STABLE ISOTOPE ANALYSES IN OTOLITHS OF COD (*Gadus morhua* L., 1758): IMPLICATION FOR LONG-TERM ENVIRONMENTAL CHANGES IN THE CANADIAN ATLANTIC

By

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ABSTRACT

Stable isotopic analysis has been widely used in study of proxy materials with concentric layers, such as fossil shells, corals, and tree rings, and has recently begun to blossom in fish otoliths. This study describes some new developments of stable isotopic analyses in otoliths of cod (*Gadus morhua*) from the northeast Scotian Shelf, Nova Scotia, and demonstrates how powerful those analyses are in study of marine environmental changes.

It is known that the relationship between oxygen isotope ($\delta^{18}O_W$) and salinity is very useful in study of coastal settings, especially for study of the dynamic processes, water currents and possible sources of mixing, and the estimation of isotopic composition in paleo-oceans. In this study, I found that the best equation for describing this relationship in the 4Vs area is: $\delta^{18}O_W = 0.524S - 18.38$. I also found that this relationship varies vertically with water depth, especially at depths greater than 200 m. The oxygen isotopic records in 4Vs over the last 20 years show that the variation trend for surface water was steadily more negative in $\delta^{18}O_W$ and lower in salinity. However, at depths greater than 200 m, a warmer water mass appeared around 1985, indicating that some distinct environmental events happened during that period.

Sagittal otoliths of cod were analyzed based on seasonal translucent (winter) and opaque (summer) aragonite zones, by a newly-established micromilling technique (*DM 2800*). The $\delta^{18}O$ values range from 0 to +3.0 ‰, with minor isotopic variations between seasons. However, there were significant differences in the growth of the first-year, indicating that juvenile cod are more active than adults at the early stage of their life history. Comparing opaque otolith zones of the 4-5 year group from different years of capture, the secular isotopic variation gradually increased by about 1 ‰ over the period of 1984-94, corresponding to a temperature decrease in the surrounding seawater of about 5 ^{0}C. This result is in agreement with the data from summer groundfish trawling survey and with other independent investigations. Based on the $\delta^{18}O$ values in otoliths (greater or less than 0.75 ‰), two types of cod in 4Vs were recognized. It suggests that about 20 % of the total cod were recruited from the Gulf of St. Lawrence (migratory) where the $\delta^{18}O$ was relatively lower, whereas the other 80 % were indigenous to the 4Vs area. Such a classification may have the potential for using $\delta^{18}O$ signals from different water masses to study recruitment and migration of cod, and possibly other fish species.

The life-long $\delta^{13}C$ variation in otoliths of cod shows a consistent increase during the first 4 to 6 years, from the minimum values between -5 and -2.5 ‰ to a maximum near 0 ‰. The early rise in $\delta^{13}C$ is partly due to the fraction of metabolic HCO_3^- in the cod's blood, and partly due to a dietary shift or trophic effect. The maximum in $\delta^{13}C$ is attained at an age (4-5 y) close to that of the age of maturity, and used to determine the age of sexual maturity of the 4Vs cod. Furthermore, the maximum $\delta^{13}C$ variation attained by otoliths is in synchrony with the coeval temperature decrease in seawater from 1984 to 1994, during which the cod stocks experienced a drastic decline in the Canadian Atlantic.

In this study, I also analyzed otoliths of known-history Norwegian cod from experiments. These cod were reared in ocean-pens for as long as 6 years, and provide a unique opportunity to examine their isotopic variations and environmental influences. Comparing these to otoliths from the 4Vs field cod, there are distinct differences in the range of isotopic composition, the life-long variations in $\delta^{18}O$ and $\delta^{13}C$, and the degree of correlation between $\delta^{18}O$ and $\delta^{13}C$. These differences probably arise from the living environmental constraints and food supply in the two different settings. The isotopic temperature estimation was very close to the records during rearing, suggesting that temperature is a controlling factor in the forming of seasonal otolith zones.

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Title: Spatial and temporal variations of the oxygen isotope-salinity relationship: Implications for environmental changes in the northeast Scotian Shelf

Authorship: Y. W. Gao, H. P. Schwarcz, U. Brand, P. M. Strain and G. L. Bugden

Status: Submitted to Earth and Planetary Science Letters

Candidate's contribution: All seawater sampling, laboratory analyzing, and paper writing were performed by the candidate, with comments and editorial input by the co-authors.

Chapter 3

Title: Oxygen isotopic variations in otoliths of cod (*Gadus morhua*): A potential thermometer and migration indicator in the Canadian Atlantic

Authorship: Y. W. Gao, H. P. Schwarcz, U. Brand and S. E. Campana

Status: Submitted to Geochimica et Cosmochimica Acta

Candidate's contribution: All otolith sampling, laboratory analyzing, and paper writing were performed by the candidate, with comments and editorial input by the co-authors.

Chapter 4

Title: Carbon isotopic variations in otoliths of cod (*Gadus morhua*): Effects of maturation and environmental change

Authorship: H. P. Schwarcz, Y. W. Gao, S. E. Campana, D. Browne, M. Knyf and U. Brand

Status: Submitted to Canadian Journal of Fisheries and Aquatic Sciences

Candidate's contribution: All otolith sampling, laboratory analyzing and diagram preparing were performed by the candidate. The paper was written by the first author.

Chapter 5

Title:	Oxygen and carbon isotopes of known-history otoliths of Norwegian cod (*Gadus morhua*), in comparison with the field cod from the northeast Scotian Shelf
Authorship:	Y. W. Gao, H. P. Schwarcz, U. Brand and S. E. Campana
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CHAPTER ONE

Introduction

1.1 Overview and objectives

People in Maritime Provinces have a long-standing interest in cod (*Gadus morhua*). In the early 1990's, however, the cod fisheries suddenly collapsed to the detriment of many fishermen in Newfoundland. There is little agreement on the cause of the cod stock collapse. Nevertheless, from the biological point of view, at least three dominant factors might account for the decline of cod: (1) seals eating cod; (2) overestimating the stocks and consequent overfishing; and (3) marine environmental changes. It is the principal objective of the present study to deal with the third factor in the Canadian Atlantic, by using stable isotopic analyses on otoliths, or "ear stones" in cod.

Otoliths are mm-sized, pearl-like structures deposited in bony fish's inner ears. Three pairs of otoliths, namely sagittae, lapilli and asterisci, function mainly as an equilibrium system and sound detector (Moyle and Cech, 1988). Of these otoliths sagittae are the largest and are routinely used for aging in fisheries (Beamish and McFarlane, 1987). All otoliths are composed of pure aragonite ($CaCO_3$), and their total organic matter ranges from 0.2 to 10% (Degens et al., 1969). Comparison between fish otoliths and molluscan shells shows that both have similar constituents of organic matrix and calcium carbonate, and a similar mode of growth (Pannella, 1971). Therefore, many research advances in carbonate shells can be applied to fish otoliths, particularly in the theory and practice of stable isotopes.

The principle of using stable isotopes in otoliths is rooted in Urey's (1947) hypothesis that calcium carbonates are precipitated in oxygen isotopic equilibrium with the surrounding waters in which the organisms lived, and thus the isotopic temperature is given as a function of the difference between $\delta^{18}O$ in calcite or aragonite and $\delta^{18}O_W$ in seawater (Epstein et al., 1953). The first attempt at stable isotopic analyses in fish otoliths was by Devereux (1967), whose data suggested that the habitat of fish can be deduced from oxygen isotope ratios. Further investigations confirm that the mineralogy of fish otoliths is aragonite, and that the isotopic interpretation requires a careful assessment of the biological record (Degens et al., 1969). The carbon isotopes in fish otoliths, in general, are considered to be in disequilibrium with ambient seawater (Mulcahy et al., 1979), and the increasing amount of $\delta^{13}C$ reflects a slowdown of metabolic activity with age. These early studies have provided the cornerstones for stable isotopic analyses in fish otoliths, and are far from out-of-date.

More recent studies have extended our horizons and gained insights into many more fish species (Radtke, 1984; Nelson et al., 1989; Kalish, 1991; Northcote et al., 1992; Iacumin et al., 1992; Patterson et al., 1993; Gauldie et al., 1994; Radtke et al., 1996; Thorrold et al., 1997; Schwarcz et al., 1997; Gao et al., 1997a, 1997b). It is accepted that teleost otoliths are deposited at, or very close to oxygen isotopic equilibrium with the ambient waters (Kalish, 1991; Patterson et al., 1993; Thorrold et al., 1997), and that the "vital effect" is not important. However, two problems deserve to be raised: (1) most stable isotopic analyses have been carried out on whole otoliths and little has been achieved on seasonal otolith zones; (2) the potential of using stable isotopes for temporal (in terms of decades) and spatial (recruitment and migration) analyses has been thoroughly examined in laboratory experiments, but little has been done from historical otolith collections. Therefore, the present study will address the first problem by using a computer-driven micromilling technique for seasonal sampling, and focus on the second by examining the field otoliths of cod from the northeast Scotian Shelf, Nova Scotia.

1.2 The basic habits and characteristics of cod

Atlantic cod (*Gadus morhua*) is one of 59 species of the family Gadidae (Lear, 1993), with large head and wide gill openings. Adults are about 60 to 70 cm in length and 2 to 3 kg in weight. The cod spawns in winter and is basically a cold-water species. Mature fish do not occur in quantity in water with a temperature above 10 ^{0}C (Leim and Scott, 1966). Theoretically, cod can live in temperatures ranging from -2 to +20 ^{0}C, but they seem to prefer temperatures between 0 and 11 ^{0}C (Jensen, 1972). In addition, cod usually swim near or sometimes even touching the ocean floor, but they are found most often in depths of from 40 to 270 m (Jensen, 1972). Cod are sensitive to water temperature changes of about 0.05 ^{0}C. If the water temperature is too low or too high they grow sluggish and may die. It might be the decline in water temperature and the subsequent period of cold that triggers the spawning urge, or the preference for certain water temperature that drives cod's migration (Jensen, 1972).

It is well known that cod grow at different rates in different areas. There are also annual differences of growth in the same area depending on water temperature, salinity, availability and quality of food, population stress, and so on (Jensen, 1972). In the Canadian Atlantic, cod's distribution has been divided into different stocks, each stock with recognizable characteristics. In this project, we chose cod of the northeast Scotia Shelf or 4Vs stock as the target (cf. Chapter 3).

Cod are voracious creatures. As young cod, they eat copepods, barnacle larvae, amphipods, and other small crustaceans (Leim and Scott, 1966). Juvenile cod eat shrimp, amphipods, euphausiids, and fish and shellfish larvae. Adult cod feed mainly on capelin, herring, sand launce, flounders, crabs, shrimp, and the host of other species of fish and shellfish (Lear, 1993).

In brief, the habits and characteristics of cod as described above provide possibilities for us to use stable isotopes in detecting population dynamics and the fish's behavior, and thus extracting environmental information from isotopic analyses.

1.3 Thesis format

This thesis adopts the format where each chapter represents a paper either in press or in the process of publication. It does not include all the work in the research, such as the trace elemental analysis, although that is part of the project. The remainder will be prepared for publication later with almost the same principal authorship in identifying the co-authors' contribution to the study.

As a basis and background information for paleotemperature studies, Chapter 2 defines the relationship between oxygen isotope and salinity in the 4Vs area, which can be used for the best estimation on $\delta^{18}O_W$ in the oceans. Chapter 3 documents the methodology for seasonal otolith microsampling and oxygen isotopic variations over the past decade, suggesting that the oxygen isotopic signals can be potentially used as a thermometer and migration indicator in marine environmental studies. Chapter 4 demonstrates that carbon isotopic variations can provide a means by which to determine the age of maturity of cod, and that the maximum $\delta^{13}C$ values attained by otoliths decrease with the coeval decline of the 4Vs cod stock. Chapter 5 extends the isotopic analysis to known-history otoliths of Norwegian cod, indicating that the distinct differences in both $\delta^{18}O$ and $\delta^{13}C$ variations between the pen-reared and 4Vs field cod are probably due to the different living environmental constraints and food supply.

CHAPTER TWO

Spatial and temporal variations of the oxygen isotope-salinity relationship in the northeast Scotian Shelf

2.1 Abstract

The relationship between oxygen isotope ($\delta^{18}O_W$) and salinity is a useful but controversial oceanic parameter in the paleotemperature studies. In addition to the geographic variations, the relationship was also found to vary with the water depth, particularly in the water column deeper than 200 m. The oxygen isotopic records over about 20 years in 4Vs seawater showed that the trend of surface variation was more negative in $\delta^{18}O_W$ and lower in salinity. At depths greater than 200 m, however, a warmer water mass appeared around 1985, indicative of some environmental events happening during that period. Overall, the best equation describing this relationship in 4Vs, from the surface to a depth of about 500 m, can be expressed as: $\delta^{18}O_W = 0.524S - 18.38$. The long-term isotopic comparisons at different depths provide meaningful information on secular changes for 4Vs, and are in good agreement with the data from other independent investigations.

2.2 Introduction

The oxygen isotope-salinity relationship plays an important role in oceanographic studies. It can be used for: (1) identifying the binary mixing processes from the different sources of marine and fresh waters, assuming that the isotopic composition and the salt concentration of both end-members are fixed (Epstein and Mayeda, 1953; Craig and Gordon, 1965; Fairbanks, 1982; Tan and Strain, 1988); (2) determining the concentrations of sea-ice meltwater and meteoric waters quantitatively in polar circulation studies (Redfield and Friedman, 1969; Tan and Strain, 1980), and the seasonal variation in the upper water column (Strain and Tan, 1993); and (3) estimating the oxygen isotopic composition of seawater ($\delta^{18}O_W$) for calculations of isotopic temperature, because the fractionation of oxygen isotopes between carbonate and seawater is temperature dependent (Epstein et al., 1953; Grossman and Ku, 1986). It is the principal aim of the present study to examine the last application because the $\delta^{18}O_W$-salinity relationship is a major source of errors in reconstructing the thermal history of paleo-oceans. We can measure the $^{18}O/^{16}O$ ratios of many calcite or aragonite proxies (e.g., fossil shells, corals, fish otoliths), but we have no direct means of measuring the $\delta^{18}O_W$ in past oceans. A common approach is to estimate the $\delta^{18}O_W$ from the relationship of oxygen isotope-salinity in known areas. Nevertheless, due to the fact that the $\delta^{18}O_W$-salinity relationship varies from area to area, and from depth to depth in the same water column (Craig and Gordon, 1965; Tan and Strain, 1980; 1988), such an estimation usually encounters many challenging problems.

We are at present studying the variations in paleotemperature as obtained from $\delta^{18}O$ variations in otoliths of cod (*Gadus morhua*). We are concerned that some of the apparent variations in paleotemperature of cod otoliths may be due to salinity-correlated variations in mixing of fresh and sea waters. Using the oxygen isotopic tool, we hope to establish the long-term temperature profile in the northeast Scotian Shelf, or 4Vs area in particular. Thus, it is necessary to clearly define the $\delta^{18}O_W$-salinity relationship in the area, and to use it for the best estimation on $\delta^{18}O_W$ in the past. Fairbanks (1982) reported a $\delta^{18}O_W$-salinity relationship for the Scotian Shelf as:

$$\delta^{18}O_W = 0.442S - 15.55 \qquad (1)$$

from 4 samples. Tan et al. (1988) reported a $\delta^{18}O_W$-salinity equation from Browns Bank ($r^2 = 0.98$; $n = 19$):

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

but did not give detailed relevant information. There are also historic collections on stable isotopic data in the database of the Bedford Institute of Oceanography (BIO) for 1976 and 1985 in the same area, which makes it possible to use the $\delta^{18}O_W$ measurements to evaluate the long-term isotopic variation in the seawater directly, but these samples are mainly scattered along the Laurentian Channel and concentrated around Cape Breton Island.

Therefore, the primary objective of the present research is to examine the relationship between $\delta^{18}O_W$ and salinity in the 4Vs area, in an attempt to define the correlation which could be used for estimating the past $\delta^{18}O_W$ in our otolith studies; the secondary goal is to compare the isotopic record over the last 20 years (1976, 1985 and 1996) to see if there were any secular variations at the 4Vs water column, and to try to link these changes to the causes of decline in cod stocks.

2.3 Materials and methods

Seawater samples analyzed for the present study were collected on BIO Cruise 96-014 aboard CSS Hudson, from June 22 to 28, 1996. The cruise track and sampling locations are presented in Figure 2.1. Stations from 2 to 19 of the cruise were taken in the Laurentian Channel while stations 22-28 were a cross section over the major fishing banks in 4Vs. Some scattered samples from the southwest Scotian Shelf were also collected for comparison and reference, but are not presented here (Fig. 2.1). The depth interval of sampling for each station was from 25 to 50 m in the upper water column, and every 100 m below 200 m depth. Other background information such as temperature, salinity, and dissolved oxygen were taken by CTD (Conductivity, Temperature and Depth; Pederson, 1984) *in situ*. Immediately after the sampling bottles (10 L) came aboard, 20 mL subsamples of seawater were stored in glass bottles, tightly capped and then sealed with a 3M Scotch™ vinyl electrical tape. These seawater sample bottles were kept refrigerated and in dark boxes until oxygen isotopic analysis.

Samples were analyzed for their $^{18}O/^{16}O$ ratios in the Stable Isotope Laboratory of the Department of Geology, McMaster University. Small seawater samples of 0.2 mL were prepared in groups of six glass tubes using a procedure modified from Epstein and Mayeda (1953). The samples were equilibrated with CO_2 in the 25 ^{0}C water bath for at least one week, and were then analyzed on a VG Optima mass spectrometer. All results are expressed with respect to standard mean ocean water (SMOW; Craig, 1961) in the common

$\delta^{18}O$ notation. The overall analytical precision, as determined by 6 replicate analyses as an internal reference standard for every two groups of tubes (12 samples), is 0.15 ‰ (standard error).

2.4 Results and discussion

The results of seawater analyses in this study are summarized in Table 2.1. From surface to deep waters, both salinity and oxygen isotopic contents increase monotonically, while the temperature gradient shows a minimum which is known as the intermediate cold layer between 50-150 m (Bugden, 1991). The mean $\delta^{18}O_W$ value of the 4Vs surface water was -2.19 ‰, corresponding to a mean salinity value of 30.7 psu (practical salinity unit; Lewis, 1980). At depths of greater than 200 m (mainly along the Laurentian Channel) the mean $\delta^{18}O_W$ values reached -0.02 ‰ and the mean salinity reached 34.8 psu, very close to the normal mean isotopic composition of world seawater (Craig and Gordon, 1965). However, the surface $\delta^{18}O_W$ values in 4Vs from this study are more ^{18}O-depleted than those of the nearby Gulf of St. Lawrence (-1.8‰; Tan and Fraser, 1976), indicating that the freshwater sources between these two localities might be different. As presented in Figure 2.2, all our samples from the surface water to the depth of about 500 m can be filtered to a single linear correlation ($r^2 = 0.92$; n = 73):

$$\delta^{18}O_W = 0.524S - 18.38 \qquad (3)$$

which would be the best equation over the entire 4Vs area and averaged over all depths. It is not surprising that equation 3 differs from equation 1 because of the regional and sampling differences. However, Tan et al.'s (1988) equation 2 is very similar to the equation we obtained, although the sampling locations and depths were not specified in their paper. As we shall see, however, equation 3 obscures significant variations in the $\delta^{18}O_W$-salinity relationship that occur as a function of depth.

2.4.1 $\delta^{18}O_W$ tracer on water sources

Extrapolation of Figure 2.2 to zero salinity gave a $\delta^{18}O_W$ value for the fresh water of -18.5 ‰. It is known that the $\delta^{18}O_W$ value of the St. Lawrence river is -10.3 ‰, which would be too ^{18}O-rich to produce equation 3 through mixing with the Laurentian Channel deep warm water (+0.15 ‰; Tan and Strain, 1988). Waters forming in the Labrador Sea at higher latitudes appear to contain a much lighter $\delta^{18}O_W$ freshwater component (-22 ‰, Tan and Strain, 1980) which would qualify as a source candidate for the 4Vs waters.

Waters obtained at different stations within 4Vs appear to be mixtures of about the same seawater with freshwaters of markedly different $\delta^{18}O_W$ values. Figure 2.3 shows some representative stations in the present study. Station 24 had a $\delta^{18}O_W$ value of -13 ‰, very close to Station 2 (-15 ‰) which was located in the mouth of Gulf of St. Lawrence. Station 22 in the margin of the continental shelf, however, gave a $\delta^{18}O_W$ value of -20.5 ‰. Much lighter $\delta^{18}O_W$ values in 4Vs region were also seen in Station 11(-25 ‰), far north from the major fishing banks (Fig. 2.1). These $\delta^{18}O_W$ intercept values convince us, therefore, that the freshwater component of the 4Vs waters might have multi-sources which include the local Gulf inputs, the Laurentian Channel deep water, and a source from the Labrador Sea. Bugden (1991) noticed that in the region east of the Scotian Shelf variations in temperature-salinity were generally related to location. He concluded that the deeper waters of the Laurentian Channel were made up of a mixture of Labrador and North Atlantic waters in varying proportions. Drinkwater (1996) gave a detailed description on the general circulation of water flows in the Northwest Atlantic. From his results we can see that the near-surface circulation pattern in 4Vs consists of Labrador Current, the outflow from the Gulf of St. Lawrence, and the northeastward Gulf Stream. Our isotopic analyses, therefore, are in good agreement with those independent investigations.

2.4.2 Vertical variation or depth effect

The vertical variations of the $\delta^{18}O_W$-salinity relationship show that, in the upper water column, the correlation is generally good, with the surface $\delta^{18}O_W$ values more negative than those in the deeper (Fig. 2.4a). There was a sharp change at depths greater than 200 m corresponding to salinity about 33 psu (Fig. 2.4c). Although the correlation between $\delta^{18}O_W$ and salinity was much improved (r^2=0.81) the slope was greatly changed. Thus from the surface to about 500 m, the $\delta^{18}O_W$ values were gradually getting heavier, the salinity getting higher and the slope of the correlation getting larger or vertical (Fig. 2.4). This is not surprising because at depths greater than 200 m salinity was almost static (cf. Table 2.1). Consequently, if we use the salinity and equation 3 to estimate the $\delta^{18}O_W$ of the past, the isotopic temperature will be suspect. Specifically in our project, since fish are mobile creatures we cannot expect that cod always live in certain layers of the water column, even though they are called "groundfish". This would be another source of error in reconstructing the paleo-temperature profile. Fig. 2.5 demonstrates a rough comparison between calculated isotopic temperatures in 1985 for cod otoliths (*Gadus morhua*) and the measurements *in situ* in the 4Vs area, assuming that the fish lived at the same depths as they were captured, and laid down the opaque otolith zones (summer) in the same year. In general, the calculated isotopic temperatures were higher than those from the summer survey, in particular at depths less than 100 m. As the depth approaches 200 m, the mean isotopic temperatures approximate the survey data (Fig. 2.5). The example strongly suggested that, in addition to considering geographical differences, the vertical variation of the $\delta^{18}O_W$-salinity relationship, or simply the depth effect, should also be taken into account when using salinity in estimating $\delta^{18}O_W$ values which were affected by stream or meltwater inputs.

The weak $\delta^{18}O_W$-salinity correlation at depths greater than 200 m could be replaced by other chemical tracers. In comparison of the vertical variation profiles in 4Vs for $\delta^{18}O_W$, Sr and salinity, the isotopes are more consistent with Sr contents than those of salinity at depths greater than 200 m. This suggests that in the deeper water column Sr concentrations might be a better tracer in relation to $\delta^{18}O_W$ estimation, and more importantly, we can measure Sr values from fish otoliths directly.

2.4.3 Secular changes for 4Vs

Comparing the oxygen isotopic data from BIO database in the same area (Fig. 2.6), the slope of the 1976 data is quite similar to the present study, but with an offset of about 1 psu in salinity. The 1985 data, however, show a distinctly different $\delta^{18}O_W$-salinity correlation. The values for the mid-depth at about 50 m break away from the main trend (middle right in Fig. 2.6). The oxygen isotopic variation for about 20 years (time lag about 10 years) and at different depths was plotted in Figure 2.7. Since the $^{18}O/^{16}O$ ratios were measured from seawater directly, this comparison at different water depths would provide meaningful information on the secular changes in the Scotian Shelf. For the surface waters it demonstrates a gradual decrease in both $\delta^{18}O_W$ and salinity (Fig. 2.7a), with variations of about 2.5 psu in salinity and of about 1.5‰ in $\delta^{18}O_W$. However, as we examine the deeper water layers (e.g., >200 m), the trend is strikingly changed. In Figure 2.7c it is clearly seen that the 1976 and 1996 data overlap, but the 1985 data are distinctly different. This variation in the $\delta^{18}O_W$-salinity relationship can be interpreted as: (1) the surface shelf waters sank deeper into the continental slope so that the $\delta^{18}O_W$ values in 1985 deep waters were distinctly lighter; (2) warmer currents injected into the water column by environmental changes in the mid 1980's. The former is unacceptable because we have no evidence that 1985 year was unusual in precipitation or sea ice melting (Tan and Strain, 1988), and if sinking occurred the salinity would reach the maximum values (cf. Fairbanks, 1982). Thus, the distinct trend in the 1985 data would indicate that some environmental changes happened during or before 1985, corresponding to lower $\delta^{18}O_W$ and salinity values by about 1‰ and 3 psu, respectively.

Drinkwater (1996) reported that the temperature trends in the Canadian Atlantic diverged around 1985, and since then the bottom water (>200 m) became warmer. Although the causes of this temperature change are still in question, our $\delta^{18}O_W$ analyses are in good agreement with Drinkwater's interpretation, and suggest that the divergence may have begun a couple of years earlier. Schwarcz et al. (1997) investigated the $\delta^{13}C$ measurements from 4Vs cod and also found that the maturity age of cod as represented by a maximum in $\delta^{13}C$ of otoliths decreased abruptly between 1984 and 1985. All these studies combined suggest that the environmental change might be a factor in recent population dynamics of the 4Vs stock, and that this change might have started around 1985.

In summary, it may be helpful for us to use the $\delta^{18}O_W$-salinity relationship as a tracer in marine environmental studies because these isotopic variables have a direct link to the dynamics of seawater. Craig and Gordon (1965) even suggested that oxygen isotopes can be used as a routine oceanographic tool. Both salinity and $\delta^{18}O_W$ are influenced by evaporation, precipitation and seasonal changes as discussed previously. The magnitudes of these effects and their interrelation vary with water depths. Therefore, these variations must also be taken into account when using $\delta^{18}O_W$ values to calculate paleotemperatures.

