant and Brown 1998b). Cod of age 1 and older tend to spend more time in deeper water and exhibit opposite diurnal behaviour from age 0 cod. The $\delta^{18}O_{oto}$ data from otoliths of the same age 1 fish used in this study indicates that they experience uniform temperature and salinity conditions (Jamieson *et al.* 2001). This evidence suggests that changes in $\delta^{13}C_{DIC}$ are less likely to affect individuals in this population. Changes in $\delta^{13}C_{DIC}$ would likely occur over a wider spatial scale which would affect the entire population.

Individual preferences in diet might also cause differences in $\delta^{13}C_{oto}$ between individual cod. The lack of variability in $\delta^{13}C_M$ values in each age group indicates that fish of a similar size are probably eating a similar diet (Jamieson and Schwarcz 2001). Only the age 1 cod show a larger variation in $\delta^{13}C_M$ (SD = 0.8‰) and a large range (-20.1 to -16.9‰). It is conceivable that since the Bonavista A and Bonavista B cod

were caught in separate years, there may have been some temporal variation in the isotopic composition of the food web. The two groups, however, show consistent $\delta^{13}C_M$ behaviour and therefore differences in $\delta^{13}C_{oto}$ would have to be associated with changes in M or $\delta^{13}C_{DIC}$. The variation of $\delta^{13}C_{oto}$ within each group is similar as well, indicating that temporal differences are probably not the most important source of variation.

If changes in diet and $\delta^{13}C_{DIC}$ can be ruled out, the relative input of metabolic carbon must be the cause of the individual variations. It has already been shown that a 20% change in M can explain most of the variation in $\delta^{13}C_{oto}$ with size. Within each age group, M varied by about 10%. Equation (4) can be used to evaluate the potential effects of varying M on $\delta^{13}C_{oto}$. If it is assumed that $\delta^{13}C_M$ and $\delta^{13}C_{DIC}$ are constant, a change in M of 10% can change $\delta^{13}C_{oto}$ by approximately 2‰. This is consistent with the individual variations observed in $\delta^{13}C_{oto}$ and therefore changes in metabolism could potentially explain the full range of variation.

5.5.4 Seasonal Variation

Seasonal variation may help to reveal the influence of some of the potential variables on $\delta^{13}C_{oto}$. The enrichment in ^{13}C of winter growth zones can be as much as 2‰ which is as large or larger than the variations seen between individuals. This could be explained by a winter decrease in metabolic input, enrichment in ^{13}C in the

food source, or increase of $\delta^{13}C_{DIC}$, possibly due to changes in living depth in response to changing temperature. The variation of $\delta^{13}C_{oto}$ over the first year of growth seems to reveal an ontogenetic diet effect as well as a seasonal one (Figure 5.6). Young cod would be expected to demonstrate the largest variations in $\delta^{13}C_M$ and they are also likely to be subject to the greatest variability in environmental conditions. This combination makes it difficult to interpret the variation associated with seasonal differences. In general, however, ^{13}C enrichment occurs in winter, and is therefore associated with colder temperatures.

Weidman and Millner (2000) observed a positive relationship between mean $\delta^{13}C_{oto}$ and regional temperature for cod from the northeast Atlantic. Trends for individual otoliths however, had negative relationships. They believed that the mean $\delta^{13}C_{oto}$ may be determined by a latitudinal control of diet $\delta^{13}C_M$, but in individuals, the effect of temperature on aragonite precipitation may become important. This should however be a relatively small effect, and probably not capable of explaining all the variations seen in $\delta^{13}C_{oto}$.

There is some independent evidence for changes in diet for cod at different times of the year. Grant and Brown (1998b) found that the species of zooplankton eaten by juvenile cod varied with the season. Cod have also traditionally been known to follow the capelin inshore in the spring and therefore this may become a more important source of variation in $\delta^{13}C_M$ at those times (Lilly 1987). Weidman and

Millner (2000) hypothesized that seasonal plankton blooms might cause seasonal variations in $\delta^{13}C_M$ by influencing the $\delta^{13}C$ of the base of the food web. This would be more likely to affect younger cod as they feed at a lower trophic level The present data are not sufficient, however, to assess the magnitude of any potential change in $\delta^{13}C_M$ due to seasonal diet shifts.

We suppose that changes in metabolic rate have a large role in controlling these variations in $\delta^{13}C_{oto}$. If M is changed by only 5%, this can lead to $\delta^{13}C_{oto}$ variations of 1‰ which would largely explain the seasonal variations.

5.6 Conclusions

Comparison of muscle and otolith $\delta^{13}C$ from the same fish has demonstrated that trophic level change associated with ontogenetic diet change accounts for most of the increase seen in the $\delta^{13}C_{oto}$ of individual cod with age. Age-related increases of $\delta^{13}C_{oto}$ seen in other species may potentially be explained by this as well. Part of the size-related enrichment in ^{13}C is most likely related to decreasing metabolic rate with increasing age, resulting in a decrease in the incorporation of metabolic carbon in DIC of the endolymph. Calculations showed that 16 to 36% of the otolith carbon may come from metabolic sources, with an overall decrease of 20% from age 0 to adult cod. Understanding the interaction between changes in $\delta^{13}C_M$ and M can help to reveal physiological and behavioural changes in cod. Importantly, we have seen

that by measuring $\delta^{13}C_{oto}$ and $\delta^{13}C_M$ for the same fish, it is possible to calculate M which gives us the ability to track changes in metabolism between different groups of fish. These interpretations though, are complicated by individual variation. Despite the fact that the otoliths in this study were from a single location, shifts of up to 2‰ between otoliths of equivalent age-classes were observed. These differences are likely explained by differences in metabolism which may be related to size, especially in rapidly growing, younger cod, or the condition of individual cod including reproductive stage. Seasonal variation in $\delta^{13}C_{oto}$ may also be related to changes in metabolism, diet, or environment. The data however, are not detailed enough at this time to assess the relative impact of these variables.

The results of this study could be more tightly constrained by measuring $\delta^{13}C_{DIC}$ in the study area and also the seasonality of $\delta^{13}C_M$. A total understanding of the overall relationship between $\delta^{13}C_{oto}$ and metabolism, diet, and environment will require further independent assessment of these variables.

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CHAPTER SIX

Carbon isotopic records from the otoliths of Atlantic cod (*Gadus morhua*) from the northern cod stock.

6.1 Abstract

Individual lifetime $\delta^{13}C$ records were reconstructed from the otoliths of Atlantic cod (*Gadus morhua*) of the northern cod stock off eastern Canada (3KL). The individual trends were remarkably similar between all areas and consistent with cod from other regions indicating that ontogenetic changes in physiology and behaviour are important for cod as a species. At least three stock components could be identified based on their characteristic lifetime patterns of $\delta^{13}C_{oto}$. The deviations in these patterns indicate that juvenile cod in different areas of the stock are feeding at different trophic levels.

There is some indication of temporal variation in the 3L otoliths. During the mid-1980s, cod from this area seem to undergo a shift to lower $\delta^{13}C_{oto}$ values. This shift however, is only seen in cod born after 1980 and not in older contemporaries captured in the same area. There appears to be no clear relationship between depth of capture, age, or location which can explain this shift and the exact cause remains uncertain.

6.2 Introduction

Otolith $\delta^{13}C$ records seem to hold the key to understanding the physiological and behavioural life history of many fish species. Otoliths are small aragonite stones found inside the ears of teleost fish (bony fish) and serve as sound and gravity sensors. There are actually three pairs of otoliths within a fish, the sagittae, lapilli, and the asterisci (Panella, 1980). The sagittae are the largest of the three and are therefore the most widely studied. Otoliths grow continuously over the life of the fish forming concentric growth layers. Since they do not undergo remodelling, otoliths preserve in their isotopic composition a record of the environmental and physiological conditions experienced by a fish throughout its life (Campana and Neilson 1985).

It has been well documented that the carbon of otolith aragonite represents a mixture of dietary carbon and ambient dissolved inorganic carbon (DIC) the proportions of which are controlled by the metabolism of the fish (Degens *et al.* 1969; Radtke *et al.* 1987; Kalish 1991a; Kalish 1991b; Iacumin *et al.* 1992; Radtke *et al.* 1996; Thorrold *et al.* 1997; Schwarcz *et al.* 1998; Weidman and Millner 2000; Jamieson and Schwarcz 2001b). In simple terms, the otolith represents an inorganic carbonate to which metabolic carbon is added in different proportions depending on the metabolic level of the fish. Inorganically precipitated aragonite will reflect the isotopic composition of dissolved inorganic carbon (DIC). The addition of metabolic carbon causes the otolith signature to become depleted in ^{13}C. Changes in $\delta^{13}C_{oto}$ can result from changes in the environment or physiology of the fish including ontogenetic

changes in diet, changes in metabolism, or changes in $\delta^{13}C_{DIC}$ (Jamieson and Schwarcz 2001b; Schwarcz *et al.* 1998).

The northern cod stock consists of cod within NAFO divisions 2J3KL off the east coast of Newfoundland and Labrador. This fishery was once one of the most important cod fisheries in the world. In the early 1990s, this stock experienced a drastic decline which led to a moratorium on fishing in 1992. Since that time, the stock has shown no signs of recovery. During the early 1990s, the northwest Atlantic also experienced a period of very cold temperatures (Colbourne *et al.* 1994). Some researchers have speculated that these environmental changes may have impacted the behaviour of cod and which in turn influenced the collapse of the stocks (de Young and Rose 1993; Rose *et al.* 1994; Rose *et al.* 2000). Environmental changes may also effect their ability to recover from collapse. In order to assess this hypothesis, it would be beneficial to have some method of monitoring the current conditions experienced by the fish. The fact that otolith $\delta^{13}C$ is related to environmental conditions as well as fish physiology makes it a valuable potential tool for monitoring the condition of cod stocks as well as assessing past conditions.

Two previous studies have shown that cod otolith $\delta^{13}C$ values increase with age. Cod captured from the Scotian Shelf, off eastern Canada, increased from approximately -5‰ to -2.5‰ during the first 4 or 5 years of life, reaching a maximum of approximately 0‰ at maturity (Schwarcz *et al.* 1998). Adult cod could

demonstrate constant, decreasing or even increasing $\delta^{13}C_{oto}$ during the rest of their life. Weidman and Millner (2000) examined eleven otoliths from Atlantic cod collected across the northeast Atlantic. They also found that cod otolith $\delta^{13}C$ values increase with age by as much as 0.94 to 2.64‰ at two years of age. These patterns have been shown to be related to ontogenetic changes in diet which lead to the increase of $\delta^{13}C_{diet}$ (Jamieson and Schwarcz 2001a). Changes in cod $\delta^{13}C_{oto}$ values are therefore largely related to diet and its affect on metabolic carbon. Part of this increase is also caused by a decrease in metabolism with age. It should therefore be possible to use $\delta^{13}C_{oto}$ values to reveal information about changes in feeding behaviour affecting trophic level and also changes in metabolism which may be related to environmental conditions.

Adult $\delta^{13}C_{oto}$ should largely be free of the ontogenetic changes in diet and metabolism which complicate the $\delta^{13}C_{oto}$ interpretation in younger cod. Metabolism decreases at a much slower rate and the isotopic composition of metabolic carbon ($\delta^{13}C_M$) has been shown to be quite consistent for adult cod (-18.6 ± 0.3‰) (Jamieson and Schwarcz 2001a). Changes in the carbon isotopic composition of otoliths during the adult stage are more likely to reflect environmental changes or major behavioural changes. Schwarcz *et al.* (1998) found that, in the mid-1980s, the average age at which Scotian Shelf cod reached their maximum or mature $\delta^{13}C_{oto}$ value decreased. They also found that the maximum $\delta^{13}C_{oto}$ which a cod could be expected to attain at

maturity had decreased in the late 1980s. It was felt that this was most likely a reflection of movement of cod to deeper waters with lower $\delta^{13}C$ values of dissolved inorganic carbon.

The Canadian Department of Fisheries and Oceans holds an archive of Atlantic cod otoliths which have been collected during research and commercial trawls carried out every year since the 1940s. This collection holds the potential to provide a long-term record of behavioural, physiological and environmental changes experienced by Atlantic cod during this century. The objective of this study was to analyze the lifetime records of $\delta^{13}C_{oto}$ for otoliths from the northern cod stock and to describe the spatial and temporal variation in order to evaluate if otolith $\delta^{13}C$ could be used within this stock to see whether changes in the environment may have affected stock level. The data from this study can be compared with the work in other areas of the Atlantic to examine regional differences within cod as a species. Most of the otoliths analyzed were from cod captured during the 1990s. This allows us to examine what was going on with the stock at the time of the collapse. Some otoliths were also selected from the 1950s, 1970s, and 1980s for comparison to examine spatial and temporal variation.

This study is one of only a few to use micro-milling methods to reconstruct individual isotopic records from otoliths. This type of data is important for examining changes in cod or environmental conditions over the life of a fish. Earlier studies utilized whole otolith analysis or low resolution sampling methods. Whole otolith

isotopic measurements represent an integrated lifetime signal and do not allow high resolution analysis of temporal fluctuations. These annual or seasonal differences may provide important information about the physiology of an individual fish or of a stock. Past studies have found it difficult to prove a relationship between changes in cod behaviour and environmental conditions. Otolith isotopic composition has the potential to provide a direct link revealing how changes in the environment are affecting cod physiology and behaviour.

6.3 Methods

The otoliths selected for this study were all obtained from the archived collection held by the Department of Fisheries and Oceans in St. John's. The otoliths had been stored in dry envelopes. Thirty otoliths were analyzed in total; twenty-seven of these otoliths were from cod collected from NAFO Division 3KL while three were analyzed from Division 3NO for comparison (Figure 6.1). All of the fish whose otoliths were analyzed were five years of age or older. Other information such as age, length, sex, location and depth of capture were available for most of the selected otoliths (Table 6.1).

To prepare the otoliths for milling, they were embedded in Araldite® resin and 100-200 μm thin sections were cut through the nucleus of each otolith using a low-speed Isomet saw as described in Jamieson (2001). The otolith thin sections were then mounted on petrographic slides for milling. Individual translucent and opaque

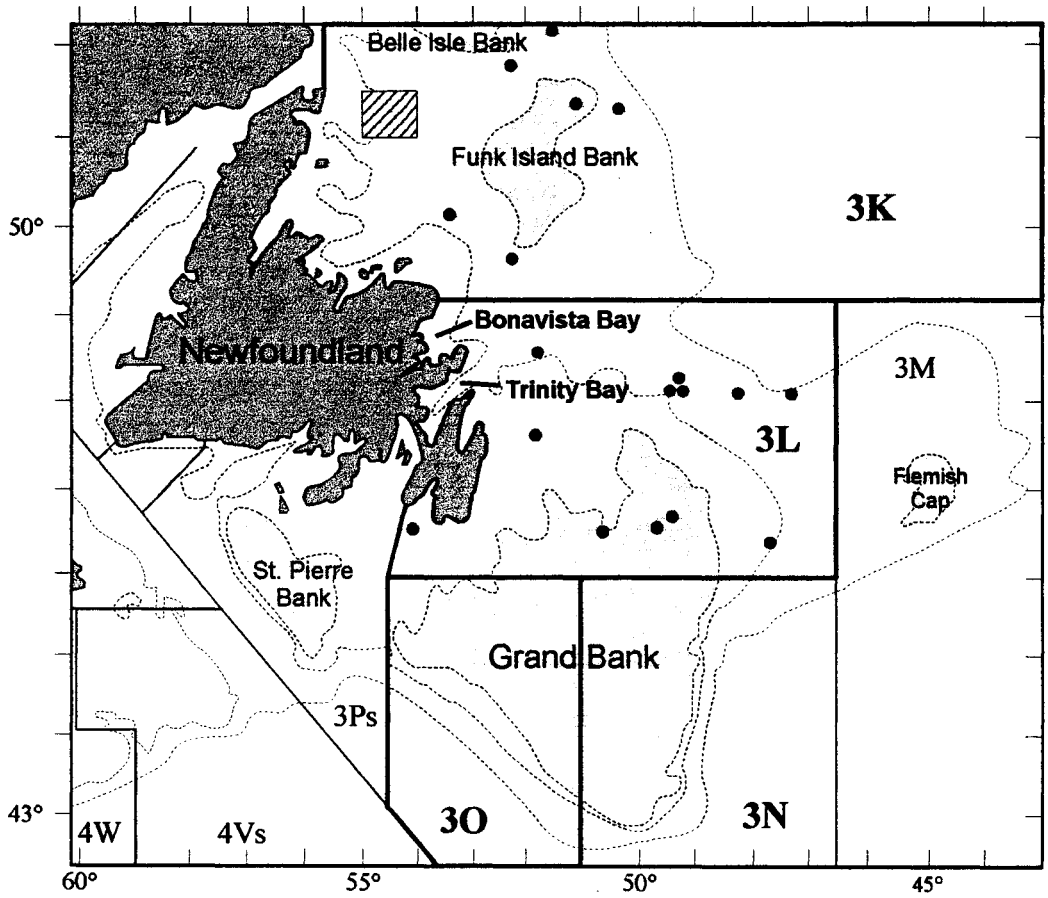

Figure 6.1 Map of offshore Newfoundland and Labrador illustrating NAFO Divs. 3KL, 3O, and the location of capture for each of the archive cod used in this study. A number of cod were also taken from Trinity and Bonavista Bays. One cod for which only a general location of capture was known is indicated by a striped box offshore.

Table 6.1 Sample information for cod collected for this study. * Depth of capture.

Otolith #	Length (cm)	Sex	Age	NAFO Div.	Depth* (m)	Latitude	Longitude
Southern 3L (n = 5)							
51-1800	94	M	18	3L	154	46° 29'	47° 39'
71-438	101	M	12	3L	82	46° 31'	50° 35'
87-5886	78	F	12	3L	67	46° 34'	49° 48'
96-4625	70	F	8	3L	68	46° 41'	49° 39'
87-5665	64	M	8	3L	181	47° 37'	51° 54'
Northern 3L (n = 7)							
79-6341	92	M	7	3L	383	48° 02'	48° 11'
87-6143	94	F	14	3L	267	48° 14'	49° 21'
83-6514	64	M	10	3L	195	48° 33'	51° 37'
93-823	66	M	8	3L	400	48° 12.9'	49° 24.5'
94-2842	67	M	8	3L	340	48° 14.2'	49° 06.6'
93-687	56	M	8	3L	905	48° 07.4'	47° 22.9'
93-847	69	F	8	3L	905	48° 07.4'	47° 22.9'
Inshore 3L (n = 7)							
96-4715	82	F	10	3L	68	46° 36'	54° 02'
96-4718	65	M	9	3L	68	46° 36'	54° 02'
96-5263	82	M	9	3L	255	47° 51'	53° 29'

Table 6.1 (cont.) Sample information for cod collected for this study. * Depth of capture.

