

Stable carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*)

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

Abstract: Stable carbon isotope ratios ($\delta^{13}\text{C}$) were measured in annual layers of otoliths of Atlantic cod (*Gadus morhua*) from the northeastern Scotian Shelf, Atlantic Canada. Layers deposited during the first 4–6 years of otolith growth increased in $\delta^{13}\text{C}$ from minimum values between $-5\text{\textperthousand}$ and $-2.5\text{\textperthousand}$ to a maximum near 0\textperthousand . This pattern of increase was independent of the years in which the fish was collected. Layers formed after reaching the maximum $\delta^{13}\text{C}$ value displayed decreasing or nearly constant isotopic ratios. Early rise in $\delta^{13}\text{C}$ may be a combined result of (i) decrease in the fraction of metabolic oxidized carbon in the fishes' blood as they mature, relative to the proportion of seawater-derived carbon, and (ii) dietary shift to higher trophic-level foods with higher $\delta^{13}\text{C}$ values. Age of maximum in $\delta^{13}\text{C}$ may be indicative of age of maturity of cod. The maximum $\delta^{13}\text{C}$ value attained by otoliths decreased steadily between 1983 and 1993, while cod stocks in Atlantic Canada were declining. Drop in age of attainment of maximum $\delta^{13}\text{C}$ between 1984 and 1985 coincides with changes in population dynamics of the 4Vs stock. This decrease, as well as the post maximum decrease in $\delta^{13}\text{C}$ values of the mature cod otoliths may represent movement of the fish to deeper waters of the shelf, where $\delta^{13}\text{C}$ of dissolved inorganic carbon is lower.

Résumé : Nous avons déterminé le rapport des isotopes stables du carbone ($\delta^{13}\text{C}$) présents dans les anneaux de croissance annuels des otolithes de morues (*Gadus morhua*) provenant du nord-est de la plate-forme Néo-Écossaise, dans la région canadienne de l'Atlantique. Dans les anneaux correspondant aux 4–6 premières années de la formation des otolithes, le rapport $\delta^{13}\text{C}$ a augmenté, passant d'une valeur minimum comprise entre $-5\text{\textperthousand}$ et $-2.5\text{\textperthousand}$ à une valeur maximum proche de 0\textperthousand . Le mode d'augmentation était indépendant de l'année où les poissons ont été capturés. Dans les anneaux formés après celui où le rapport $\delta^{13}\text{C}$ avait atteint sa valeur maximale, le rapport des isotopes diminuait ou demeurait presque constant. L'augmentation du rapport $\delta^{13}\text{C}$ observée au cours des premières années pourrait être due à l'effet conjugué des facteurs suivants : (i) durant la maturation du poisson, la concentration sanguine de carbone métabolique oxydé baisse par rapport à la concentration de carbone provenant de l'eau de mer; et (ii) le poisson change d'alimentation et sa nourriture provient d'un niveau trophique supérieur présentant un rapport $\delta^{13}\text{C}$ plus élevé. L'âge où le rapport $\delta^{13}\text{C}$ est maximum pourrait être celui où la morue parvient à maturité. Nous avons constaté que la valeur maximum du rapport $\delta^{13}\text{C}$ dans les otolithes a diminué suivant une courbe régulière de 1983 à 1993, période durant laquelle les stocks de morue ont baissé dans l'Atlantique canadien. L'âge où le rapport $\delta^{13}\text{C}$ atteignait sa valeur maximum a baissé de 1984 à 1985; cette baisse coïncide avec les changements de la dynamique des populations observés dans les stocks de la zone 4Vs. Il se peut que ce phénomène, de même que la diminution du rapport $\delta^{13}\text{C}$ observée après la pointe de valeurs maximums dans les otolithes des poissons matures, corresponde au déplacement de la morue vers les eaux plus profondes de la plate-forme, où le rapport $\delta^{13}\text{C}$ des formes de carbone inorganique dissous est moins élevé.