2.5 Conclusions

From the present study we can make the following conclusions:

1) The best relationship between $\delta^{18}O_W$ and salinity in the 4Vs area can be expressed as: $\delta^{18}O_W = 0.524S - 18.38$. This equation can be used for estimating the $\delta^{18}O_W$ values in the past ocean, and thus the calculated isotopic temperatures were in good concert with the coeval survey data *in situ*;

2) The correlation between $\delta^{18}O_W$ and salinity varies as the water depths change. In the upper column in 4Vs this correlation is reasonably good; but at the water depths greater than 200 m the relationship changed sharply. This fact means that salinity is a conditional factor for estimating the $\delta^{18}O_W$ in the isotopic paleotemperature scales;

3) The oxygen isotopic records for the past 20 years from three data sets (1976, 1985 and 1996) show that for the surface waters the trend of variation was generally towards more negative in $\delta^{18}O_W$ and lower in salinity. At depths greater than 200 m, an abnormally warmer water mass appeared around 1985, and resulted in the formation of thermal divergence in the whole region. Such an event was probably related to regional environmental changes, and partly caused the decline of the cod stocks in the 4Vs area.

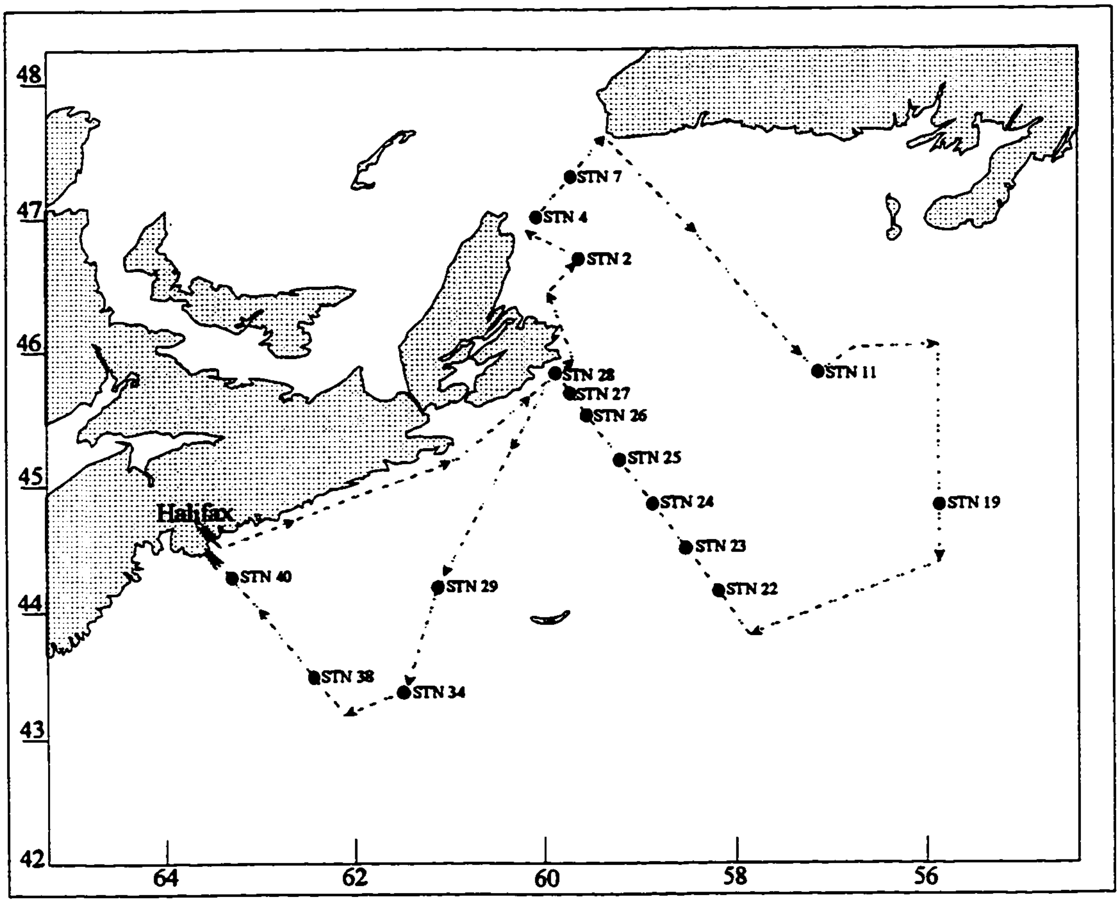

Fig. 2.1 Location map in the northeast Scotian Shelf, showing the cruise track and sampling stations for BIO Cruise 96-014, June 22-28, 1996.

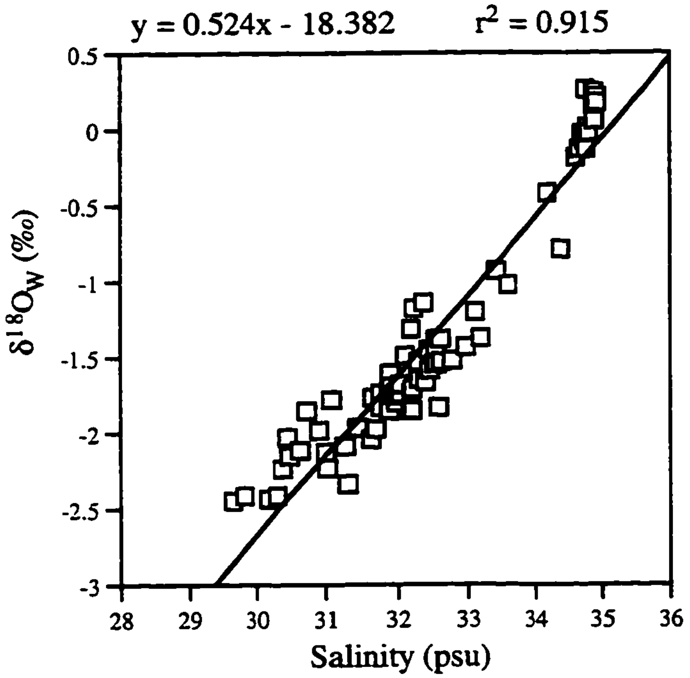

Fig. 2.2 Correlation between oxygen isotopes and salinity in the 4Vs area for 73 analyses from surface to about 500 m depth.

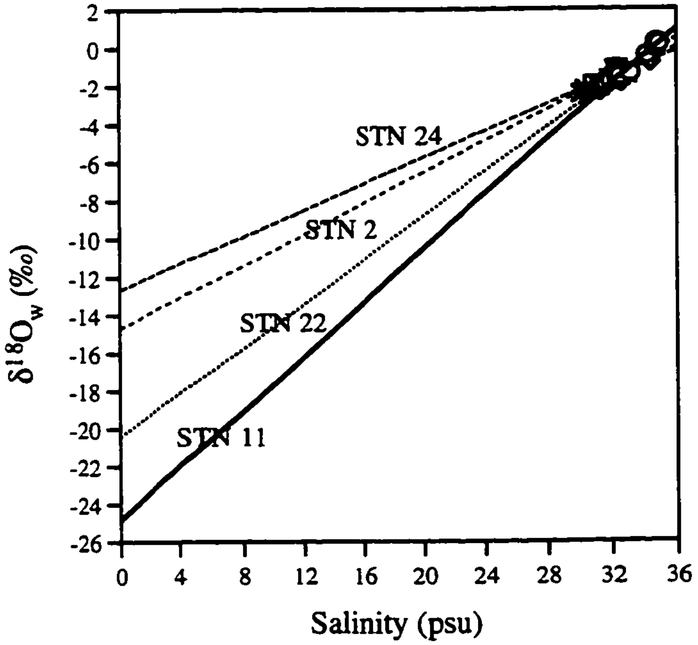

Fig. 2.3 $\delta^{18}O$ values and possible water sources by selecting representative stations in this study, showing gradual lighter $\delta^{18}O_W$ sources from the Gulf of St. Lawrence to the sea.

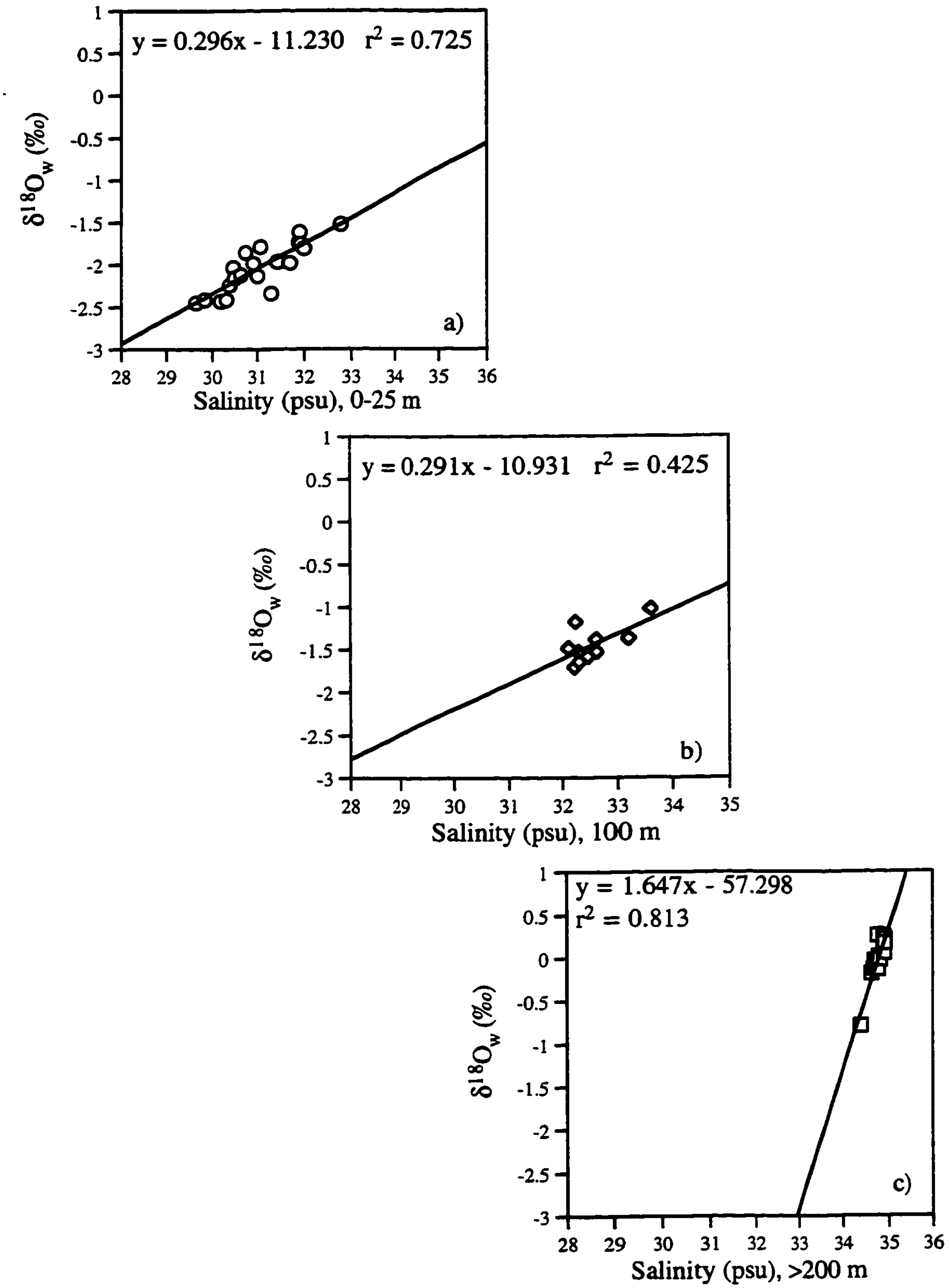

Fig. 2.4 Vertical variation of $\delta^{18}O_W$ and salinity from surface (a), 100 m (b) and the depths greater than 200 m (c), respectively. Note that at depths greater than 200 m the correlation slope changes because of static salinity.

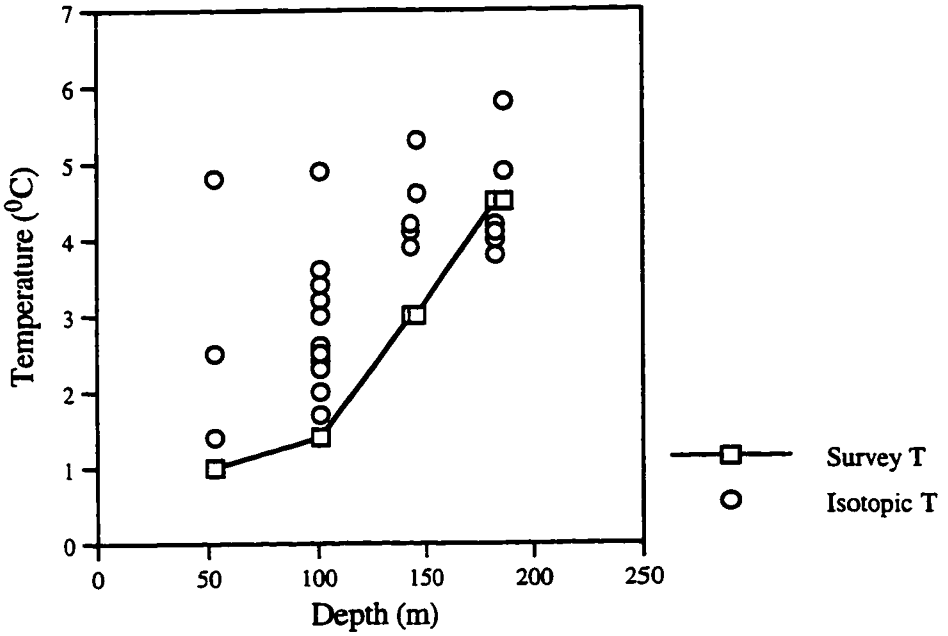

Fig. 2.5 Comparison between the calculated isotopic temperatures and the survey measurements *in situ* during the summer of 1985, assuming that the fish lived at same depths when they were captured, and laid down the opaque otolith zones in the same year.

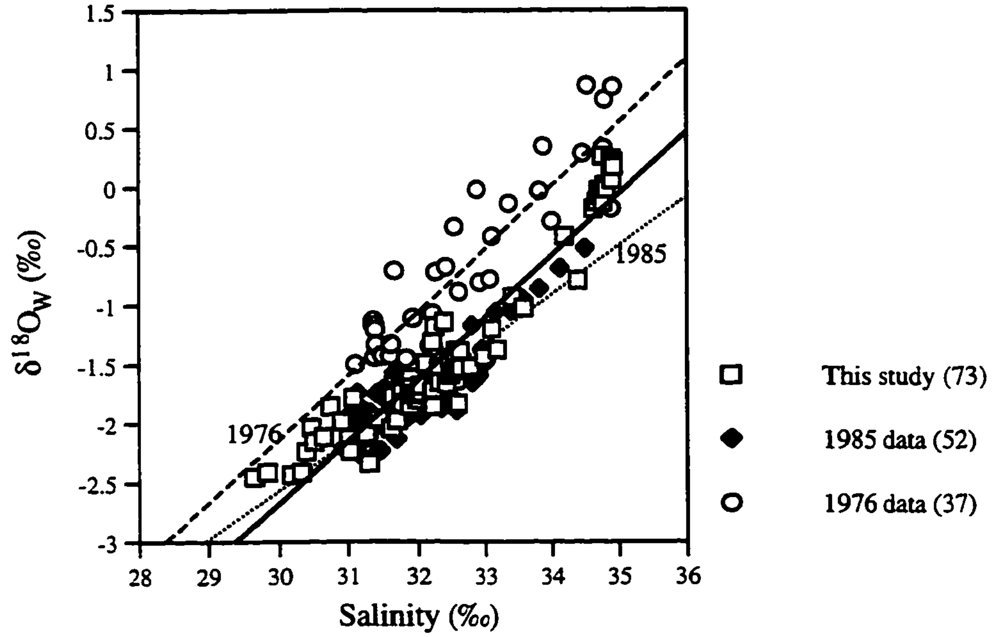

Fig. 2.6 Relationship between $\delta^{18}O_W$ and salinity from three sets of data in 1976, 1985, and 1996, with a time lag about 10 years. Note that the slope in 1976 (long dash line) is parallel to that of 1996 (solid line), while the 1985 slope (short dash line) was distinctly different. The figures in parentheses are numbers of seawater samples analyzed.

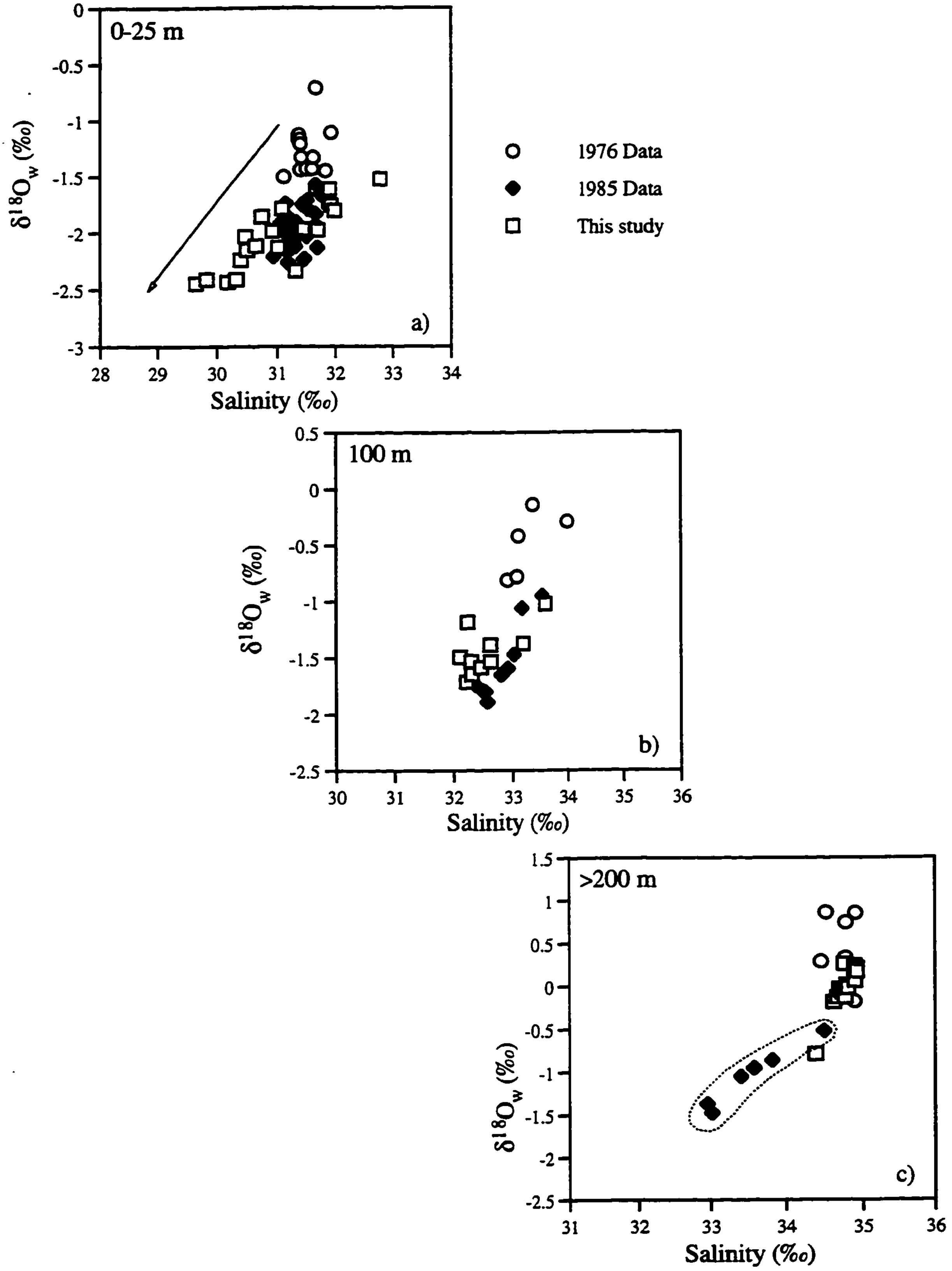

Fig. 2.7 Comparison of the $\delta^{18}O_W$-salinity variations at different depths based on three sets of isotopic data in 4Vs. (a) at the surface there was a gradual decrease in $\delta^{18}O_W$ and salinity; (b) at 100 m depth, a mixed data points; and (c) at depths greater than 200 m the 1985 data were distinctly different.

Table 2.1 Summary of seawater samples from different depths of 4Vs in the present study

Depth	$\delta^{18}O_W$ (‰)			Temperature (^0C)			Salinity (psu)		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
5m	-2.19	0.20	10	10.1	0.56	10	30.71	0.63	10
25m	-1.88	0.28	10	4.5	1.93	10	31.35	0.89	10
50m	-1.75	0.31	12	1.5	0.90	12	31.88	0.44	12
75m	-1.65	0.20	11	1.0	0.40	11	32.31	0.35	11
100m	-1.44	0.22	9	1.4	0.88	9	32.60	0.49	9
150m	-1.20	0.44	6	2.3	1.73	6	33.04	0.66	6
>200m	-0.02	0.26	15	5.8	0.63	15	34.77	0.14	15

CHAPTER THREE

Oxygen isotope variations in otoliths of cod: A potential thermometer and migration indicator

3.1 Abstract

Oxygen isotopes can be used as a thermometer in determining the environmental living temperature of the fish through its lifetime, because they are temperature-dependent during the process of isotopic fractionation between otolith aragonite and the ambient waters. If the isotopic composition of water masses is different we can then identify the recruitment and migration of the fish based on the isotopic signals stored in otoliths. These principles have been examined in the historical collection of otoliths of cod (*Gadus morhua*) from the northeast Scotian Shelf, Nova Scotia. Sagittal otoliths were analyzed seasonally by using a computer-controlled micromilling technique (*DM 2800*). The oxygen isotopic composition of these field cod ranged from 0.0 to +3.0 ‰, with a consistent variation pattern of increase up to their sexual maturation. There are no distinct isotopic variations between seasonal otolith zones, except for the first year. Based on the $\delta^{18}O$ signals in otoliths two types of cod, Type 1 (>0.75 ‰) and Type 2 (<0.75 ‰), were discriminated. It is suggested that about 20 % of the total cod were recruited from the Gulf of St. Lawrence, while the other 80 % were resident in the 4Vs area. In comparison with the opaque otolith zones of the 4-5 year cod group, the secular variation in $\delta^{18}O$ was about +1 ‰, corresponding to a decrease in temperature of the seawater by about 5 ^{0}C. This temperature change is in concert with temperatures measured in the same area from the summer groundfish trawling survey over the past decade.

3.2 Introduction

The sudden collapse of the cod fishery in Atlantic Canada shook the fisheries society, and stimulated us to learn about marine environmental changes from the cod itself by using isotopic data from otoliths (*Gadus morhua*, L., 1758). Otoliths are mm-sized, pearl-like aragonite concretions found in the membranous labyrinth of osteichthyan fish (Campana and Neilson, 1985). Although the morphology and size of otoliths are species-specific, their small size limits detailed stable isotopic analyses. This may be why so little attention has been given to otoliths since Devereux's initial work (Devereux, 1967). Little work has been done on analysis of the isotopic composition of yearly or seasonal zones. Rather, most analyses have been carried out on whole otoliths. The fact that otoliths grow in concentric layers around a nucleus suggests that it should be possible to obtain a temporal record of $\delta^{18}O$. For studies such as that of the New Zealand common smelt (*Retropinna retropinna*), the whole otolith isotopic analyses may not be a serious problem because that species lives only one or two years (Nelson et al., 1989; Northcote et al., 1992). However, for long-lived species such as Atlantic cod (e.g., Radtke, 1984), such an analysis is absolutely unacceptable because the data would average isotopic composition over the fish's whole life.

The basic principle of using fish otoliths in stable isotopic studies is that otoliths are deposited at, or very close to oxygen isotopic equilibrium with the ambient waters in which the fish lived, although indirectly through the endolymph fluid (Devereux, 1967; Mulcahy et al., 1979; Kalish, 1991; Patterson et al., 1993). However, Radtke (1984) and Radtke et al. (1996) reported a totally negative result. They argued that cod otoliths were not formed in isotopic equilibrium with the surrounding seawater, neither ^{18}O nor ^{13}C, and that the isotopic temperature scale (Epstein et al., 1953) was not applicable. Many investigators query their statements (e.g., Patterson et al., 1993) and most the recent laboratory test on Atlantic croaker *Micropogonias undulatus* (Thorrold et al., 1997) suggested that the "vital effect" is not important.

We report here on a study of seasonal $\delta^{18}O$ records in 61 cod otoliths from the northeast Scotia Shelf, Nova Scotia. Both seasonal and annual oxygen isotopic variations show that cod otoliths would be a useful thermometer for long-term marine environmental changes, and a useful indicator for the migration of the 4Vs stock. The $\delta^{13}C$ data for these otoliths has been reported elsewhere (Schwarcz et al., 1997).

3.3 Materials and methods

3.3.1 Sampling strategy

Sections of cod otolith plates used in the present study were taken from the Bedford Institute of Oceanography (BIO) at Dartmouth, Nova Scotia, Canada. It is known that Atlantic cod grow at different rates from area to area, and there is also an annual variation in the same area (Leim and Scott, 1966). The variation in growth rate is influenced by many factors such as seawater temperature, salinity, food ration, genetic background, population dynamics, and so on. Hence care must be taken in the selection of sampling area. Fortunately, tagging studies have shown that migration of mature cod on the Scotian Shelf is limited in the Canadian Atlantic, and cod of the 4Vs stock are non-migratory, particularly in the summer time (Campana et al., 1995). The water mass of the Scotian Shelf has also been investigated in detail (Smith et al., 1991). Therefore, three dominant selection criteria were followed during sampling: (1) species of Atlantic cod (*Gadus morhua*); (2) 4Vs stock of the NAFO (North Atlantic Fisheries Organization) Subdivision (Fig. 3.1); and (3) only summer captures (from June to October) were chosen each year. Although samples were collected from 1980 to 1994, only the even-year captures since 1988 are used in the present study.

The thin growth rings or zones in sagittal otoliths correspond to yearly, seasonal, weekly and even daily growth increments (Pannella, 1971). A yearly increment is composed of two thick zones: a translucent and an opaque zone as viewed under reflected light (Casselman, 1983; Campana and Neilson, 1985). For cod, the translucent material is laid down mainly in winter and the opaque one mainly in summer (Bagenal and Tesch, 1978). Since cod are spawned and hatched in winter the increments always start from a translucent nucleus. Most of the 4Vs cod we selected show the seasonal zones clearly, particularly in the younger fish (3 to 6 years old). In general, the translucent zone is thicker than the opaque one. However, in the older cod, more than 8 years of age for instance, the width of the outermost translucent and opaque zones reverses. In some abandoned samples the resolution of seasonal zones was so poor that we could not recognize any increments.