Otolith #	Length (cm)	Sex	Age	NAFO Div.	Depth* (m)	Latitude	Longitude
Inshore 3L (cont.)							
96-5301	52	F	7	3L	117	48° 19'	53° 21'
96-2518	60	M	10	3L			
96-2856	72	M	10	3L			
96-3001	71	M	10	3L			
3K (n = 8)							
51-2381	54	M	9	3K			
96-4912	48	M	5	3K	321	49° 35'	52° 15'
96-4869	51	F	5	3K	354	50° 10'	53° 22'
87-7764	69	F	8	3K	345	51° 21'	50° 21'
71-535	68	F	7	3K	232	51° 25'	51° 04'
91-6390	56	M	8	3K	500	51° 44'	52° 16'
91-6344	66	F	9	3K	322	52° 08'	51° 29'
91-6333	61	M	7	3K	322	52° 08'	51° 29'
3O (n = 3)							
96-3224	68	F	8	3O			
96-3292	77	F	7	3O			
96-3615	73	M	8	3O			

zones were milled using a Merchantek Micromill system. This system permits control of the depth and width of sampling paths which allows milling of precise samples of growth zones and control of sample size.

Although it was possible to carry out very fine scale milling, in some cases it was not possible to analyze individual growth zones in order to obtain enough sample for isotopic analysis. In these cases, annual samples were taken. For ease of comparison, most of the individual otoliths records presented are composed of annual means taken by averaging the opaque and translucent layers.

Milled aragonite samples were analyzed on an Optima duel-inlet IRMS equipped with an Isocarb carbonate analyzer. $\delta^{13}C$ values are reported with respect to the Vienna Pee Dee Belemnite (VPDB) international standard. Reproducibility of $\delta^{13}C$ analysis is $\pm 0.05‰$.

6.4 Results

Figure 6.1 is a map of offshore Newfoundland which illustrates the location of capture of each of the cod analyzed in this study. In order to compare the individual otolith records and examine spatial variations, the results have been divided into groups according to latitude (Table 6.1). In this study, there are otoliths from the NAFO divisions 3K (49° to 52°N), 3L (46° to 48°N), and 3O (south of 46°N). The otoliths from 3L have been further subdivided into southern (46° to 48°N), northern (48° to 49°N), and inshore groupings. Not all of the inshore otoliths have latitude and

longitude data associated with them. We know, however that these cod were all captured in Trinity Bay, so these are represented by a hatched area. There is also a hatched area offshore in 3K for otolith 51-2381. This otolith also does not have precise latitude and longitude information, but the approximate area of capture is known. Each of the groups includes otoliths from cod caught in different time periods, except the inshore group which consists only of otoliths from cod caught in 1996. The time span covered in each group is not identical.

Table 6.2 presents a summary of the $\delta^{13}C_{oto}$ data including the initial, maximum, and mean adult $\delta^{13}C_{oto}$ values for each otolith. All of the otolith records, regardless of group, display an increase in $\delta^{13}C_{oto}$ over the life of the fish (Figures 6.2 to 6.6). In almost every case, the initial $\delta^{13}C_{oto}$ value is the lowest. For most of the otoliths, increases occur primarily in the first two to four years. This is consistent with previous findings that ontogenetic diet change causes large changes in $\delta^{13}C_{oto}$ (Jamieson and Schwarcz, 2001a). Also included in Table 6.2 is the estimated age at which the maximum $\delta^{13}C_{oto}$ value is reached. This age has been determined by examining the record for each otolith and determining the point at which the otolith seems to reach a plateau or the slope of the increasing trend decreases. For a number of the otoliths, it was not possible to determine the age of maturity as they continually increase and do not exhibit a distinct plateau. It has been speculated that this peak in $\delta^{13}C_{oto}$ represents the time when the fish becomes mature (Schwarcz *et al.* 1998);

Table 6.2 Results of individual otolith $\delta^{13}C$ analyses.
"-" indicates that no age of maturity could be determined.

Otolith #	$\delta^{13}C_{oto}$ (‰)			Maturity
	Initial	Maximum	Adult Mean	
Southern 3L				
51-1800	-1.77	0.15	-0.34 ± 0.22	4
71-438	-1.58	0.27	-0.14 ± 0.32	5 - 6
87-5886	-1.51	0.57	0.32 ± 0.17	3
96-4625	-2.41	-0.34	-0.72 ± 0.26	4
87-5665	-1.94	-0.00	-0.19 ± 0.14	3
Northern 3L				
79-6341	-2.44	-0.59	-0.69 ± 0.12	5
87-6143	-1.77	0.01	-0.41 ± 0.22	4
83-6514	-0.75	0.59	0.06 ± 0.16	4
93-823	-2.36	-0.28	-0.43 ± 0.12	5
94-2842	-2.00	-0.40	-0.76 ± 0.22	2
93-687	-3.06	-1.29	-1.53 ± 0.19	3
93-847	-3.39	-1.28	-	-
Inshore 3L				
96-4715	-2.66	-0.58	-	-
96-4718	-2.29	-0.48	-0.79 ± 0.24	4
96-5263	-2.07	-0.46	-0.89 ± 0.24	3
96-5301	-2.44	-1.15	-	-
96-2518	-3.08	0.17	-0.43 ± 0.37	3
96-2856	-2.03	-0.64	-0.80 ± 0.14	4
96-3001	-2.62	-0.94	-	-
3K				
51-2381	-3.46	-0.85	-1.27 ± 0.31	3
96-4912	-2.02	-0.93	-	-
96-4869	-2.82	-1.14	-	-
87-7764	-1.78	0.27	-0.03 ± 0.18	3
71-535	-2.95	-1.27	-1.54 ± 0.15	3

Table 6.2 (cont.) Results of individual otolith $\delta^{13}C$ analyses. "-" indicates that no age of maturity could be determined.

Otolith #	$\delta^{13}C_{oto}$ (‰)			Maturity
	Initial	Maximum	Adult Mean	
3K (cont.)				
91-6390	-2.49	-1.00	-1.44 ± 0.31	3
91-6344	-4.05	-0.22	-0.88 ± 0.37	3
91-6333	-2.76	0.20	-	-
3O				
96-3224	-3.14	-0.64	-1.11	3
96-3292	-2.02	-0.72	-0.99	2
96-3615	-2.25	-0.79	-1.21	2

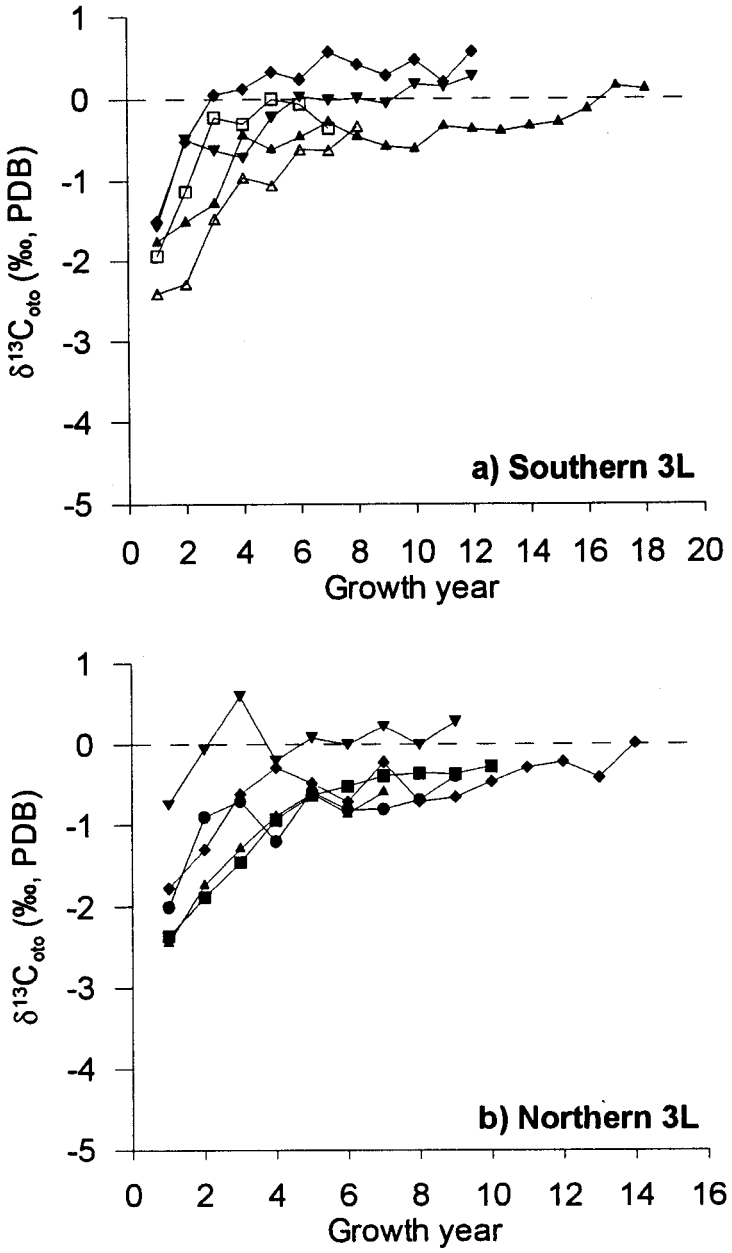

Figure 6.2 Individual $\delta^{13}C_{oto}$ records for cod from a) southern 3L and b) northern 3L.

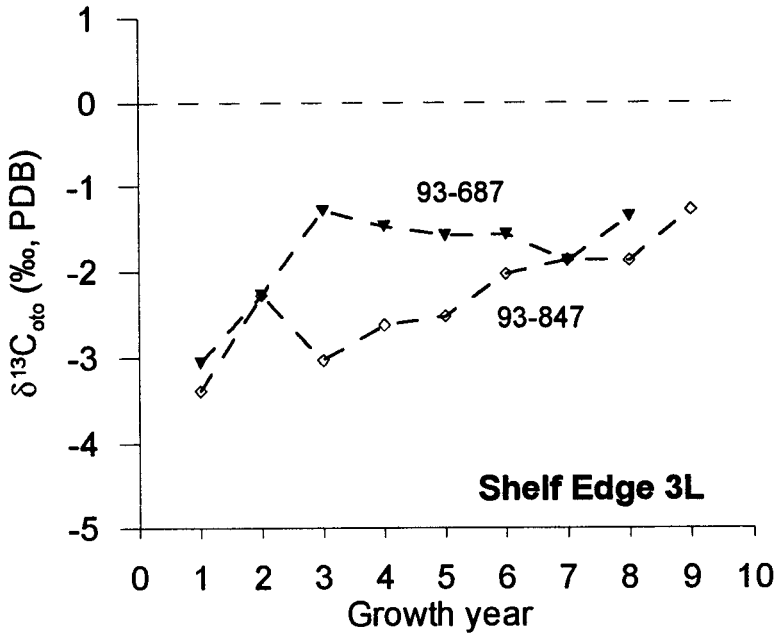

Figure 6.3 Individual $\delta^{13}C_{oto}$ records for two cod caught in deep water off the edge of the shelf in 3L.

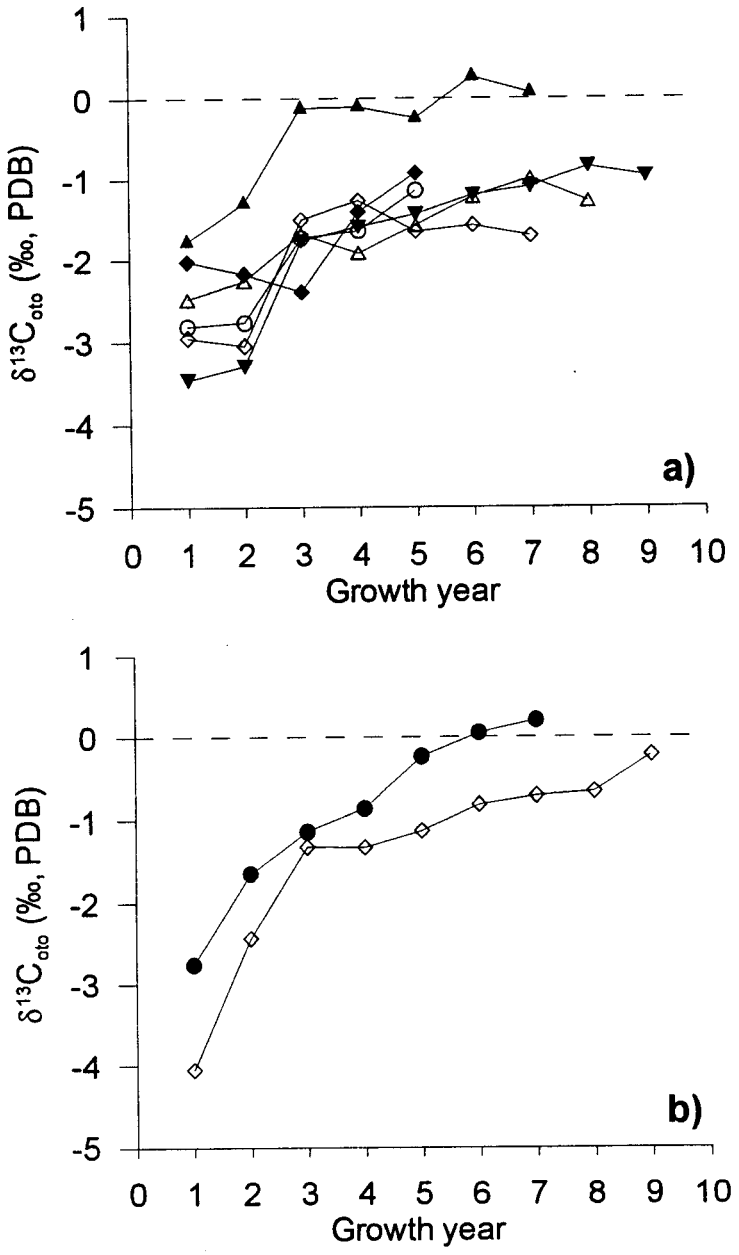

Figure 6.4 Individual $\delta^{13}C_{oto}$ records for 3K cod illustrating a) typical trends b) two apparent outliers.

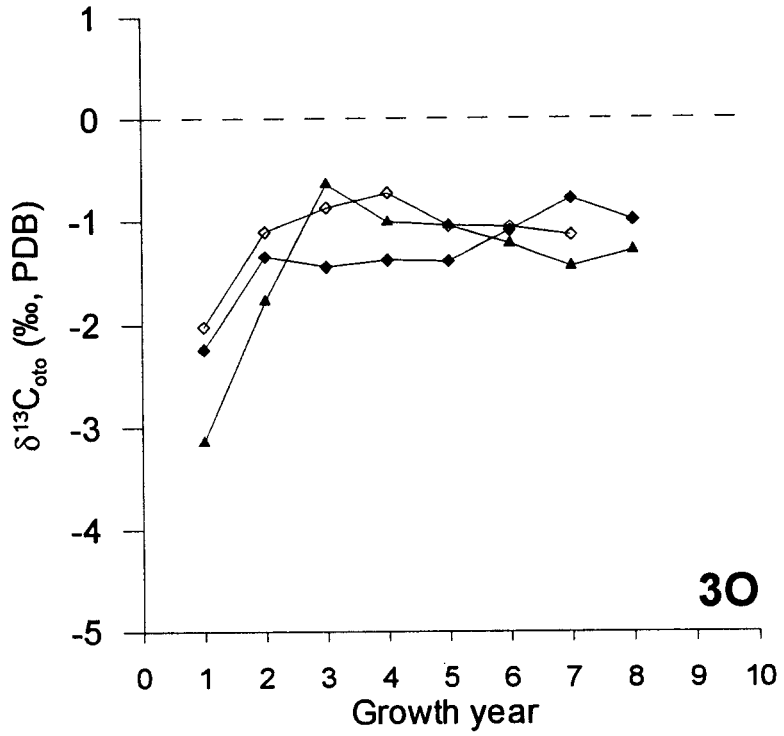

Figure 6.5 Individual $\delta^{13}C_{oto}$ records for 3O cod.

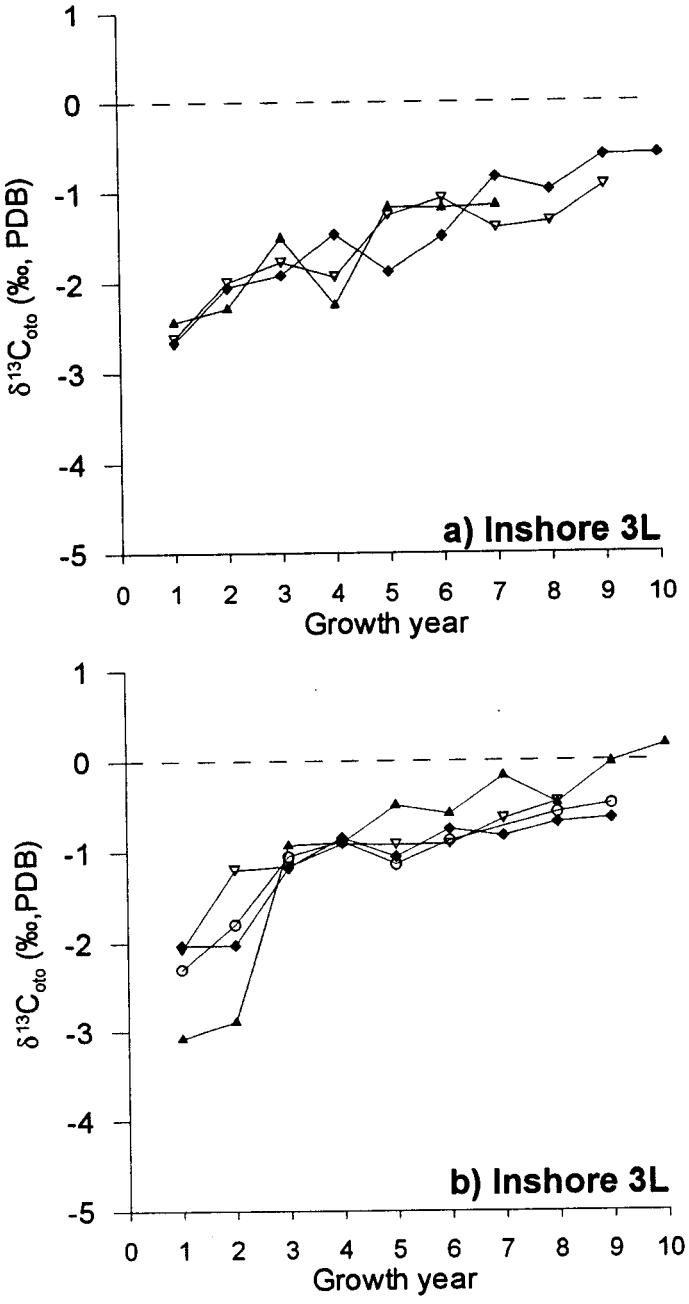

Figure 6.6 Individual $\delta^{13}C_{oto}$ records for a) inshore cod which show continually increasing $\delta^{13}C$ and b) inshore cod for which exhibit a plateau.

adopting the more stable adult diet and behaviour.