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Introduction

Teleost fish possess aragonitic otoliths, which grow from a fluid medium (endolymph) containing calcium ions and dissolved inorganic carbon (DIC), predominantly bicarbon-

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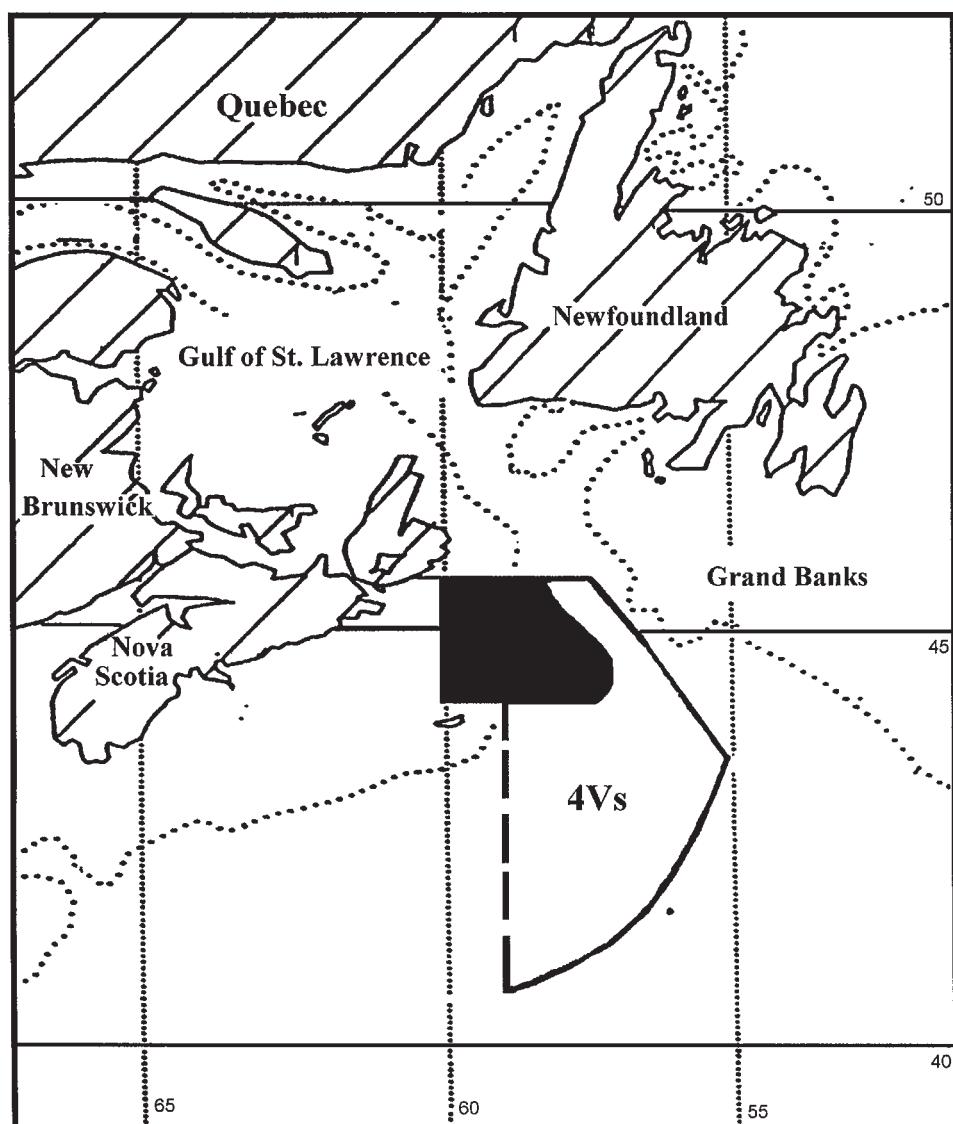
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ate (HCO_3^-). Otoliths begin to form before hatching and grow continuously through the entire life of a fish, thus preserving an uninterrupted record of the internal and external environment of the fish. Also, once otoliths have been laid down in a fish, they remain chemically (and isotopically) inert and, therefore, preserve the life-long record of otolith growth (Campana and Neilson 1985). Previous isotopic studies of otoliths have focused on the $^{18}\text{O}/^{16}\text{O}$ ratio of otolithic aragonite, which is deposited close to oxygen isotopic equilibrium with environmental water (Kalish 1991; Patterson et al. 1993; Thorrold et al. 1997) and, therefore, can be used as a measure of growth temperature. The present paper deals only with the carbon isotopic composition of otoliths, which is in equilibrium with the DIC of endolymph. A subsequent paper (Y. Gao, H.P. Schwarcz, S. Campana, and U. Brand, unpublished data) will present oxygen isotope data for the otoliths discussed here.