3.3.2 A new micromilling technique

We have established a new micromilling technique by using a computer-controlled machine (*DM 2800*) in the Department of Mechanical Engineering, McMaster University. The basic procedures can be briefly described as follows. Each sectioned sagittal plate was first cut into pieces, and then attached to a petrographic thin section slide. After polishing and photographing, the samples were examined with a *Swift* binocular microscope in both reflected and transmitted lights, in an attempt to verify the previous age determination made by image analysis. This process is critical because only visible growth zones can be further examined and measured. Some otoliths looked fuzzy under reflected light, but gave better images by transmitted light. We then designed the milling paths for the *DM 2800* machine using a *Nikon-18494* Measurescope, in which each step of milling along the translucent and/or opaque zones was measured. The precision of this measurement is better than 1 µm. After recording each step and coordinate of the paths, we wrote the milling program by using *DOS-EDIT* software. Then we put the sagitta thin section into a special jig, and automatically milled out a predetermined path at a speed of 3000-6000 rpm, depending on the brittleness of the individual specimen. The milling tool we used was about 25 µm at its tip, with three facets approximately 10 degrees off vertical. Because of the relationship between the depth and width of milled path ($W \approx 0.176D$), we can set the milling depth based on the different widths of otolith zones. When one path was finished we took the thin section out of the jig, carefully tapping the powder onto aluminum paper and then into a metal cup. At least 30 µg of aragonite powder was obtained from each seasonal zone

(Fig. 3.2). Both the thin section and milling bit were subsequently blown off by using a pure *Aero-Duster* gas.

3.3.3 Analyses for $\delta^{18}O$ and $\delta^{13}C$

The aragonite powder in the metal cups was reacted with 100 % phosphoric acid in an autocarb automatic carbonate analyzer attached to a VG Optima mass spectrometer. All the measurements for both $\delta^{18}O$ and $\delta^{13}C$ are reported in the standard δ notation (‰), $\delta^{18}O = \{[(^{18}O/^{16}O)_X / (^{18}O/^{16}O)_S] - 1\} \times 1000$, where X is sample and S is standard (VPDB via NBS-19). The precision of analyses is better than ±0.06 ‰ for both oxygen and carbon isotopes.

3.4 Results

The analyses of cod otoliths in the present study are presented in Table 3.1. These fish were aged from 2 to 11 years, with a range of fork length from 41 to 109 cm. As described previously, they were captured in summer and mainly from water depths greater than 100 m. Due to the lack of original data we do not have information about the gender and weight of these cod.

The oxygen isotopic composition of most otoliths ranged from 0 to +3.0 ‰, with a mean standard deviation of 0.538 (Table 3.1). Most cod otoliths showed a similar pattern of oxygen isotopic variation over the past decade (Fig. 3.3), namely a gradual increase of $\delta^{18}O$ with age in cod's life, although the magnitude of the variation was not identical. It was also noted that some of these cod had distinctly greater $\delta^{18}O$ variation than the majority of the stock, and could be easily picked out by their more negative first-year $\delta^{18}O$ values. After the initial 4-5 years, interestingly, the trend of $\delta^{18}O$ for all cod levelled off and was limited to the same range of 2 to 3 ‰ (Fig. 3.4a). This life-long trend in $\delta^{18}O$ of otoliths allow us to distinguish two types of cod in the 4Vs stock, based on a cutoff value of 0.75 ‰ after hatching (Fig. 3.4b). Type 1 cod had a higher initial $\delta^{18}O$ record (>0.75 ‰) with very flat and smaller $\delta^{18}O$ variation, while the starting $\delta^{18}O$ of Type 2 fish was lower (<0.75 ‰) and their life-long isotopic variation was much greater (Fig. 3.4). The cutoff value was chosen based on two facts: (1) from the life-long $\delta^{18}O$ variation, the value of 0.75 ‰ distinguishes between the majority and some "unusual" patterns of isotopic variation (Fig. 3.3); and (2) according to the isotopic temperature calculation, the value of 0.75 ‰ corresponds to 8.2-12.5 ^{0}C in 4Vs seawater by using different isotopic temperature scales, which is in concert with the surface temperature of seawater in the summer survey and would be the maximum the cod lived. Therefore, this isotopic signal was tentatively interpreted as an indicator of migration of young cod from an environment in which the $\delta^{18}O$ value of seawater was relatively lower than in 4Vs.

The seasonal oxygen isotopic composition of the 4-5 year cod group showed only minor differences (Fig. 3.5), generally a small $\delta^{18}O$ variation from 0.1 to 0.3 ‰ between winter and summer. It could be suggested that the seasonal temperature variation was not significant enough to affect the cod's life, or the minor difference in $\delta^{18}O$ was due to the seasonal change of habits and characteristics of 4Vs stock. In fact, the largest seasonal difference in the first year for all the 4-5 year group (Table 3.2) are evidence that both interpretations are possible.

We examined the $\delta^{18}O$ records of cod in the 4-5 year group, in an attempt to eliminate or minimize the age and growth effects in the whole stock. As expected, they displayed a consistent trend to heavier values among the four data sets (Fig. 3.6), despite the fact that these data were spread over 7 years. This consistency suggests that the cod otoliths deposited in corresponding growth years were comparable, because they were subjected to the same environmental changes from the same water masses. The long-term oxygen isotopic profile over the sampling period was based on the opaque zones (summer records) of the 4/5 year cod group (Fig. 3.7). It showed an increasing trend of $\delta^{18}O$ values by about +1 ‰ in the decade, and some details in variation for each year. Assuming that the cod lived at same depths as they were captured, and laid down the opaque otolith zones in the same year, the correlation between these summer $\delta^{18}O$ records in 1985 and temperatures from the summer groundfish trawling survey *in situ* (Table 3.3) yielded a positive result, similar to those of widely accepted oxygen isotopic temperature scales (Fig. 3.8). Such an estimation was apparently rough although it gave a quite good temperature equation: $\delta^{18}O_{A-W} = 4.23 - 0.240T$ (^0C). However, the high similarity and consistent decrease between calculated isotopic temperatures and summer survey data convince us that the oxygen isotopic composition in cod otoliths could be a potential thermometer in modern environmental studies (Fig. 3.9).

3.5 Discussion and conclusion

The fractionation of oxygen isotopes between otolith aragonite and seawater (δ_{A-W}) is temperature-dependent. This variation in δ_{A-W} with temperature has been successfully used in fish otolith research, and found to be close to that initially obtained by Epstein et al. (1953) for molluscs. Nelson et al. (1989) and Patterson et al. (1993) examined freshwater fish species, while Devereux (1967), Mulcahy et al. (1979), Kalish (1991) and Thorrold et al. (1997) studied marine fishes. Nevertheless, Radtke (1984) and Radtke et al. (1996) suggested that Atlantic cod (*Gadus morhua*) was an exception. They believe that the oxygen isotopic composition of cod otoliths was not deposited in equilibrium with ambient seawater (Radtke, 1984; Radtke et al., 1996). Our studies for field cod from 4Vs are in disagreement with their statements (see below).

The oxygen isotopic temperature scales (e.g., Epstein et al., 1953; Grossman and Ku, 1986) were given as a function of the difference between $\delta^{18}O$ in calcite or aragonite (δ_A) and $\delta^{18}O$ in seawater (δ_W). We can only measure δ_A from samples, unfortunately, and do not have any direct way of measuring δ_W in which cod was living when a particular zone of aragonite (whether daily or seasonally) was being laid down. If there were changes in δ_W these would lead to "apparent" changes in temperature even though temperature had remained constant. Such changes in δ_W are in fact occurring in the 4Vs area, as a result of changes in salinity (Tan and Strain, 1988; Gao et al., 1997a). Unfortunately, salinity is a conditional environmental factor, particularly at water depths greater than 200 m (Petrie and Drinkwater, 1993; Gao et al., 1997a). Thus two serious uncertainties, both δ_W and the depth in which cod was living, remain in the research of paleo-oceans when using oxygen isotopic tools. Our estimations (Fig 3.8) revealed that the $\delta^{18}O$ values of summer otolith aragonite at depths greater than 100 m were correlated well with the temperature (and δ_W measurement) *in situ*. This makes it possible to use the oxygen isotopic temperature scale in estimating the cod's thermal history from otoliths. The result is not surprising because cod are ground fish, and almost all captures in the present study are deeper than 100 m. Therefore, as a poikilothermic cold-water species, cod would not be exceptional in the process of oxygen isotopic fractionation.

One problem, perhaps, was the specimens that Radtke et al. (1996) used in their experiments. They obtained young cod of two months old and then reared them for three months more. Juvenile cod at about 5 months of age are very close to their first seasonal growth in our study. Fig. 3.5 and Table 3.2 show that the $\delta^{18}O$ variation of the first year in cod otoliths was greatest, and thus the uncertainties in the oxygen isotopic temperature scales would be maximal. In addition, because cod grow very fast during the first season the growth effect could be the dominant factor in the oxygen isotopic fractionation. As has been reported for many other marine fish species (Devereux, 1967; Mulcahy et al., 1979; Kalish, 1991; Thorrold et al., 1997), we believe that the oxygen isotopic composition of cod otoliths is deposited at, or very close to isotopic equilibrium with the ambient seawater. Any deviation might be from the uncertainties discussed above, even in controlled laboratory experiments (Patterson et al., 1993).

3.5.1 Temperature effect

The temperature effect on habitat and growth of cod has been well documented both in the field and in the laboratory. Perry and Smith (1994) developed an objective method to identify habitat association of four species of marine fish by using survey data from 1979 to 1984. They found that cod were not consistently associated with particular depths in winter and summer, so that they cannot be monitored by single temperature or salinity factor. Brown et al. (1989) conducted two experiments for 0+ cod (young-of-the-year). Their results showed that cod in the 8.3 ^{0}C group were significantly more active than those in the 4.5 ^{0}C and 0.6 ^{0}C groups before and after feeding, suggesting that seawater temperature affected food consumption and length and weight changes. The temperature effect has also been reported in otolith increment deposition, although the increment was not merely related to water temperature (Zhang and Runham, 1992). Seasonal $\delta^{18}O$ comparison in this study (Fig. 3.5) showed there were no significant changes between the translucent and opaque zones except for the first year. If we accept that the $\delta^{18}O$ variation is temperature-dependent, this result is in disagreement with Perry and Smith's (1994) objective model, and strongly suggests that cod prefer to live in certain water layers with small temperature changes. In summer, the optimum temperature was perhaps associated with a relatively lower level of food, and a very dense opaque zone was laid down. During the winter and spawning time, the lower temperature together with higher ration yielded a wide translucent zone (Zhang and Runham, 1992). Therefore, the oxygen isotopic comparison of the 4-5 year cod group between winter and summer not only reveals the significant temperature effect of seawater, but also suggests that cod may like to live in a limited range of temperature and salinity. In the 4Vs area, it is reported that cod prefer temperatures between 2 and 4 ^{0}C, and salinity between 32 and 34 ‰ (Scott, 1982; Smith et al., 1991).

3.5.2 Cod recruitment

Nelson et al. (1989) compared the $\delta^{18}O$ value of common smelt (*Retropinna retropinna*) otoliths with that of water sources, in an attempt to find possible migratory routes. Their result suggested that there was a potential to use stable isotopes, both $\delta^{18}O$

and $\delta^{13}C$, to distinguish smelt by their habitat waters and thus to examine their migration behavior. Since there are differences in δ_W values between different water masses (Tan and Strain, 1988) it is possible to use oxygen isotopes as a tracer for migratory studies in cod. Therefore, the distinct $\delta^{18}O$ variation in the present study was mainly accounted for a regional or stock migration although other effects (e.g., climate and environmental changes; metabolic differences as fish growth) might also be important.

Some cod showed a remarkable increase in $\delta^{18}O$ in the first few years of growth (Fig. 3.3), after which their $\delta^{18}O$ values varied little and in concert with those of the rest of the stock (Fig. 3.4). For example, the $\delta^{18}O$ values of sample 92-3881 initially started from about -0.5 ‰. The more negative $\delta^{18}O$ values could be interpreted as either higher temperature (greater than 12 ^0C) or lower salinity which was correlated with δ_W. The former was improbable because cod is a ground fish, only very occasionally moving near the surface. Thus the more negative $\delta^{18}O$ is an indicator of young cod from an environment in which the equilibrium $\delta^{18}O$ values of otolith aragonite were consistently lower. According to Tan and Strain (1988), the $\delta^{18}O$ values for surface waters in the St. Lawrence Estuary range from -10.3 to about -2.0 ‰, much lower than those in the 4Vs area (about -1.72 ‰) because of mixing with St. Lawrence river water. We believe that cod recruitment is coming from the Gulf of St. Lawrence. After three to four years' growth and migration the fish reached the 4Vs area, simultaneously increasing its $\delta^{18}O$ to about 2.5 ‰. Once the cod moved into 4Vs, it appears to follow the variation trend of the 4Vs stock (Fig. 3.4).

This might be a very useful technique to figure out how many migratory fish were in the whole stock, even though fish are mobile creatures. Using the life-long isotope records we recognized two types of 4Vs cod in the present study based on a cutoff value of 0.75 ‰ in $\delta^{18}O$, corresponding to the isotopic temperature of about 10 ^0C which is generally the average maximum temperature of surface water. Of the 61 cod investigated, about 20 % were identified as Type 2 fish. The classification could be very helpful in fisheries biology and stock management. Rose (1993) even proposed that for the Newfoundland cod, a change of migration patterns might account for the population declines in the early 1990s because the constancy of the temperature chosen by cod might direct their migration routes. Thus, an isotopic study of these fish would be of interest.

3.5.3 Environmental change

The long-term temperature decrease of the 4Vs seawater can be illustrated by the 4-5 year cod group (Fig. 3.6). We tentatively investigated the 4-5 year cod group due to the following reasons: (1) there is an important relationship between the characteristics of water mass and age-4 year cod in 4Vs (Smith et al., 1991); (2) the $\delta^{13}C$ variation showed that the age of maturity of the 4Vs cod had changed from the previous 7-8 years to 4-5 years (Beacham, 1982; Gao et al., 1996), and after age 4-5 years there were much less or no distinct variations in $\delta^{18}O$ because adult cod like to live in certain water layers with constant temperature (see above); and (3) about half the total samples are 4-5 years old in the present study so that this group would represent the 4Vs stock and would be suitable for oxygen isotopic examinations. Fig. 3.7 shows that the summer average $\delta^{18}O$ value was gradually increasing by about +1 ‰ over a period of 11 years. This could be caused by either a decrease in seawater temperature or an increase in salinity. The latter was unlikely because at the beginning of this period the cod were already living in water with maximum salinity for this region. Therefore, the 1 ‰ increase in $\delta^{18}O$ would be interpreted as a secular decrease of seawater temperature by about 5 ^{0}C based on the temperature equation of Grossman and Ku (1986). Further, there are many data from groundfish trawling observations conducted by BIO each year, which, fortunately, provide primary evidence either supporting or rejecting our isotopic interpretations. According to the long-term data analysis there were no striking salinity changes, but a temperature decline in the last several decades. The largest temperature and salinity changes were 4.6 ^{0}C and 0.7 ‰, respectively (Petrie and Drinkwater, 1993). Most of these changes happened at about 100 m depth over the Scotian continental slope. The temperature trends in the bottom seawater (>200 m) and shallower areas diverged around 1985 (Drinkwater, 1996). A cold intermediate layer (*CIL*) then remained yearly at depth from 50 to 100 m. The $\delta^{18}O$ variation in opaque otolith zones (Fig. 3.9) was in good agreement with the above summer survey, revealing a secular temperature decrease from about 10-11 ^{0}C at the beginning to about 5-6 ^{0}C in 1994. The apparent differences between isotopic and survey records are mainly due to the water depths in which cod lived (cf. Fig. 3.9), because the isotopic data are records of the temperature shift actually experienced by the cod whereas the hydrographic records are "spot" measurements. We cannot be sure that individual cod were continuing to live in the same water masses while temperatures in seawater declined through the last decade. This might be debatable as the cause of the collapse of cod fisheries in the Canadian Atlantic. However, with a relatively stable variation of salinity the secular temperature decrease in seawater could be partly accounted for the drastic decline of the cod stock in the early 1990s.

In summary, we have demonstrated that the seasonal $\delta^{18}O$ composition of cod otoliths (*Gadus morhua*) is a promising tool in the study of marine environmental changes. The oxygen isotopic markers in otoliths not only describe the temperature effect in the cod's life, but also display the possible migration track into the 4Vs stock. Using the 4-5 year cod group, the seasonal and long-term isotopic temperature variations have been verified by both the summer records of temperature and salinity, and the independent results of oceanographic observations.

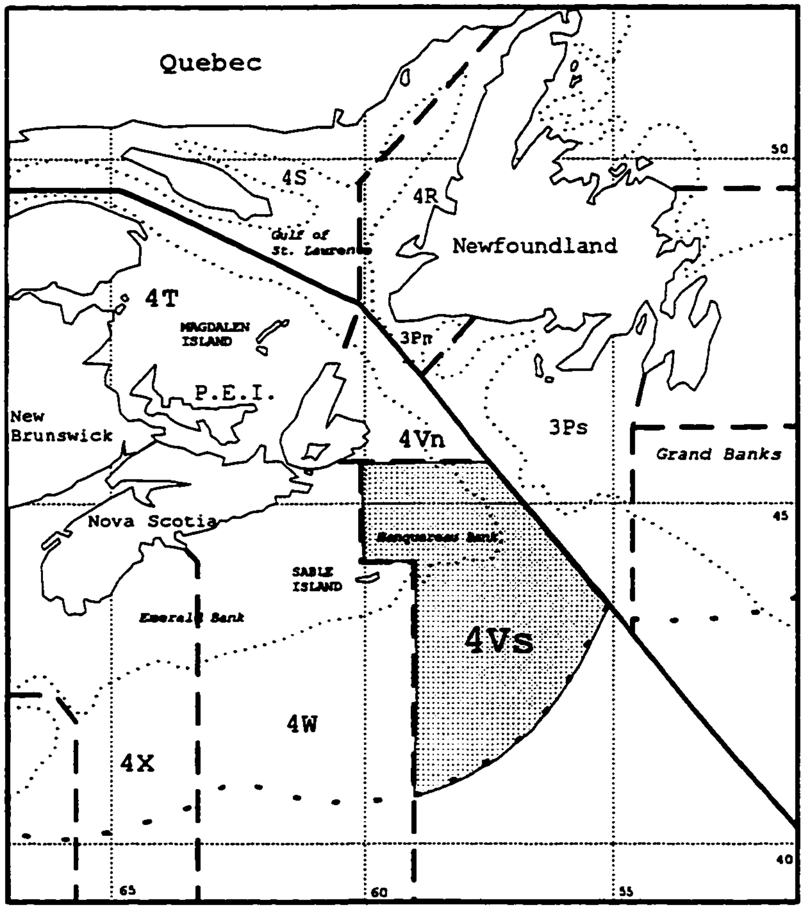

Fig. 3.1 Location map showing the 4Vs research area.

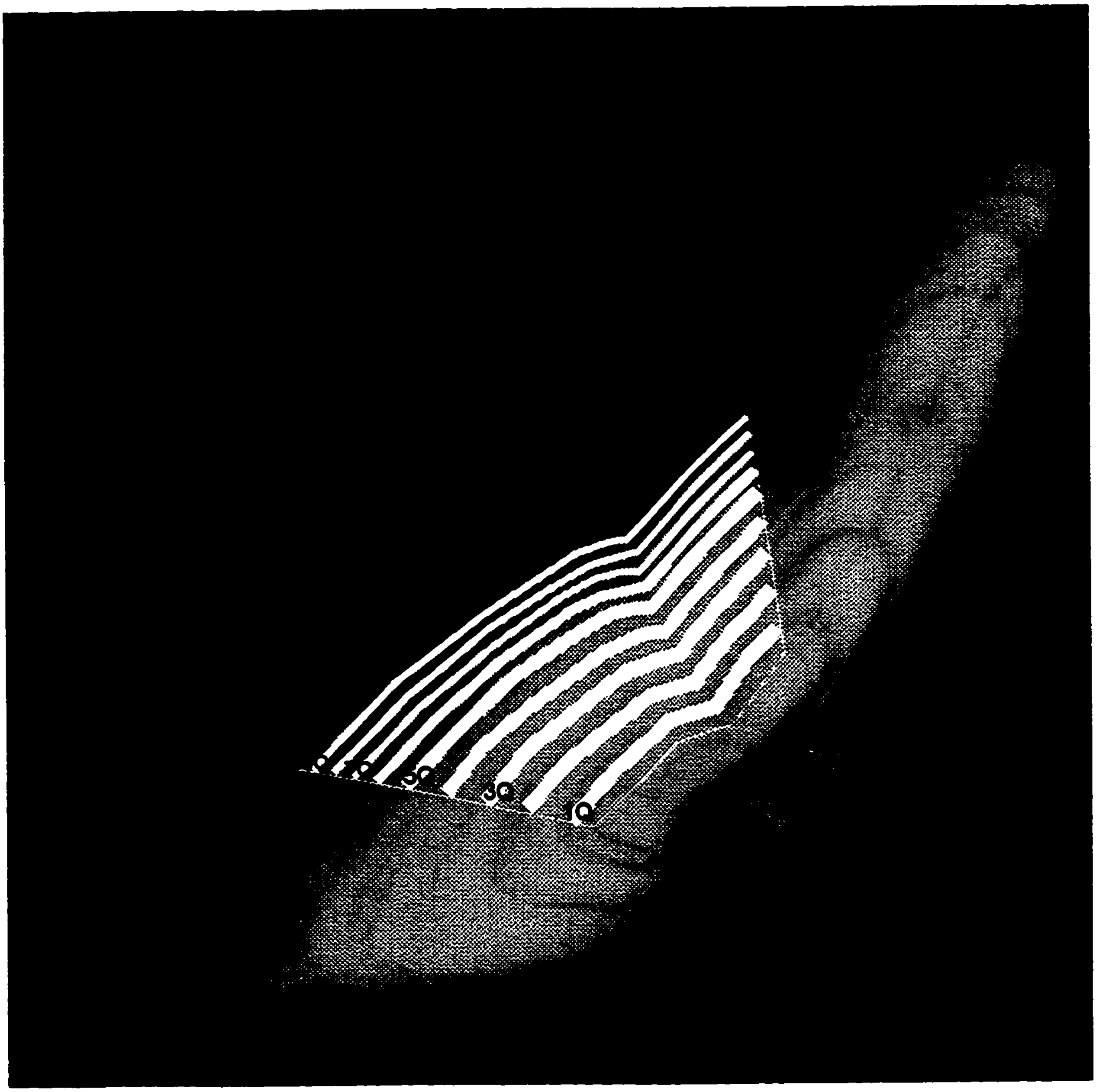

Fig. 3.2 Microsampling of a 9-year sagittal otolith of cod, showing the milling paths of opaque (Q) and translucent seasonal zones. Scale 1/20.

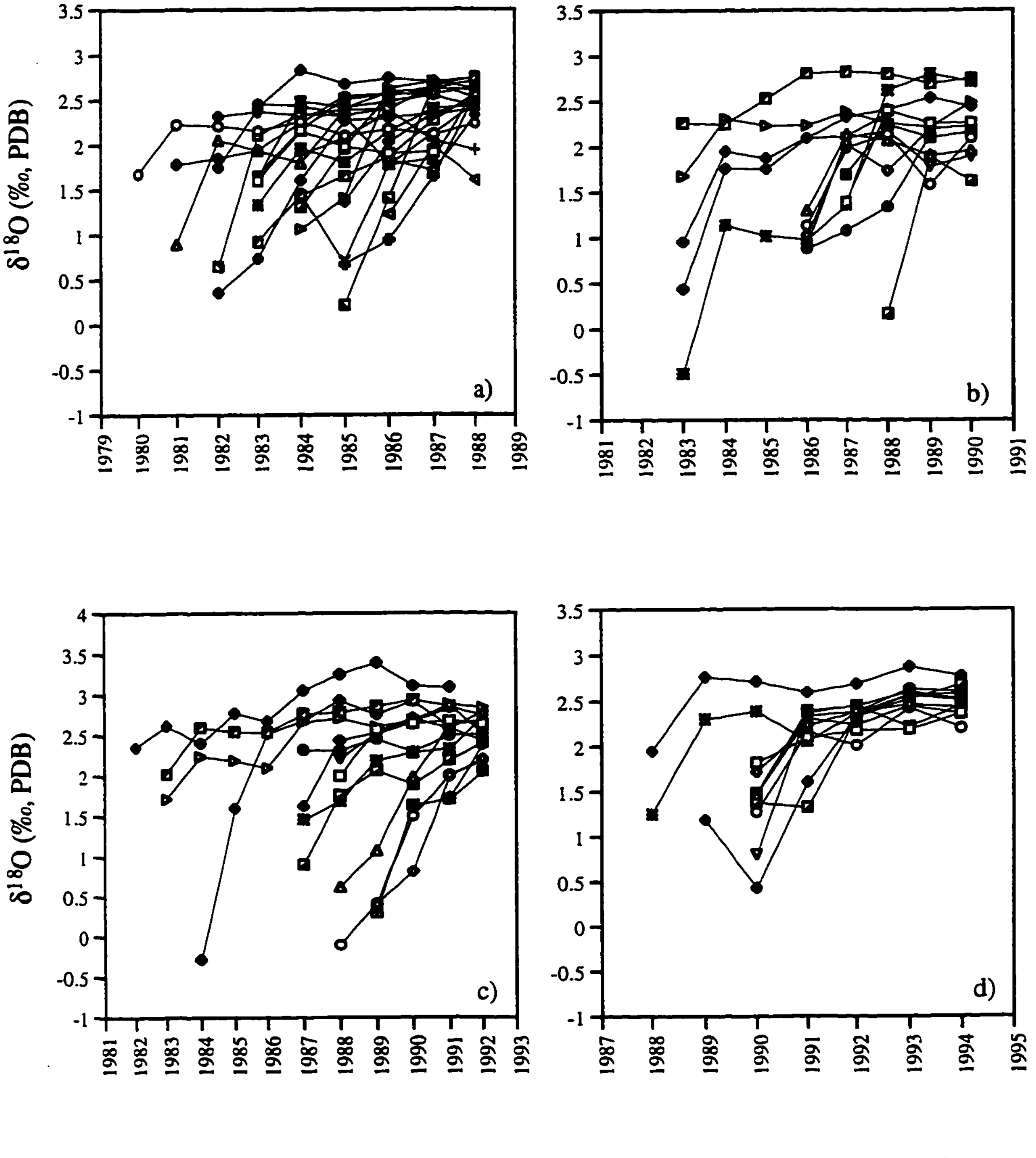

Fig. 3.3 Annual oxygen isotopic variation of cod otoliths (*Gadus morhua*) from captures of 1988 (a), 1990 (b), 1992 (c) and 1994 (d), respectively.