6.4.1 Spatial Variability

There does not appear to be much variation in $\delta^{13}C_{oto}$ records across the 3L area. Although there is some variability, the otoliths of the southern 3L group and the northern 3L group are remarkably similar (Figures 6.2a and b). Two of the otoliths from 3L (93-687 and 93-847), however, seem to stand out from the majority (Figure 6.3). They appear to be more ^{13}C depleted than the otoliths of either 3L group. Most of the cod used in this study were caught at less than 500 m depth, these two cod were unique because they were caught at the edge of the shelf in very deep water (905 m).

Table 6.3 summarizes $\delta^{13}C_{oto}$ values for each group. Considered without the otoliths with the lowest $\delta^{13}C$ values, the initial values are almost identical between the southern and northern 3L groups (-1.84 ± 0.32‰ and -1.84 ± 0.61‰). The adult means are not significantly different (-0.21 ± 0.34‰ and -0.45 ± 0.29‰), and the overall ranges are almost identical as well. On average, there is a lifetime increase in $\delta^{13}C_{oto}$ of approximately 1.5‰ in 3L. The average age at maximum $\delta^{13}C_{oto}$ seems to be slightly higher in the north, but this is probably not significant in light of the number of otoliths being considered. It seems therefore that cod from across 3L have relatively consistent otolith $\delta^{13}C$ patterns and, for the purpose of this study, these

Table 6.3 Summary of the mean $\delta^{13}C_{oto}$ values for each latitude grouping.

		$\delta^{13}C_{oto}$ (‰)				
	n	Initial	Range	Adult mean	n*	Age
Southern 3L	5	-1.84 ± 0.32	-2.41 to 0.57	-0.21 ± 0.34	4	3.5 ± 0.5
Northern 3L	5	-1.87 ± 0.61	-2.44 to 0.59	-0.45 ± 0.29	5	4.0 ± 1.1
3K	8	-2.79 ± 0.69	-4.05 to 0.27	-1.03 ± 0.55	5	3.0 ± 0.0
3O	3	-2.47 ± 0.48	-3.14 to -0.64	-1.10 ± 0.09	2	2.5 ± 0.5
Inshore 3L	7	-2.46 ± 0.34	-3.08 to 0.17	-0.73 ± 0.18	4	3.5 ± 0.5

* number of samples for which an age of maximum $\delta^{13}C_{oto}$ could be determined.

groups can probably be considered together as one offshore 3L group. Otoliths with the lowest $\delta^{13}C$ values have initial $\delta^{13}C_{oto}$ values (-3.06‰ and -3.39‰) that are 0.5 to1‰ lower than any of the other otoliths in this group. Maximum $\delta^{13}C_{oto}$ values are also much lower than any other the mean adult values. There is a weak correlation between depth and the adult mean $\delta^{13}C_{oto}$ ($r^2 = 0.399$). This, however, is mainly controlled by these deep cod (96-687). Without these otoliths, the correlation is less significant ($r^2 = 0.253$).

Within the 3K group, the individual otolith records are also generally consistent (Figure 6.4). One otolith (87-7764) has particularly high $\delta^{13}C$ values compared to the others while two other otoliths (91-6333 and 91-6344) do not seem to follow the same increasing trend as the others. As a group, the 3K otoliths vary slightly from the offshore 3L group. These cod tend to have lower initial values, averaging -2.79 ± 0.69‰ with a range between -4.05 and -1.78‰. Adult mean $\delta^{13}C$ values are also lower with an average of -1.03 ± 0.55‰. An average lifetime increase of approximately 1.9‰ is slightly higher, but not significantly different from the 3L cod. The age of maximum $\delta^{13}C_{oto}$, when it was possible to determine, was consistently 3 years in this group. Although it seems that there is some decrease in the 3K otoliths compared with 3L, if we look at the correlation between the mean adult $\delta^{13}C_{oto}$ and latitude for all offshore otoliths, we find a very weak relationship ($r^2 = 0.241$). The relationship for initial $\delta^{13}C_{oto}$ is no stronger ($r^2 = 0.292$), and the slopes

for each of these relationships is essentially zero.

While there is considerable overlap between the 3K and 3L groups in terms of their absolute $\delta^{13}C_{oto}$ values, if we examine the pattern and rate of increase over the life of each cod, there appears to be some contrast between the groups. The 3K cod generally have consistent values for the first couple of years, and then quickly increase. In general, $\delta^{13}C$ of these otoliths also continues to increase after reaching maturity. The 3L otoliths have much smoother records. They gradually increase over the first few years and then attain a plateau in $\delta^{13}C_{oto.}$

Something which has not been examined before is the effect that the sex of a fish may have on its lifetime $\delta^{13}C_{oto}$ record. It appears from this study that there is no significant difference between males and females. The mean adult values for females and males were -0.54 ± 0.60‰ and -0.61 ± 0.53‰ respectively.

Three otoliths from NAFO division 3O, which lies directly to the south of 3L (Figure 6.1) were analyzed as a further test of north to south spatial variability in this area. The average initial value for this group was -2.47 ± 0.48‰, similar to the 3K cod. The adult mean was also similar to 3K at -1.10 ± 0.09‰. The three individual records are very consistent as indicated by the standard deviation of the adult mean and all remain well below 0‰ as adults (Figure 6.5).

Otoliths from the inshore 3L cod, commonly display less variation than the offshore otoliths. Of the 7 otoliths in this group, age of maturity could not be

determined for 3. These three exhibited a very gradual increase throughout their lives and the overall records are very consistent (Figure 6.6a). The remaining four otoliths have the typical pattern of increase over the first three or four years and then generally continue to increase at a very slow rate during their adult years (Figure 6.6b).

These otoliths seem to somewhat resemble the offshore 3K otoliths as they have somewhat lower values compared to the 3L otoliths. The average initial value is -2.46 ± 0.34‰ while the mean adult $\delta^{13}C_{oto}$ is -0.73 ± 0.18‰. All of these otoliths reach maximum $\delta^{13}C_{oto}$ values well below 0‰. The standard deviations are lower than the other groups and indicate less variability in the overall isotopic records. Although they appear low, the average lifetime increase is close to 2‰, similar to both offshore groups and the overlap of the individual records between all groups is large.

6.4.2 Seasonal Variation

Although most of the individual records are shown as average annual values, seasonal samples were taken where possible. Adjacent opaque and translucent growth zones (142 pairs) were analyzed and averaged according to growth year (Figure 6.7). While the translucent (winter) zone generally has higher $\delta^{13}C$ values than the opaque (summer) zone for the first three years, the amount of variation is very large and the relationship is not very significant. Figure 6.8 illustrates the almost normal distribution of the values for the difference between adjacent opaque and

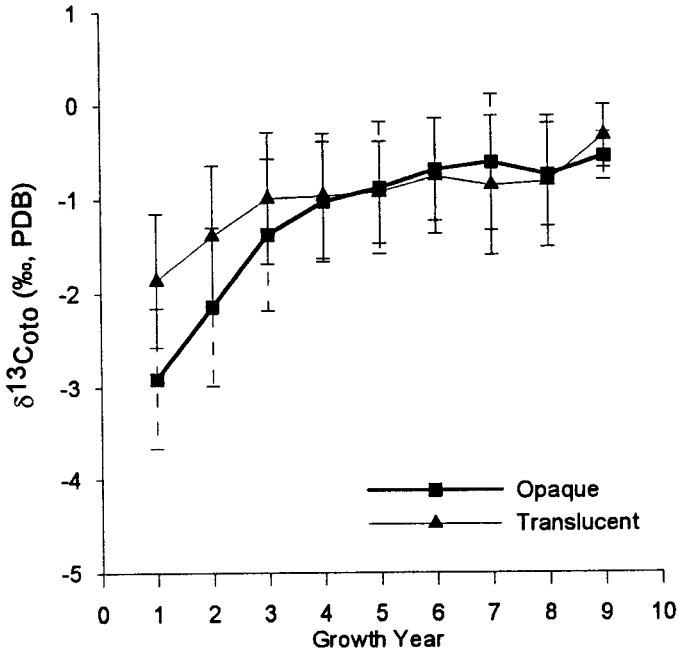

Figure 6.7 Average $\delta^{13}C_{oto}$ for opaque and translucent growth zones for each year of growth. Error bars represent the SD.

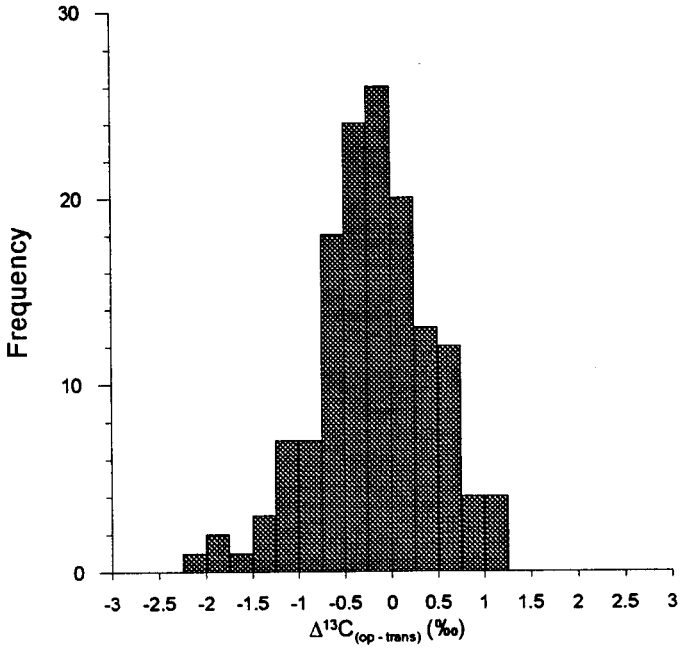

Figure 6.8 Histogram of the $\Delta^{13}C_{(opaque - translucent)}$ for 142 pairs of adjacent opaque and translucent growth zones.

translucent zones ($\Delta^{13}C_{op-trans}$). The average for the entire data set is -0.18 ± 0.62 ‰. If we look only at the first 3 years of otolith growth, the seasonal difference appears to be more significant, the average $\Delta^{13}C_{op-trans}$ values become progressively smaller through the first three years (-1.05 ± 0.58‰, -0.75 ± 0.62‰, and -0.39 ± 0.50‰) and less significant.

6.4.3 Temporal Variation

The available data set does not allow us to assess temporal variation very well. While the otoliths from both 3K and 3L cover relatively comparable time spans, all of the otoliths from the inshore were from cod collected in 1996. This may account for some of the consistency between the inshore records compared with the others.

Figure 6.9 has two plots of the mature portion of each otolith record (otoliths for which an age of maximum $\delta^{13}C_{oto}$ values could be determined) versus the calendar year in which the growth occurred. From this diagram, it seems that there has been shift towards lower mature values in the mid to late 1980s. Most of this variation is in the 3L otoliths which appear to be shifting towards the 3K otoliths. The 3K otoliths seem to display less temporal variation, although one otolith (87-7764) has higher $\delta^{13}C$ values resembling the 3L otoliths. The four otoliths from before 1970 (Figure 6.9a), show a wide spread for $\delta^{13}C_{oto}$ comparable to the magnitude of the shift seen in the 1980s. Few otoliths were analyzed from this early period. The 3L otoliths

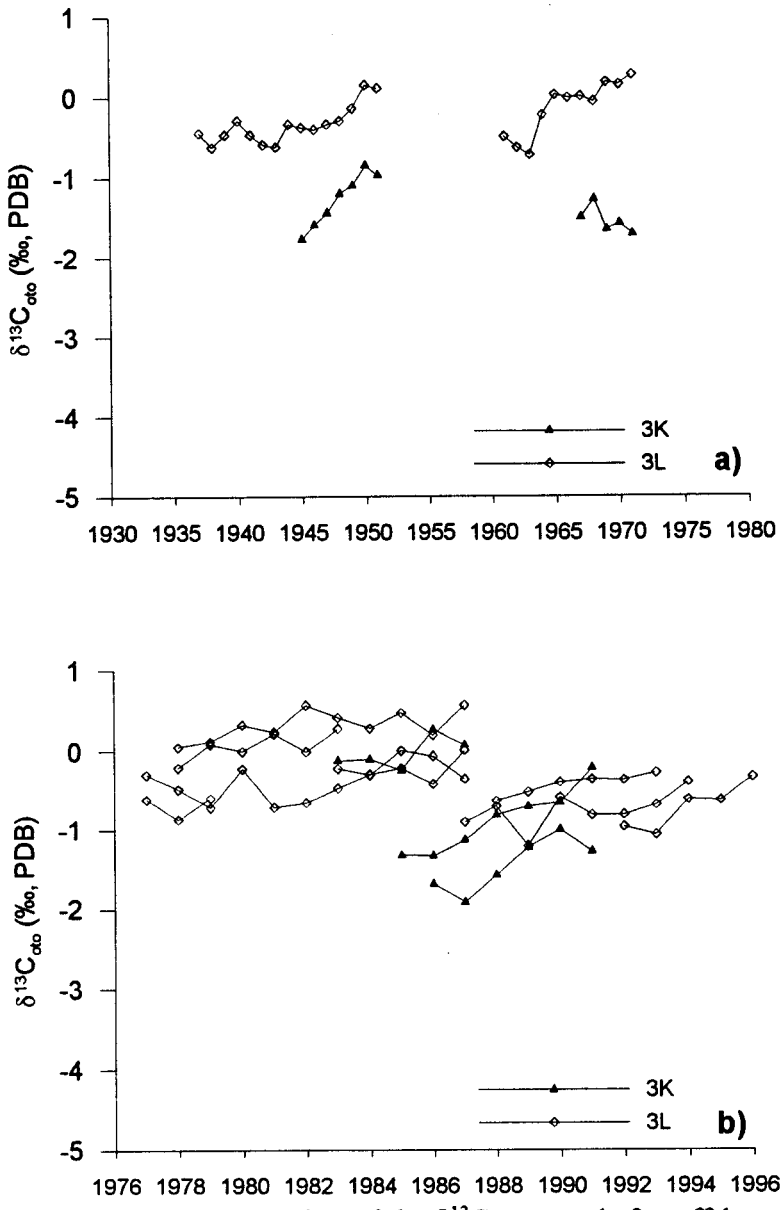

Figure 6.9 Mature portion of the $\delta^{13}C_{oto}$ records for offshore 3K and 3L otoliths a) pre-1970s period b) 1976 to 1996 period.

have consistently higher $\delta^{13}C$ values compared with the contemporary 3K otoliths from the 1940s to the 1960s. Mean adult $\delta^{13}C_{oto,}$ in the 1975 to 1996 period, when plotted versus the year the maximum was reached gives a weakly negative correlation for the offshore otoliths ($r^2 = 0.310$).

6.5 Discussion

6.5.1 Spatial Variation

A number of variables, including diet, metabolism, and DIC may affect the carbon isotopic composition of otoliths. In order to interpret environmental or behavioural changes affecting cod through isotopic analysis of otoliths, the relative influence of these variables must be considered. The results of this study have shown that there is a great deal of similarity between otoliths taken from northern cod. Cod from different areas within the stock, however, can be characterized by particular lifetime patterns of $\delta^{13}C_{oto}$ demonstrating that there are probably separate stock components. These patterns seem to hold up despite the fact that these otoliths came from cod which were caught over a fifty-year time span. All of the cod examined exhibit the same overall trend of ^{13}C enrichment with age. Typical lifetime $\delta^{13}C$ increases fall between 1.5 and 2‰ and most of this ^{13}C enrichment occurs during the first 4 or 5 years. This demonstrates that otolith $\delta^{13}C$ is largely controlled by ontogenetic changes in diet and metabolism as described by Jamieson and Schwarcz

(2001b). The reconstructed lifetime trends in otolith $\delta^{13}C$ also agree well with the findings of other studies on cod indicating that ontogenetic changes are important for cod in all regions of the north Atlantic (Schwarcz *et al.* 1998; Weidman and Millner 2000). Relative to this, most of the other fluctuations seen in the otolith records are small.

Most of the otoliths seem to conform to two different basic lifetime patterns. The otoliths from 3L have a more gradual $\delta^{13}C$ increase during the first 4 or 5 years and then reach a plateau, while those from 3K tend to be level for the first two years and then rapidly increase at 3 or 4 years. They then continue to become ^{13}C enriched at a decelerated rate during their adult life. Otoliths from division 3O more closely fit the pattern of 3L cod with gradual $\delta^{13}C$ increase. A gradual increase conforms with the idea that these fish are experiencing a gradual shift in trophic level with size. The 3K otoliths, however, indicate that young cod are feeding at the same trophic level up to age 2. These differences suggest important differences in the feeding behaviour of these two groups. Studies of the feeding behaviour of cod have found differences in prey between regions (Lilly 1987). The dominant prey is often determined by abundance and may vary seasonally (e.g. Grant and Brown 1998). It is possible that cod remain dependent on smaller prey such as copepods for a longer period in this area until they become large enough to feed on fish prey at a higher trophic level.

A feature which has not been seen in this data set is the trend of declining

adult $\delta^{13}C_{oto}$ values with age. Schwarcz *et al.* (1998) noted this for Scotian Shelf cod and Begg and Weidman (2001) saw this trend in haddock otoliths. If cod reach a maximum trophic level as they mature and experience a decline in metabolism with age, it would be expected that an increasing trend of $\delta^{13}C_{oto}$ would be observed. Many of the otoliths examined here display constant or increasing $\delta^{13}C_{oto}$ as adults. Increasing $\delta^{13}C_{oto}$ would seem to indicate that decreases in metabolic rate in these cod are still important, even up to 7 or 8 years old. It is also possible that the cod are feeding at successively higher trophic levels or that they are preferentially retaining ^{13}C as they age. ^{13}C enrichment of the entire food web due to some environmental change seems unlikely as increasing $\delta^{13}C_{oto}$ trends are not observed in all fish examined in this study.