Fig. 1. Map of the Scotia Shelf, showing the position of the 4Vs subdivision; solid area is the part of 4Vs from which fish were taken for this study. Dotted line is the 200-m depth contour.

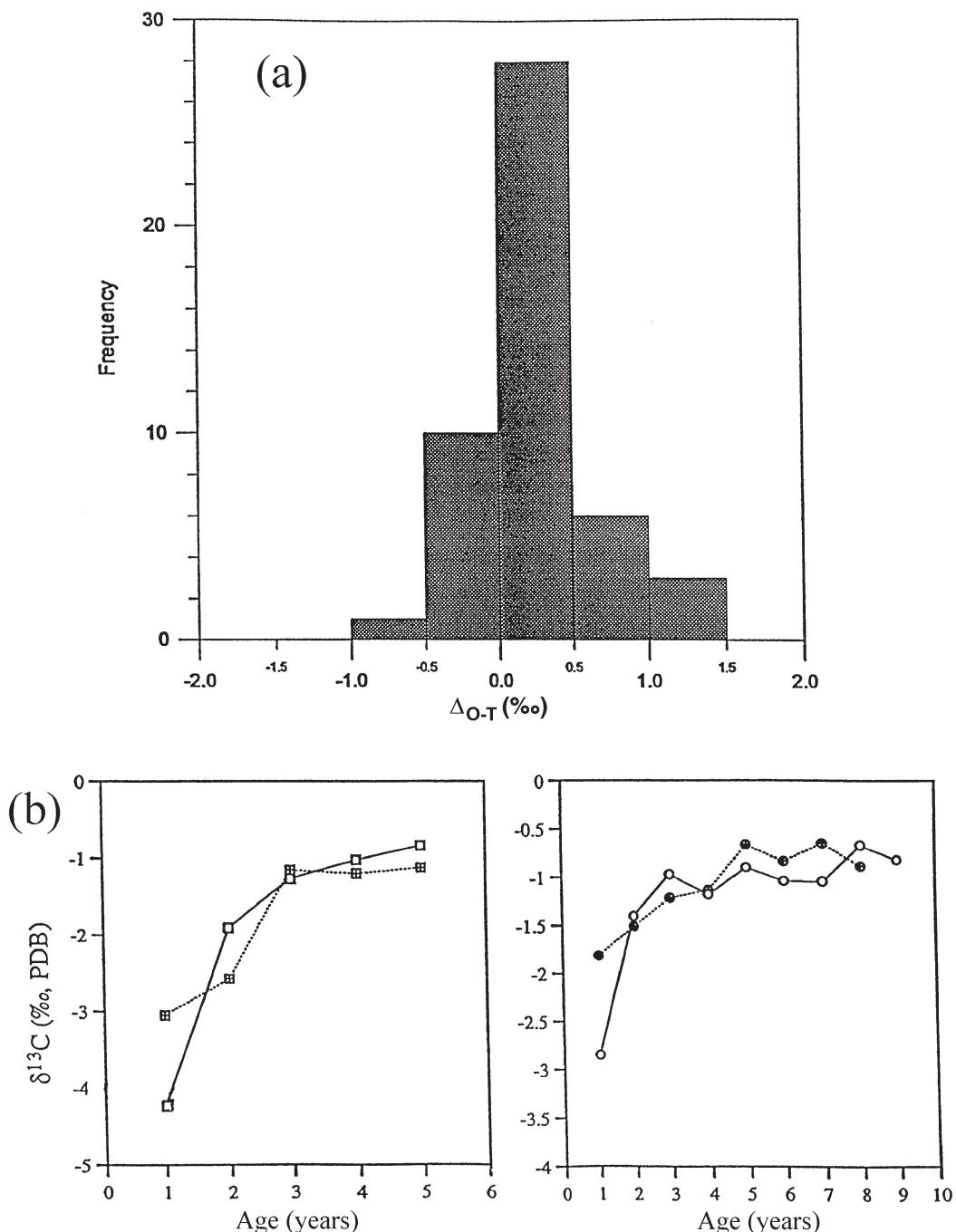


The $^{13}\text{C}/^{12}\text{C}$ ratio of DIC is determined by a balance between bicarbonate formed during oxidative metabolism and isotopic exchange with seawater through the gills (via CO_2). A small amount of HCO_3^- is absorbed through the gut from ingested seawater (Loretz 1995). Mulcahy et al. (1979) showed a correlation between $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios in a benthopelagic Pacific fish (*Coryphaenoides acrolepis*). They found that $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 may reflect changes in habitat (as a result of depth-dependent changes in $^{13}\text{C}/^{12}\text{C}$ of DIC of seawater) and changes in nutrient sources used by the fish, which might affect $^{13}\text{C}/^{12}\text{C}$ of metabolic carbon.

The otoliths of Atlantic cod (*Gadus morhua*) are large enough to permit the extraction of sequential records of changing environmental conditions through the life of a cod. In cross section, the largest of the otoliths in each fish, the sagittae, display a regular series of alternating growth bands called translucent and opaque zones. It is generally believed that the opaque zones are deposited during periods of faster growth in warmer summer waters, while the translucent zones represent winter deposition (Blacker 1974). The age of the otolith (and therefore of the fish) can be determined from analysis of the zone sequence.

In this paper we describe life-long variations in $^{13}\text{C}/^{12}\text{C}$ of otoliths of cod from Atlantic Canada. Our first goal was to study the variation in isotopic ratios within single otoliths to evaluate the comparative roles of isotopic exchange and metabolic activity on the isotopic composition of seasonally and annually deposited otolith zones. The second purpose was to compare the isotopic composition of otoliths deposited over the last two decades to see what, if any, changes could be detected over time and to see if these changes

Fig. 2. Interseasonal isotopic differences $\Delta_{O-T} = \delta^{13}\text{C}(\text{opaque}) - \delta^{13}\text{C}(\text{translucent})$. (a) Histogram showing average differences for 48 adjacent opaque-translucent zones. (b) Typical graphs of successive opaque and translucent zones. Open symbols are the translucent zones; solid symbols are the opaque zones.