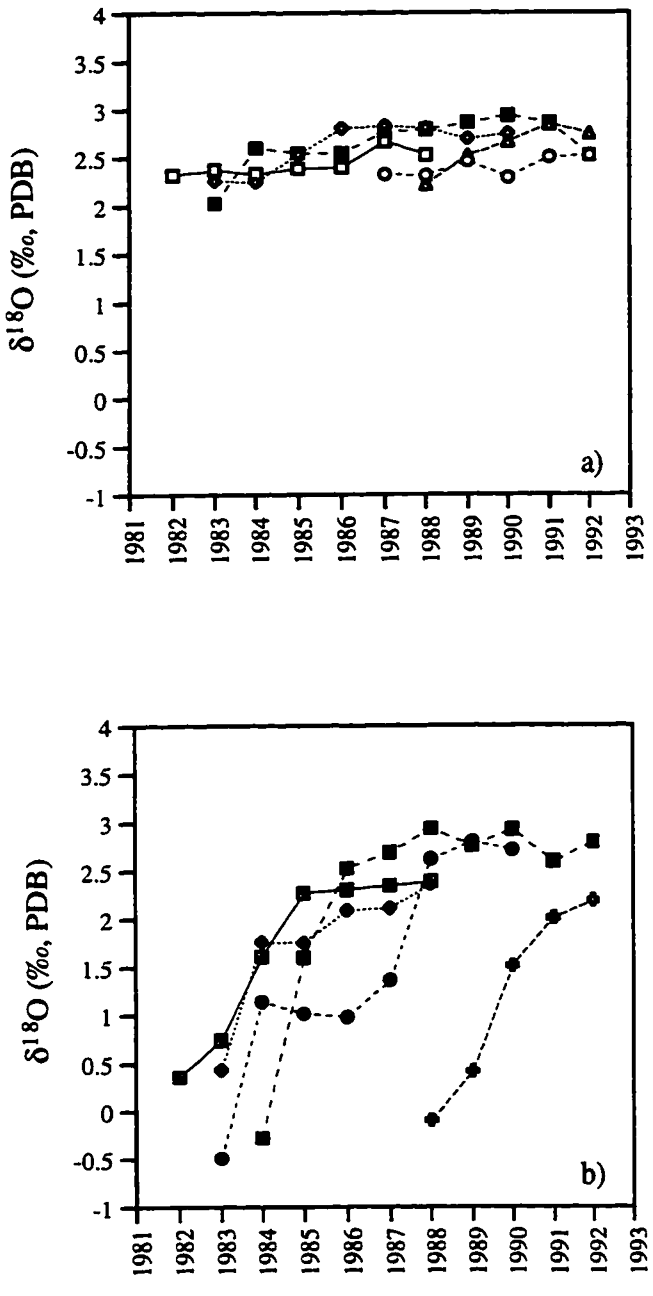

Fig. 3.4 Typical Type 1 (a) and Type 2 (b) fish in the 4Vs region based on a cutoff value of 0.75‰ in their oxygen isotopes.

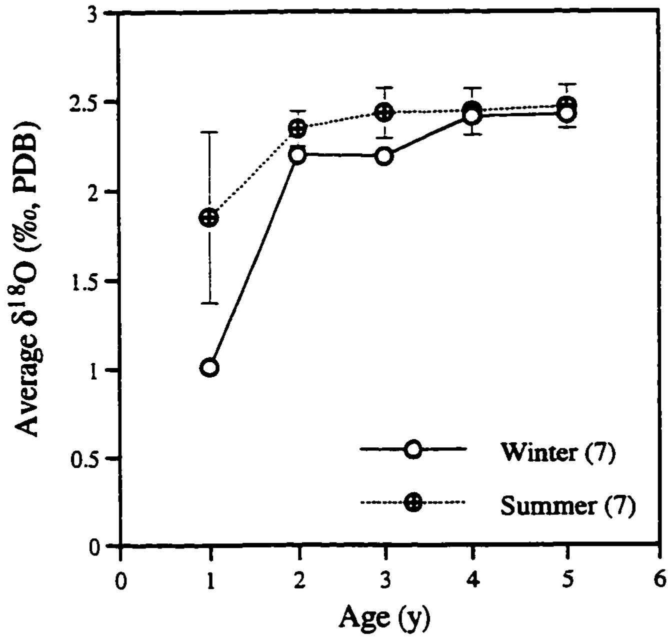

Fig. 3.5 Comparison of average $\delta^{18}O$ values between winter and summer otolith zones in a 5-y cod group from 1994 capture. The figures in parentheses are numbers of otoliths averaged.

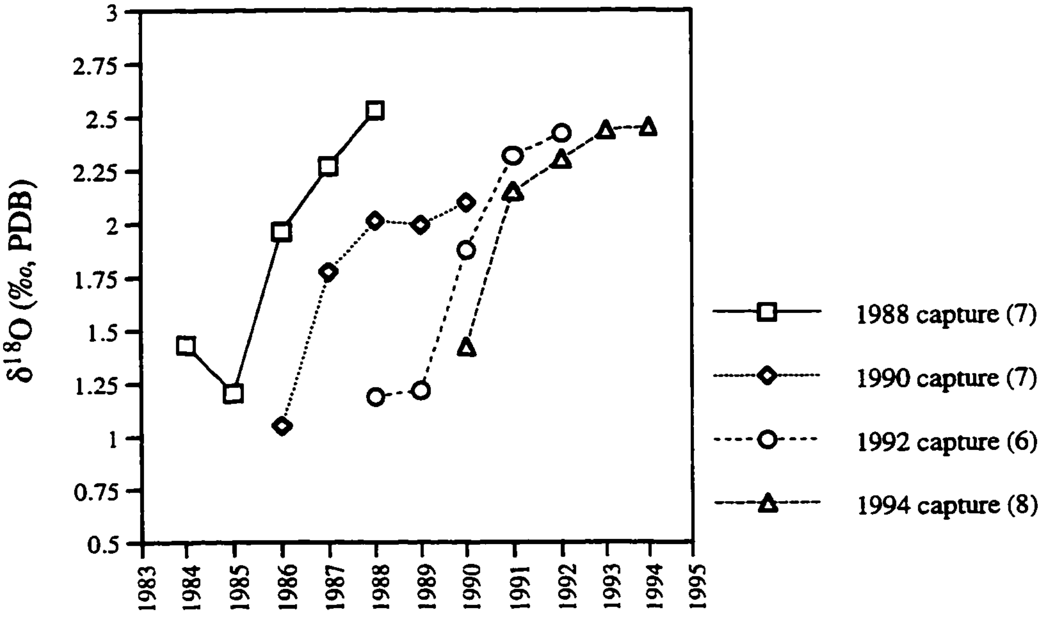

Fig. 3.6 Comparison of the 4-5 year cod group from the mean annual contents. The figures in parentheses are numbers of otoliths analyzed.

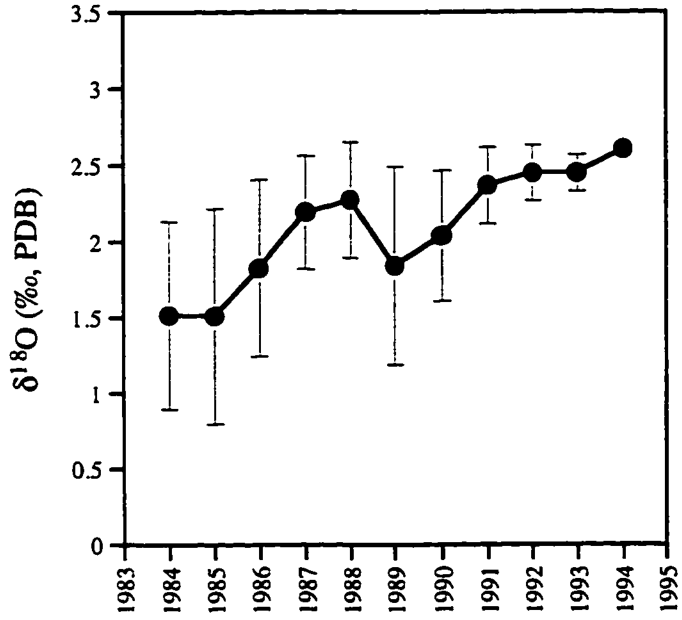

Fig. 3.7 Average oxygen isotopic variation of the 4-5 year cod group from their opaque otolith zones (*summer*).

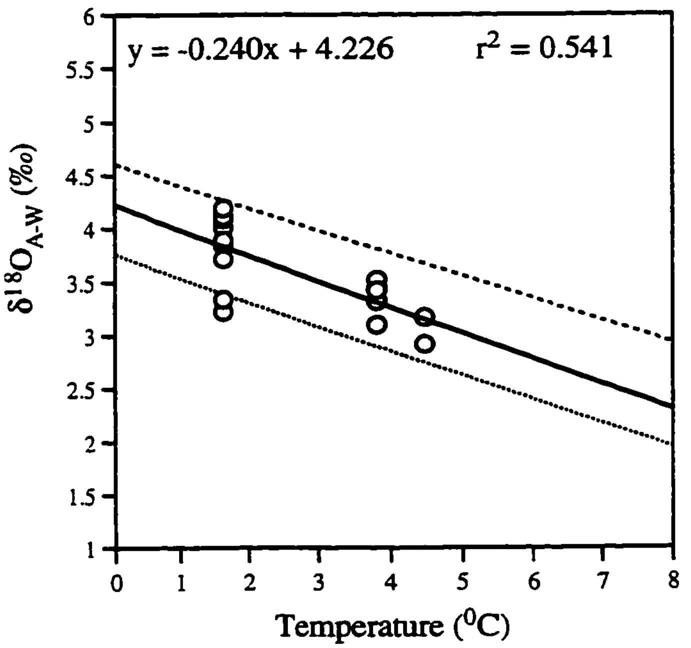

Fig. 3.8 Correlation between the $\delta^{18}O$ values of opaque otolith zones and the summer survey data (*in situ*) in 1985 (cf. Table 3.3). The δ_W data were from Tan and Strain (1988), and the dashed lines from isotopic temperature scales of Epstein et al. (1953; *low*) and Grossman and Ku (1986; *top*).

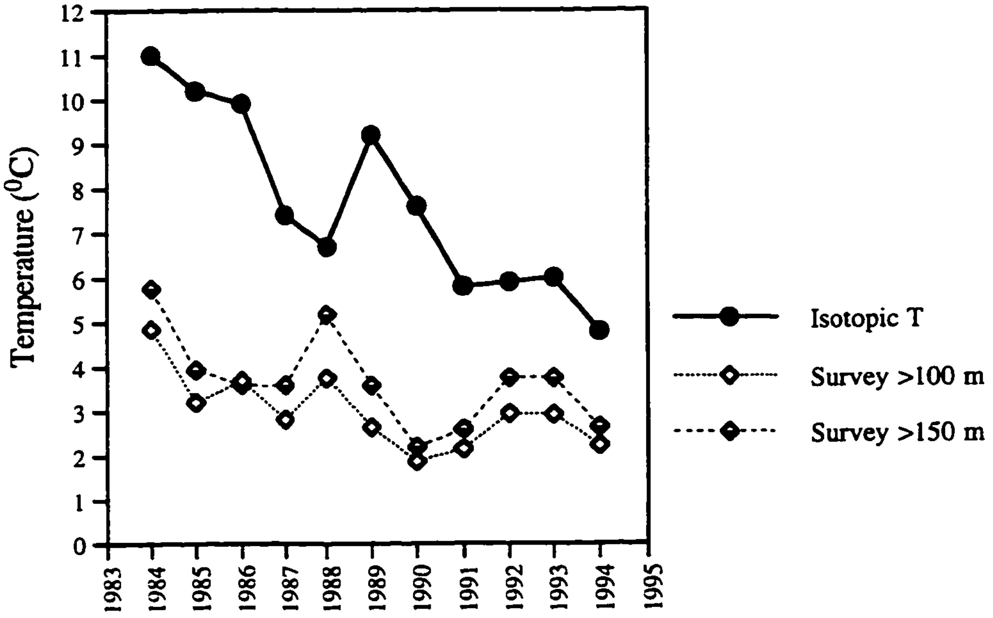

Fig. 3.9 Comparison between isotopic records of the 4-5 year cod group (*opaque zones*) and summer survey data (*average*) from the same area.

Table 3.1 Summary of 61 cod (*Gadus morhua*) captured from 4Vs Subdivision (1988-94)

Sample#	Date	Depth (*m*)	Length (*cm*)	Age (y)	$\delta^{18}O$(‰) range	SD
653	31/08/88	146	75	6	1.49-2.26	0.22
659	"	"	44	3	1.66-2.35	0.32
660	"	"	79	9	1.44-2.31	0.20
771	2/09/88	101	87	8	0.90-2.60	0.50
772	"	"	59	6	1.25-2.69	0.41
775	"	"	74	8	1.63-2.57	0.36
779	"	"	53	6	1.20-2.56	0.41
787	"	"	66	6	0.93-2.66	0.52
791	"	"	50	5	0.79-2.81	0.63
792	"	"	70	7	1.75-2.87	0.30
795	"	"	53	6	1.19-2.44	0.40
796	"	"	49	5	0.59-2.72	0.82
810	"	"	47	6	0.78-2.57	0.54
811	6/09/88	143	60	7	0.13-2.45	0.87
812	"	"	45	4	0.29-2.63	0.78
814	"	"	43	3	1.23-2.03	0.36
820	"	"	65	7	0.45-2.63	0.72
822	"	"	54	7	2.08-2.73	0.18
961	14/09/88	187	48	5	1.86-2.38	0.20
962	"	"	47	5	0.45-2.58	0.76
965	"	"	45	4	-0.07-2.78	1.19
967	"	"	54	4	0.51-2.57	0.66
1481	18/06/90	53	52	4	0.55-2.43	0.62
1483	"	"	49	5	0.77-2.33	0.49
1487	"	"	50	5	1.12-2.18	0.41
1489	"	"	58	5	1.30-2.19	0.26
1491	"	"	47	4	1.04-2.36	0.42
1492	"	"	61	8	0.81-2.54	0.58
1494	"	"	52	5	0.42-2.21	0.59
1496	"	"	55	5	0.60-2.25	0.50
1502	"	"	43	3	0.16-1.91	0.73
1510	"	"	69	8	0.41-2.36	0.76
1518	"	"	78	8	-0.65-2.82	1.12
1522	"	"	86	8	1.57-2.49	0.25
1530	"	"	77	8	2.24-2.86	0.24
3834	22/07/92	101	49	5	1.57-2.68	0.40
3835	"	"	46	4	0.28-2.35	0.88
3836	"	"	45	5	-0.13-2.24	0.93
3838	"	"	43	5	0.63-2.70	0.82
3839	"	"	44	4	0.31-2.38	0.79
3840	"	"	41	2	0.98-2.58	0.74
3845	"	"	61	6	2.22-2.53	0.15
3850	"	"	57	5	1.97-2.85	0.26
3856	"	"	72	6	0.89-2.52	0.55
3869	"	"	67	6	0.98-2.94	0.47
3870	"	"	70	6	1.03-2.83	0.53
3872	"	"	83	10	1.56-2.88	0.39
3880	"	"	88	10	1.81-2.94	0.28
3881	"	"	86	9	-0.59-3.09	1.07
3883	"	"	109	11	2.04-3.40	0.37
731	2/09/94	124	56	5	1.11-2.52	0.41
732	"	"	57	5	1.20-2.50	0.41
733	"	"	51	5	1.27-2.43	0.44
734	"	"	56	5	0.89-2.60	0.52
737	"	"	62	5	0.96-2.62	0.52
739	"	"	59	6	0.24-2.70	0.89
741	"	"	63	5	1.30-2.66	0.42
742	"	"	54	5	0.34-2.50	0.74
744	"	"	63	5	0.71-2.68	0.68
748	"	"	67	7	1.63-2.89	0.35
752	"	"	69	7	0.94-2.63	0.47

Table 3.2 The 4-5 year cod group and their $\delta^{18}O$ values used in the present study

Sample#	Age (y)	Differences in $\delta^{18}O$ (‰)		Mean $\delta^{18}O$ (‰)	SD
		(Seasonal)	*(First year)*		
88-791	5	0.06-1.06	1.06	2.24	0.63
88-796	5	0.08-0.96	0.96	2.03	0.82
88-812	4	0.69-0.86	0.86	1.62	0.78
88-961	5	0.00-0.29	0.04	2.07	0.20
88-962	5	0.03-0.99	0.55	1.46	0.76
88-965	4	0.06-1.57	0.56	1.76	1.19
88-967	4	0.16-1.73	1.73	2.06	0.66
90-1481	4	0.05-1.69	1.69	2.08	0.62
90-1483	5	0.00-0.51	0.51	1.84	0.49
90-1487	5	0.02-0.30	0.02	1.79	0.41
90-1489	5	0.03-0.10	0.10	1.94	0.26
90-1491	4	0.01-1.32	1.32	2.05	0.42
90-1494	5	0.16-0.91	0.91	1.36	0.59
90-1496	5	0.08-0.65	0.65	1.77	0.50
92-3834	5	0.00-0.88	0.88	2.45	0.40
92-3835	4	0.26-0.65	0.26	1.44	0.88
92-3836	5	0.09-1.09	1.09	1.35	0.93
92-3838	5	0.09-0.20	0.17	1.84	0.82
92-3839	4	0.28-1.49	1.49	1.59	0.79
92-3850	5	0.01-0.49	0.49	2.60	0.26
94-731	5	0.12-1.41	1.41	2.10	0.41
94-732	5	0.07-1.04	1.04	2.22	0.41
94-733	5	0.01-0.17	0.01	2.01	0.44
94-734	5	0.04-1.16	1.16	2.14	0.52
94-737	5	0.04-1.04	1.04	2.26	0.52
94-741	5	0.05-0.33	0.33	2.29	0.42
94-742	5	0.10-0.92	0.92	2.02	0.74
94-744	5	0.03-1.23	0.11	1.98	0.68

Table 3.3 Oxygen isotopic composition of cod otoliths used for the temperature estimation

Sample#	Depth (m)	δ_A (‰)	δ_W (‰)	δ_{A-W} (‰)	T (^{0}C)
653	150	1.969	-1.167	3.097	3.82
660	150	2.193	-1.167	3.321	3.82
771	100	2.305	-1.59	3.843	1.64
772	100	2.476	-1.59	4.014	1.64
775	100	2.553	-1.59	4.091	1.64
779	100	2.351	-1.59	3.889	1.64
787	100	2.576	-1.59	4.114	1.64
791	100	2.177	-1.59	3.715	1.64
792	100	2.655	-1.59	4.193	1.64
795	100	1.686	-1.59	3.224	1.64
796	100	1.801	-1.59	3.339	1.64
811	150	2.312	-1.167	3.440	3.82
820	150	2.392	-1.167	3.520	3.82
822	150	2.296	-1.167	3.424	3.82
961	200	1.984	-0.97	2.921	4.48
967	200	2.236	-0.97	3.173	4.48

CHAPTER FOUR

Carbon isotope variations in otoliths of cod: Effects of maturation and environmental change

4.1 Abstract

We measured the life-long variations in $^{13}C/^{12}C$ of otoliths of cod (*Gadus morhua*) from the northeast Scotian Shelf, Atlantic Canada. Samples were milled out of each sagitta in a radial sequence representing seasonal growth over periods ranging from 2 to 11 years. Aragonite layers deposited during the first 4 to 6 years of growth increased in $\delta^{13}C$ from minimum values between -5 ‰ and -2.5 ‰ to a maximum near 0 ‰. This pattern of increase was independent of the years in which the fish was collected. Layers formed after reaching the maximum $\delta^{13}C$ value displayed decreasing or constant isotopic ratios. The early rise in $\delta^{13}C$ is partly due to decrease in the fraction of metabolic oxidized carbon in the fish's blood as they mature, relative to the proportion of seawater derived dissolved inorganic carbon (DIC), and partly due to a dietary shift to higher trophic-level foods with higher $\delta^{13}C$ values. The maximum in $\delta^{13}C$ is attained at an age close to that of the age of maturity of cod, and may be indicative of that transition. The maximum $\delta^{13}C$ value attained by otoliths decreased steadily from 1984 to 1993, a period during which cod stocks in Atlantic Canada experienced a drastic decline. The age of attainment of maximum $\delta^{13}C$ decreased abruptly between 1984 and 1985, approximately coeval with changes in population dynamics of the 4Vs stock. This decrease, as well as the post-maximum decrease in $\delta^{13}C$ values of the mature cod otoliths may represent movement of the fish to deeper waters of the shelf, where $\delta^{13}C$ of DIC is lower.

4.2 Introduction

Stable carbon and oxygen isotope ratios of biogenic carbonates can provide information on the life history of marine organisms and the conditions under which carbonate deposition occurs. Teleost fish possess aragonitic otoliths which are formed close to oxygen isotopic equilibrium with their environmental water (Kalish, 1991; Patterson et al., 1993; Thorrold et al., 1997). As a result, the $^{18}O/^{16}O$ ratio of otolithic aragonite can be used as a measure of growth temperature; with increasing T, $\delta^{18}O$ decreases (Kalish, 1991). In addition, we note that otoliths grow from a fluid medium (endolymph) containing calcium ions and dissolved inorganic carbon (DIC), dominantly bicarbonate (HCO_3^-). The carbon isotopic composition of DIC in blood is determined by a balance between bicarbonate formed during oxidative metabolism and isotopic exchange with seawater DIC through the gills (via CO_2). A small amount of HCO_3^- is absorbed through the gut from ingested seawater (Loretz, 1995). Mulcahy et al. (1979) showed a correlation between $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios in a benthopelagic Pacific fish (*Coryphaenoides acrolepis*). They found that $\delta^{13}C$ of the otolith increased with increasing age of the fish; they attributed this increase to a decrease in metabolic activity with age. As a result, DIC of the blood in older fish is dominated by seawater derived carbon. Mulcahy et al.'s study suggests the possibility of using $\delta^{13}C$ of aragonite to trace the change in metabolic activity through the lifetime of a fish as reflected in the change in the balance between endogenous, metabolic carbonate and isotopic exchange with seawater DIC. As well, these changes can reflect changes in habitat (as a result of depth-dependent changes in $^{13}C/^{12}C$ of DIC of seawater) and changes in nutrient sources used by the fish, which might affect $^{13}C/^{12}C$ of metabolic carbon.

In this paper we present a study of life-long variations in $^{13}C/^{12}C$ of otoliths of cod (*Gadus morhua*) from Atlantic Canada. The first purpose of the study was to study the variation in $\delta^{13}C$ within single otoliths in order to evaluate the comparative roles of isotopic exchange and metabolic activity on the isotopic composition of seasonally and annually deposited zones. The second purpose was to compare the isotopic composition of otoliths deposited over the last two decades to see that if any secular changes could be detected, and to see if these changes could be related to the decline in cod stocks over this interval.

It is important to note that otoliths grow continuously through the entire life of the fish and thus preserve an uninterrupted record of the metabolic state of the fish. Also, once otoliths have been laid down in a fish, they remain chemically (and isotopically) inert and therefore preserve the life-long record of otolith growth (Campana and Neilson, 1985). We also note that these same samples of otolith aragonite which give us a record of $^{13}C/^{12}C$ also give a simultaneous record of variation in oxygen isotope ratios. The latter ratios are only in some instances correlated with $^{13}C/^{12}C$ and appear to vary principally in response to changes in the external environment (temperature and salinity). We will discuss these effects in a accompanying paper (Gao et al., 1997b).

4.3 Materials and methods

Otolith samples were taken from collections by the Marine Fish Division at the Bedford Institute of Oceanography (BIO), from the 4Vs Subdivision (Fig. 3.1) between the even-year captures from 1988 to 1994 (cf. 3.3.1). These otoliths range in age from 2 to 11 years and their background information was already presented elsewhere (see Table 3.1). After embedding in resin, transverse sections 700-800 µm thick, passing through the nucleus, were made of each sagittal pair, using a high-speed diamond saw. The sectioned otolith plates were then prepared and each translucent and/or opaque zone was milled out by using a computer-controlled micromilling technique (*DM 2800*) as described previously (Gao et al., 1997b). The trace of the milling typically consisted of tracks about 150-300 µm deep, 25-60 µm wide, extending along a single growth zone. The samples were then analyzed on an Autocarb attached to a VG Optima mass spectrometer. All the analyses are given in the common δ notation (‰) with respect to the VPDB standard, and the precision of the analyses for the present study is better than ± 0.06‰.

4.4 Results

We analyzed a total of 61 otoliths whose zones were deposited over the years 1980 to 1994. Isotopic differences between adjacent opaque and translucent (summer and winter) zones were very small compared to the life-long trend of each otolith. Figure 4.1 shows the distribution of these seasonal differences in $\delta^{13}C_{S\text{-}W}$ for a random subsample of 48 pairs. The distribution is approximately normal around a mean value of 0.26 ± 0.39 ‰ (1σ). Typical trends of $\delta^{13}C$ for opaque and translucent zones are shown in Figure 4.2; although some otoliths display consistent $\delta^{13}C$-enrichment of summer zones with respect to winter, in others the inter-seasonal differences are quasi-random. These do not, of course, represent the maximum interseasonal differences that exist within an otolith because the milled-out samples average over almost the entire thickness of each zone, especially for samples taken after the fifth year of growth. In view of the small interseasonal difference, we shall represent annual and secular variations in terms of the averages of adjacent translucent and opaque zones.

Every otolith which we measured from the 4Vs stock displayed a similar trend of $\delta^{13}C$ with age over the first 4 to 6 years of growth; typical samples are shown in Fig. 4.3. In each otolith, $\delta^{13}C$ increases through life starting at an initial value of between -5 to -2.5‰. The rate of yearly increase in $\delta^{13}C$ gradually diminishes as the cod matures, and the majority of the otoliths reach a maximum value near 0 ‰ at age 4 to 6 y. For those fish which exhibit a maximum in the curve of $\delta^{13}C$ *vs* age (n = 36 out of 61), the subsequent pattern of $\delta^{13}C$ values differs between fish, either remaining constant or decreasing steadily until the date of the capture.

In Figure 4.4 we have combined the data for all otoliths to show the similarity in the life-long trends of $\delta^{13}C$ through the periods of sampling. Each mesh point on the surface fitted to these data is the average of all analyses of a given age (ranging from 1 to 10) that was deposited in a given calendar year (1980 to 1994). The individual mesh points on the surface incorporate data from between one and four analyses. A small part of the spread around the fitted surface arises from the difficulty in milling out samples which precisely correspond to a given year but this introduces an error in age of less than one year. The figure shows the general trend of exponentially rising $\delta^{13}C$ values prevalent in the population over the entire period of sampling, reaching a maximum near 0 ‰ at age about 5 and then gradually declining. Figure 4.4 shows that the early (0-5 y) behavior of otolith $\delta^{13}C$ values is similar in all cod, independent of the calendar years in which growth occurred (with the exception of those fish hatched after 1987 and which had not yet lived long enough to reach a maximum). As we shall see below, this is probably due to a combination of effects including life-long changes in diet and metabolic rate.

The majority of the otoliths analyzed (70 %) exhibit a maximum in the plots of $\delta^{13}C$ *vs* age. The remainder did not display a maximum, that is, the slope of the $\delta^{13}C$ *vs* age plot was positive to the end of the isotopic record. Some of these fish may have been captured before they reached that stage in their development, but others lived up to 8 years without exhibiting a reversal in the age trend of $\delta^{13}C$. For those fish which do display a maximum, both the age and the value of this maximum appears to have been changing through time. Figure 4.5 shows a plot of the age at which maximum $\delta^{13}C$ was reached in a series of cod which were hatched between 1980 and 1990. The age at maximum $\delta^{13}C$ appears to have decreased from about 5 years before 1984 to about 3 years after 1985. Figure 4.6 shows the average maximum $\delta^{13}C$ recorded for those cod which reached that maximum in a given year *vs* the year in which the maximum occurred. There is a gradual but significant decrease in maximum $\delta^{13}C$ values through the period from 1984 to 1993.