The inshore cod exhibited $\delta^{13}C_{oto}$ values and trends similar to those in the offshore cod. Again, in this population it is possible to divide the otoliths according to two rather distinct basic patterns; one with a clear ontogenetic increase and one with a gradual increase in $\delta^{13}C_{oto}$ over the life of the fish. It is interesting that the pattern exhibited is independent of the inshore area in which the fish was caught. Cod from both the south coast and Trinity Bay locations display these patterns. It is not clear whether a particular trend can be related to the place of origin of these fish. Cod have been known to migrate between the inshore bays along the east coast of Newfoundland (Brattey 2000). It would therefore not be unexpected to see mixing

between populations. The overall consistency between otolith records for the inshore may result from living under more uniform conditions. Cod are highly mobile, however if distinct populations exist which remain inshore, they may experience more uniform conditions than those living offshore. It is also possible that because the inshore samples were all collected in 1996, a lack of secular variation may lead to greater agreement between the individual records. Jamieson and Schwarcz (2001b) examined otoliths of cod from Bonavista Bay, Newfoundland captured in 1997 and 1998. Generally, these cod had lifetime patterns and $\delta^{13}C_{oto}$ values similar to the archive cod otoliths.

It is not only the overall pattern of ^{13}C enrichment that differs between areas, but also the range of observed $\delta^{13}C_{oto}$ values. The otoliths of the 3L group seem to be 0.5 to 1‰ higher in comparison with the 3K, inshore, and 3O groups. Higher values could result from a number of factors 1) eating at a higher trophic level, 2) ^{13}C enrichment at the base of the food web, 3) a lower metabolic rate, or 4) living in water with a higher $\delta^{13}C_{DIC}$. The pattern of lifetime increase has already suggested that these cod have different feeding habits from the 3K cod. It is possible that fish are a more important component of their diet, placing these cod at a higher trophic level. For example, (Lilly 1987) reported that in the summer and autumn, cod off southern Labrador and northeastern Newfoundland (2J3K) fed on capelin. Cod from the northern Grand Bank (3L) fed primarily on capelin throughout the year. These cod also come from the northern Grand Bank and therefore they may be spending more

time in shallow water which has higher $\delta^{13}C_{DIC}$ values.

The most extreme variation over the entire study area was found in the two otoliths caught in very deep water on the shelf edge (Figure 6.3). The $\delta^{13}C$ values for these otoliths are clearly lower compared to most of the others studied. It seems clear that these cod must have had a different life history from the other 3L cod and the most likely explanation for their lower $\delta^{13}C_{oto}$ values is that they have spent their lives in deep water which would be characterized by lower $\delta^{13}C_{DIC}$ values. At a depth of 900 m, the decrease in $\delta^{13}C_{DIC}$ could be as much as 2‰ compared with surface water (Kroopnick 1980). These two otoliths suggest that a separate stock component may exist at the edge of the shelf.

While the overall trends are similar, there is still variation between individual otoliths. A range of at least 0.5 to 1‰ is generally seen for any given year of growth. Understanding these differences may be key to interpreting otolith $\delta^{13}C$ records in terms of environmental and physiological changes. While some of the differences may be due to secular changes, it is clear that, even when cod are captured at the same time and place, they may not have uniform $\delta^{13}C_{oto}$ trends. It is possible that these fish may have come from different areas. In the case of 91-6333 and 91-6344 (Figure 6.4b) from the 3K group, these cod have markedly different lifetime $\delta^{13}C_{oto}$ trends from the rest of the cod in this area. They seem to more closely resemble those from 3L and may represent migrants. Comparing these otoliths however, there is as much

as a 1‰ difference between them for any given growth year. One is two years younger than the other, so it is possible that secular variations may play a role. Even when they are compared by calendar year however, there is quite a bit of variation, especially in the later years. It is possible that individual diet preferences or differences in metabolism may account for some of this variation among cod from the same area. If these changes are related to the depth at which these fish were living, it is not evident from the depths of capture which seem to hold no relationship with either the initial $\delta^{13}C_{oto}$ or the adult mean $\delta^{13}C_{oto}$ values except in the case of the two otoliths caught at 905 m. Other studies have found a large amount of variability in the $\delta^{13}C_{oto}$ of wild fish and this may be a general feature which must be taken into account when using otoliths for environmental studies (Kalish 1991a).

One important aspect of each individual record is the age at which maximum $\delta^{13}C$ is achieved. This seems to vary between 2 and 5 years of age while other otoliths do not have any particular peak in $\delta^{13}C_{oto}$. This does not appear to be related to when or where a cod was captured. The claim by previous researchers that the $\delta^{13}C_{oto}$ peak is related to the age at which the fish reaches maturity (Schwarcz *et al.* 1998; Begg and Weidman 2001) is not supported by these data. From our results, it is not clear if this is the case. It seems just as likely that this peak is related to size. When cod reach approximately 35 cm in length, they achieve their maximum $\delta^{13}C_M$ as they adopt a largely piscivorous adult diet (Jamieson and Schwarcz 2001a). In

1996, the median age at maturity (A50) for female cod in NAFO divisions 2J3KL was 5.44 years and 4.34 years for males (Lilly *et al.* 1999). These ages are slightly higher than the peak $\delta^{13}C_{oto}$ ages found here. The small number of otoliths examined, however, prevents a true comparison. There also does not appear to be any relation between sex and the age of peak $\delta^{13}C_{oto}$. In fact, the median age at maturity has been decreasing in recent years (Lilly *et al.* 1999), but this is not reflected in our results. This contrasts with the findings of Schwarcz *et al.* (1998) who found a decrease in the age of maximum $\delta^{13}C_{oto}$ during the early to mid-1980s. These differences may be related to differences in the regions in which the cod were living (Nova Scotia vs Newfoundland).

6.5.2 Seasonal Variation

Seasonal variation does not seem to be an important factor for northern cod. The differences between opaque (summer) and translucent zones (winter) are not consistent or large, especially for adult cod. Generally the translucent zones are ^{13}C enriched compared to the opaque zones. Weidman and Millner (2000) found that $\delta^{13}C$ and $\delta^{18}O$ co-varied in northeast Atlantic cod also indicating that the highest $\delta^{13}C_{oto}$ values corresponded with the coldest temperatures. Schwarcz *et al.* (1998) however, found opposite results for Scotian Shelf cod, although the absolute differences were again small.

Cod have traditionally undergone large seasonal migrations between the inshore and offshore (Rose 1993). Fish in the 2J3KL area migrate between offshore spawning grounds in winter and their inshore feeding areas in summer. During this time, their living depth may change and also their diet will be shifting with prey availability. These factors may act to confound any large scale fluctuations which would lead to seasonal variations. Juvenile cod from Bonavista Bay were shown to experience large seasonal fluctuations (Jamieson and Schwarcz 2001b). It has been suspected that inshore areas may be important nursery grounds for cod. The early years of otoliths growth would therefore reflect this increased seasonal variation for the inshore part of their lives. This seems to be the case as the $\Delta^{13}C_{op\text{-}trans}$ values increase to an average of -1‰ during the first year of growth in the archive otoliths.

6.5.3 Temporal Variation

One of the main reasons for studying otolith $\delta^{13}C$ was to test for links between changes in environmental conditions and the physiology, and behaviour of cod. We expected that these might result in changes in the cod stocks contributing to the collapse of the early 1990s. To do this, it is necessary to examine the temporal variation in $\delta^{13}C_{oto.}$ It does appear that there has been a shift, mainly evident in the 3L otoliths, during the mid-1980s. The exact reason for this shift is not clear. It seems that these 3L cod are approaching the low values seen in the 3K cod. Decreases in $\delta^{13}C_{oto}$ have been linked to declining mean-length-at-age in haddock from Georges

Bank (Begg and Weidman 2001). There appears to be no link between low $\delta^{13}C$ values and depth of capture, length, or age in this group. In fact, the extent of this decrease is not out of the normal range seen in years prior to 1980 (Figure 6.9a). The decrease appears to be most closely linked with the year of birth for the period of 1970 to 1996. Cod spawned in the 1980s never seem to reach the same $\delta^{13}C_{oto}$ values as older cod. If this transition represents a change in the environment, it would seem reasonable that this would be reflected in all cod in the area. It is not, however, apparent in the older cod living in the area at the same time. A similar decrease found by Schwarcz *et al.* (1998) in Scotian Shelf cod was interpreted as a shift of cod to deeper water. If this is the case, however, it only seems to be affecting younger cod. The observed trend could be accounted for by younger fish moving away from the areas inhabited by older cod. It is also possible that it is related to some factor of prey availability, but it is again difficult to imagine how this would only affect younger cod.

6.6 Conclusions

It is clear from the results of this study that the lifetime trends of otolith $\delta^{13}C$ are remarkably similar both within a particular stock and between regions. This similarity reveals the importance of ontogenetic changes in behaviour and physiology to the development of otolith $\delta^{13}C$ signatures. Despite this similarity, it is possible to distinguish different stock components based on characteristic patterns of $\delta^{13}C_{oto}$.

Differences in the feeding behaviour in juvenile cod seem to be indicated by deviations in these patterns. There also appears to be some variation in the relative increase of different stock components, but the large amount of overlap between groups makes this a less effective tool for differentiating cod from different areas. Cod living in very deep water off the edge of the shelf appear to have most distinct $\delta^{13}C_{oto}$ patterns with isotopic decrease being related to the lower $\delta^{13}C_{DIC}$ of these deep waters.

Seasonal variations do not seem to play an important role in determining $\delta^{13}C_{oto}$ trends. It is likely that seasonal changes in prey and living environment act to confound any seasonal signal.

As well as spatial variation, there appears to be some temporal trend towards lower $\delta^{13}C_{oto}$ values during the mid-1980s evident in cod living in division 3L. The exact reason for this decline is uncertain and seems to be most clearly linked with the year the cod was born. Further information on the isotopic composition of potential prey as well as DIC will be necessary to better interpret these types of fluctuations.

Micro-milling of cod otoliths has provided us the opportunity to examine short time-scale changes in otolith isotopic composition. The uniformity of cod $\delta^{13}C_{oto}$ on this time scale is surprising considering that these are highly mobile fish. Recognizing the possible variables affecting otolith $\delta^{13}C$ will aid in the design of future studies targeted on reconstructing changes in the environment experienced by cod over their lifetime. Careful selection of otoliths in order to avoid the influence of spatial

variation will help to clarify the effects of the environment. This, combined with a better understanding of the isotopic composition of potential prey and environments, also provides hope that future research will be able to link changes in otolith $\delta^{13}C$ more clearly with environmental changes.

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CHAPTER SEVEN

Life history of Atlantic cod (*Gadus morhua*) from the $\delta^{18}O$ records of otoliths.

7.1 Abstract

Since the collapse of the northern cod stock in the early 1990s, speculation has surrounded the theory that environmental changes may have influenced this decline. In order to assess the spatial and temporal variations in the individual $\delta^{18}O$ records of cod otoliths, otoliths were collected from an inshore location at Bonavista Bay, Newfoundland and also from an archive collection of northern cod otoliths spanning more than half a century. The most striking feature in almost all the otolith records is the effect of ontogenetic change in habitat. Most of the cod have low $\delta^{18}O_{oto}$ values early in life which increase as the cod move to waters of higher salinity offshore. The inshore Bonavista cod display a clear correlation between $\delta^{18}O_{oto}$ and length which again is related to an ontogenetic shift in habitat. Based on their lifetime pattern of $\delta^{18}O_{oto}$ increase, as many as three different populations of cod can be identified in the 3KL area. Using the relationship between $\delta^{18}O_{oto}$ with temperature and salinity it is possible to trace the movements of cod between inshore and offshore. Although a clear seasonal signal is observed in young cod from Bonavista Bay, seasonal variation does not appear to be an important influence in older cod.

While there does not appear to be a clear correlation between changes in

temperature during the later half of the 1900s, temporal variations in both the mean adult $\delta^{18}O_{oto}$ and initial $\delta^{18}O_{oto}$ values are observed. These are most likely related to changes in habitat. Initial $\delta^{18}O_{oto}$ peaked in the early 1970s and has been declining since that time. This may reflect a change in the importance of different recruitment areas to northern cod stock. Mean adult $\delta^{18}O_{oto}$ decreased in the early 1990s and may be related to a southerly shift which was observed in the early 1990s due to the cold temperatures in the northwest Atlantic at that time.

7.2 Introduction

7.2.1 Otolith $\delta^{18}O$

Lifetime variations in the oxygen isotopic composition of otoliths provides a potential method of directly measuring the temperatures which a fish has experienced throughout its lifetime. Otoliths are small aragonite structures found in the inner ear of teleost fish. They provide a direct measurement of temperature because the isotopic composition of this aragonite is related to the temperature and isotopic composition of ambient water. It should therefore be possible to use otolith $\delta^{18}O$ values to determine changes related to environmental change or migration between different water masses. Carbonates secreted by other organisms have been used by geologists for many years in order to assess past climate change. Otoliths have only found a use more recently. They have been used in both paleontological studies

(Patterson *et al.* 1993; Patterson 1998; Patterson 1999) and environmental studies of fish biology (Gao 1997; Dufour *et al.* 1998; Stephenson *et al.* 2001; Edmonds *et al.* 1999; Weidman and Millner 2000; Begg and Weidman 2001).

Calculating temperature from the oxygen isotopic composition of carbonate requires a known relationship between oxygen fractionation and temperature. The first attempt to use the isotopic composition of otoliths to determine environmental conditions was by Devereux (1967). This study compared temperatures determined from fossil otoliths with those from benthic foraminifera and found that the otoliths were deposited in equilibrium with seawater. The relationship between oxygen isotopic fractionation and temperature in otoliths has been determined in a number of studies. Studies on wild fish in known habitats were the first to directly demonstrate that otolith oxygen is in equilibrium with the environment (Degens *et al.* 1969; Kalish 1991; Iacumin *et al.* 1992; Patterson *et al.* 1993). Laboratory studies have since confirmed these findings (Radtke *et al.* 1996; Thorrold *et al.* 1997). The $\delta^{18}O$ of otolith aragonite is therefore essentially the same as if it had been deposited inorganically. In the past, the fractionation equation derived by Grossman and Ku (1986) for foraminifera was commonly used to calculate temperatures from otolith $\delta^{18}O$. Recently however, it has been suggested that the more recent equation of Kim and O'Neil (1997) for calcite precipitation is more appropriate for use with otoliths after correcting for the ^{18}O enrichment between calcite and aragonite of +0.6‰ (Campana 1999). This fractionation equation is:

$$1000 \ln \alpha_{a\text{-}w} = 18.03\ (1000\ T^{-1})\ -\ 31.82 \qquad (1)$$

where the temperature, T, is in Kelvins (K). There is not a great of difference between these equations and therefore the later will be used in the present study.

Another important consideration is the isotopic composition of the ambient water. Often the $\delta^{18}O$ of the fluid from which the carbonate has been precipitated is not known. Generally, ocean water has a $\delta^{18}O$ of 0‰, but it varies from place to place depending on factors such as the relative amounts of evaporation and condensation, inputs of fresh water from rivers or ice melt, and the mixing of waters from different areas (Craig and Gordon 1965). The mobility of fish can also complicate the interpretation of isotopic results (Begg and Weidman 2001). Differences between water masses have actually been utilized by a number of studies to trace stock structure or migration (Begg and Weidman 2001; Edmonds *et al.* 1999; Stephenson *et al.* 2001). It was therefore necessary to assess the $\delta^{18}O$ of the ocean water in the study area in order to accurately calculate temperature and to evaluate stock structure and migration. As a companion to this study, a survey of the relationship between water isotopic composition, salinity, and temperature in the study area was carried out and is discussed in detail in Jamieson and Schwarcz (2001a).

The relationship between salinity and $\delta^{18}O_{SW}$ leads to an interesting observation, namely that changes in salinity may have a larger impact on $\delta^{18}O_{oto}$ values

than changes in temperature. A change in temperature of 1°C will only result in a change of 0.2‰ in $\delta^{18}O_{oto.}$ A change in salinity of 1 psu will result in a change in $\delta^{18}O_{SW}$ of 0.6‰ which in turn affects $\delta^{18}O_{oto}$. Within the observed range of salinity and temperature change in the study area, it is possible for either variable to cause fluctuations in $\delta^{18}O$ (Jamieson and Schwarcz 2001a).

Advances in micro-milling have allowed otolith researchers to take advantage of the zoned nature of otolith growth (Gao 1997; Schwarcz *et al.* 1998; Gao and Beamish 1999; Weidman and Millner 2000; Begg and Weidman 2001). Otoliths exhibit seasonal banding with translucent zones forming in winter and opaque zones forming in summer. Low resolution sampling methods such as drilling or whole otolith analysis can obscure the temporal fluctuations which may be evident over the life of a fish and which may prove important for understanding short and long-term variations in environmental conditions or behaviour.

7.2.2 Northern Cod

This study was undertaken to examine the otoliths of Atlantic cod from the northern cod stock off the east coast of Newfoundland and Labrador, Canada (NAFO Division 2J3KL). This stock has traditionally supported one of the most important cod fisheries in the world. In the early 1990s however, this stock underwent a drastic decline in population and has shown no signs of recovering (Taggart *et al.* 1994). While overfishing has certainly played a large role in this decline, some researchers

have speculated that cod may have been influenced by changes the environment. In the early 1990s, the north Atlantic experienced a period of very cold temperatures (Colbourne *et al.* 1994). At the same time, the distribution of cod seemed to shift towards the south and seaward (Rose *et al.* 1994; Atkinson *et al.* 1997; Rose *et al.* 2000; de Young and Rose 1993). These movements may have been prompted by temperature directly or indirectly by changes in prey abundance. Cod may have become more vulnerable to the efforts of the offshore fishing fleets as a result of this redistribution.

The first attempt to measure lifetime variations in $\delta^{18}O$ in cod otoliths was by Gao (1997). This work focussed on cod from the Scotian Shelf off Nova Scotia. Gao (1997) found $\delta^{18}O_{oto}$ differences in the first 4 or 5 years of life could be used to distinguish two populations of cod in the 4Vs area. These values could be related to recruitment of fish from the Gulf of St. Lawrence. The $\delta^{18}O$ of water in this area would be lower due to the influence of fresh water and therefore the otolith $\delta^{18}O$ values for cod maturing in this area would be lower. Once they were recruited into the 4Vs population, their $\delta^{18}O$ values conform with the other fish. Cod which grew up on the Scotian Shelf (Type 1) had fairly constant values throughout their lives.