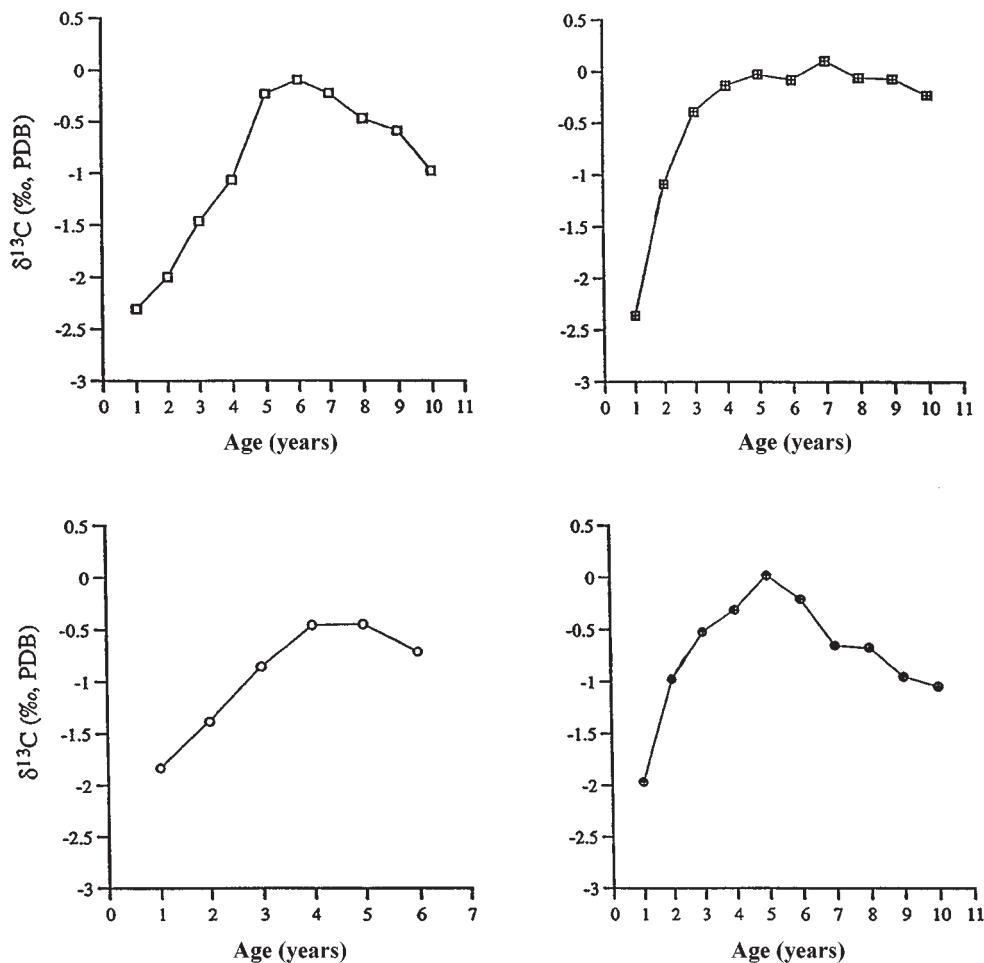


could be correlated with the decline in cod stocks over this interval. The data presented here complement studies of changes in the marine environment over time as obtained by oceanographic surveys. The significant difference and advantage of the present data is that they are recorded inside the fish themselves, and record environmental changes where the fish were living.

Materials and methods

Otoliths for this study were taken from collections made by the

Marine Fish Division of the Canadian Department of Fisheries and Oceans (DFO) at the Bedford Institute of Oceanography, from the Northwest Atlantic Fisheries Organization (NAFO) Division 4Vs stock, caught on the continental shelf off Nova Scotia (Fig. 1) between 1988 and 1994. The sampled fish ranged in age from 6 to 11 years. After the otoliths had been embedded in resin, transverse sections 700–800 μm thick, passing through the nucleus, were made of each saggital pair using a low-speed saw. The sections were then mounted on glass slides and polished to reveal the translucent and opaque zones. Samples of 30–50 μg were milled out of each zone using a computer-driven micromilling machine (DM 2800) in the Department of Mechanical Engineering, McMaster

Fig. 3. Carbon isotope ratios of otoliths plotted versus age for four typical otoliths from 4Vs subdivision.