4.5 Discussion

The isotopic data described above appear to fall into two categories: effects that are essentially independent of the year in which cod was living; and secular effects which change in a consistent manner on a year-to-year basis, and therefore appear to be driven by changes in the environment or by long-term changes in the behavior of the fish. As well we have noted an absence of a large seasonal cycle in $\delta^{13}C$(oto).

4.5.1 Time-independent changes: variation in $\delta^{13}C$ of immature cod

Variation of $\delta^{13}C$ in cod otoliths is strikingly uniform during the first 4 to 6 years of growth in all the fish studied here. The trends with age are similar to those shown by Mulcahy et al. (1979) for *Coryphaenoids acrolepis* from the Pacific. Cod otoliths of unknown origin from around Nova Scotia, investigated in a pilot study (Browne, 1995) showed identical trends of increasing $\delta^{13}C$ in their first 5 years. These trends are almost independent of the years in which growth occurred, and suggest that this trend is a result of persistent characteristics of the fish, which may be a blend of genetically controlled physiological changes together with habitual, life-long changes in living environment and/or food preferences of the cod. Their persistent, repetitive character suggests that they are not a consequence of changing regional environment. In general, $\delta^{13}C$ increases steeply from initial values between -5 and -2.5 ‰, and gradually levels off at a value near 0 ‰ after age 4 to 6 y. There are no deviations from the trend of increasing $\delta^{13}C$ values in any of the 4Vs otoliths. In order to account for this increase in $\delta^{13}C$ we must consider the principal factors which determine the carbon isotopic composition of the otoliths.

We assume that aragonite is in carbon isotope equilibrium with dissolved CO_3^{2-} ions in the fluid from which it grows. Thus, $\delta^{13}C$ of each successive layer of aragonite is determined by the equilibrium fractionation factor, $\Delta_{ac} = \delta^{13}C(arag) - \delta^{13}C(HCO_3^-)$ and by the isotopic composition of the bicarbonate, $\delta^{13}C(HCO_3^-)$, which would be the dominant species of DIC in the endolymph. We can assume that $\delta^{13}C(HCO_3^-)$ of the endolymph is equal to that of bicarbonate in the blood of the cod since there is little metabolic activity in the endolymph itself and no isotopic fractionation during transport of bicarbonate into the auricular chamber where the otolith grows. The $\delta^{13}C$ value of blood bicarbonate is mainly determined by the balance between two processes: (1) metabolic generation of oxidized carbon; and (2) uptake of DIC from seawater. A small amount sea water DIC can be taken up through the digestive tract as a result of the ingestion of seawater (Loretz, 1995). We suppose, however, that the main route of entry of marine carbon is through the gills. Only neutral (uncharged) molecules can pass between seawater and the fish's blood, so DIC exchange must occur as a result of diffusion of neutral CO_2 molecules from seawater into the blood. Even though the outward diffusive flux of CO_2 is much greater, and molecular CO_2 makes up less than 1 % of seawater DIC, the constant exposure of the blood to the inexhaustible reservoir of DIC that is in the ocean assures that the blood's DIC approaches a steady-state with respect to inward diffusion of CO_2. As a result of equilibrium isotopic fractionation, CO_2 that diffuses through the gills from seawater will have a $\delta^{13}C$ value about 7 ‰ less than that of bicarbonate (Friedman and O'Neil, 1977). However, this dominant DIC species in blood is also HCO_3^-, and it will be about 7 ‰ enriched in ^{13}C (also due to isotopic equilibration) with the net result that blood DIC will tend to approach the $\delta^{13}C$ value of DIC in seawater. The other component of the blood's DIC is presumed to be metabolic carbon, principally in the form of bicarbonate, which would have a $\delta^{13}C$ value ($\delta^{13}C(met)$) very close to that of the fish's diet. This two-component model requires that the $\delta^{13}C$ value of HCO_3^- in the endolymph must lie somewhere between the value of metabolic carbon (which is assumed to be oxidized without isotopic fractionation) and the $\delta^{13}C$ value of DIC in seawater. We can then write an expression to describe the variation in $\delta^{13}C$ of the otolithic aragonite ($\delta^{13}C(oto)$) as follows.

We assume that, whatever is the $\delta^{13}C$ of blood DIC, it is related to that of otolith aragonite by the fractionation factor Δ_{ac} which varies with temperature (T) as shown by Grossman and Ku (1986):

$$\delta^{13}C(oto) = \delta^{13}C(DIC) - 0.131T + 2.66 \qquad (1)$$

For our purposes, we can assume an average temperature value of 5 ^0C, so that

$$\delta^{13}C(oto) = \delta^{13}C(DIC) + 2.0 \qquad (2)$$

From the T coefficient in equation 1 we see that a 5 ^{0}C change in temperature would change δ^{13}C(oto) by only about 0.7 ‰ and we can practically neglect the influence of temperature on δ^{13}C of the aragonite deposits (whereas temperature is the dominant control on δ^{18}O). Thus δ^{13}C(oto) is mainly dependent on δ^{13}C(DIC), which depends in turn on the balance between the two sources:

$$\delta^{13}C(DIC) = M\delta_d + (1\text{-}M)\ \delta_{sw} \qquad (3)$$

where δ_d is the δ^{13}C value of the diet, δ_{sw} is δ^{13}C of DIC in seawater, and M is the fraction of metabolic carbon in the blood DIC.. Combining equations (2) and (3),

$$\delta^{13}C(oto) = M\ \delta_d + (1\text{-}M)\ \delta_{sw} + 2 \qquad (4)$$

Therefore, the variation in δ^{13}C(oto) is dependent on variations in all three variables on the right side of this equation. We shall use this approach to account for the life-long change in δ^{13}C(oto). First, we can estimate the variability in each of the terms in this equation and how they contribute to the life-long variation in δ^{13}C(oto).

δ_{sw}: The δ^{13}C of marine DIC varies greatly in the upper few hundred meters of the sea (Kroopnick, 1980), from near-surface of about +2 ‰, to slightly negative values at depths of about 1 km. In general, δ^{13}C(DIC) also varies seasonally in response to changes in photosynthetic activity of phytoplankton (which is the process responsible for the enrichment of ^{13}C in near-surface waters; Kroopnick, 1974). If most otolith growth occurs in the upper 300 m of the water column then the marine component of the fish's DIC will have a δ^{13}C value of about 1±1‰, but can be expected to change as the living depth of the fish changes through its lifetime and seasonality.

δ_d : The δ^{13}C of the diet of cod is determined by the nature of the foods which the cod eats through its lifetime. Young cod fry are believed to live on a variety of smaller marine organisms including copepods, amphipods, and other small crustaceans (Lear, 1993). Juvenile cod feed on shrimp, amphipods, euphausiids, and the larvae of fish and shellfish. Mature fish feed on a variety of larger marine organisms including fish and shellfish. Thus, like other marine fish, their diet changes from lower to higher trophic-level organisms within the lifetime. There is a tendency for the $\delta^{13}C$ of the flesh of marine organisms to increase with trophic level, by slightly less than 1 ‰ for each trophic level (Peterson and Fry, 1987). We might therefore expect a life-long increase in $\delta^{13}C$ by as much as 3 ‰, which is a large fraction of the total observed shift.

M: It is determined by the rate of metabolic production of DIC relative to the rate of exchange of CO_2 between blood and seawater in the gills. To a large extent, these are independent processes, even though one of the functions of gas exchange in the gills is to dispose of excess DIC from the blood. However, the exchange of dissolved CO_2 between seawater and blood is essentially passive and only controlled by the rate of blood flow. The latter is not likely to change with age (as a fraction of total mass of the fish) whereas the metabolic rate decreases with age. For example, Edwards et al. (1972) showed that oxygen consumption, Q_{O2}, of cod varied as a function of weight (W, in grams)

$$Q_{O2} = 0.245\ W^{0.82}\ (\mathrm{mg/h}) \qquad (5)$$

This shows that oxygen consumption decreases more slowly than weight, and therefore the O_2 consumption per gram decreases as the fish ages.

We can now estimate the contributions of variation in M, δ_d, and δ_{sw} to the observed life-long variations in $\delta^{13}C$(oto). To a first approximation this variation can be described as a steep, quasi-linear increase in $\delta^{13}C$ over the first 3-5 y, followed by a continued increase at gradually decreasing rate, in many cases reaching a maximum. It is probable that during successive stages in the life-history of $\delta^{13}C$(oto), successively different terms in equation 4 are controlling the variation in isotopic composition.

The steep initial increase in $\delta^{13}C$(oto) by up to 4 ‰ is most likely due to a change in trophic level. It is well known that smaller fish tend to feed at lower trophic levels than larger fish in the same ecosystem (Peterson and Fry, 1987). We might reasonably suppose that the same would be true of the same fish at successive stages of its life. That is, as a given fish grows to successively larger sizes, its trophic level increases and, as a consequence, its tissues would increase in δ_d. From equation 4, we see that this would lead to a steady increase in $\delta^{13}C$(oto), even if the metabolic fraction M remains constant with age. We do not yet have any direct evidence of this increase in trophic level. This could be observed by studying $\delta^{13}C$ of the flesh of cod of successive increasing age, but we know of no data on this subject or on the age-dependent increase in $\delta^{13}C$ of the tissues of any fish. A possible alternate approach to this problem is to analyze the collagen fraction of some bony tissue of the fish which gradually accretes through the life of the fish, such as the operculum or the cleithrum. The $\delta^{13}C$ value of collagen in bones is typically equal to that of the diet of the animal plus a constant offset (in mammals $\Delta^{13}C$(coll-diet) = 5 ‰).

Let us consider the possible effects of changing values of M, the metabolic fraction of bicarbonate in the fish's blood. For the moment, let us assume that δ_{sw} is held constant at an average value of +1.0 ‰, typical of seawater at depths of 100 to 300m. At maturity, most cod otoliths reach a maximum $\delta^{13}C$ value close to 0 ‰, after which $\delta^{13}C$ changes little or decreases slightly. This suggests that blood DIC has reached a steady state with respect to diffusion and metabolic input. From the form of equation 5 we see that M must tend to decrease with age, correlated with the increasing weight of the fish. This suggests that at the maximum value of $\delta^{13}C$(oto), M has reached a minimum value. At the minimum limiting value of M = 0, equation 2 would give $\delta^{13}C$(oto) = 3 ‰; clearly the fish could never reach this state which would require zero metabolic contribution. The maximum value of $\delta^{13}C$(oto) actually observed is about 0.8 ‰, which we can use in equation 4 to estimate the minimum value of M. For this purpose we can use the $\delta^{13}C$ of the flesh of mature cod as an estimate of δ_d. A cod caught in the Bay of Fundy, gave a $\delta^{13}C$ value of -17.0 ‰, while another cod obtained in Halifax (presumed to have been caught in the Scotian Shelf region) gave a $\delta^{13}C$ value of -18.4 ‰. These are similar to values reported for other North Atlantic fish (Fry, 1988), and should be close to values for the flesh of 4Vs cod. Assuming a mean value of δ_d = -17.5 ‰, equation 4 gives M = 0.12.

Conversely, M would have achieved its maximum value in the juvenile life-stage of the cod, when the fish was eating at a lower trophic level. We shall assume that juvenile cod consume a diet which is 3 ‰ lighter than that of mature cod. Then (still assuming $\delta_{sw} = +1‰$) the lower limit to our observed range of δ^{13}C(oto), about -5 ‰, corresponds to M = 0.37, showing that even in young fish, more than half the DIC in the plasma is externally derived. From these calculations, it appears that a significant part of the changing δ^{13}C(oto) may also due to a decrease in M. Further resolution of this partitioning of the variation must await better estimates of the variation δ_d with age. Clearly, both factors contribute significantly to the observed variation and at this point it is impossible to quantify the separate contributions of each to the general pattern of life-long increase in δ^{13}C.

Let us now consider the contribution of variation in δ_{sw} (δ^{13}C of DIC in seawater) to variation in δ^{13}C(oto). Because cod are known to change their living depth both seasonally and as a function of age, they will therefore experience varying δ_{sw}. However, the maximum variation to the expected is ±1 ‰. Therefore, this effect will be small compared to the effects of changes in δ_d and M.

These calculations show that the rise in δ^{13}C(oto) over the first 4-6 y of life could be partly accounted for a combination of a progressive decrease in M, the fraction of metabolic bicarbonate in endolymph and an increase in δ^{13}C of the average diet, the δ_d due to an increase in trophic level. The effect on δ^{13}C(oto) of decreasing metabolic activity of the fish with increasing age was also suggested by Mulcahy et al. (1979). However, typically δ^{13}C(oto) increases approximately linearly with age (Fig. 4.3) whereas the variation of M with age is non-linear (eq. 5). Therefore it appears that most of the early variation in δ^{13}C(oto) is a result of change in diet, rather than declining metabolic rate. Decreases in δ^{13}C after about 6 y are most likely due to decrease in δ_{sw} as the living depth of the cod increases.

As noted, δ^{13}C values of most otoliths reach a maximum between year 4 and 6, after which there is generally a decrease in δ^{13}C with age within individual cod (Fig. 4.4), although in some otoliths the isotope ratio remains essentially constant after reaching a maximum value (e.g., Fig. 4.3b). The attainment of this maximum in the δ^{13}C value of otolithic aragonite marks some changes in the physiological characteristics of the fish. Typically we see this occurring close to age about 6 y, when cod reach sexual maturity (Lear, 1993). It is possible that the attainment of this isotopic steady state is an indicator of that stage in the life of the cod. If so, carbon isotopic analyses would provide a means by which to determine when maturity had been reached in a given individual or population. Further study needs to be made of the correlation between this maximum and physiological correlates of sexual maturity. Three otoliths which we studied did not exhibit a maximum at all, but showed a continuously rising record of $\delta^{13}C$(oto) up to the time of their capture, even after seven years of growth. Two of these individuals had exceptionally high initial $\delta^{13}C$(oto) values (> -1.5 ‰), which may be significant in explaining the continuously rising $\delta^{13}C$(oto) signal of these fish.

4.5.2 Lack of Interseasonal difference in $\delta^{13}C$(oto)

There is a surprisingly small difference between $\delta^{13}C$ of opaque and translucent zones (0.26 ± 0.39 ‰). The metabolic rate of Atlantic cod doubles with a 10 ^{0}C rise in temperature (Brander, 1996). We would therefore expect a large increase in M with increasing temperature in the summer, leading to a corresponding decrease in $\delta^{13}C$ of DIC and of otolithic aragonite. In fact, the average seasonal difference in $\delta^{13}C$(oto) is positive, and for the majority of fish studied here, the $\delta^{13}C$ of aragonite laid down in the summer is significantly enriched in ^{13}C with respect to adjacent winter deposits. While $\delta^{13}C$(oto) does not give a precise thermometric value for the fish's environment, we are surprised to see that even the sign of this isotopic difference is the opposite of expectation.

Two factors may contribute to this phenomenon. First, we know that cod would prefer to stay in waters of constant temperature, and will therefore migrate vertically in the water column to a depth at which the temperature is maintained at a constant low value. Therefore, the temperature changes experienced by marine fish (and 4Vs cod in particular) would be much less than those experienced by lake or river fish, where most studies of metabolic effects of temperature have been done (Patterson et al., 1993). For example, Patterson (in press) shows a seasonal fluctuation in $\delta^{13}C$(oto) of up to 4 ‰, with δ(summer) < δ(winter) as expected.

Secondly, we know that there is a seasonal cycle in the gradient of $\delta^{13}C$ of DIC with depth. The enrichment of near-surface seawater bicarbonate in ^{13}C is a result of photosynthetic activity by plankton, which preferentially reduce HCO_3^-, leaving the residue enriched in ^{13}C. During the summer the $\delta^{13}C$ of DIC in seawater at a given depth will be higher than that in the winter, due to lower photosynthetic activity at the lower diurnal light levels experienced in winter. Both of these effects will tend to oppose the temperature-driven shift to lower $\delta^{13}C$(oto) values expected in summer.

4.5.3 Time-dependent changes: variation in $\delta^{13}C$ of older cod

After reaching a maximum value, $\delta^{13}C$ gradually decreases in most otoliths (Fig. 4.4). But in addition, the maximum $\delta^{13}C$ attained by each otolith gradually decreased through the period 1984-93 (Fig. 4.6). This decrease could be due to a number of causes. In principle this could be the result of a decrease in the metabolic activity during this period. Compana et al. (1995) have shown that the growth rate of cod in 4Vs had declined over this period; this is likely to have been coupled with a decrease in the metabolic rate. Changes in seawater temperature could also have lowered the metabolic activity. However, the shift to lower $\delta^{13}C$(oto) values which we observed would correspond to an *increase* in M during this period. An accompanying study of oxygen isotope ratios in these otoliths (Gao et al., 1997b) suggests that the opposite has occurred, namely a decrease in environmental temperatures experienced by the cod. A decrease in $\delta^{13}C$ of the nutrient sources could also account for this shift. Local variations have been recorded in $\delta^{13}C$ of phytoplankton in the Atlantic (Fontugne and Duplessy, 1981) but we have no evidence that the distribution pattern of $\delta^{13}C$ values has changed over recent times. In particular, there is no reason to expect such a shift over the past decade.

Another, more likely cause of this downward shift in $\delta^{13}C$ is a decrease of the $^{13}C/^{12}C$ ratio of DIC in the seawater to which the cod is exposed. This would happen if the cod began to live at greater depths (Kroopnick, 1980). We would expect this change in living depth to be correlated with other environmental changes. For cod living at progressively deeper levels of the cold intermediate layer (Drinkwater, 1996) the fish would also experience a progressive decrease in average water temperature, which is somewhat borne out by our accompanying study of $\delta^{18}O$ (Gao et al., 1997b). Measurements are now in progress of the local depth gradient in $\delta^{13}C$ of seawater DIC for the lower water mass.

Coupled to this decrease in the value of the maximum $\delta^{13}C$(oto) during this interval is a decrease in the age at which this maximum occurs. We have suggested that this may be the time of sexual maturity of the cod (Fig. 4.5). Fish hatched prior to 1985 reach maximum $\delta^{13}C$ at age 5-6 y, while after 1985, they reach their maximum value at an average age of 3 y. This is a striking change in the behavior of the cod, and would suggest that this was a period of major environmental stress. The year 1985 also marks a period of abrupt change in the population dynamics of the 4Vs stock. Note however, that the decrease in growth rate during this period observed by Campana et al. (1995) would be expected to lead to an *increase* in the age of sexual maturation, so the data of Fig. 4.6 cast some doubts on the interpretation of the attainment of maximum $\delta^{13}C$ as a mark of maturity.

4.6 Conclusions

We have shown that single otoliths of cod can be sequentially sampled to provide $\delta^{13}C$ records of seasonal growth zones. Studying otoliths of cod from the 4Vs region of the Atlantic coast, we have seen some striking trends in the behaviour of $\delta^{13}C$ of the otoliths, both over the lifetime of the individual fish, and also as secular trends in the population as a whole, over the period from 1980 to 1994.

There is a single consistent trend within all otoliths that we have studied: $\delta^{13}C$ of the otolithic aragonite gradually increases from values around -5 to -2.5 ‰ to near 0 ‰ over the first 5 years of life of the fish. We can account for this in terms of a life-long decrease in the amount of metabolic DIC in the blood plasma of the fish as compared with the amount of CO_2 exchanged between blood and seawater through the gills, coupled with a shift in diet from lower to higher trophic levels. Change in living depth may also have some influences on this shift. The DIC component of the plasma is assumed to be transmitted unaltered into the endolymph. The proportion of metabolic DIC in the endolymph drops from nearly 50 % of total DIC in 1 y-old cod, to a minimum near 12 % in 5 y-old cod. The form of the increase in $\delta^{13}C$ of the otoliths, linear at first and later exponential approaching an asymptotic limit, suggests that the early increase (0-5 y) is dominated by a shift in dietary $\delta^{13}C$, whereas the later shift (> 5 y) appears to be related to the decrease in metabolic activity which is exponentially related to body weight and therefore to age. The $\delta^{13}C$ values of the otoliths reach a maximum in most fish that we have studied, between ages 4 to 6. Subsequently, $\delta^{13}C$ either stabilizes or decreases, with the majority showing a slight decrease.

As opposed to these uniform life-long trends observed in each cod, we have also observed some secular trends in the population as a whole. The maximum $\delta^{13}C$ value for 4Vs cod has been gradually decreasing over the decade prior to 1992. As well, the age at which the cod reaches this maximum value has dropped, most rapidly during the period 1984-85. Both of these trends must reflect the changing environment during this period, but the specific environmental effects responsible for these secular trends have not yet been clearly defined. Decrease in the food supply could have partially starved the cod, and effectively reduced their metabolic level; a decrease in growth rate has also been observed in this period. A simpler explanation is that the cod have been progressively shifting their habitat to waters with lower $\delta^{13}C$ of DIC. This could be achieved by the cod spending a longer time in deeper water which are also known to be more ^{13}C-depleted.

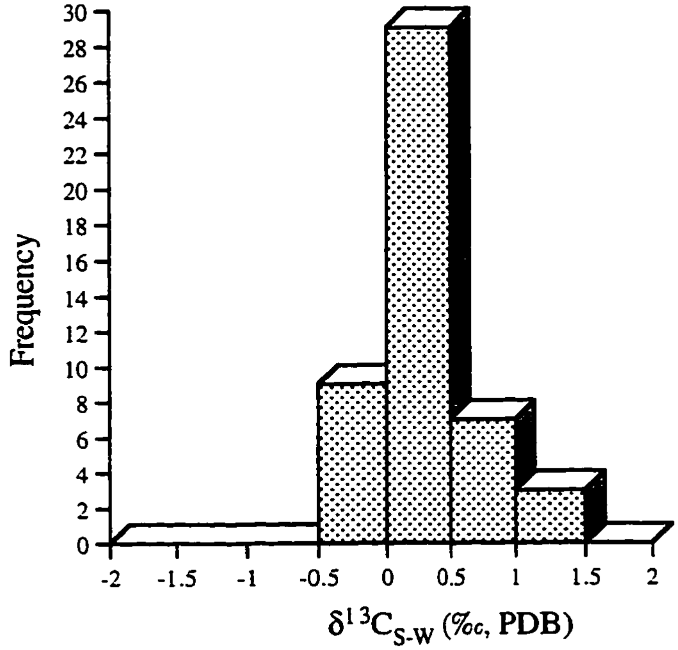

Fig. 4.1 Histogram showing the seasonal carbon isotopic differences (*summer-winter*) for 48 pairs.

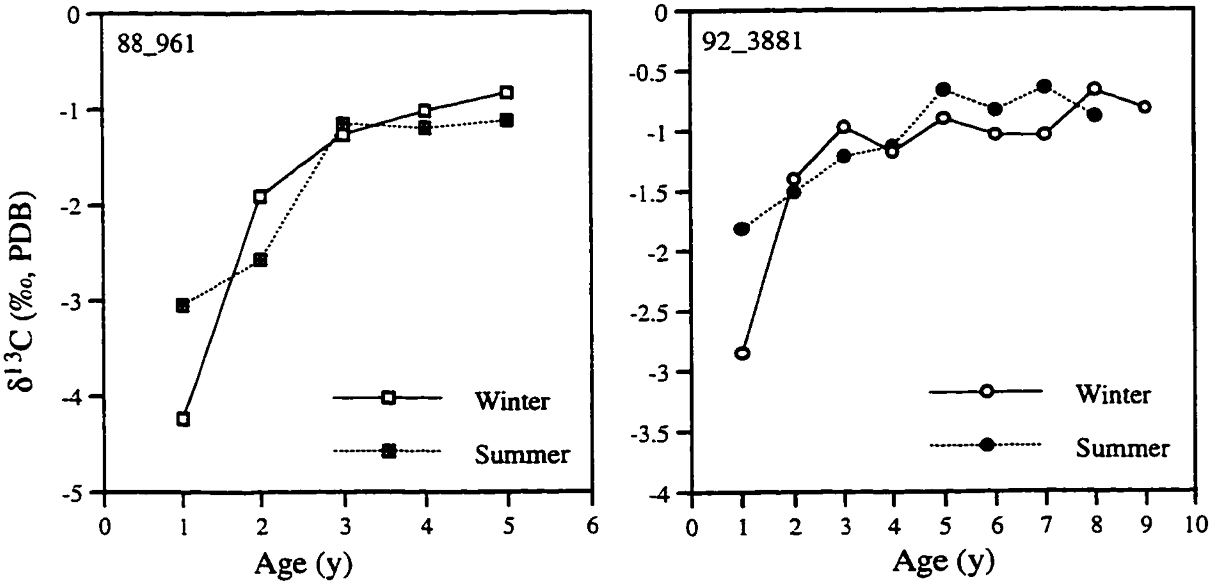

Fig. 4.2 Typical graphs of interseasonal $\delta^{13}C$ differences between the translucent (*winter*) and opaque (*summer*) otolith zones from 4Vs cod.

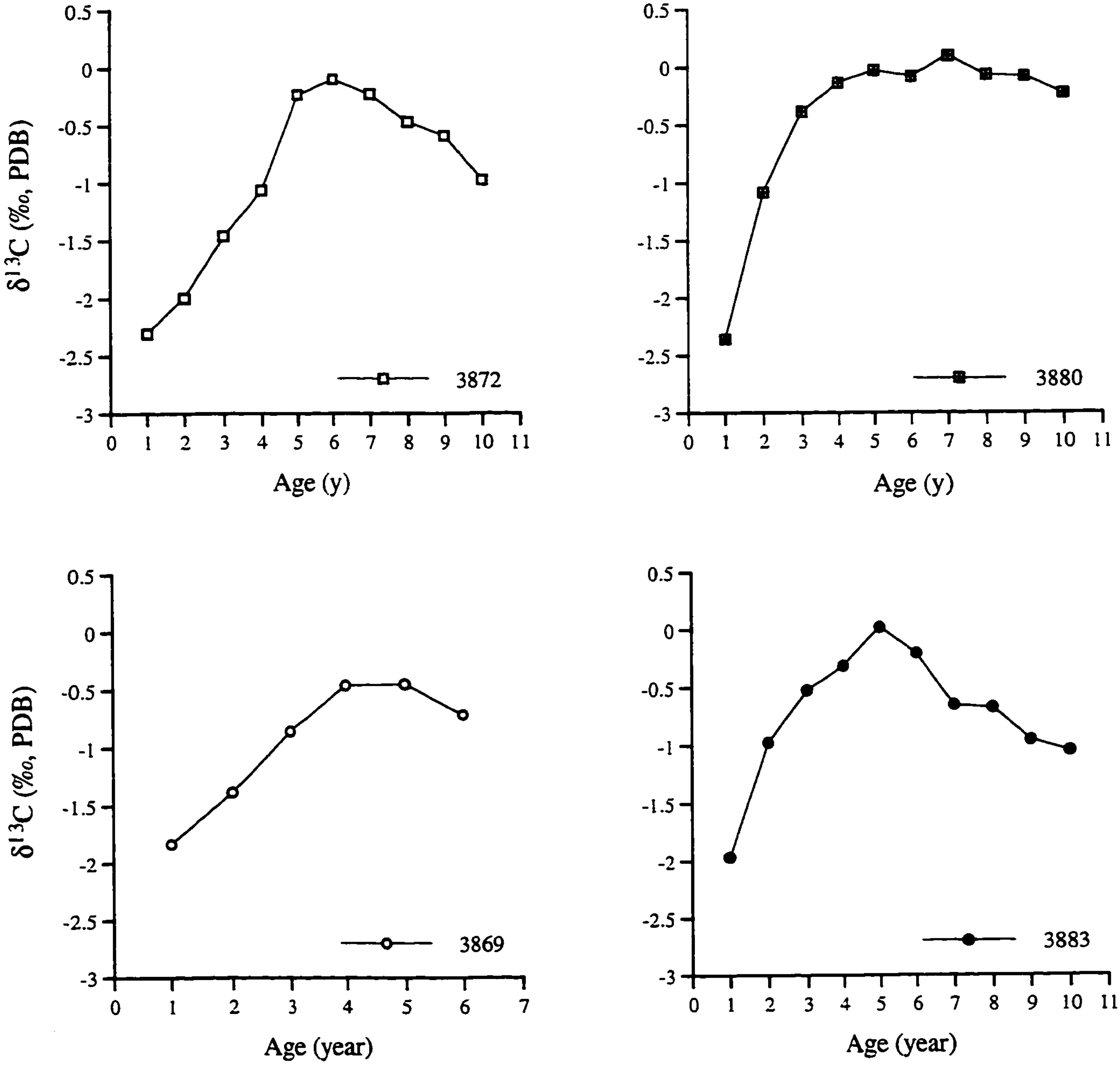

Fig. 4.3 Carbon isotopic ratios of otoliths plotted versus year of deposition for four cod otoliths from the 4Vs Subdivision.