Weidman and Millner (2000) examined Atlantic cod otoliths from the northeast Atlantic. They found that the $\delta^{18}O_{oto}$ values were significantly correlated with latitude of capture between 50° and 70°N with ^{18}O enrichment toward the pole.

They also found regional differences which suggested that otoliths could be used to distinguish different stock components.

The purpose of this study is to examine the variations in individual $\delta^{18}O$ records from northern cod in order to determine the ability of isotopes to distinguish regional differences in the stock and evaluate the potential influence of environmental and distribution changes. This work will compare two different groups of otoliths: those obtained from captured Bonavista Bay cod; and archive otoliths from cod captured during research and commercial trawls off the coast of Newfoundland and Labrador over more than half a century. Analyzing an inshore population should reduce the uncertainty associated with the archive otoliths which are mainly from offshore cod and should elucidate features such as seasonal and ontogenetic $\delta^{18}O_{oto}$ variations. The archive otoliths were obtained from a collection held by the Department of Fisheries and Oceans (DFO) in St. John's, Newfoundland.

7.3 Methods

The inshore samples were taken from fish caught in Bonavista Bay, on the east coast of Newfoundland (Figure 7.1). Twenty-two cod were collected in November 1997 and another thirty-two were collected in September 1998. The first set (Bonavista B) were mainly larger, older fish ranging in size from 18 to 75cm. The second group (Bonavista A) consisted of younger cod from the age 0 (4.9 to 9.8cm) and age 1 (13.4 to 17.4cm) groups. Of the 54 fish caught, otoliths were extracted

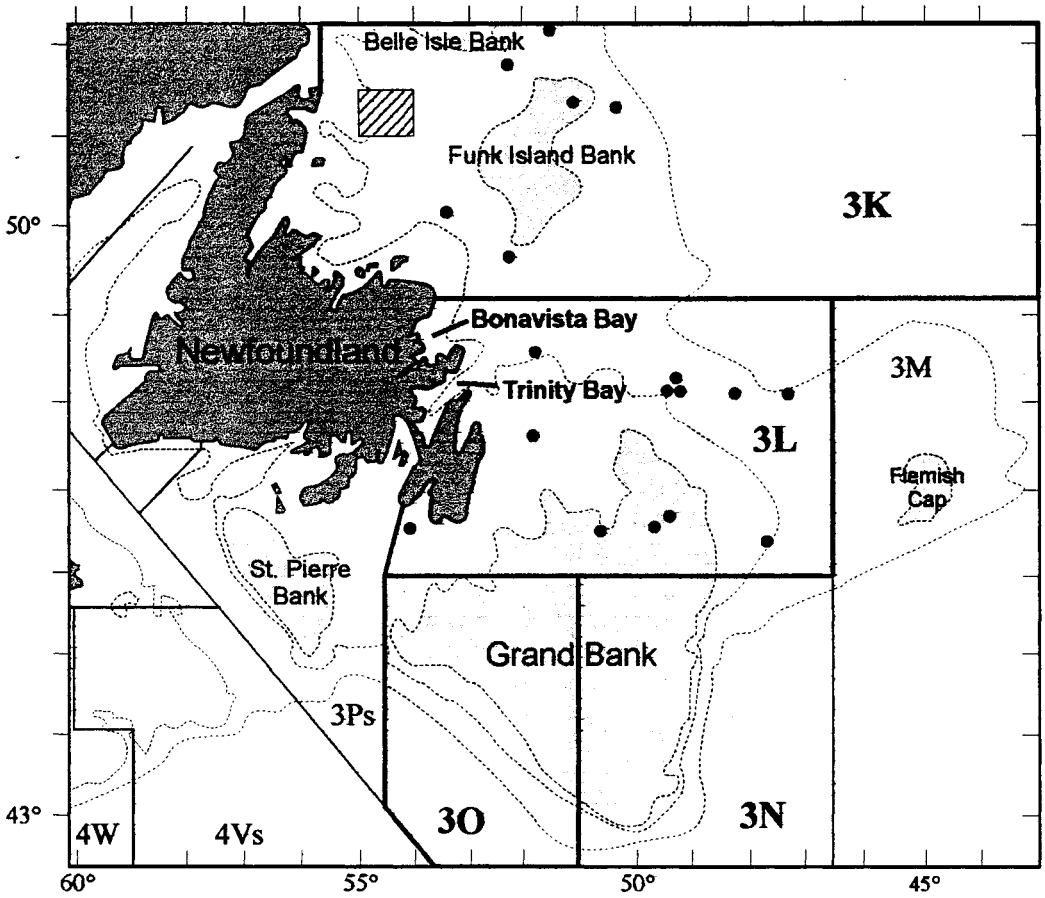

Figure 7.1 Map of offshore Newfoundland and Labrador illustrating NAFO Divs. 3KL, 3O, and the location of capture for each of the archive cod used in this study. A number of cod were also taken from Trinity and Bonavista Bays. One cod for which only a general location of capture was known is indicated by a striped box offshore.

from 41 and were analyzed for $\delta^{18}O_{oto}$. The otoliths were cleaned with a dilute bleach solution and deionized water after extraction to remove any surficial organic material. A second group of thirty otoliths was selected from the DFO collection. All were from cod of age five years or older. Most of these otoliths were from cod captured in the 1990s, but for comparison a number were selected from the 1950s, 1970s and 1980s. Twenty-seven of these otoliths were from cod collected from NAFO Division 3KL while three came from Division 3O (Figure 7.1). The archive inshore cod otoliths came from cod captured at two locations. Most were captured in Trinity Bay on the east coast of Newfoundland, while two others were caught on the southern coast (Figure 7.1). Most of the selected otoliths had corresponding information on their age, length, sex, location and depth of capture (Table 7.1).

All of the milling procedures are more fully described in Jamieson (2001). While micro-milling was the preferred method of sampling, some of the otoliths from the age 0 and age 1 cod were too small and therefore were ground whole for isotopic analysis. The $\delta^{18}O$ values from these fish therefore represent an integrated lifetime value. It was possible to mill some of the larger age 1 otoliths, and seven were chosen to provide a higher resolution examination of seasonal variation during the first year of growth. The larger archive otoliths were all analyzed by milling.

Preparation for milling involved embedding the otoliths in Araldite® resin and sectioning them through the nucleus to produce thin sections 100-200 μm thick using a low-speed Isomet saw. These thin sections were then mounted on petrographic

Table 7.1 Sample information for cod collected for this study. * Depth of capture.

Otolith #	Length (cm)	Sex	Age	NAFO Div. Depth* (m)		Latitude	Longitude
Southern 3L (n = 5)							
51-1800	94	M	18	3L	154	46° 29'	47° 39'
71-438	101	M	12	3L	82	46° 31'	50° 35'
87-5886	78	F	12	3L	67	46° 34'	49° 48'
96-4625	70	F	8	3L	68	46° 41'	49° 39'
87-5665	64	M	8	3L	181	47° 37'	51° 54'
Northern 3L (n = 7)							
79-6341	92	M	7	3L	383	48° 02'	48° 11'
87-6143	94	F	14	3L	267	48° 14'	49° 21'
83-6514	64	M	10	3L	195	48° 33'	51° 37'
93-823	66	M	8	3L	400	48° 12.9'	49° 24.5'
94-2842	67	M	8	3L	340	48° 14.2'	49° 06.6'
93-687	56	M	8	3L	905	48° 07.4'	47° 22.9'
93-847	69	F	8	3L	905	48° 07.4'	47° 22.9'
Inshore 3L (n = 7)							
96-4715	82	F	10	3L	68	46° 36'	54° 02'
96-4718	65	M	9	3L	68	46° 36'	54° 02'
96-5263	82	M	9	3L	255	47° 51'	53° 29'

Table 7.1 (cont.) Sample information for cod collected for this study. * Depth of capture.

Otolith #	Length (cm)	Sex	Age	NAFO Div.	Depth* (m)	Latitude	Longitude
Inshore 3L (cont.)							
96-5301	52	F	7	3L	117	48° 19'	53° 21'
96-2518	60	M	10	3L			
96-2856	72	M	10	3L			
96-3001	71	M	10	3L			
3K (n = 8)							
51-2381	54	M	9	3K			
96-4912	48	M	5	3K	321	49° 35'	52° 15'
96-4869	51	F	5	3K	354	50° 10'	53° 22'
87-7764	69	F	8	3K	345	51° 21'	50° 21'
71-535	68	F	7	3K	232	51° 25'	51° 04'
91-6390	56	M	8	3K	500	51° 44'	52° 16'
91-6344	66	F	9	3K	322	52° 08'	51° 29'
91-6333	61	M	7	3K	322	52° 08'	51° 29'
3O (n = 3)							
96-3224	68	F	8	3O			
96-3292	77	F	7	3O			
96-3615	73	M	8	3O			

slides. Individual translucent and opaque zones were milled using a Merchantek Micromill system. This system permits control of the depth and width of sampling paths which allows milling of precise samples of growth zones and control of sample size. The thickness of the growth zones dictates the number of samples that could be collected. In some cases it was not possible to obtain enough carbonate for isotopic analysis by milling individual summer and winter zones and therefore annual samples were collected. In order to obtain high-resolution seasonal records in some of the smaller inshore otoliths, four to six samples were taken from the opaque core and one to two were taken from the outer translucent zone.

Milled aragonite samples were analyzed on an Optima dual-inlet IRMS equipped with an Isocarb carbonate analyzer. $\delta^{18}O$ values are reported with respect to the VPDB international standard. Reproducibility of $\delta^{18}O$ analysis is ±0.05‰.

7.4 Results

7.4.1 Inshore Cod

The inshore population should experience roughly similar conditions, except for those which result from ontogenetic changes in behaviour. Figure 7.2 (a-b) presents the seasonal variation for the first year of growth for seven cod from Bonavista Bay. The curves for the individual otoliths have been matched assuming that the highest $\delta^{18}O_{oto}$ values correspond with the same period of coldest temperature. The individual curves are remarkably similar within and between the

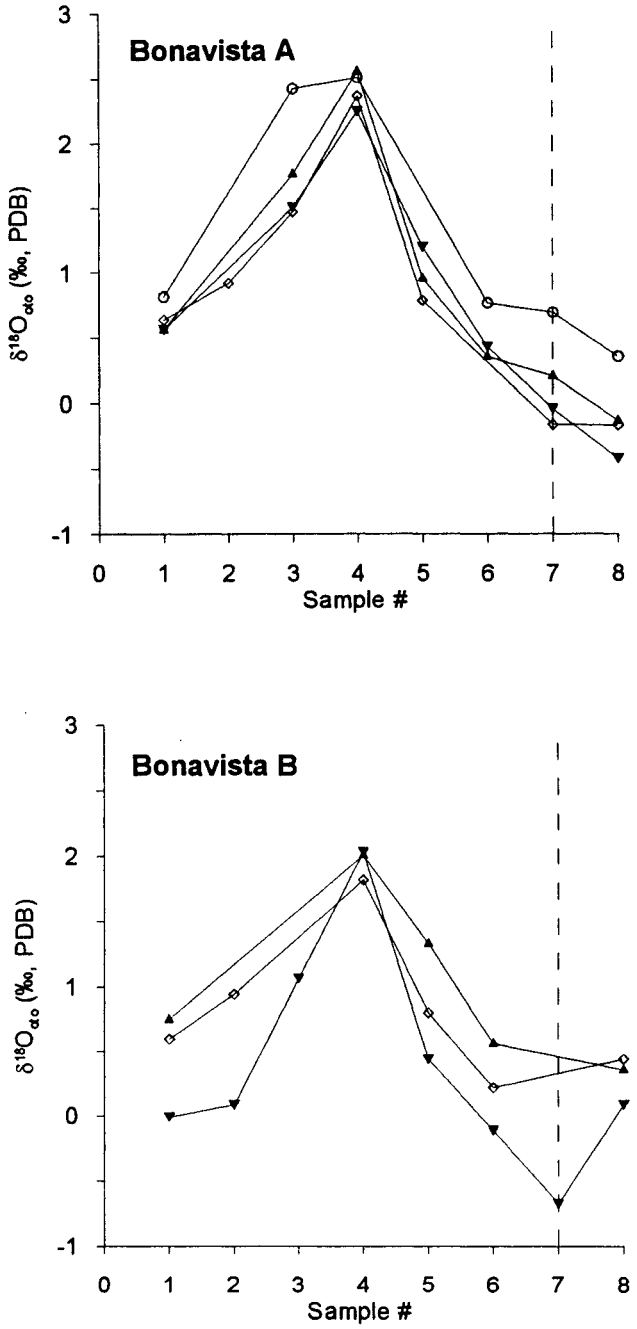

Figure 7.2 Seasonal variation over the first year of growth for individual cod from the Bonavista A and Bonavista B groups. The dashed line represents the boundary between the opaque and translucent zones.

two groups of otoliths. The average maximum $\delta^{18}O_{oto}$ value is 2.22 ± 0.26‰ for both groups. The maximum for Bonavista A is slightly higher than Bonavista B (2.42 ± 0.12‰ and 1.95 ± 0.09‰ respectively). The minimum values, which should correspond with the highest temperatures, have slightly more scatter but are also generally consistent with an overall average of -0.07 ± 0.37‰ for both groups.

Using the equation of Kim and O'Neil (1997) it is possible to use these results to calculate the fluctuation in temperature experienced by these cod. The range of $\delta^{18}O_{oto}$ values indicates an average seasonal temperature fluctuation of approximately 11°C. The average fluctuation for Bonavista A (12.1 ± 1.0°C) is slightly higher than that recorded by the Bonavista B otoliths (9.6 ± 2.5°C). In order to calculate absolute temperature values, it is necessary to know the isotopic composition of the water in the area. Assuming that the waters of Bonavista Bay are relatively lower in salinity and are isotopically similar to the offshore waters of 2J3KL (Jamieson and Schwarcz 2001a), the $\delta^{18}O_{sw}$ value should lie between -1.5 and -2‰. Using a value of -2‰ gives unreasonably cold temperature values on the order of -3°C. A value of -1.5‰, however, yields an overall range in temperatures from -1.7 to 14.0°C which is well within the observed temperature range of this area (Narayanan *et al.* 1996). The long term temperature monitoring station at Stock Cove, Bonavista Bay records a maximum variation of between -1.80 and 15.80°C at 9m depth.

These inshore cod can also be assessed for ontogenetic changes by comparing $\delta^{18}O_{oto}$ with fish length. Figure 7.3 is a plot of $\delta^{18}O_{oto}$ versus length for the Bonavista

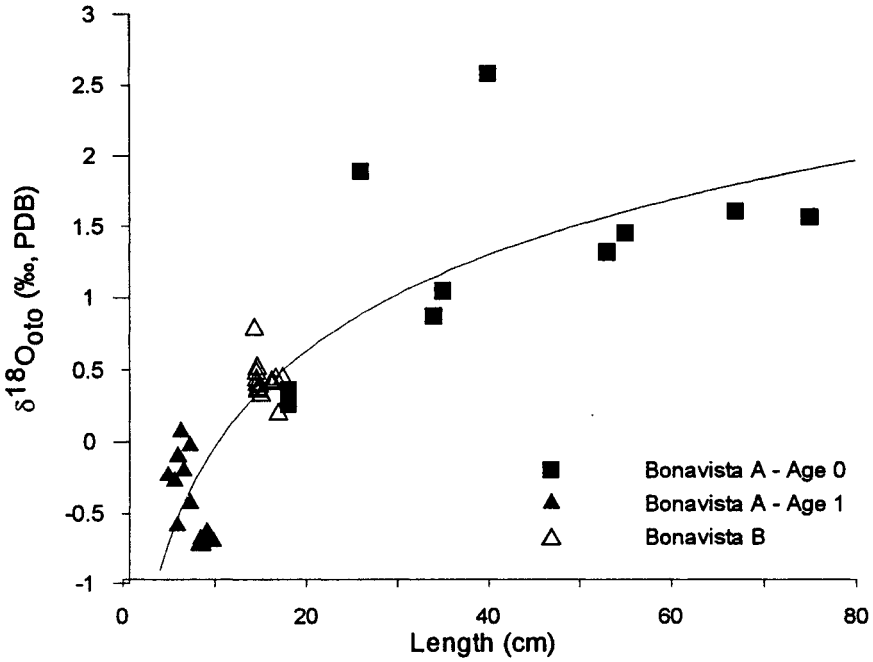

Figure 7.3 Relationship between $\delta^{18}O_{oto}$ and length for the Bonavista A and Bonavista B otoliths. The thin line represents the log fit of the data represented by the equation: $\delta^{18}O_{oto} = 2.2\text{Log}(L) - 2.23$ ($r^2 = 0.728$).

A and Bonavista B cod. The $\delta^{18}O_{oto}$ values for the older cod (Bonavista B) are values for the most recent annual growth zone while those for the age 0 and age 1 cod are lifetime averages. It is clear from this plot that the otoliths are becoming enriched in ^{18}O as the fish grows. The overall data can be fitted to a curve with the equation:

$$\delta^{18}O_{oto} = 2.2 \log(L) - 2.2 \qquad (r^2 = 0.728)$$

where L is the length in centimetres. The isotopic results for each size class are presented in Table 7.2. The age 0 and age 1 cod cluster into separate groups with only a small amount of deviation. There is also no overlap between the isotopic values of the different size groups. The two smaller Bonavista B cod which belong to an age 1 size class fall within the Bonavista A age 1 cod group, giving an average of 0.44 ± 0.13‰ for the group as a whole. Most of the older Bonavista B cod fall along the regression line except for two obvious outliers.

7.4.2 Archive Cod

7.4.2.1 Seasonal Variation

Seasonal variation can be partly assessed by examining the difference between adjacent opaque and translucent growth zones. Pairs of growth zones (n = 142) were analyzed and averaged according to growth year (Figure 7.4). Although the difference between translucent zones and opaque zones seems to be relatively consistent through the life of the fish (≈ 0.3‰), with the translucent zones ^{18}O enriched relative to the opaque zones, there is a significant amount of variation, and

Table 7.2 Summary of $\delta^{18}O_{oto}$ results for Bonavista cod divided by size class.

		$\delta^{18}O_{oto}$ (‰)	
	n	**Average**	**Range**
Bonavista A	29		
age 0 (4.9 to 9.8 cm)	15	-0.44 ± 0.27	-0.72 to 0.07
age 1 (13.4 - 17.4)	14	0.43 ± 0.12	0.21 to 0.80
Bonavista B	10		
18 cm	2	0.31 ± 0.05	0.26 to 0.36
26 to 75 cm	8	1.54 ± 0.49	0.87 to 2.58

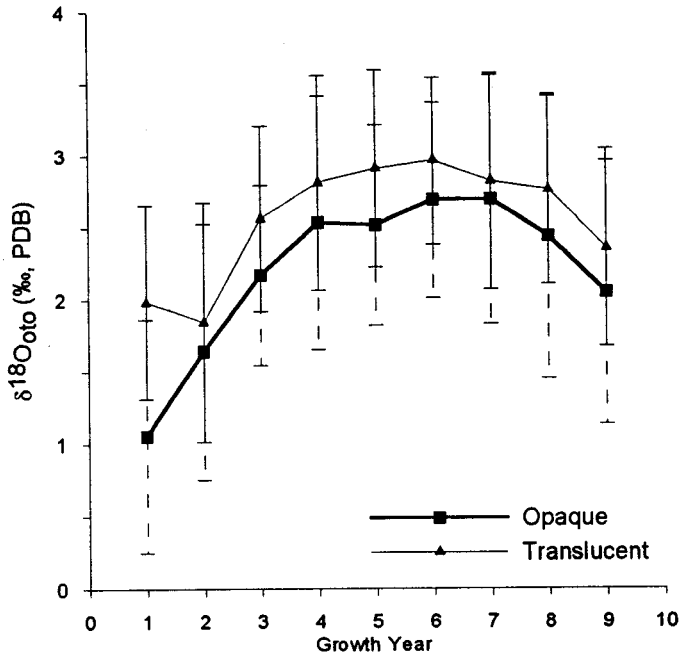

Figure 7.4 Average $\delta^{18}O_{oto}$ for opaque and translucent growth zones for each year of growth. Error bars represent the SD.

considerable overlap of the two data sets. The distribution of values for the difference between adjacent opaque and translucent zones ($\Delta^{18}O_{op-trans}$) is almost normal around the average value of -0.32 ± 0.62 ‰ (Figure 7.5). The difference during the first year seems to be slightly more significant (-0.93 ± 0.69‰), but by the second year it is no longer significant (-0.20 ± 0.54‰). Since seasonal variation does not seem to be a significant factor, for the remainder of this discussion individual otolith records will be presented as annual averages.

7.4.2.2 Spatial Variation

In order to assess the geographic variation of the archive otoliths, they have been divided into groups according to the latitude at which they were caught. Eight cod were caught in NAFO Div. 3K while nineteen came from 3L. The 3L cod were further subdivided into a southern group (between 46° and 48°N), a northern group (between 48° and 49°N), and an inshore group. Three cod were also analyzed from the south of 3L in NAFO Div. 3O. The location of capture for each cod is shown in Figure 7.1.

All of the plots comparing individual $\delta^{18}O$ records are plotted against calendar year. This is done knowing the age of the otolith and the year in which the cod was captured. The results for individual growth layers can be assigned to the year in which they were laid down. This allows us to compare growth zones from different otoliths which had been laid down at the same time.

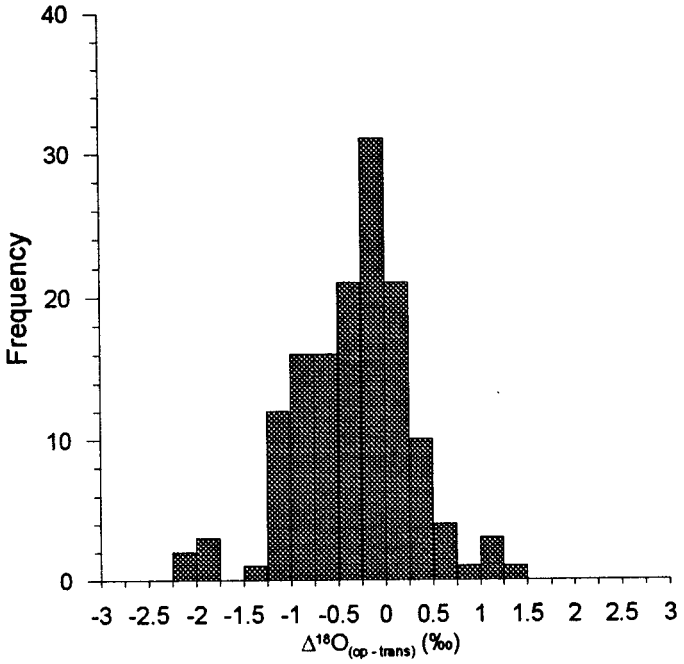

Figure7.5 Histogram of the $\Delta^{18}O_{(opaque - translucent)}$ for 142 pairs of adjacent opaque and translucent growth zones.

The overall range of values observed for $\delta^{18}O_{oto}$ was from 0.05 to 3.92‰. Generally, the individual records begin with relatively low $\delta^{18}O_{oto}$ values which increase as the fish grows. The amount of variability seen in the $\delta^{18}O_{oto}$ data is larger than that seen in the carbon data for the same otoliths (Jamieson *et al.* 2001).

Figures 7.6a and 7.6b present the individual otolith $\delta^{18}O$ records for the southern and northern 3L cod respectively. Records for two of the otoliths (93-687 and 93-847) from the northern 3L group have been presented separately in Figure 7.6c. The $\delta^{18}O_{oto}$ trends for these otoliths are distinct compared to the rest of the 3L otoliths and these will be treated as a separate group. A summary of the results for individual otoliths can be found in Table 7.3. Table 7.4 summarizes the data for each group. In both groups, the minimum value for each record usually corresponds with the initial value, in agreement with the observation that these otoliths are, in general, continually increasing with age. The mean initial values for southern 3L (2.10 ± 0.46‰) and northern 3L (1.84 ± 0.76‰) are not significantly different. Most seem to come to a plateau at some point during their adult life. A mean adult value was calculated by taking the average of the last three years of growth for all mature cod. This should represent an average for the plateau. The average adult $\delta^{18}O_{oto}$ values for the southern (3.28 ± 0.17‰) and northern groups (2.96 ± 0.42‰) are also not significantly different although there is more scatter in the north. It should be noted that the timing of these plateaus is not consistent with that observed for $\delta^{13}C_{oto}$ in

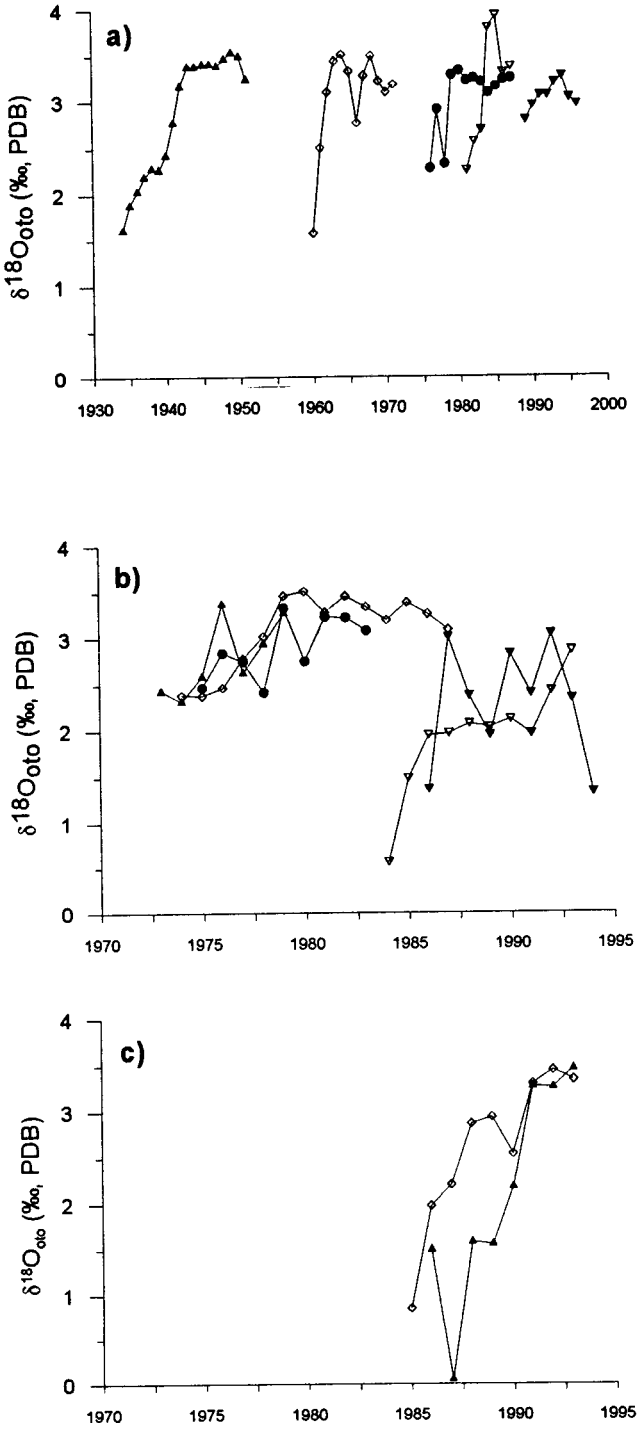

Figure 7.6 Individual $\delta^{18}O$ records for cod from a) southern 3L b) northern 3L and c) shelf edge of 3L. Note that a) covers a longer time span.

Table 7.3 Results of individual otolith $\delta^{18}O_{oto}$ analyses.

Otolith #	$\delta^{18}O$ (‰, PDB)		
	Initial	Maximum	mean adult
Southern 3L			
51-1800	1.61	3.53	3.42 ± 0.13
71-438	1.57	3.5	3.16 ± 0.05
87-5886	2.28	3.32	3.21 ± 0.04
96-4625	2.79	3.26	3.08 ± 0.13
87-5665	2.25	3.92	3.53 ± 0.28
Northern 3L			
79-6341	2.43	3.37	2.94 ± 0.26
87-6143	2.38	3.5	3.24 ± 0.12
83-6514	2.47	3.33	3.17 ± 0.07
93-823	0.57	2.85	2.41 ± 0.37
94-2842	1.36	3.04	2.23 ± 0.70
93-687	1.5	3.46	3.33 ± 0.09
93-847	0.85	3.45	3.36 ± 0.06
Inshore 3L			
96-4715	1.25	3.01	2.03 ± 0.06
96-4718	1.92	2.88	2.23 ± 0.00
96-5263	-	2.61	2.08 ± 0.17
96-5301	2.19	2.93	2.55 ± 0.43
96-2518	1.5	3.19	1.79 ± 0.25
96-2856	0.41	3.09	1.29 ± 0.12
96-3001	1.06	2.18	1.41 ± 0.48
3K			
51-2381	0.85	3.35	3.25 ± 0.12
96-4912†	1.2	2.72	-
96-4869†	1.88	2.56	-
87-7764	1.84	3.6	3.52 ± 0.08
71-535	2.24	3.8	3.35 ± 0.40

† Not matured

Table 7.3 (cont.) Results of individual otolith $\delta^{18}O_{oto}$ analyses.

Otolith #	$\delta^{18}O$ (‰, PDB)		
	Initial	Maximum	Mean adult
3K (cont.)			
91-6390	1.69	3.63	3.54 ± 0.06
91-6344	1.66	3.44	3.09 ± 0.11
91-6333	1.22	3.62	3.36 ± 0.19
3O			
96-3224	0.86	2.62	2.36 ± 0.07
96-3292	1.72	2.98	2.55 ± 0.30
96-3615		3.27	3.16 ± 0.08

Table 7.4 Summary of the mean $\delta^{18}O_{oto}$ values for each latitude grouping.

		$\delta^{18}O_{oto}$			
	n	**Range**	**Mean**	**Mean**	**Mean**
Southern 3L	5	1.57 to 3.92	2.10 ± 0.46	3.51 ± 0.23	3.28 ± 0.17
Northern 3L	5	0.57 to 3.50	1.84 ± 0.76	3.22 ± 0.24	2.96 ± 0.42
3K	8	0.81 to 3.80	1.58 ± 0.44	3.57 ± 0.14	3.35 ± 0.15
3O	3	0.86 to 3.27	1.49 ± 0.45	2.96 ± 0.27	2.69 ± 0.34
Inshore 3L	7	0.41 to 3.19	1.39 ± 0.58	2.84 ± 0.32	1.91 ± 0.41

these same otoliths and is generally later. In carbon, plateaus have been related to maturity. There is a large amount of interannual variability which does not appear to be related to ontogenetic changes, especially in the northern 3L group. The maximum increase between these groups is similar with average observed maximum values of 3.51 ± 0.23‰ and 3.22 ± 0.24‰ for the southern and northern groups respectively. These high values are generally associated with the adult plateau.

The two shelf edge otoliths have very distinct isotopic trends. Early in life, they appear to be much more ^{18}O depleted than the other otoliths (as low as 0.05 ‰) and they become continuously ^{18}O enriched through out their lives. The individual curves seem to converge once the cod have reached five to six years of age. Their mean adult values of 3.33 ± 0.09‰ and 3.36 ± 0.06‰ reflect this consistency late in life.

The otoliths of the 3K group, generally seem to have much steeper $\delta^{18}O_{oto}$ trends through the first five or six years of growth (Figure 7.7). Most of the individual records seem to reach a plateau during adulthood, as was observed in the 3L cod. This is not always the case as one otolith (71-535) continually increases. The two otoliths from 1996 (96-4912 and 96-4869) are both 5 years old and have not reached maturity which may explain why they don't reach the same level of ^{18}O enrichment as the other otoliths in this group. Despite their overall increase with age, the initial $\delta^{18}O_{oto}$ values do not always correspond with the minimum value in this group. Some of the otolith $\delta^{18}O$ values decrease during their second year after which

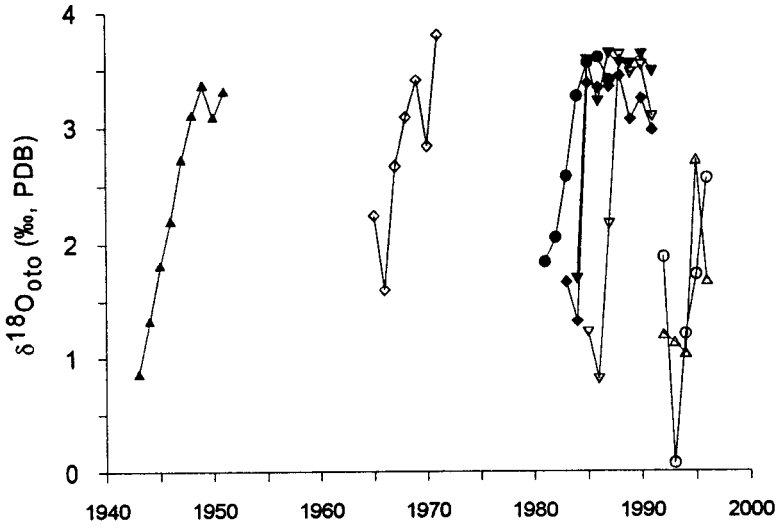

Figure 7.7 Individual otolith $\delta^{18}O$ records for cod from 3K.

$\delta^{18}O_{oto}$ values rise steeply. The mean adult $\delta^{18}O_{oto}$ for 3K is 3.35 ± 0.15‰ which is slightly higher than the 3L averages. The minimum and initial values however, seem to be lower than 3L. The average initial value is 1.58 ± 0.44‰ and the mean minimum value is 1.35 ± 0.40‰. Most of the initial values are < 2‰ whereas in 3L they were >2‰.

As a further test of spatial variability, three otoliths from NAFO Division 3O were analyzed. These records are presented in Figure 7.8. Again, these otoliths become ^{18}O enriched during their early life and level off with age or increase at a slower rate. The average adult value for this group is 2.69 ± 0.34‰ which seems slightly lower than the other groups, but the amount of variation makes it impossible to distinguish them. Minimum $\delta^{18}O$ values are the initial value for each of the three otoliths, and these values also seem to be slightly lower with respect to the other groups.

The final group studied is composed of the inshore 3L otoliths. There is a large amount of variability within this group and the individual records have been separated according to their general shape in Figure 7.9 (a-c). The otoliths in Figure 7.9a and 7.9b, are all from Trinity Bay, on the east coast of Newfoundland (Figure 7.1). The two otoliths in shown in Figure 7.9c are both from the south coast of Newfoundland near St. Mary's Bay. Within each group, there seems to be a fair amount of consistency. The overall range of observed values spans from 0.41‰ to 3.19‰ and the mean adult $\delta^{18}O_{oto}$ is 1.91 ± 0.41‰. In general, these otoliths seem

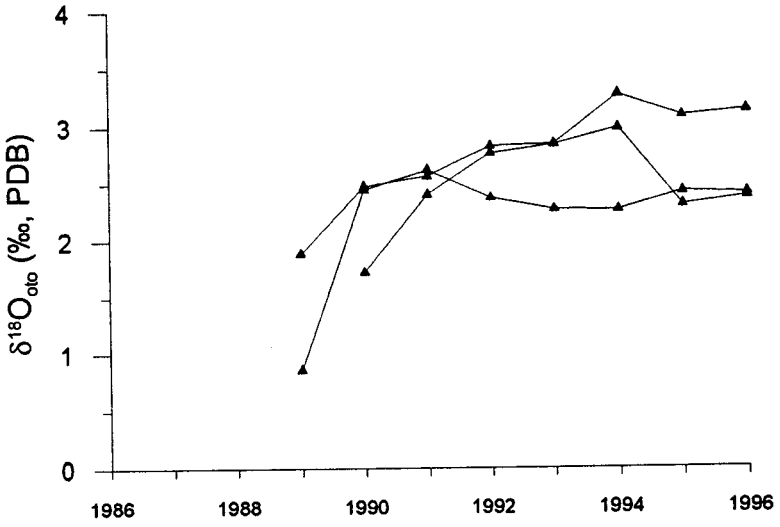

Figure 7.8 Individual otolith $\delta^{18}O$ records for cod from 3O.