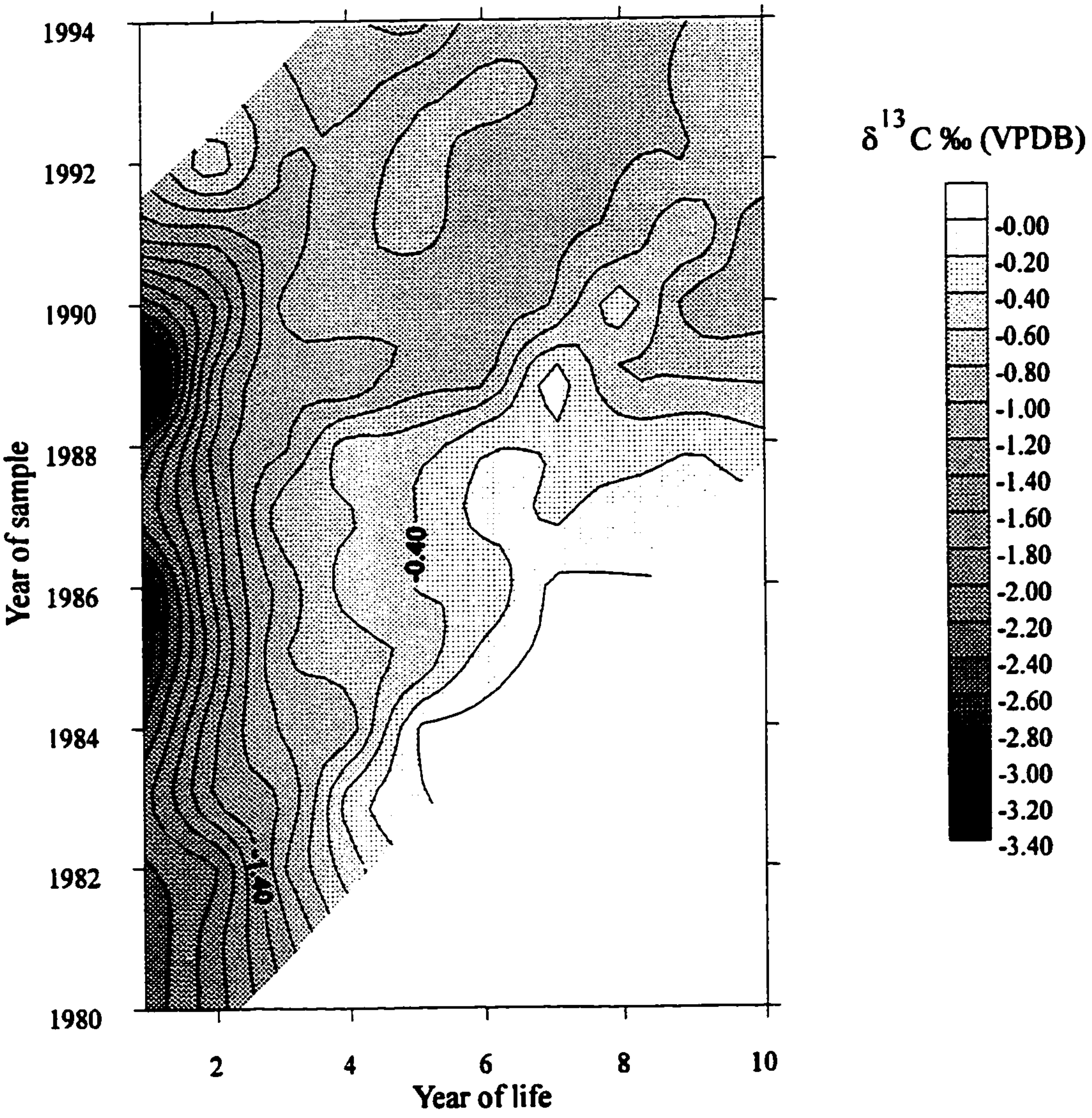

Fig. 4.4 Polynomial regression surface for total data set of $\delta^{13}C$ values for 61 otoliths from the 4Vs Subdivision.

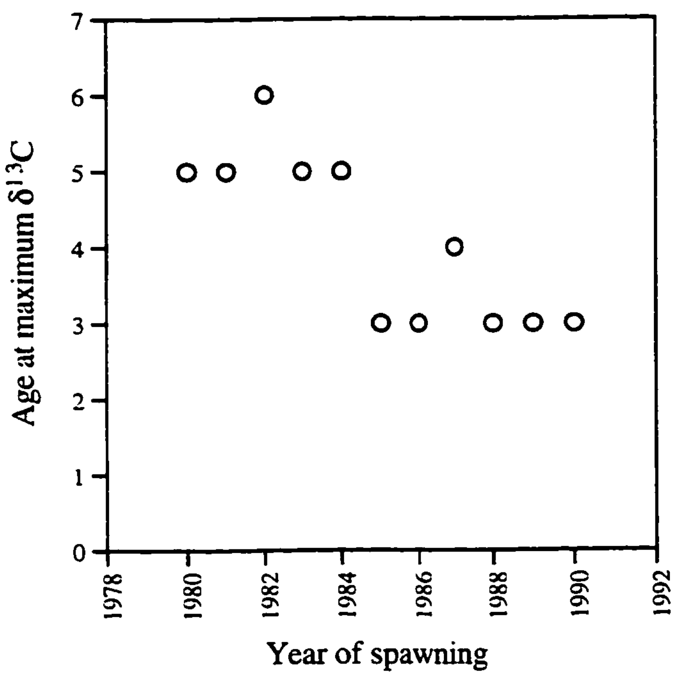

Fig. 4.5 Age at which maximum $\delta^{13}C$ was reached in a series of cod which were hatched between 1980 and 1990 as a function of age at which maximum occurs. The age at maximum $\delta^{13}C$ drops abruptly between 1984 and 1985.

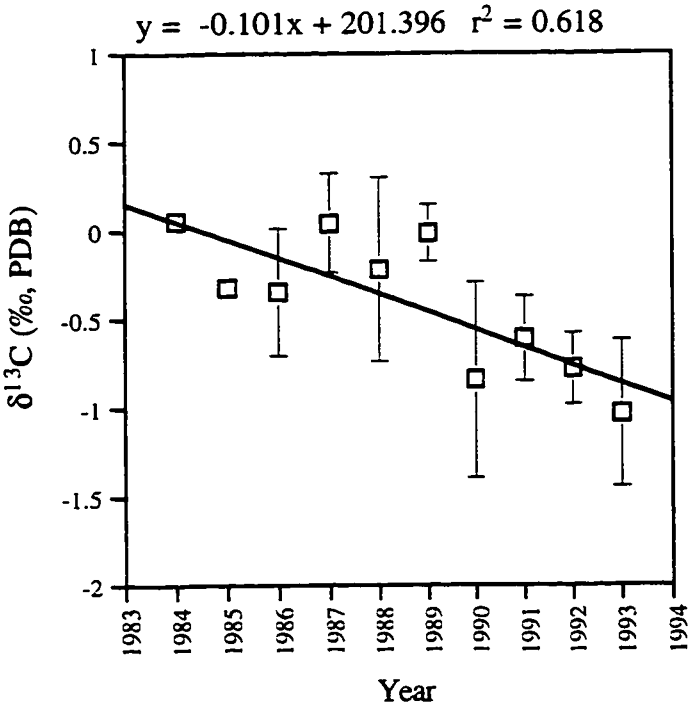

Fig. 4.6 Average maximum $\delta^{13}C$ value recorded for all the cod which reached their maximum in a given year versus the year in which the maximum occurred.

CHAPTER FIVE

Oxygen and carbon isotopes of known-history otoliths of cod, in comparison with the field cod from 4Vs

5.1 Abstract

The known-history otoliths of Norwegian cod (*Gadus morhua*), reared in the ocean-pens up to 6 years old, provide a unique opportunity for us to study the life-long history of the fish and population dynamics. We analyzed 18 pairs of these otoliths for stable oxygen and carbon isotopes, and compared them with the results from the field cod at the same age group from the northeast Scotia Shelf. There were distinct differences in the range of isotopic composition, the life-long variations in both $\delta^{18}O$ and $\delta^{13}C$, and the correlation between $\delta^{18}O$ and $\delta^{13}C$. These differences were probably attributed to the different living environmental constraints and food supply, indicating that the laboratory experiments might be too limited in study of the influence of environmental parameters. There is also evident that the isotopic temperature estimations were generally in agreement with the records during rearing, suggesting that temperature is a dominant factor in the forming of seasonal otolith aragonite, and the $^{18}O/^{16}O$ ratio can be potentially used as a thermometer in study of the temperature history of the ambient seawater.

5.2 Introduction

Rearing fish under controlled temperature and salinity and then coring their otoliths for microstructural studies is generally considered as one of the best methods for determining the optimal growth and early life-history of the fish, because larvae are good indicators of marine environmental quality (e.g., Laurence, 1978; Brown et al., 1989; Clark and Green, 1991; Lambert et al., 1994) and otoliths can store the initial information about the teleost development (e.g., Brothers & McFarland, 1981; Lecomte-Finiger, 1992; Hare & Cowen, 1994). However, there are also some disadvantages with the experimental approach: (1) in comparison with the fish's life-long record, the information derived from the larval stage might be very useful for aquaculture, but too limited to decipher the adult population and recruitment dynamics since most laboratory experiments were conducted only a couple of months; (2) according to our oxygen isotopic studies on Atlantic cod (*Gadus morhua*), the isotopic variations between winter and summer are greatest in the first year, suggesting that the variations in the fish's living conditions and vertical migration at the larval stage would be maximal (Gao et al., 1997b). Otoliths of ocean-pen reared cod surviving as long as 6 years, therefore, would be especially valuable in study of the life-long history and the influence of environmental factors.

In the present study, we have collected 32 pairs of known-history cod otoliths (*Gadus morhua*) from Norway. These cod were hatched from eggs in the laboratory and then transferred to meshed pens in the ocean when they were a few months old and about 10 cm long. They were reared there for the next 6 years (1989-1994) to a length of about 70 cm. Recording thermometers and salinity gauges were mounted in each pen during the rearing. The temperature in the pens was naturally homogeneous and the salinity varied by natural fluctuations of the seawater. Thus, the pen environment was superior to the situation in tanks used in many laboratories, and thus was suitable for stable isotopic analyses.

Epstein et al. (1953) showed that it is possible to determine the living temperature of an organism from the oxygen isotope fractionation between biogenic calcite or aragonite ($CaCO_3$) and ambient seawater. Since then a number of experiments have followed and more temperature equations have been generated for a variety of organisms (e.g., Horibe and Oba, 1972; Killingley et al., 1981; Grossman and Ku, 1986). The first attempt for otoliths of cod, among others, was conducted by Radtke et al. (1996). From their experiments, Radtke (1984) and Radtke et al. (1996) concluded that otoliths were not deposited in isotopic equilibrium with the seawater in which the cod were reared, which was in disagreement with the laboratory work for other marine fish species (Kalish, 1991; Thorrold et al., 1997). In the present study, known-history cod up to 6 years old provide a unique opportunity to broaden our horizons with respect to the previous isotopic studies in otoliths of cod (Schwarcz et al., 1997; Gao et al., 1997b), and gain insights into some aspects of the temperature dependence of otolith-seawater fractionation. Further, it is also possible for us to compare between the known-history Norwegian cod otoliths and the field collections from the northeast Scotian Shelf, Nova Scotia.

The objectives of the present study are to: (1) analyze the stable isotopic ratios ($^{18}O/^{16}O$, $^{13}C/^{12}C$) in known-history otoliths, and determine the range of their isotopic composition, the variation trend as fish growth, and the age of maturity; (2) examine the $\delta^{18}O$ variation and resultant isotopic temperature, and compare with known temperature readings in the pens; (3) study the interseasonal differences in $\delta^{13}C$ between opaque (summer) and translucent (winter) otolith zones, to see if there were large decreases in $\delta^{13}C$ of DIC (dissolved inorganic carbon) and of otolith aragonite, because the ocean-pens were greatly influenced by the seasonal temperature shift and close to the freshwater environment; and (4) compare the pen-reared and field cod in the same age group (6 years), to address the life-long isotopic variations and environmental imprints to which cod were exposed.

5.3 Materials and methods

The sagittal otolith pairs were gathered from Flødevigen Marine Research Station, Norway, and prepared at the Bedford Institute of Oceanography in Dartmouth. All samples clearly show seasonal zones of growth, except for three, which display the annual aragonite layers for less than 6 years. All the cod were living in similar ocean-pen conditions. The typical morphological features of these otoliths are shown in Fig. 5.1.

Strips of sectioned otoliths were carefully cut with a jewelry saw, and then mounted on petrographic glass slides. After gently polishing the slices were measured and portions were milled out of each seasonal zone by using a computer-controlled micromilling technique (*DM 2800*; Gao et al., 1997b), and then analyzed on an autocarb carbonate analyzer attached to a VG Optima mass spectrometer. Of 32 pairs of sagittal otoliths, we only analyzed 18 pairs because of the quality of the sample slices. All the analyses are given in the common δ notation (‰) with respect to the VPDB standard, and the precision of the analyses is better than ±0.06 ‰ for both oxygen and carbon isotopes.

In order to examine the temperature function in oxygen isotopes, 12 water samples from one pen were collected by Steve Campana in November, 1996. These seawater samples were prepared by using a modified procedure for small samples (0.2 mL) from Epstein and Mayeda (1953), and were equilibrated with CO_2 in the 25 ^{0}C water bath for at least one week before analyzing on the Optima mass spectrometer (cf. Gao et al., 1997a). The overall analytical precision, as determined by 6 replicated analyses as an internal reference standard for every two groups of tubes (12 samples), is 0.15‰ (standard error). The salinity of the seawater were also determined in the Lab of Environmental Chemistry in the Department of Geology, McMaster University, by measuring the specific conductivity at 21^{0}C (Weyl, 1964). Each water sample was measured twice and reported in average (Table 5.1). After making adjustment and corrections 12 samples yield a poor linear correlation between oxygen isotopes in seawater and salinity as: $\delta^{18}O_W = 0.027x - 1.876$ ($r^2=0.13$). The quality of the relationship was not satisfied, but it gave a fairly close estimation for $\delta^{18}O_W$ in the pens.

5.4 Results

The isotopic analyses of the known-history otoliths of Norwegian cod were summarized in Table 5.2. In general, the range of $\delta^{18}O$ values was from 0.0 to 2.0 ‰, whereas the $\delta^{13}C$ values range from -6.1 to -1.0 ‰. There was no correlation between fork length or body weight of the cod and their isotopic components (Table 5.2). During the analysis sample 2B3 was too brittle to provide enough aragonite powder for analysis, and was discarded from the data series in the discussion.

The average $\delta^{18}O$ variation of the Norwegian cod was presented in Fig. 5.2. Comparing with the recorded temperatures there was a parallel variation in $\delta^{18}O$ in the first 4 years of growth, but in the last two years it was constant. This pattern of $\delta^{18}O$ variation is very homogeneous amongst all samples and essentially independent of the number of samples as examined (cf. figures in parentheses in Fig. 5.2b). However, the very flat trend in the last two years may conceal the true variation because we averaged 10 and/or 17 samples, and the variation after 4 years is in fact very small. In an attempt to compute the corresponding oxygen isotopic temperatures we selected two representative specimens, 2B7 and 3B18, and adopted equations from Grossman and Ku (1986) for aragonite (Table 5.3). It seems that the results were close to the summer temperature records (Fig. 5.3). This might be due to the larger salinity fluctuation in summer (24-32 ‰) and the poor correlation between salinity and seasonal temperature changes (Fig. 5.4), or due to most of the aragonite deposited in the summer season.

The life-long $\delta^{13}C$ variation trend showed that the cod reached a maturity at the age 4, similar to the behavior of 4Vs cod in the same age group (Fig. 5.5). Before reaching maturity, however, the variation curve between the two settings was slightly different: the Norwegian cod showed somewhat seasonal variation up to year 4, whereas the field cod increased their $\delta^{13}C$ values steadily. This pattern in $\delta^{13}C$ is also independent of the sample numbers. The seasonal isotopic differences in $\delta^{13}C$ between the opaque (summer) and translucent (winter) zones are presented in Fig. 5.6. The 4Vs otoliths showed somewhat consistent ^{13}C-enrichment in summer with respect to winter, but there were no such seasonal variation in the reared cod.

There is a strong correlation between $\delta^{18}O$ and $\delta^{13}C$ in the samples studied. The ratios of good correlation ($r^2 \geq 0.4$, in general) between $\delta^{18}O$ and $\delta^{13}C$ from the Norwegian otoliths were 47 % (8 out of 17 otoliths), while the same ratios in the 4Vs cod were 86 % (6 out of 7 otoliths in the same age group). These significant differences were shown in Fig. 5.7, indicating that the mechanisms in interpreting the positive $\delta^{18}O$ and $\delta^{13}C$

relationship might be more complex. Because of the dimensional constraints in the pens, the degree of activity of reared cod and their vertical migration would be different from cod living in the field.

5.5 Discussion

5.5.1 $\delta^{18}O$ variation and temperature

Based on the seasonal $\delta^{18}O$ comparison between the opaque (summer) and translucent (winter) zones in otoliths, Gao et al. (1997b) suggested that cod prefer to live in certain water layers with constant temperature. They demonstrated a gradual $\delta^{18}O$ increase in the 4-5 year group over the period of 1984-1994 in the 4Vs area by about 1 ‰, corresponding to a decrease in the living temperature of the seawater by about 5 ^{0}C. The ocean-pen reared Norwegian cod show very clearly that the seasonal $\delta^{18}O$ variation can be divided into two stages: before age 4 (maturity) the $\delta^{18}O$ varies in synchrony with ambient temperature (Fig. 5.2b), while after age 4 the $\delta^{18}O$ is almost constant. This pattern is remarkably consistent with Gao et al.'s study, assuring that it is a good strategy to use juvenile cod group for comparison with different years of otolith collections, and cod do choose certain water layers to live after maturation (> 4-5 y). It is much clear now that the oxygen isotope in juvenile cod varies with seasonal temperature changes in seawater, whereas $\delta^{18}O$ in mature cod is almost constant (cf. Chapter 3). However, there are still some mysteries for the reared cod after maturation, because the dimensions of the pen are quite limited.

The lack of $\delta^{18}O_W$ measurements in pen waters during rearing makes it difficult to discuss the oxygen isotopic equilibrium between otolith aragonite and seawater. However, it is known that teleost otoliths are deposited very closely to the isotopic equilibrium with the surrounding waters (Kalish, 1991; Patterson et al., 1993; Thorrold et al., 1997). The large bias between the isotopic calculation and recorded temperature (Fig. 5.3b) was probably due either to sampling effect (Leder et al., 1996) or the poor correlation between

$\delta^{18}O_W$ and salinity (see 5.3). The former is negligible because the seasonal zones of reared otoliths do not change too much from year 2 to 6 (Fig. 5.1) and our milling paths varied with the width of each zone (cf. 3.3.2). Therefore, the bias would result from the quality of pen water samples we used for isotopic computation. Fortunately, there was essentially no large variation in salinity as measured (29.6 to 31.8 ‰; Table 5.1). From Fig. 5.3b we see that the isotopic temperatures in general match the summer records, and thus the bias does not hinder our main purpose to examine how well the isotopic variations capture the variation in temperature for the reared cod. Nevertheless, the seasonal temperature variations are much different over years (Table 5.3). The largest change in both $\delta^{18}O$ and temperature happened in a transition from the first to the second year of growth (Fig. 5.3a), suggesting that there was a significant variation over that period in the reared cod, whether drastic environmental changes in pens or very fast growth in the cod's early life. Overall, the isotopic temperature profile displayed a very close trend to the temperature records, indicating that temperature is a dominant factor in the forming of seasonal otolith aragonite.

5.5.2 The life-long $\delta^{13}C$ variation

The life-long $\delta^{13}C$ variation in otoliths of 4Vs cod had been studied thoroughly (Schwarcz et al., 1997). Experimental rearing displayed that the Norwegian cod reached sexual maturity at age 4 as indicated by their maximum $\delta^{13}C$ values at about -2.2 ‰, which is in good agreement with the sexual maturity age from 4Vs field cod (Gao et al., 1996; Schwarcz et al., 1997). Nevertheless, there were two distinct differences from the two settings: (1) the range of variation in $\delta^{13}C$ in both environments was almost identical (about 2 ‰), but the absolute values in the reared (-4 to -2 ‰) and field cod (-2 to 0 ‰) were totally divergent (Fig. 5.5). Furthermore, the maximum $\delta^{13}C$ values at maturity in the reared cod were much lower than the field cod at the same age group; (2) before reaching the maximum $\delta^{13}C$, the Norwegian cod showed some seasonal variation whereas the field cod increased their $\delta^{13}C$ values smoothly. Since the environmental conditions did not change the fundamental pattern of the life-long $\delta^{13}C$ variation (cf. Fig. 5.5), these differences suggest that the diet of the fish and metabolic effect might play a dominant role in $\delta^{13}C$ values (DeNiro and Epstein, 1978). Schwarcz et al. (1997) argued that at maturity the $\delta^{13}C$ in otoliths approaches equilibrium with seawater DIC, while still including some metabolic HCO_3^-. Thus, the lower $\delta^{13}C$ values observed in this study may be simply due to the fact that the average $\delta^{13}C$ of the diet in rearing was much lower than in 4Vs.

It is somewhat surprising that seasonal differences in $\delta^{13}C$ between the opaque (summer) and translucent (winter) zones in otoliths of reared cod were also small (Fig. 5.6), although the pen environments were generally considered similar to lakes and the metabolic effects of temperature changes should be significant. Schwarcz et al. (1997) discussed two possible factors that might contribute to reducing the seasonal differences in $\delta^{13}C$. They suggested that since cod preferred to stay in waters of constant temperature, the temperature-driven seasonal shift experienced by marine fish would be much less than reported for freshwater fish (Patterson et al., 1993). Another possible factor would be the ^{13}C-enrichment of near surface seawater bicarbonate because of the higher photosynthetic activity in summer than in winter (Schwarcz et al., 1997). Comparing with the seasonal $\delta^{13}C$ differences from the 4Vs cod, however, there were no consistent ^{13}C-enrichment of summer otolith zones with respect to winter in the reared cod (Fig. 5.6). This result reveals that even in the shallower coastal settings the metabolic effects experienced by lake or river fish may not be comparable with those of marine fish. In addition to the environmental differences that cod experienced, the factor of paramount importance between the 4Vs and pens is probably the food supply, which is in turn related to the metabolic effect and the life-long $\delta^{13}C$ variation. Thus, integrating with the life-long $\delta^{13}C$ variation discussed in the last section and the small range of seasonal $\delta^{13}C$ variation (Fig. 5.6), the lower seasonal mean $\delta^{13}C$ values in the reared cod may not be properly interpreted as the temperature-driven photosynthesis activity in summer.

5.5.3 Correlation between $\delta^{18}O$ and $\delta^{13}C$

It was previously found that the seasonal differences in $\delta^{18}O$ for the 4-5 year cod group in the 4Vs Subdivision were from 0.1 to 0.3 ‰, suggesting that there were no significant temperature changes during the otolith aragonite laid down in seasons. There were, however, distinct seasonal $\delta^{18}O$ variations in the first year of growth of 4Vs larval cod (Gao et al., 1997b), which would have resulted from vertical migration behavior and living strategy such as looking for food and minimizing predation risk (Clark and Green, 1991). Our current research on the reared Norwegian cod is in general consistent with those studies, but no seasonal $\delta^{18}O$ changes in the first year (cf. Fig. 5.2). This indicates that the ocean-pen environments do restrain the vertical movement of the fish during rearing so that there are no significant $\delta^{18}O$ variations, nor strong correlations between $\delta^{18}O$ and $\delta^{13}C$.

Among the 17 Norwegian otoliths examined, in fact, only 8 cod had relatively strong correlation ($r^2 \geq 0.40$) between $\delta^{18}O$ and $\delta^{13}C$, whereas ratios in the same criteria for the 4Vs field cod in the same age group were much higher (Fig. 5.7). Other independent research even reported that there was no such relationship seen in the laboratory reared fish (e.g., Kalish, 1991). Correlation between $\delta^{18}O$ and $\delta^{13}C$ has been extensively discussed such as in corals (McConnaughey, 1989), Australian salmon (Kalish, 1991) and Atlantic cod (Radtke et al., 1996), as being either kinetic or metabolic effects. Our $\delta^{13}C$ data increase with age before maturity and we know it is not due to a kinetic isotope effect. Metabolic effects are favored by many authors and result in incorporation of metabolic CO_2 during the otolith aragonite precipitation by the biological processes, although some studies also argued that the positive correlation between $\delta^{18}O$ and $\delta^{13}C$ in otoliths of marine fish may be related to temperature (Kalish, 1991; Thorrold et al., 1997). Since the seasonal $\delta^{13}C$ differences from otolith zones in both field and pen settings were quite small, it in turn indicates that there was no distinct photosynthesis activity in our case. Thus, an interpretation of metabolic effects might also be farfetched. It was suggested from the present study that some other mechanisms, say the activity of fish (Brown et al., 1989), might account for the highly positive correlation between $\delta^{18}O$ and $\delta^{13}C$ (Fig. 5.7) in otoliths of cod. Gao et al. (1997b) defined two types of cod in the 4Vs area, and the correlation between $\delta^{18}O$ and $\delta^{13}C$ in Type 2 fish (migratory) was much higher than Type 1 which was considered as 4Vs residents. The pen-reared cod with lower $\delta^{18}O$ and $\delta^{13}C$ correlation were probably evident that the degree of activity of the fish was limited by their living environments, and the fish need not to look for food.

5.6 Conclusions

We have demonstrated that comparing the reared Norwegian cod otoliths with the field collections from the northeast Scotian Shelf, there were marked differences in the range of isotopic composition, the life-long isotopic variations in both $\delta^{18}O$ and $\delta^{13}C$, and the degree of correlation between $\delta^{18}O$ and $\delta^{13}C$. These differences were probably arisen from the different living environmental constraints and food supply, indicating that the stable isotopic signals derived from the otoliths were sensitive and useful in fisheries biology. There were also some similarities between the two sources of cod otoliths, such as the age of maturity and small seasonal $\delta^{13}C$ changes. These similarities were all related to $\delta^{13}C$, suggesting that carbon isotopes might be useful markers in study of genetic and metabolic mechanisms.

The $\delta^{18}O$ variation and resultant isotopic temperature calculation matched the temperature records reasonably well, indicating that temperature is a dominant factor in deposition of seasonal otolith aragonite. Because of the poor correlation between oxygen isotopes and salinity in the present study, which in turn affects the quality of isotopic estimation, future analysis for pen water samples was strongly suggested.

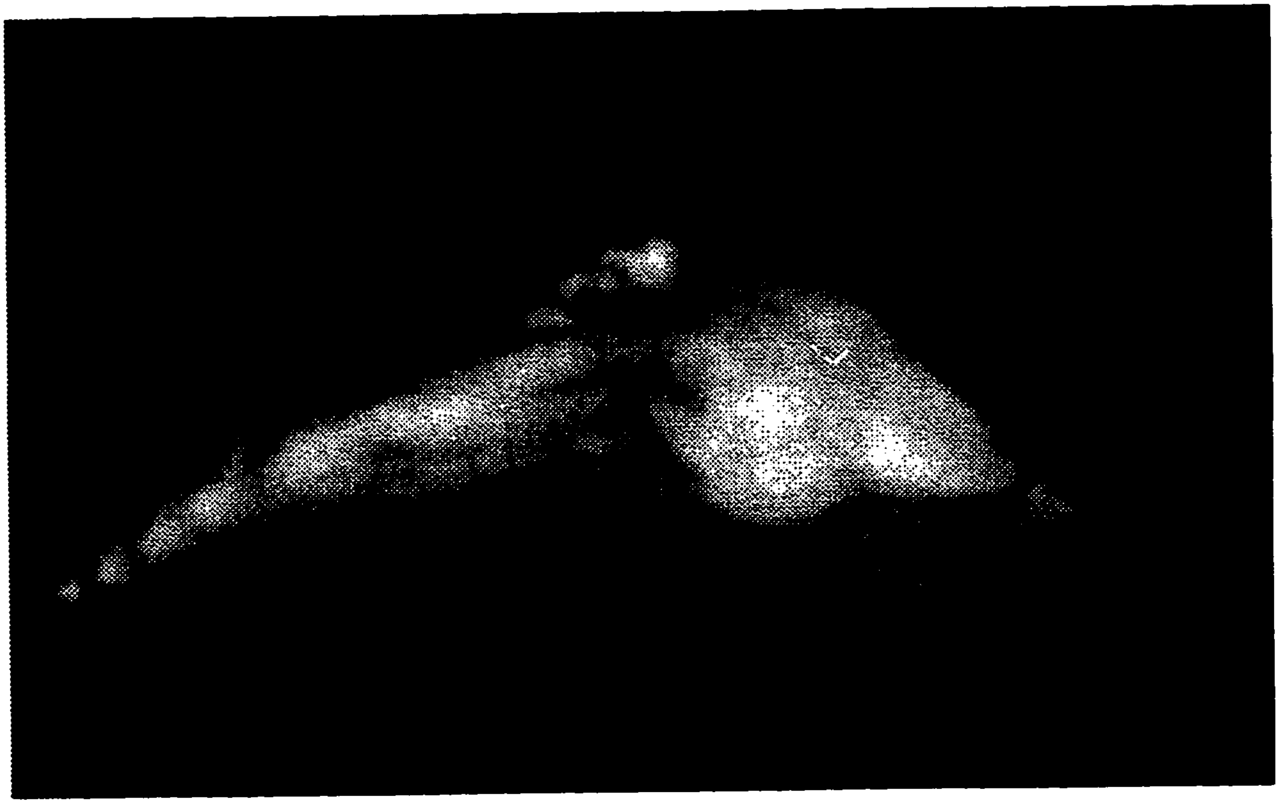

Fig. 5.1 A typical sagittal section of the Norwegian cod, showing some morphological features such as outline and clearness of the seasonal zones. Scale 1/20.

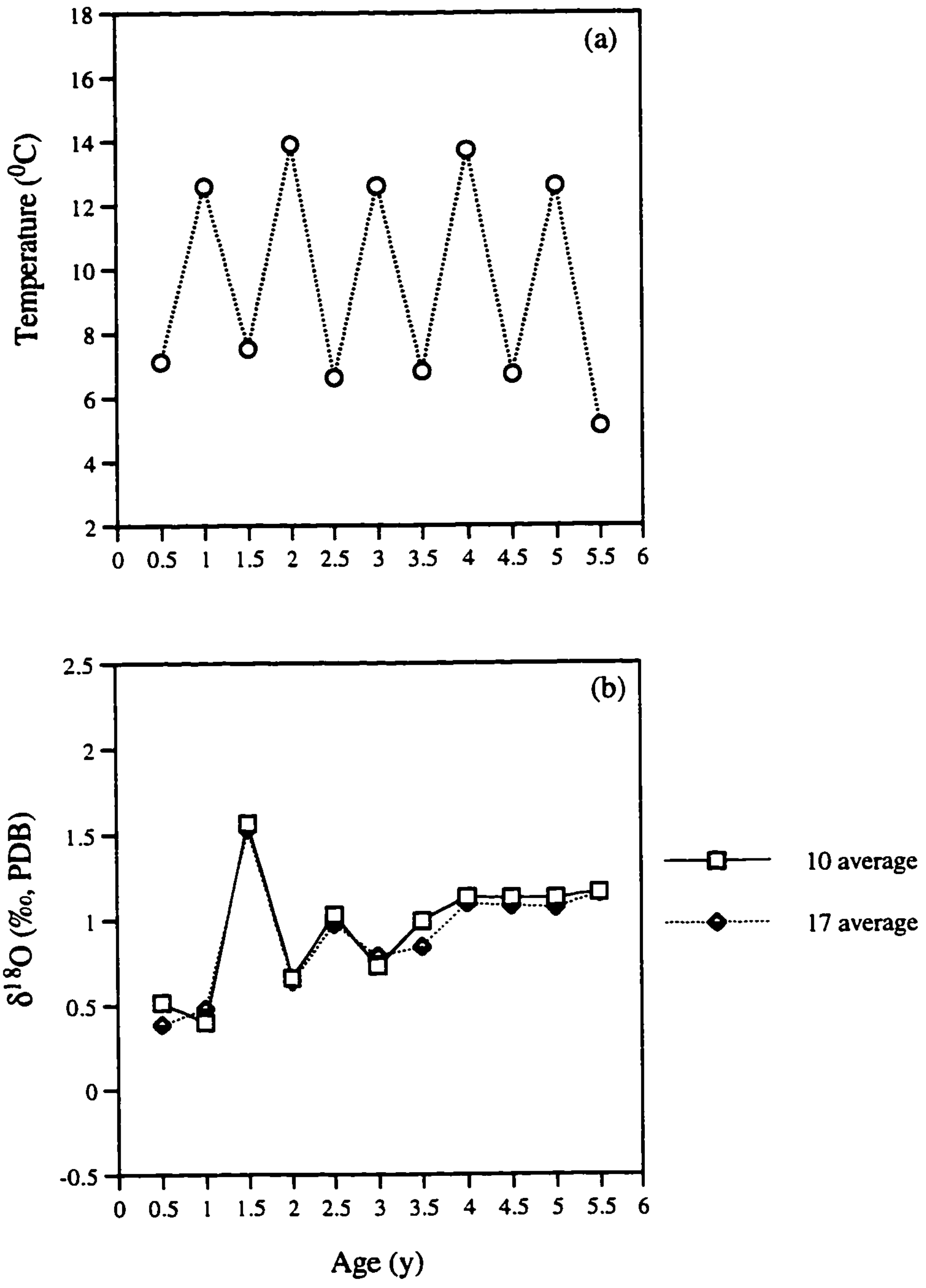

Fig. 5.2 The $\delta^{18}O$ variation in otoliths of Norwegian cod, corresponding to the temperature records during rearing. The temperature profile is based on average readings from winter and summer (a), whereas the $\delta^{18}O$ values are based on seasonal isotopic analyses (b).

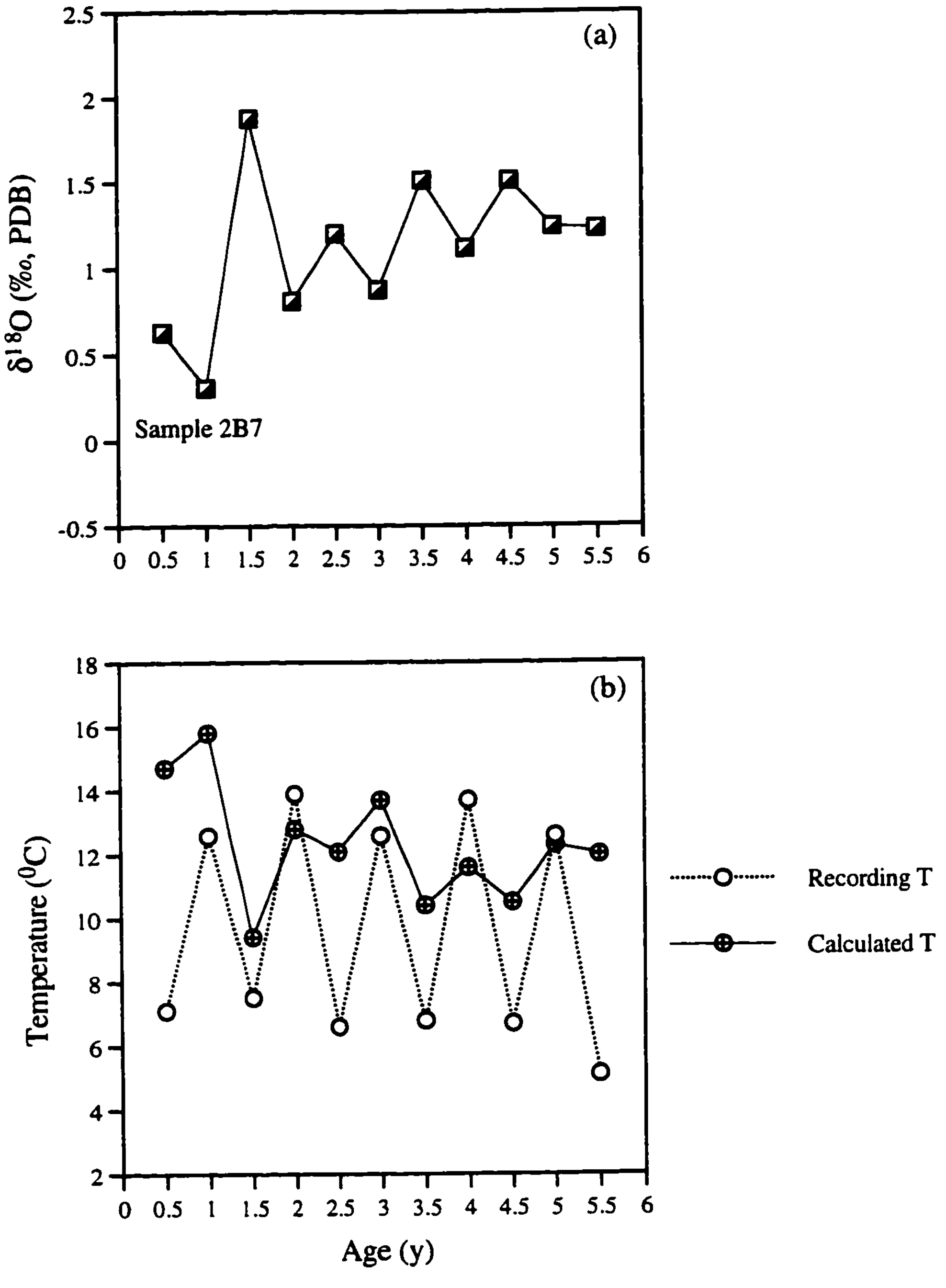

Fig. 5.3 Comparison of the calculated isotopic temperatures with the recordings. The calculation is based on equations of Grossman and Ku (1986), and on average $\delta^{18}O$ value of two representative samples, 2B7 (a) and 3B18. Note that the result was close to the summer records (b).

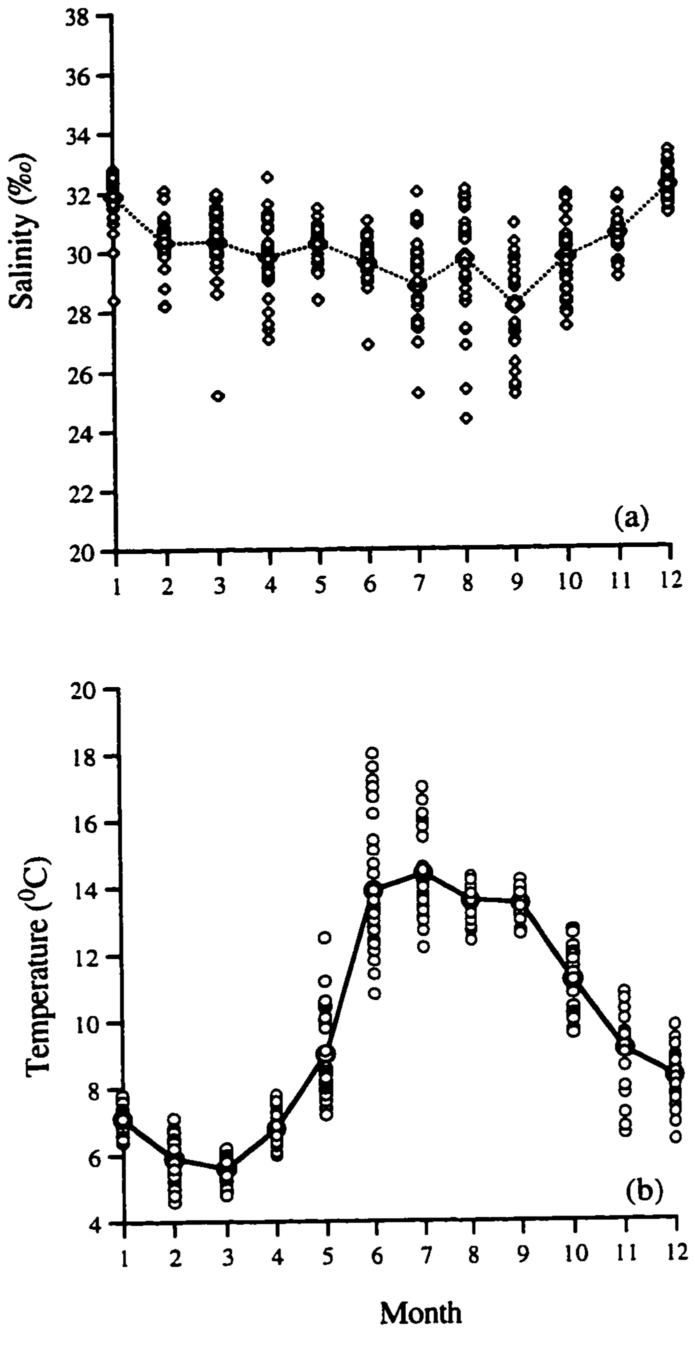

Fig. 5.4 Recorded monthly variations of salinity (a) and temperature (b) in 1989, the first year during rearing. Note that the salinity fluctuation is quite large (24-32 ‰) in summer and is not well correlated with seasonal temperature changes.

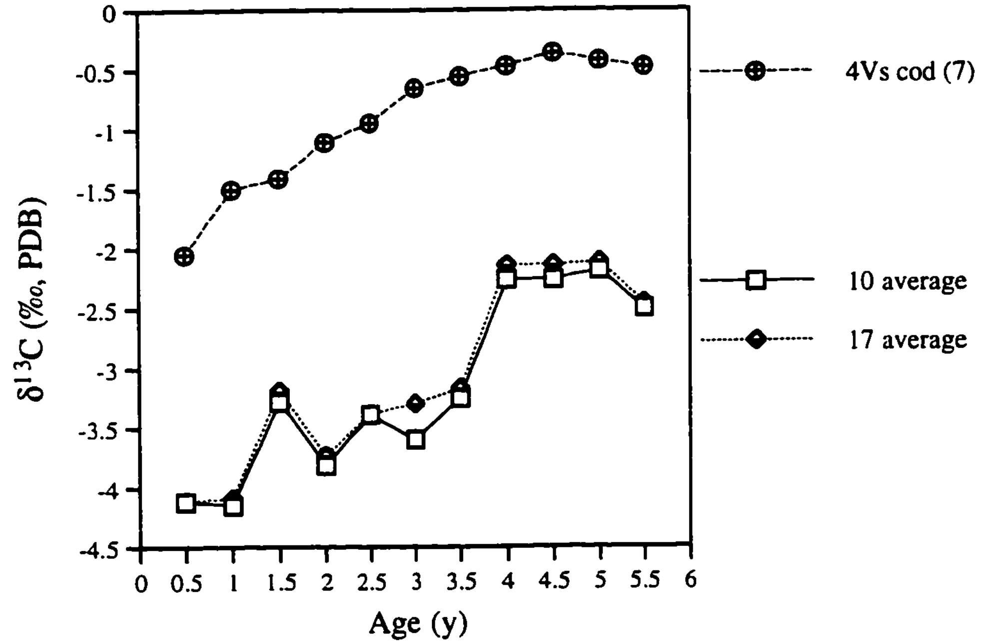

Fig. 5.5 The life-long $\delta^{13}C$ variation in both reared and field cod, showing the same age of maturity (4-5 y) as they reach the maximum $\delta^{13}C$ values.

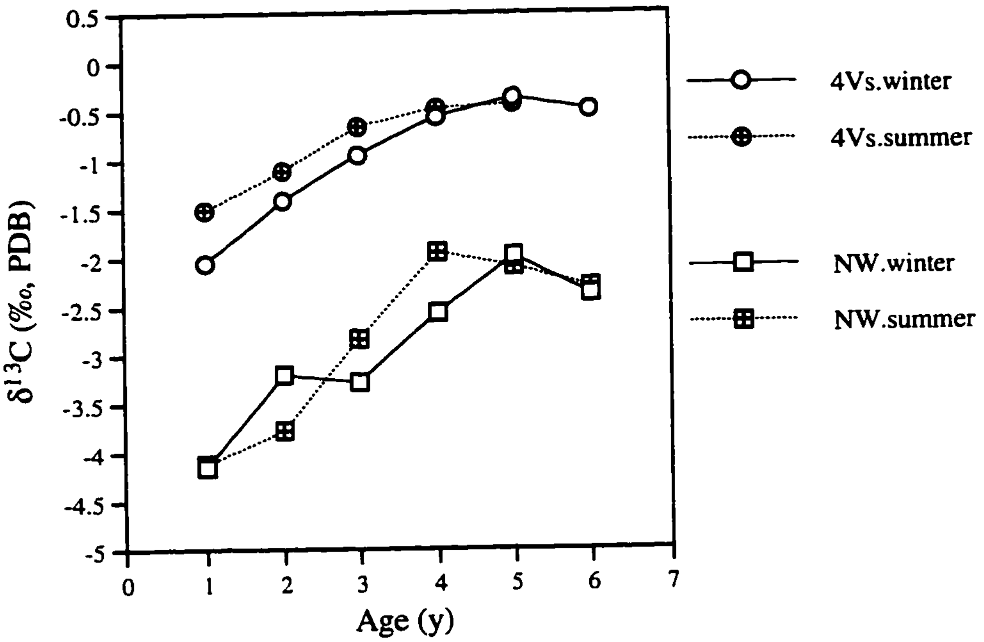

Fig. 5.6 Seasonal $\delta^{13}C$ differences between the opaque (*summer*) and translucent (*winter*) otolith zones. The 4Vs cod otoliths show slightly consistent ^{13}C enrichment in summer with respect to winter, but no such seasonal variation was observed in the Norwegian cod.

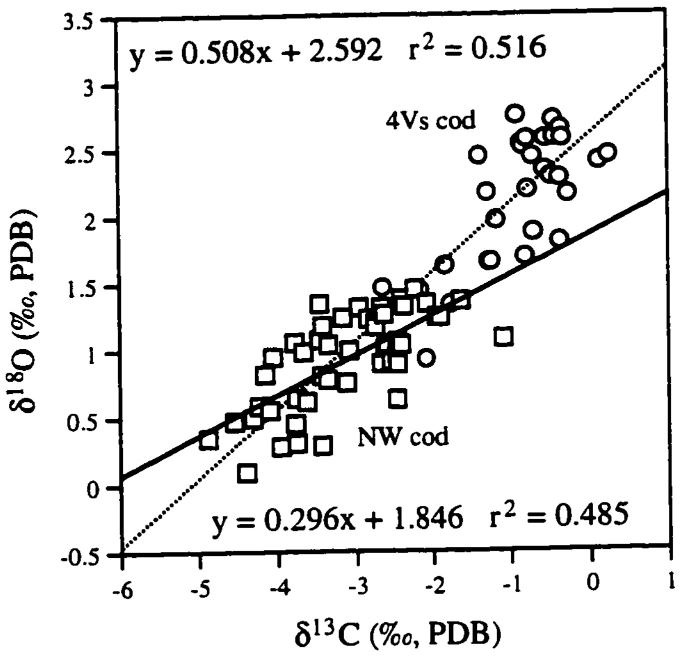

Fig. 5.7 Comparison of the $\delta^{18}O$ and $\delta^{13}C$ correlation between otoliths of reared and 4Vs cod. The degree of $\delta^{18}O$ variation in the field (0.5-3‰) was much higher than the reared (0-1.5‰), indicating some constraints in the pen environment.

Table 5.1 Oxygen isotope and salinity measurements for the pen water samples

Sample#	$\delta^{18}O_W$ (‰)	Mean $\delta^{18}O_W$ (‰)	Salinity (‰)
NW4-2	-0.906	-0.906	32.90
NW5-1	-0.978		
NW5-2	-1.105	-1.042	31.28
NW6-1	-1.042		
NW6-2	-0.933	-0.988	30.72
NW7-2	-1.023	-1.023	30.43
NW8-1	-0.998		
NW8-2	-1.110	-1.054	32.37
NW10-1	-1.252		
NW10-2	-0.981	-1.117	29.60
NW12-1	-1.071		
NW12-2	-1.275	-1.173	31.85

Table 5.2 Summary of 18 otoliths of Norwegian cod (*Gadus morhua*) in this study

Sample#	L (cm)	W (g)	δ^{13}C range (‰)	Mean (‰)	δ^{18}O range (‰)	Mean (‰)
2B2	69	2640	-5.13 to -2.39	-3.70	0.03 to 1.75	0.80
2B3	74	3435	-3.72 to -1.36	-2.33	0.80 to 1.13	0.96
2B5	72	3875	-4.85 to -2.23	-3.30	0.16 to 1.40	0.78
2B6	80	4140	-4.40 to -2.07	-2.86	0.10 to 1.98	0.59
2B7	81	4905	-4.83 to -2.28	-3.30	0.31 to 1.88	1.12
2B8	84	4790	-4.15 to -1.29	-2.58	0.24 to 1.87	0.99
3B9	68	3455	-3.79 to -1.33	-2.50	0.16 to 1.37	0.90
3B13	76	4895	-4.52 to -1.41	-2.77	0.49 to 1.55	1.14
3B14	83.5	5490	-3.86 to -1.66	-2.76	0.31 to 1.56	0.92
3B16	73	5395	-4.74 to -2.01	-3.22	0.47 to 1.53	1.01
3B17	72.5	3210	-6.08 to -2.41	-3.87	-0.07 to 1.73	0.81
3B18	88	5515	-4.12 to -1.67	-3.04	0.17 to 1.43	0.91
4C20	73	3430	-4.34 to -1.42	-2.69	0.20 to 1.61	1.03
4C21	80.5	4420	-3.91 to -1.01	-2.51	-0.04 to 1.59	0.76
4C22	72	4720	-4.42 to -2.12	-3.07	-0.21 to 1.76	0.93
5B25	80	4110	-4.51 to -1.82	-2.80	0.29 to 1.47	1.06
5B26	89	6710	-5.32 to -3.01	-3.84	0.28 to 1.81	1.04
5B28	84.5	6630	-4.92 to -1.66	-2.89	0.26 to 1.89	1.01

Table 5.3 Isotopic temperature calculation using two representative samples (2B7 and 3B18) with respect to the temperatures recorded during rearing

Age (y)	T_K (^{0}C)	S_K (‰)	δ_A (‰)	δ_W (‰)	$\delta_{A\text{-}W}$ (‰)	T_C (^{0}C)
0.5	7.1	30.9	0.507	-1.042	1.514	14.7
1	12.6	29.4	0.239	-1.082	1.285	15.8
1.5	7.5	31.6	1.659	-1.023	2.644	9.4
2	13.9	28.9	0.866	-1.096	1.925	12.8
2.5	6.6	31.6	1.076	-1.023	2.064	12.1
3	12.6	30.8	0.713	-1.044	1.722	13.7
3.5	6.8	31.4	1.432	-1.028	2.425	10.4
4	13.7	29.4	1.121	-1.082	2.167	11.6
4.5	6.7	31.8	1.429	-1.017	2.412	10.5
5	12.6	30.7	1.007	-1.047	2.019	12.3
5.5	5.1	31.8	1.105	-1.017	2.088	12.0

T_K: Known temperature from seasonal average.
S_K: Known salinity from seasonal average.
T_C: Calculated temperature based on equation of Grossman and Ku (1986).

CHAPTER SIX

Conclusions

6.1 Stable isotopic signals from otoliths of cod

This thesis systematically examined the stable isotopes from otoliths of cod (*Gadus morhua*) in the northeast Scotian Shelf, by combining historical field collections, seawater, and known-history Norwegian cod otoliths. Through Chapter 2 to 5, we have defined and recognized the following stable isotopic signals which would be of importance in study of marine environments.