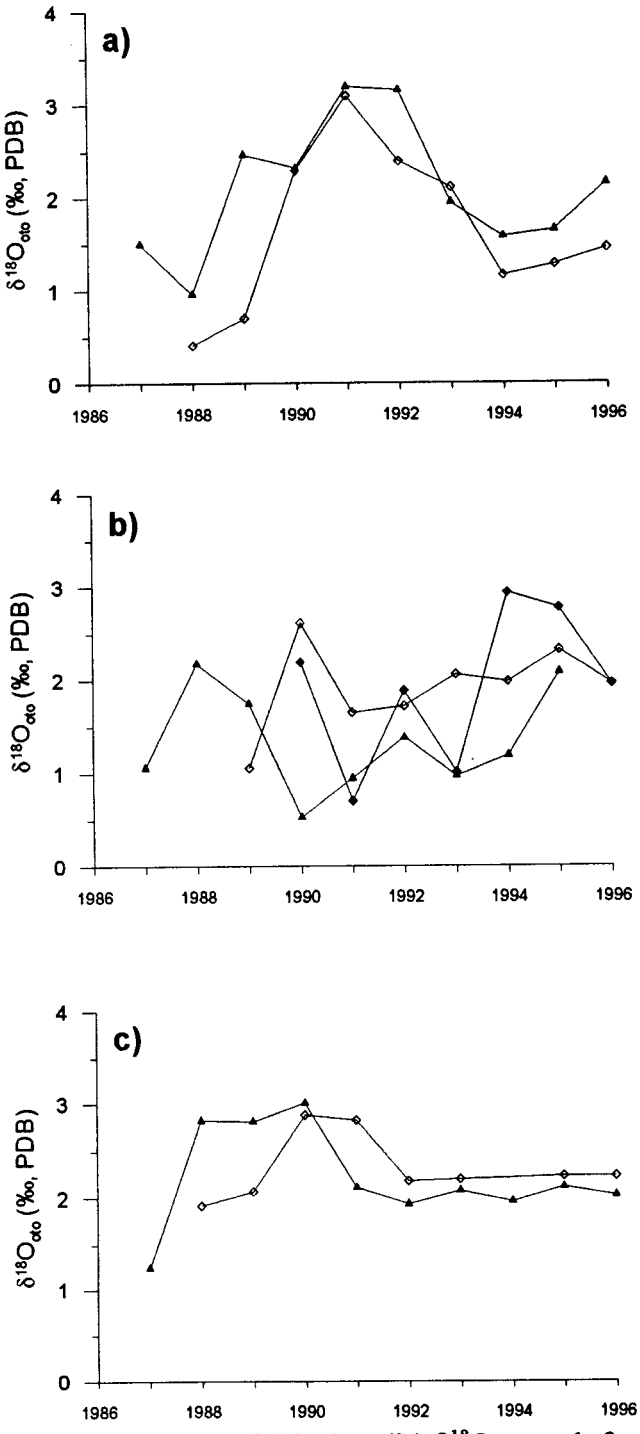

Figure 7.9 (a-c) Individual otolith $\delta^{18}O$ records for cod from inshore 3L

to be depleted in ^{18}O with respect to the offshore otoliths. The two otoliths in Figure 7.9a, however, have a very distinctive increase in the middle of their lives, reaching $\delta^{18}O_{oto}$ values greater than 3‰. There seems to be some indication in both the second and third sets of an increase during the first four years and then decreasing to a plateau with age which is perhaps related to ontogenetic changes. The mean maximum in this group is approximately 0.5‰ lower than the 3L and 3K groups while the mean minimum is also lower although not significantly so at 0.98 ± 0.47‰.

If we now reconsider the offshore groups as a whole, there does not appear to be a great deal of difference between them in terms of the overall observed $\delta^{18}O_{oto}$ values. Regression fails to identify any significant relationships between latitude and the mean adult, maximum, or minimum $\delta^{18}O_{oto}$ values. The data do not significantly correlate with longitude either. A weakly significant correlation was found between the depth of capture and the minimum observed $\delta^{18}O_{oto}$ ($r^2 = 0.460$). Without the two ^{18}O depleted shelf edge otoliths, this correlation is even weaker ($r^2 = 0.180$). The cod were caught in depths between 65 to 905 m although the majority were between 300 and 400 m. Cod were caught at an average depth of about 200 m in 3L while the 3K cod were mainly between 300 and 350 m. Length, age, or sex also seemed to have no significant impact on $\delta^{18}O_{oto}$ values.

Otolith growth zones laid down in the same year range from very consistent, which would be expected if the fish were experiencing similar conditions, to as much

as a 1 to 1.5‰ variation. Despite this variation, examining temporal fluctuations in $\delta^{18}O_{oto}$ of offshore otoliths seems to suggest that there may be an underlying environmental signal. If the mean adult value is plotted against the year of capture, there seems to be a slight shift towards more negative values in cod caught in the early 1990s (Figure 7.10a). An even stronger decline seems to occur in the initial $\delta^{18}O_{oto}$ values. In Figure 7.10b, initial $\delta^{18}O_{oto}$ for each otolith is plotted against the year of birth; the year in which this growth occurred. In the early 1970s, initial $\delta^{18}O_{oto}$ values increase to a peak and then decline again to the mid-1980s although there is one anomalously high otolith in 1988. In the period from 1972 to 1988 where the decline is greatest, the correlation between $\delta^{18}O_{oto}$ and year of growth has an r^2 value of 0.789. There does not seem to be any difference between the 3L and 3K otoliths in this respect.

7.5 Discussion

7.5.1 Ontogenetic Change

The most striking feature of the $\delta^{18}O$ records from the offshore is the fact that almost all of them demonstrate a clear increase with age. While there is some amount of interannual variability, there is a common trend which seems to be related to ontogenetic changes of habitat. It had been suspected for some time, and only recently shown, that the carbon isotopic composition of otoliths could be influenced

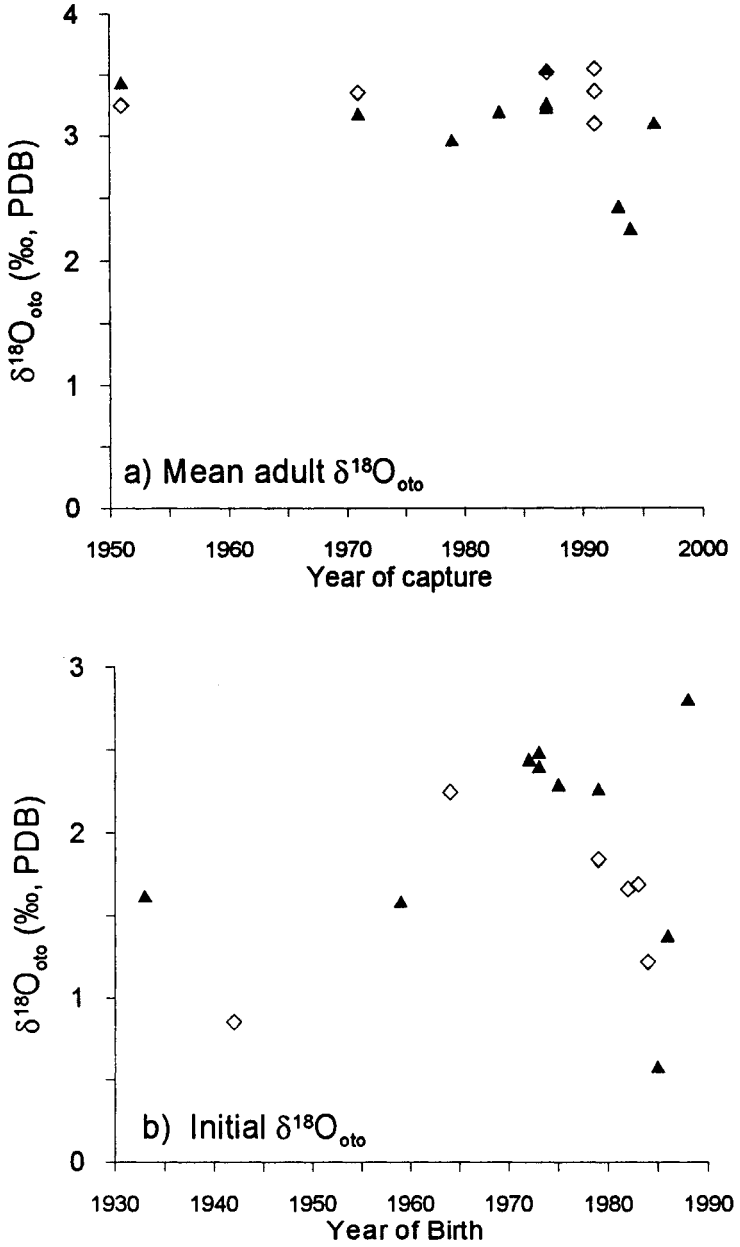

Figure 7.10 a) Mean adult $\delta^{18}O_{oto}$ versus year of capture and b) initial $\delta^{18}O_{oto}$ versus year of birth for 3K and 3L otoliths. The solid triangles represent 3L otoliths and the open diamonds represent 3K otoliths.

by ontogenetic shifts in diet (Jamieson and Schwarcz 2001b). Shifts in the oxygen isotopic composition with age have been noted in other fish populations including cod (Gao 1997) and haddock (Begg and Weidman 2001) although Gao (1997) noted that some Scotian Shelf cod had consistent $\delta^{18}O_{oto}$ values throughout their lives.

This increase in $\delta^{18}O_{oto}$ might be taken to mean that cod move to colder water as they grow, but other studies have shown that young cod tend to inhabit colder water while older cod move to warmer and deeper water (Dalley and Anderson 1997). The ^{18}O enrichment of otoliths must therefore be related largely to changes in salinity. Atlantic cod in the Newfoundland and Labrador area become more widely distributed offshore during the first 3 to 4 years of life and thus encounter waters of increased salinity (Dalley and Anderson 1997; Anderson and Gregory 2000). The increase seen in the archive otoliths in some cases seems to take much longer than four or five years. The changes which affect the plateau in $\delta^{18}O_{oto}$ with age are clearly not related to the changes affecting $\delta^{13}C_{oto.}$

Analysis of the inshore population of cod from Bonavista Bay provides an opportunity to study this ontogenetic shift in a group which should experience relatively consistent environmental conditions if we assume that they are part of a resident inshore population. There is some indication that distinct inshore populations do exist although the extent of these populations is not fully understood (Lawson and Rose 2000). This should limit the uncertainty associated with the interpretation of

$\delta^{18}O_{oto}$ of offshore cod which may be more likely to experience a range of conditions as they move over the shelf. The $\delta^{18}O_{oto}$ data shows clearly that these cod are occupying particular habitats during different life stages. Within each age class there is very close agreement, but there is no overlap between the age classes. It is clear that waters in certain areas of the Newfoundland and Labrador shelves have distinct isotopic and temperature conditions which may impart distinct signatures to fish living there. It may be possible therefore to at least narrow down the location where a fish has been living by combining these sources of information.

Jamieson and Schwarcz (2001a) showed that there was a strong linear relationship between salinity (S) and $\delta^{18}O$ of seawater in the offshore waters of Newfoundland. Figure 7.11 shows this relationship and also lines representing the isotopic composition of an otolith deposited at a particular temperature through a range of salinity values (based on eq. 1). The $\delta^{18}O_{SW}$ data used to calculate $\delta^{18}O_{oto}$ were derived using an average $\delta^{18}O_{SW}$ equation representing the average regression for the spring and summer water data sets taken together. Temperatures over the Newfoundland and Labrador Shelves range between approximately -1.8 and 15°C. Both salinity and temperature tend to vary with $\delta^{18}O_{SW}$ across the shelf. This is related to the relative influence of the waters of the cooler and fresher Labrador Current inshore and the warmer and more saline waters of the North Atlantic Current offshore. Summer heating of the surface layers causes scatter in the relationship

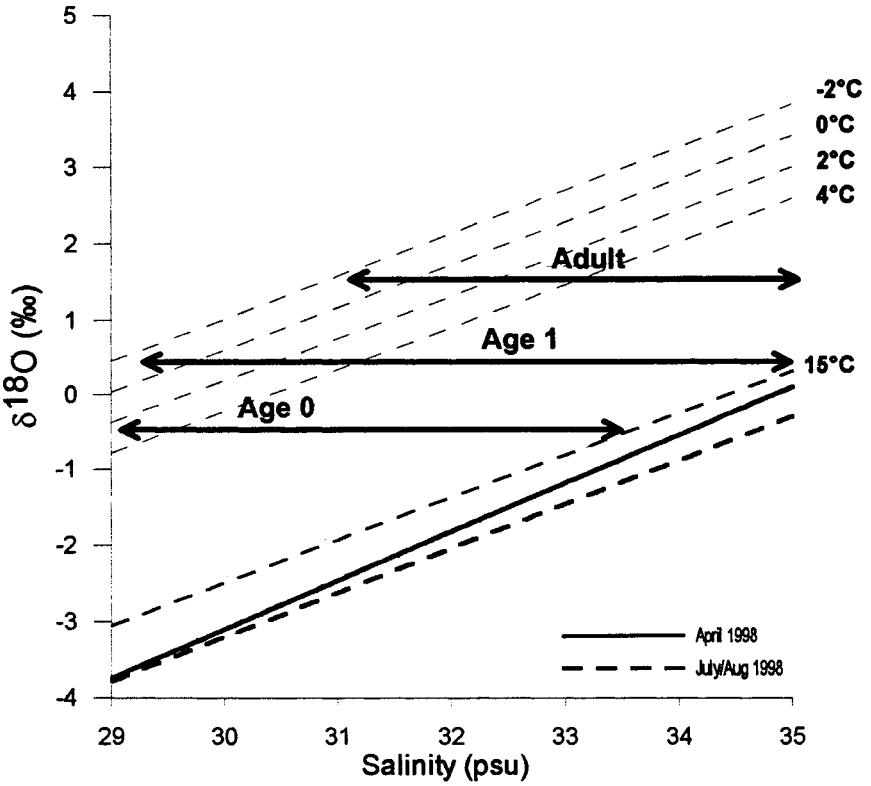

Figure 7.11 A $\delta^{18}O$ - salinity diagram for Bonavista Bay inshore cod otoliths. Each thin dashed line represents the $\delta^{18}O$ produced at a given temperature over a range of salinity. The thick lines at the bottom are the spring and summer regressions for the $\delta^{18}O_{sw}$ - salinity relationship in the 2J3KL area from Jamieson and Schwarcz (2001a). The average $\delta^{18}O_{oto}$ for each age group within the Bonavista Bay inshore cod group are represented by the arrows.

between $\delta^{18}O_{sw}$ and temperature at low $\delta^{18}O_{sw}$. These waters are not as likely to be occupied by cod except in inshore regions. A more realistic upper temperature limit is probably 4°C which is the temperature found in deep waters near the continental slope. Salinities range between approximately 30 and 35 psu increasing towards the offshore. Jamieson and Schwarcz (2001a) did not extend their study into the inshore bays and it is possible that salinity decreases even further in these areas although there are no large freshwater inputs in these areas. At very high salinity (≈34.8 psu) the linear relationship between $\delta^{18}O_{sw}$ and salinity breaks down somewhat and there is an almost vertical change in $\delta^{18}O_{sw}$ at a constant salinity in the deepest waters. Within these boundaries it should be possible to use Figure 7.11 to trace the ontogenetic movement of cod.

The arrows on Figure 7.11 represent the average $\delta^{18}O_{oto}$ values for each age group of otoliths from Bonavista Bay. It is clear that each $\delta^{18}O_{oto}$ could be produced by a wide range of possible salinity and temperature combinations. The graph indicates, however, that the conditions for each age group do not completely overlap. This makes sense in the context of cod moving to deeper water with age. We know that, in the 3KL area, the salinity of shallow inshore regions should be lower (30 to 31 psu) than the deep offshore (33 to 34 psu). If these adults are remaining inshore all year, as is perhaps indicated by the general coherence of the $\delta^{18}O_{oto}$ - length data, the experienced salinity may be relatively consistent between age classes. If the cod

were living in constant salinity conditions though, they must have experienced large variations in temperature in order to accommodate the observed range in $\delta^{18}O_{oto}$. Adult cod would have lived at temperatures as low as 0 to -2°C. Inshore juvenile cod have been shown to tolerate colder temperatures because they have blood antifreeze proteins, but older cod are expected to move to warmer water (Dalley and Anderson 1997). It seems more likely that older cod must be experiencing slightly higher salinity conditions, perhaps in deeper areas of the bay or offshore. There are two outliers in the Bonavista Bay data (Fig. 7.3). These two cod have significantly higher $\delta^{18}O_{oto}$ values (2 to 2.5‰) relative to the average adult value of approximately 1.5‰. These may represent cod which are not native to the area but have migrated in from elsewhere; such behaviour has been previously documented by Brattey (2000).

We can continue this type of analysis for the offshore cod. Figure 7.12 is again a plot of $\delta^{18}O$ versus salinity. In this case the range of maximum $\delta^{18}O_{oto}$ observed in the offshore cod has been plotted as a shaded box and the initial values for each offshore group has been plotted as an arrow indicating the range of possible salinity and temperature combinations which could produce these values. As with the inshore cod, extreme increases in $\delta^{18}O_{oto}$ must correspond to high salinity, to avoid positing impossibly low growth temperatures. We know that such conditions only exist at depth and towards the edge of the shelf in offshore Newfoundland and Labrador (Jamieson and Schwarcz 2001a). In these areas, temperatures are often

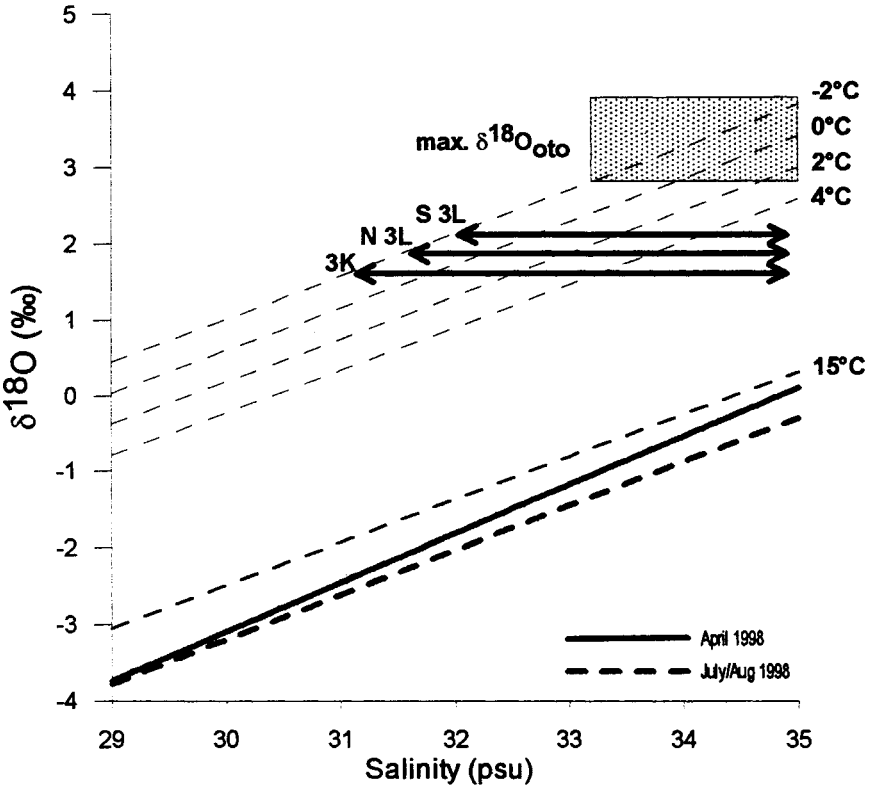

Figure 7.12 A $\delta^{18}O$ - salinity diagram for offshore cod otoliths. This diagram displays the range of temperature and salinity conditions which could produce the initial $\delta^{18}O_{oto}$ values for offshore cod otoliths (represented by arrows) and also the range of maximum $\delta^{18}O_{oto}$ values (the shaded box).

between 3 and 4°C. There is some discrepancy between predicted and observed values at very high salinity as colder temperatures are required to obtain the maximum observed $\delta^{18}O_{oto}$ values. This can be explained by the large degree of scatter which is observed around the $\delta^{18}O_{SW}$ - salinity curves, especially at high salinity (Jamieson and Schwarcz 2001a). This can lead to a corresponding dispersion of $\delta^{18}O_{oto}$ values. A variation in $\delta^{18}O_{SW}$ of 1‰ will correspond to a change in $\delta^{18}O_{oto}$ of 1‰, equivalent to a change of 4 °C in temperature. The general consistency of the adult mean $\delta^{18}O_{oto}$ values reveals that adult cod tend to live under similar conditions. The lack of seasonal variation and the relative stability of $\delta^{18}O_{oto}$ observed in some of the adult cod indicates that cod are living under these conditions throughout their adult life and are perhaps adjusting their behaviour to maintain these optimum conditions.