The seasonal $\delta^{18}O$ variation of otoliths of cod is useful in determining the living temperature of the fish and in reconstructing the paleothermal profile that the fish experienced. In 4Vs there was a gradual increase in $\delta^{18}O$ (about 1‰), corresponding to a temperature decrease by about 5 ^{0}C over the period of 1984-94. This secular isotopic shift is in agreement with the temperature change expected (at -0.25 ‰/ ^{0}C), and with the result from summer groundfish trawling survey at depths greater than 100 m (Fig. 3.9). The minor isotopic variations between winter and summer otolith zones suggest that cod may have somewhat regulation to temperature changes in seawater by moving to certain layers in which the temperature are constant. This is especially true as cod reach sexual maturity (cf. Fig. 5.2), and experimental and field evidence show that young cod (<3 y) have greater tolerance to cold than adult (Kao and Fletcher, 1988).

The life-long variation pattern in $\delta^{13}C$ of otoliths of cod shows a consistent increase to a maximum value near 0 ‰, and then levelling off or decreasing. The maximum in $\delta^{13}C$ is attained at the age (4-5 y) close to that of the age of maturity, and was tentatively interpreted as a symbol of sexual maturity of cod. The maximum $\delta^{13}C$ value attained by cod otoliths also gradually decreased from 1984 to 1993 (Fig. 4.6).

The living environmental effects on cod are also embodied in the isotopic variations of reared Norwegian otoliths. Comparing with the 4Vs field cod in the same age group (6 y), there were significant differences in the range of isotopic composition, the life-long variations in $\delta^{18}O$ and $\delta^{13}C$, and the degree of correlation between $\delta^{18}O$ and $\delta^{13}C$. There is direct evidence that the calculated isotopic temperatures were very close to the records during rearing (cf. Fig 5.3), suggesting that temperature is a dominant factor during which the seasonal aragonite material was laid down.

In summary, there is no simple answer for why the cod stock collapsed suddenly in the Canadian Atlantic, as mentioned in Chapter 1. However, based on the isotopic signals obtained from otoliths of cod, it suggests that some environmental changes should be responsible for the significant shifts in the decrease of seawater temperature, the decrease of the age of maturity of cod, and the decline of the cod stock over the same period (1984-94).

6.2 Problems and comments

It would be worth discussing some problems remaining in the present study. We selected and sampled cod otoliths with distinct increment zones, and discarded those with faint or indistinct zones. Questions were raised, for instance, did such specimens represent the whole population? Some studies suggest that when growth rate is fast, the otolith zones will be clear (Campana and Neilson, 1985). If this is the case, our specimens would represent conditions favorable for fast growth. Bagenal and Tesch (1978), nevertheless, reported that the clearness of otolith zones was attributed to the seasonal temperature differences, and the greater the seasonal temperature differences, the clearer the annual marks. If this is true, our specimens would represent the maximal of the seasonal temperature changes. Since the 4Vs Subdivision has shown large temperature differentials between seasons (Campana et al., 1995), we favor the latter explanation for our specimens. Secondly, there is some organic matter (protein) concentrated in the opaque zones of cod otoliths, although the amount is only 0.2-10 % by weight (Degens et al., 1969; Radtke, 1984). Some investigators roasted the sample powder at 200 ^{0}C or higher in a vacuum for a couple of hours to remove the organic contamination (Degens et al., 1969; Nelson et al., 1989; Patterson et al., 1993). However, our own test on this procedure showed that there were no differences with or without heating. The result may be partly due to the tiny amount of powder (about 30 μg) we collected during sampling, or partly due to the relatively small amount of organic matter in cod species (0.29 %, Degens et al., 1969). In short, the organic material in the cod otoliths could be negligible. Finally, of the three pairs of otoliths that occur in cod, only the sagittae were used for stable isotopic analyses. Recent advances suggest that lapilli are more valuable in microstructural research (Secor et al., 1991), and that asterisci provide the most accurate estimates of age among the three otolith pairs (David et al., 1993). Because the size and increment of lapilli and asterisci are beyond our detection, we have no comments on those otolith pairs.

6.3 Future work

Many interesting topics and research opportunities in the fisheries geochemistry of cod are deserved to be worked out continuously. One of the big challenges, perhaps, is to deal with the long-term climate change and cod fisheries. Since marine ecosystems are very sensitive to the climate change, particularly in the mid and high latitudes, it makes some oceanographers believe that the secular Northwest Atlantic cooling is a buffer for the current global warming (Buch and Stein, 1989). Nevertheless, a number of fisheries biologists argued that it is not easy to link the long-term temperature fluctuation with cad catches. Over long-time periods, variability in cod catches is largely ascribed to fishing efforts and management of fisheries, although over shorter periods the temperature of some areas is out of phase (Brander, 1996). The close correlation between global climate change and fish catches has been noted from other fish species, such as salmon and halibut in the Pacific coast. With the surface temperature in seawater decreasing in the late 1970's, the salmon and halibut catches steadily decline (D. J. Noakes, 1997, personal communication). Thus, research in this field is far from completed. We may easily get a time series by examining archived otoliths of long-lived fish species, assuming there are some historical collections like cod. We may also look at geochemical records (both stable isotopes and trace elements) in different scales, such as seasonal (opaque and translucent zones), annual, or 5-year's average to get information about the oceanic process and climate change over a period of many decades.

As we claimed in Chapter 4, the metabolic rate of Atlantic cod doubles with each 10 ^{0}C rise in temperature (Brander, 1996). Therefore, with the average 6.2 ^{0}C in the reared Norwegian cod, we would expect metabolism to increase about 1.2 times. This would cause δ^{13}C to be lower in summer and higher in winter, if the metabolic fraction model is correct (cf. Equation 4). We observed the seasonal δ^{13}C variation in the ocean-pen reared cod (Fig. 5.6), but the magnitude of the shift is very small compared to what is expected. The failure to get a drastic change in δ^{13}C(HCO3$^-$) of the endolymph remains a mystery in carbon isotopic fractionation of fish. Since we have no original data about the gender and weight of the 4Vs cod in this study (cf. Chapter 3), we cannot test the temperature effect on metabolic rate by the oxygen isotopic temperature scales. However, such work in the future is strongly recommended, and an experimental test to see the δ^{13}C variation in fish's blood while raising the temperature has been proposed (H. P. Schwarcz, 1997, personal communication).

The food supply or trophic-effect is crucial to the range and variation of carbon isotopes, from both experimental and field evidence (Chapter 5). Nevertheless, since cod are voracious feeders (cf. Chapter 1) and their diet changes with age, it makes very difficult to examine the trophic-effect on metabolism of the fish. Some investigators set the criteria by body size (35 cm) or weight (1 kg) to define the food choices of cod (e.g., Lilly and Fleming, 1981). Thus, it might be necessary to examine the food supply for cod covering their life-long history in the future studies, and to see how temperature affects the growth and their carbon isotopic variation.

REFERENCES

Bagenal T. B. and Tesch F. W. (1978) Age and growth. In *Methods for Assessment of Fish Production in Fresh Waters* (ed. T. B. Bagenal), pp. 101-136. Blackwell Scientific Publication.

Beacham T. P. (1982) Size and age compositions of Atlantic cod, American plaice and witch flounder in the landings of Canadian otter trawlers, Danish seiners, or long-liners in the Northwest Atlantic Ocean. *Can. MS Rep. Fish. Aquat. Sci.* 1679: 73p.

Beamish R. J. and McFarlane G. A. (1987) Current trends in age determination methodology. In *Age and Growth of Fish* (eds. R. C. Summerfelt and G. E. Hall), pp. 15-42. Iowa State University Press.

Blacker R. W. (1974) Recent advances in otolith studies. In *Sea Fisheries Research* (ed. F. R. H. Jones), pp. 67-90. Wiley.

Brander K. (1996) Effect of climate change on cod stocks. In *Global Warming: Implications for Freshwater and Marine Fish* (eds. C. M. Wood & D. G. McDonald), pp. 255-278. Cambridge University Press.

Brothers E. D. and McFarland W. N. (1981) Correlations between otolith microstructure, growth and life history transitions in newly recruited French grunts (*Haemulon flavolineatum*). *Rapp. P.-V. Reun. Cons. Int. Explor. Mer.* 178, 369-374.

Brown J. A., Pepin P., Methven D. A. and Somerton D. C. (1989) The feeding, growth and behavior of juvenile cod, *Gadus morhua* L., in cold environments. *J. Fish Biol.* 35, 373-380.

Buch E. and Stein M. (1989) Environmental conditions off West Greenland, 1980-85. *J. Northw. Atl. Fish. Sci.* 9, 81-89.

Bugden G. L. (1991) Changes in the temperature-salinity characteristics of the deeper waters of the Gulf of St. Lawrence over the past several decades. In *The Gulf of St. Lawrence: Small Ocean or Big Estuary* (ed. J. C. Therriault), pp. 139-147. Department of Fisheries and Oceans, Canada.

Campana S. E., Mohn R. K., Smith S. J. and Chouinard G. A. (1995) Spatial implications of a temperature-based growth model for Atlantic cod (*Gadus morhua*) off the eastern coast of Canada. *Can. J. Fish. Aquat. Sci.* 52, 2445-2456.

Campana S. E. and Neilson J. D. (1985) Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* 42, 1014-1032.

Casselman J. M. (1983) Age and growth assessment of fish from their calcified structures-techniques and tools. In *Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks* (eds. E. D. Prince and L. M. Pulos), pp. 1-17. NOAA Tech. Rep. NMFS 8.

Clark D. S. and Green J. M. (1991) Seasonal variation in temperature preference of juvenile Atlantic cod (*Gadus morhua*), with evidence supporting an energetic basis for their diel vertical migration. *Can. J. Zool.* 69, 1302-1307.

Craig H. (1961) Standard for reporting concentrations of deuterium and oxygen-18 in natural waters. *Science* 133, 1833-1834.

Craig H. and Gordon L. I. (1965) Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. *Consiglio Nazionale Delle Ricerche*, 1-122.

David A. W., Isely J. J. and Grimes C. B. (1993) Differences in the sagitta, lapillus, and asteriscus in estimating age and growth of juvenile red drum. *Abstract.* International Symposium on Fish Otolith Research and Application, Jan. 23-27, Hilton Head Island, South Carolina, USA.

Degens E. T., Deuser W. G. and Haedrich R. L. (1969) Molecular structure and composition of fish otoliths. *Mar. Biol.* 2, 105-113.

DeNiro M. J. and Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42, 495-506.

Devereux I. (1967) Temperature measurements from oxygen isotope ratios of fish otoliths. *Science* 155, 1684-1685.

Drinkwater K. F. (1996) Atmospheric and oceanic variability in the Northwest Atlantic during the 1980s and early 1990s. *J. Northw. Atl. Fish. Sci.* 18, 77-97.

Edwards R. C., Finlayson D. M. and Steele J. H. (1972) An experimental study of the oxygen consumption, growth and metabolism of the cod (*Gadus morhua* L.). *J. Exp. Mar. Biol. Ecol.* 8, 299-309.

Epstein S., Buchsbaum R., Lowenstam H. A. and Urey H. C. (1953) Revised carbonate-water isotopic temperature scale. *Geol. Soc. Am. Bull.* 64, 1315-1326.

Epstein S. and Mayeda T. K. (1953) Variation of ^{18}O content of waters from natural sources. *Geochim. Cosmochim. Acta* 4, 213-224.

Fairbanks R. G. (1982) The origin of continental shelf and slope water in the New York Bight and Gulf of Maine: Evidence from $H_2{}^{18}O/H_2{}^{16}O$ ratio measurements. *J. Geophys. Res.* 87, 5796-5808.

Fanning L. P., Mohn R. K. and MacEachern W. J. (1995) Assessment of 4VsW cod in 1994 with consideration of ecological indicators of stock status. *DFO Atlantic Fisheries Research Document* 73, Canada.

Fontugne M. R. and Duplessy J. C. (1981) Organic carbon isotopic fractionation by marine plankton in the temperature range -1 to 31 ^{0}C. *Oceanogica Acta* 4, 85-90.

Friedman I. and O'Neil J. R. (1977) Compilation of stable isotope fractionation factors of geochemical interest. In *Data of Geochemistry* (ed. M. Fleischer). *U.S. Geol. Surv. Prof. Paper* 440K.

Fry B. (1988) Food web on Georges Bank from stable C, N, and S isotopic compositions. *Limnol. Oceanogr. 33*, 1182-1190.

Gao Y. W., Schwarcz H. P. and Brand U. (1996) Long-term marine environmental changes from stable isotopic analyses of cod otoliths (*Gadus morhua*) in the Canadian Atlantic. 30th International Geological Congress, *Abstracts volume* 2, 247.

Gao Y, W., Schwarcz H. P., Brand U., Strain P. M. and Bugden G. L. (1997a) Spatial and temporal variations of the oxygen isotope-salinity relationship: Implications for environmental changes in the northeast Scotian Shelf. *Earth Planet. Sci. Lett.* (in review).

Gao Y. W., Schwarcz H. P., Brand U. and Campana S. E. (1997b) Seasonal oxygen isotopic composition of cod otoliths (*Gadus morhua*): A potential thermometer and migration indicator in the Canadian Atlantic. *Geochim. Cosmochim. Acta* (in review).

Gauldie R. W., Thacker C. E. and Merrett N. R. (1994) Oxygen and carbon isotope variation in the otoliths of *Beryx splendens* and *Coryphaenoides profundicolus*. *Comp. Biochem. Physiol.* 108A, 153-159.

Grossman E. L. and Ku T.-L. (1986) Oxygen and carbon isotope fractionation in biogenic aragonite: Temperature effects. *Chem. Geol.* 59, 59-74.

Hare J. A. and Cowen R. K. (1994) Ontogeny and otolith microstructure of bluefish *Pomatomus saltatrix* (Pisces: Pomatomidae). *Mar. Biol.* 118, 541-550.

Horibe Y. and Oba T. (1972) Temperature scales of aragonite-water and calcite-water systems. *Fossils* 23, 71-80.

Iacumin P., Bianucci G. and Longinelli A. (1992) Oxygen and carbon isotopic composition of fish otoliths. *Mar. Biol.* 113, 537-542.

Jensen A. C. (1972) *The cod*. Crowell.

Kalish J. M. (1991) Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). *Mar. Biol.* 110, 37-47.

Kalish J. M. (1991) ^{13}C and ^{18}O isotopic disequilibria in fish otoliths: Metabolic and kinetic effects. *Mar. Ecol. Prog. Ser.* 75, 191-203.

Kao M. H. and Fletcher G. L. (1988) Juvenile Atlantic cod (*Gadus morhua*) can be more freeze resistant than adults. *Can. J. Fish. Aquat. Sci.* 45, 902-905.

Killingley J. S., Johnson R. F. and Berger W. H. (1981) Oxygen and carbon isotopes of individual shells of planktonic foraminifera from Ontong-Java Plateau, equatorial Pacific. *Palaeogeogr. Palaeoclimatol. Palaaeoecol.* 33, 193-204.

Kroopnick P. (1974) The dissolved O_2-CO_2-^{13}C system in the eastern equatorial Pacific. *Deep Sea Res.* 21, 211-227.

Kroopnick P. (1980) The distribution of ^{13}C in the Atlantic Ocean. *Earth. Planet. Sci. Lett.* 49, 469-484.

Lambert Y., Dutil J. and Munro J. (1994) Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). *Can. J. Fish. Aquat. Sci.* 51, 1569-1576.

Laurence G. C. (1978) Comparative growth, respiration and delayed feeding abilities of larval cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) as influenced by temperature during laboratory studies. *Mar. Biol.* 50, 1-7.

Lear W. H. (1993) Atlantic cod. *Underwater World.* DFO, Canada.

Lecomte-Finiger R. (1992) Growth history and age at recruitment of European glass eels (*Anguilla anguilla*) as revealed by otolith microstructure. *Mar. Biol.* 114, 205-210.

Leder J. J., Swart P. K., Szmant A. M. and Dodge R. E. (1996) The origin of variations in the isotopic record of scleractinian corals: I. Oxygen. *Geochim. Cosmochim. Acta* 60, 2857-2870.

Leim A. H. and Scott W. B. (1966) Fishes of the Atlantic Coast of Canada. *Fish. Res. Board Can. Bull.* 155.

Lewis E. L. (1980) The practical salinity scale 1978 and its antecedents. IEEE J. Oceanic Engineering, OE-5, 3-8.

Lilly G. R. and Fleming A. M. (1981) Size relationships in predation by Atlantic cod. *NAFO Sci. Coun. Stud.* 1, 41-45.

Loretz C (1995) Electrophysiology of ion transport in teleost intestinal cells. In *Cellular and Molecular Approaches to Fish Ionic Regulation* (eds. C. M. Wood & T. J. Shuttleworth) Vol. 14 In *Fish Physiology* (ed. W. S. Hoar et al.), pp. 25-56. San Diego, Academic Press.

McConnaughey T. (1989) ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: I. Patterns. *Geochim. Cosmochim. Acta* 53, 151-162.

McConnaughey T. (1989) ^{13}C and ^{18}O isotopic disequilibrium in biological carbonates: II. *In vitro* simulation of kinetic isotope effects. *Geochim. Cosmochim. Acta* 53, 163-171.

Moyle P. B. and Cech Jr. J. J. (1988) *Fishes: An Introduction to Ichthyology.* Second Edition. Prentice Hall.

Mulcahy S. A., Killingley J. S., Phleger C. F. and Berger W. H. (1979) Isotopic composition of otoliths from a bentho-pelagic fish, *Coryphaenoides acrolepis*, Macrouridae: Gadiformes. *Oceanol. Acta* 2, 423-427.

Nelson C. S., Northcote T. G. and Hendy C. H. (1989) Potential use of oxygen and carbon isotopic composition of otoliths to identify migratory and non-migratory stocks of the New Zealand common smelt: a pilot study. *New Zealand J. Mar. & Freshwater Res.* 23, 337-344.

Northcote T. G., Hendy C. H., Nelson C. S. and Boubee J. A. T. (1992) Tests for migratory history of the New Zealand common smelt (*Retropinna retropinna* (Richardson)) using otolith isotopic composition. *Ecology of Freshwater Fish* 1, 61-72.

Pannella G. (1971) Fish otoliths: Daily growth layers and periodical patterns. *Science* 173, 1124-1126.

Patterson W. P. (1997) North American continental seasonality during the last millennium: High resolution analysis of sagittae. *Palaeogeog. Palaeoclim. Palaeoecol.* (in press).

Patterson W. P., Smith G. R. and Lohmann K. C. (1993) Continental paleothermometry and seasonality using the isotopic composition of aragonitic otoliths of freshwater fishes. In *Climate Change in Continental isotopic Records* (eds. P. Swart, K. C. Lohmann, J. McKenzie and S. Savin), pp. 191-202. Geophysical Monograph 78.

Pederson A. M. (1984) A modular high resolution CTD system with computer-controlled sample rate. Mar. Tech. Soc. 1984 STD Conf., San Diego, 1-7.

Perry R. I. and Smith S. J. (1994) Identifying habitat associations of marine fishes using survey data: An application to the northwest Atlantic. *Can. J. Fish. Aquat. Sci.* 51, 589-602.

Peterson B. J. and Fry B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics* 18, 293-320.

Petrie B. and Drinkwater K. (1993) Temperature and salinity variability on the Scotian Shelf and in the Gulf of Maine 1945-1990. *J. Geophy. Res.* 98, 20079-20089.

Radtke R. L. (1984) Cod fish otoliths: Information storage structures. In *The Propagation of Cod Gadus morhua* L. (eds. E. Dahl, D. S. Danielssen, E. Moksness and P. Solemdal), pp. 273-298. Floedevigen Rapportser 1.

Radtke R. L., Showers W., Moksness E. and Lenz P. (1996) Environmental information stored in otoliths: insights from stable isotopes. *Mar. Biol.* 127, 161-170.

Redfield A. C. and Friedman I. (1969) The effect of meteoric water, melt water and brine on the composition of Polar Sea water and of the deep waters of the ocean. *Deep Sea Res.* 16, 197-214.

Rose G. A. (1993) Cod spawning on a migration highway in the north-west Atlantic. *Nature* 366, 458-461.

Schwarcz, H. P., Gao, Y. W., Campana, S., Browne, D., Knyf, M. and Brand, U. (1997) Stable carbon isotope variations in otoliths of cod (*Gadus morhua*): Effects of maturation and environmental change. *Can. J. Fish. Aquat. Sci.* (in press).

Secor D. H., Dean J. M. and Laban E. H. (1991) *Manual for Otolith Removal and Preparation for Microstructural Examination.* The Electric Power Res. Ins. and Belle W. Baruch Ins. for Mar. Biol. and Coast Res..

Scott J. S. (1982) Depth, temperature and salinity preference of common fishes of the Scotian Shelf. *J. Northw. Atl. Fish. Sci.* 3, 29-39.

Scott J. S. (1983) Inferred spawning areas and seasons of groundfishes on the Scotian Shelf. *Can. Tech. Rep. Fish. Aquat. Sci.* 1219: 14p.

Sinclair, A. (1992) Fish distribution and partial recruitment: The case of eastern Scotian Shelf cod. *J. Northw. Atl. Fish. Sci.* 13, 15-24.

Smith S. J., Perry R. I. and Fanning L. P. (1991) Relationships between water mass characteristics and estimates of fish population abundance from trawl. *Environmental Monitoring and Assessment* 17, 227-245.

Strain P. M. and Tan F. C. (1979) Carbon and oxygen isotope ratios in the Saguenay Fjord and the St. Lawrence Estuary and their implications for paleoenvironmental studies. *Estuarine Coastal Mar. Sci.* 8, 119-126.

Strain P. M. and Tan F. C. (1993) Seasonal evolution of oxygen isotope-salinity relationships in high-latitude surface waters. *J. Geophys. Res.* 98, 14589-14598.

Tan F. C., Cai D. and Roddick D. L. (1988) Oxygen isotope studies on sea scallops, *Placopecten magellanicus*, from Browns Bank, Nova Scotia. *Can. J. Fish. Aquat. Sci.* 45, 1378-1386.

Tan F. C. and Fraser W. D. (1976) Oxygen isotope studies on sea ice in the Gulf of St. Lawrence. *J. Fish. Res. Board Can.* 33, 1397-1401.

Tan F. C. and Strain P. M. (1980) The distribution of sea ice meltwater in the eastern Canadian Arctic. *J. Geophys. Res.* 85, 1925-1932.

Tan F. C. and Strain P. M. (1988) Stable isotope studies in the Gulf of St. Lawrence. *In Chemical Oceanography in the Gulf of St. Lawrence* (ed. P. M. Strain), 59-77.

Thorrold S. R., Campana S. E., Jones C. M. and Swart P. K. (1997) Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta* (in press).

Urey H. C. (1947) The thermodynamic properties of isotopic substances. *J. Chem. Soc.*, 562-581.

Weyl P. K. (1964) On the change in electrical conductance of seawater with temperature. *Limnol. Oceanogr.* 9, 75.

Zhang Z. and Runham N. W. (1992) Temporal deposition of incremental and discontinuous zones in the otoliths of *Oreochromis niloticus* (L.). *J. Fish Biol.* 40: 333-339.

IMAGE EVALUATION
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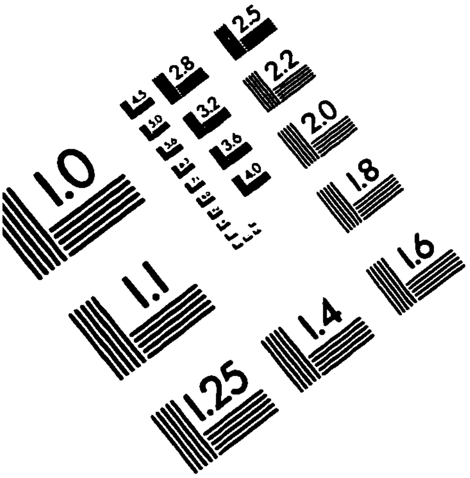

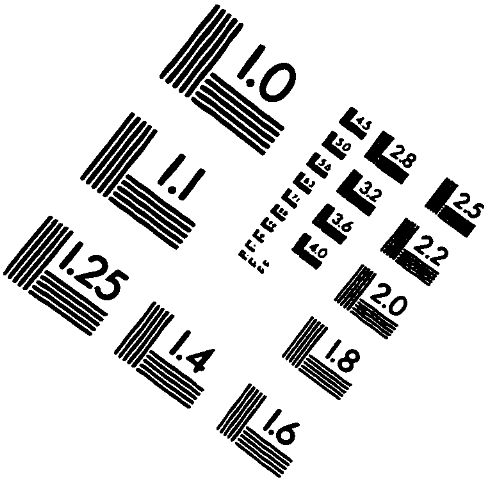

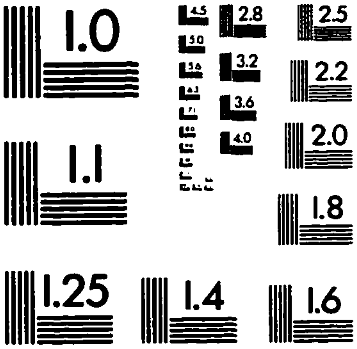

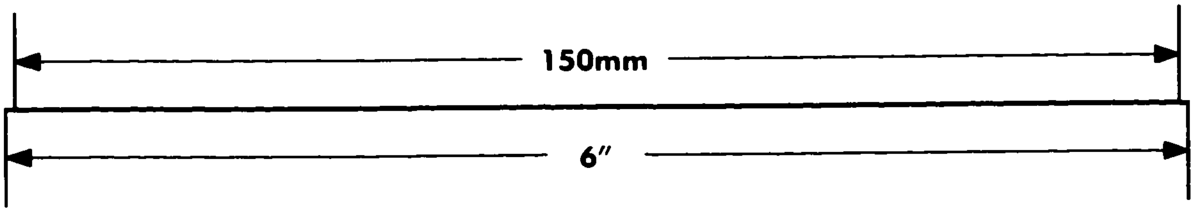

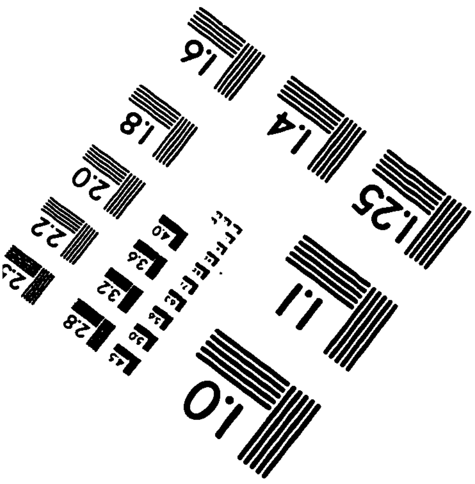

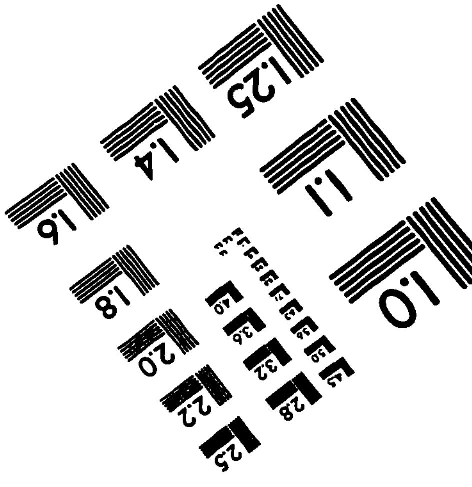

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