Initial $\delta^{18}O_{oto}$ values can be produced by a much wider range of conditions within the observed field of variation of T and S in the study area. The very slight trend of decreased initial values towards the north is not consistent with a lowering of temperature. Changes in salinity are also not particularly obvious between the north and south. In fact, cod living on the northern Grand Bank (3L) have been observed to inhabit colder waters (to -1°C) than cod on the northeast Newfoundland Shelf (Rose *et al.* 1994). Inshore areas have been suggested as important nursery areas for northern cod (Anderson and Gregory 2000). It seems that these low initial $\delta^{18}O_{oto}$ are indicating that almost all the cod studied here have come from inshore

areas where the appropriate combination of low temperature and low salinity can be found.

The log linear relationship found between $\delta^{18}O_{oto}$ and length for the Bonavista inshore cod would seem to mean that it would be possible to calculate the expected $\delta^{18}O_{oto}$ for a cod based on its length. This does not appear to be the case, at least for the offshore cod. Using the same equation, it is not possible to recreate the $\delta^{18}O_{oto}$ values for offshore cod based on their length. $\delta^{18}O_{oto}$ values are consistently underestimated and there is much more scatter in the observed data. The scatter can be explained because the offshore cod can potentially be exposed to a much broader range of environmental conditions. Bonavista Bay inshore cod are also exposed to lower salinity conditions than the offshore cod which would explain why there is a consistent underestimation. Agreement is much better for the inshore archive cod with the difference between predicted and observed $\delta^{18}O$ values ranging between 0.02 and -0.59‰. It seems therefore that this may be a consistent relationship among inshore populations.

Two of the archive otoliths exhibit particularly strong $\delta^{18}O$ increases over their entire lives; very different from the other offshore otoliths (Figure 7.6c). These cod were caught together in the same trawl in 905 m water depth and appear to constitute a separate population of cod living at the very edge of the shelf in 3L. It was previously found that the $\delta^{13}C_{oto}$ results from these otoliths were also very different

when compared with the rest of the offshore population (Jamieson *et al.* 2001). More study will be needed on this group to determine if they do indeed constitute a separate population.

While seasonal variation seems to be important for young cod, it does not appear in the $\delta^{18}O_{oto}$ records of older cod. This difference is confirmed by the large seasonal variations observed in age 1 cod and also the comparison of opaque and translucent zones in archive otoliths. The only significant difference between opaque and translucent growth zones was found in the first growth year for the archive group. The translucent or winter growth zone does appear to be ^{18}O enriched throughout the life of the fish which is consistent with the expected ^{18}O enrichment at lower temperatures. This is similar to the findings of Gao (1997) in cod and Begg and Weidman (2001) for haddock. A lack of seasonality is not necessarily surprising since older cod are living in deeper water where seasonal variations in temperature and $\delta^{18}O_{sw}$ are small. It is also possible that the cod are adjusting to the environment by moving to more favourable waters (Rose *et al.* 1994). A similar decrease in interseasonal variation in $\delta^{18}O$ was reported by Gao *et al* (2001) for pen-reared cod.

7.5.2 Inshore Archive Cod

Inshore archive cod display much more variety in the types of lifetime trends than the offshore cod. The cod in Figure 7.9a seen to reach a peak and then decline quite rapidly while those in Figure 7.9c maintain high values for a couple of years

before decreasing again. While this increase in early life is similar, the return to lower $\delta^{18}O_{oto}$ values seen later in life is quite unusual compared to all the other groups. The maximum values for most of these cod, as high as 3 to 3.5‰, are much higher than corresponding values for Bonavista inshore cod (1.5‰), but the low mean adult $\delta^{18}O_{oto}$ values in older cod seem consistent with an inshore life at lower salinity. From Figure 7.11, we can see that it would be impossible to produce the maximum $\delta^{18}O_{oto}$ values at lower temperatures in inshore areas and therefore this would seem to indicate that these cod must be moving offshore where salinities of 34 to 35‰ can be found.

Northern cod seem to have undergone changes in distribution during the 1990s (de Young and Rose 1993; Rose *et al.* 1994; Kulka *et al.* 1995; Atkinson *et al* 1997). This shift has generally been towards the south of the 2J3KL area and to deeper offshore water. Also, as cod have been disappearing from offshore, large aggregations of inshore cod have been found (Anderson and Rose 2001). There has been some speculation that cod previously living in the offshore moved inshore and ceased their traditional migration during the fall (Taggart 1996). Otolith $\delta^{18}O_{oto}$ records indicate that it is possible that some of these cod, now living inshore, had at one time lived in the offshore. All of the inshore otoliths examined were from cod captured in 1996, which makes it difficult to assess whether these shifts are a regular feature of inshore otoliths or represent an aberration specific to the late 1980s and

early 1990s. The behaviour exhibited by these cod is also not specific to a certain year because these increases occur during different calendar years in different otoliths. It is possible that this is simply another type of ontogenetic change which simply indicates that these are a distinct population of cod.

7.5.3 Environmental monitoring

In analyzing otolith $\delta^{18}O$ lifetime records, one of the main purposes was to assess temporal changes in environmental conditions as experienced by the fish. During the mid 1900s, there appears to be very little variation in $\delta^{18}O_{oto}$ which shows that these cod were living in relatively consistent conditions. It is only during the 1970s to 1990s that $\delta^{18}O_{oto}$ variation becomes evident. Both the adult mean $\delta^{18}O_{oto}$ and the initial $\delta^{18}O_{oto}$ values decrease during this time as shown in Figures 7.10a and 7.10b. A clear peak is seen in the data for initial $\delta^{18}O_{oto}$ values in the early 1970s which was followed by a continuous decline to the late 1980s. The north Atlantic experienced a cold period during the 1970s which would be consistent with an increase in $\delta^{18}O_{oto}$. Mean adult $\delta^{18}O_{oto}$ values, however, decline slightly in the early 1990s but have no corresponding peak in the 1970s and therefore this change appears to only be affecting younger cod. Colder temperatures occurred during the mid-1980s (Colbourne *et al.* 1994) which is not consistent with this trend to decreased $\delta^{18}O_{oto}$. The decrease in initial $\delta^{18}O_{oto}$ is also very large (1.5 to 2‰) and seems more

likely to represent a change in salinity related to habitat than simply variation in temperature at one location. It may therefore, represent some change in the area of recruitment for this population, possibly with more cod coming from lower salinity areas. The shift towards lower maximum $\delta^{18}O_{oto}$ does not seem particularly significant because there are very few data at the time of the shift. If valid, this trend would again indicate lower salinity and higher temperature. The southerly shift in cod distribution could explain this observation. The findings are not consistent however, with the observation of cod moving towards the shelf edge which would have higher salinity values. Clearly, more data are needed to assess the importance of these apparent temporal trends.

7.6 Conclusions

This study has demonstrated that ontogenetic changes in habitat have a clear influence on $\delta^{18}O_{oto}$. Initial $\delta^{18}O_{oto}$ values seem to vary both between areas and temporally. All of the offshore cod otoliths however, experience increasing $\delta^{18}O$ over the first few years of life and seem to converge upon similar maximum $\delta^{18}O_{oto}$ values. We have shown that this can only be accomplished by increasing the salinity conditions and therefore this indicates a preference for the warm, high salinity waters at the edge of the shelf in adult cod. These results demonstrate the power of combining otolith $\delta^{18}O$ analysis with analysis of the variations of salinity and

temperature within a study area for detailed examination of lifetime $\delta^{18}O_{oto}$ records. Using this combination we can also say that there are two or possibly three different populations of cod in this area. Inshore cod do not exhibit the same clear ontogenetic shifts observed in the offshore. While they do increase early in life they later decrease in $\delta^{18}O_{oto}$ values as adults. This likely represents an excursion into the higher salinity waters offshore and later return to the inshore, but the exact reason for these movements is unknown. A third population of cod may exist in very deep water offshore at the edge of the shelf. The $\delta^{18}O_{oto}$ records of these cod do not resemble any of the other groups. Further analyses will be required to determine if this is the case.

The observed temporal variations in the initial and mean adult $\delta^{18}O_{oto}$ values of offshore cod during the later part of the 1900s seems to indicate that there was some fluctuation in habitat used by cod. This change is particularly evident in young cod and may therefore represent some change in habitat and perhaps recruitment behaviour for this population. It seems less likely to represent a change in the actual environment conditions as this would be out of the range of observed temperature or salinity anomalies in this area. In light of the ontogenetic changes in $\delta^{18}O_{oto}$ observed in this study, the importance of using lifetime $\delta^{18}O$ records versus whole otolith analysis is emphasized. Whole otolith analysis would potentially average the isotopic composition resulting from many potential habitats and therefore obscure any true environmental change.

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CHAPTER EIGHT

Conclusions

In this study of Atlantic cod otoliths I have examined factors which may influence the otolith isotopic composition and then moved on to examine otolith records of the northern cod stock. Otolith carbon is a complicated non-equilibrium mixture of metabolic and environmental carbon sources. In order to interpret $\delta^{13}C$ records, there must be a clear understanding of the biological and environmental factors which may influence this carbon signature. On the other hand, the oxygen isotopic composition is related only to the temperature and the $\delta^{18}O$ composition of the precipitating fluid by a relatively well known relationship. Understanding the composition of the water, however, is not as straightforward as it might appear. Seawater $\delta^{18}O$ can be affected by the interaction of water masses with their own characteristic $\delta^{18}O$ and salinity. It becomes necessary therefore to study all these factors before undertaking the analysis of otoliths to examine questions of environmental change. The complicated nature of otolith $\delta^{13}C$ and $\delta^{18}O$ signatures can however, add to the information that can be obtained from them.

The carbon isotopic signature of otoliths has been demonstrated to be a mixture between metabolic carbon and carbon from the dissolved inorganic carbon

in the environment. Changes in this composition with age seemed to indicate that ontogenetic dietary change might be important, but this had never been clearly demonstrated before. A significant contribution of this study has been to show that this is indeed an important contribution to the lifetime otolith $\delta^{13}C$ signature. The observed change in $\delta^{13}C$ of cod muscle tissue is parallelled by a ^{15}N enrichment in muscle nitrogen which clearly demonstrates the action of a trophic effect. Understanding the effect of ontogenetic diet change on metabolic carbon then allows us to analyze the effect on otoliths.

Studies of trophic level enrichment of isotopes have been widely used to study ecosystem interactions. This type of study had not, however, been undertaken to specifically examine the change in trophic level associated with ontogenetic diet shift. Also, few food web studies had included cod. The results presented in this study are the most extensive isotopic examination of cod to date. They have revealed not only the continuous trophic level change related to ontogenetic diet shift, but also the potential input of different sources of carbon at the base of the inshore food web. While most marine food webs are generally based on phytoplankton, it has been shown that extreme increases in $\delta^{13}C$ values can occur in juvenile cod related to inputs of seagrass or macroalgae at the base of this inshore food web. After cod have grown beyond approximately 35 cm, they reach their highest trophic level indicating a relatively consistent diet between all fish. Understanding these shifts in feeding behaviour and their effect on isotopic composition may provide an additional method

of monitoring energy flow in these ecosystems and how environmental change may impact them.

Relating these changes in the metabolic component to otolith $\delta^{13}C$ was the next important step undertaken in this study. Past otolith studies have attempted to quantify the metabolic input, but none have actually measured both the otolith $\delta^{13}C$ and metabolic carbon $\delta^{13}C$ from the same fish and attempted to explain ontogenetic changes in otolith $\delta^{13}C$. Doing this has allowed us to show that most of the increase in otolith carbon is a result of diet shift with age. In turn, this allows us to quantify the input of metabolic carbon and to what extent the importance of this component varies with age. The metabolic component must decrease with age by approximately 20%. In addition to the important information this provides for studies of environmental questions using otoliths, this knowledge also indicates that otoliths can be used to examine variations in metabolic rate for different fish populations.

This study was the first to examine otoliths from the northern cod stock. It was hoped that examining these otoliths would give some insight about how cod might be responding to changes in their environment. The collection of otoliths used for this investigation spans a period of over sixty years, allowing a long-term examination of changes in otolith signatures. There was a remarkable similarity between the trends for all of the otoliths studied, irrespective of area or time. Ontogenetic change in diet is undoubtedly an important factor determining the

individual lifetime $\delta^{13}C$ records. Juveniles from different areas, however, seem to be feeding at different trophic levels. There also appears to be a temporal variation towards lower $\delta^{13}C$ values in cod from the mid-1980s. No clear correlation was found between otolith $\delta^{13}C$ values and location, depth, or age which could explain this shift. It seems, however, that this may indicate some shift which may be related to changes in the environment.

The survey of seawater $\delta^{18}O$ carried out for the Newfoundland and Labrador Shelves is the first such large scale survey carried out in this area. It has clearly demonstrated that while there is a very strong linear correlation between $\delta^{18}O_{sw}$ and salinity, large shifts in $\delta^{18}O$ and salinity can occur across the shelf. These variations must be taken into account when analyzing otolith $\delta^{18}O$ records. Large differences in $\delta^{18}O$ and salinity can also however, be used to examine migration and population structure. Other complications in the $\delta^{18}O$ - salinity relationship have also been revealed in this study. At very high salinity, $\delta^{18}O$ varies with little or no coupled salinity change.

Examining the otoliths from cod of the northern cod stock has revealed that ontogenetic shifts in habitat play a clear role in determining the lifetime record of otolith $\delta^{18}O$. This demonstrates that otolith oxygen can be used not only to determine changes in temperature experienced by fish, but can also be used to examine changes in the behaviour of juvenile fish. Cod appear to be moving between lower salinity

water inshore and higher salinity water on the shelf as they grow which is generally consistent with the known habits of cod. The $\delta^{18}O$ records also identify three populations of cod with the area. Inshore cod have very different lifetime signatures when compared with offshore cod. There is also much more variability between the inshore cod records. Offshore, there appears to be another population living in very deep water at the edge of the shelf. This is confirmed by both the oxygen and carbon data.

Otolith $\delta^{18}O$ records have not identified an unequivocal link between the cold temperatures experienced during the early 1990s and changes in the stock although temporal variations in $\delta^{18}O$ are evident. Mean adult and juvenile $\delta^{18}O$ values seem to decline during the later decades of the 1900s. The decline in mean adult $\delta^{18}O$ is only evident in the early 1990s, but the decline in juvenile $\delta^{18}O$ is clear from the early 1970s. This change indicates that juvenile cod must be living in different areas and may indicate a change in recruitment for the stock. Changes in adult values make a stronger case of environmental impact on the northern cod, and may be linked to a southerly shift in the distribution which is thought to have occurred because of colder temperatures in the early1990s.

Only a handful of studies have fully utilized the zoned nature of otolith structure to look at lifetime variations in the $\delta^{13}C$ and $\delta^{18}O$ records. It is evident from the results obtained here that micromilling is an important advance in otolith studies.

Analyzing whole otoliths or low resolution sampling would likely overlook many of the important ontogenetic changes recorded by these growth zones and observed in this study. It is also clear that it is important to compare equivalent portions of the otolith when examining environmental changes. A whole otolith analysis represents an integrated life signal, incorporating both the juvenile signal which tends to be light and the adult signal which tends to be heavy.

Future Work

This study has made great progress in revealing the controls of isotopic composition of otoliths. There are still however, some unanswered questions which future work will need to address. To interpret otolith isotopic records, it is necessary to understand whether small scale fluctuations seen in these records are a result of environmental or some other biological factor. While the $\delta^{13}C$ records tend to be consistent, interannual fluctuations in $\delta^{18}O$ can be quite large.

The carbon record has been positively linked to changes in diet. While this study has shown that ontogenetic changes in diet are important, we did not undertake analysis of the possible food sources for these particular cod. Constructing a clear food web in this area and understanding the distribution of $\delta^{13}C$ values between different prey will help us to understand the potential fluctuations in the metabolic component. The ^{13}C enrichment of carbon was also very large with respect to the normally observed trophic enrichment. This may be related to physiological factors

which are specific to cod. This may have some bearing on otolith $\delta^{13}C$ signatures as well.

While a thorough study of the metabolic component of otolith $\delta^{13}C$ was undertaken, variation in the dissolved inorganic carbon pool was not analyzed. Most studies of DIC have looked at ocean scale variation in $\delta^{13}C$. Smaller scale variation may be important to the otolith records of cod living in a particular area.

Our survey of seawater $\delta^{18}O$ revealed that the correlation between salinity and $\delta^{18}O$ could potentially be a powerful method of tracing cod movement. This survey did not, however, extend into the inshore bays in the area. These bays seem to be important nursery areas for juvenile cod. A study of the variation of seawater $\delta^{18}O$ in these areas may add important information to our present knowledge and perhaps reveal a method for identifying cod which have been recruited from different areas of the inshore.

The variation seen in both the carbon and oxygen otolith records indicates that it will be necessary to analyze large numbers of otoliths to determine if the observed temporal changes are truly related to environmental change. This along with increased knowledge of the factors influencing otolith isotopic composition has the potential to make otoliths an extremely useful tool for environmental monitoring.

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