University, Hamilton, Ont. The trace of the milling tool was recorded on drawings of the otolith and typically consisted of tracks about 150–300 µm deep, 25–60 µm wide, extending along a single growth zone. We were able to obtain between 9 and 26 samples from individual otoliths, depending on the age of the otolith. The samples were reacted with phosphoric acid in an Autocarb automatic carbonate analyser attached to a VG Optima mass spectrometer. The analyses are given in the δ notation, $\delta^{13}\text{C} = \{[(^{13}\text{C}/^{12}\text{C})_x / (^{13}\text{C}/^{12}\text{C})_{\text{s}-1}] - 1\} \times 1000$, where x is the sample and s is the standard (VPDB) in per mil (‰). Precision of the analyses was better than $\pm 0.06\text{‰}$. We shall represent $\delta^{13}\text{C}$ of otoliths by $\delta^{13}\text{C}_{\text{oto}}$.

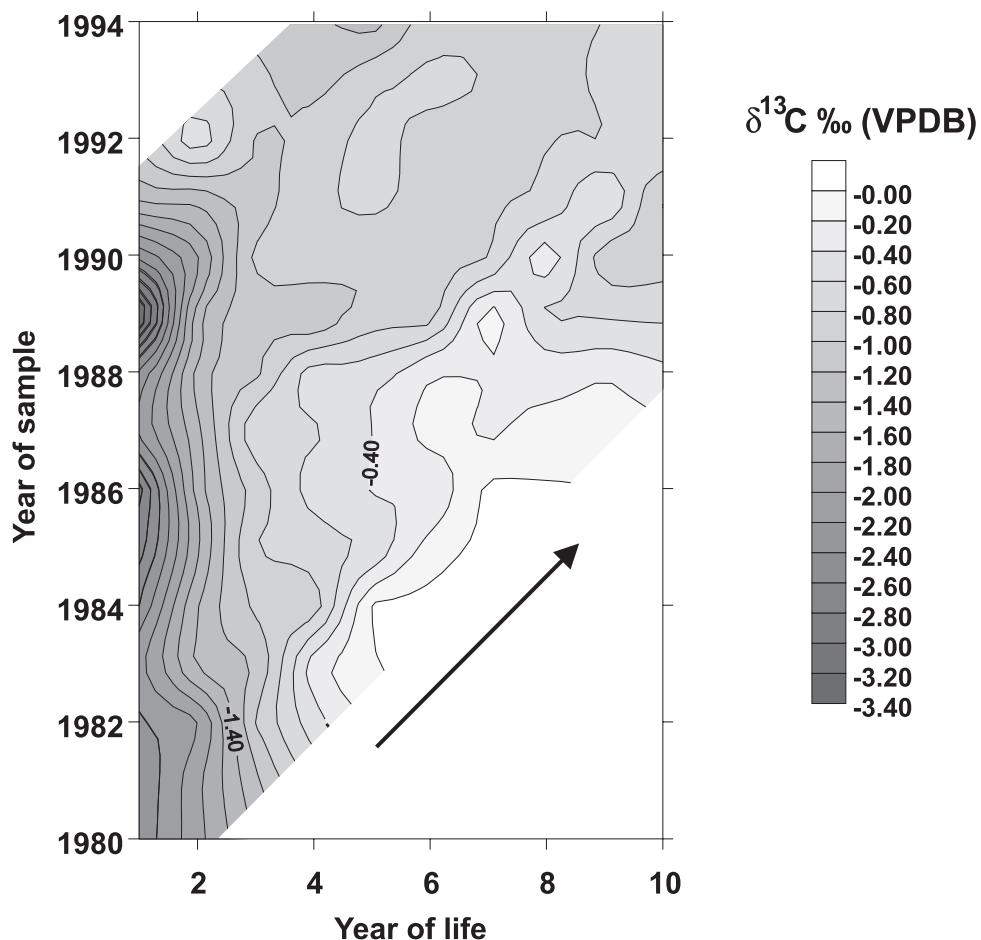
Results

We analysed a total of 61 otoliths whose growth zones were deposited over the years 1980–1994. Isotopic differences between adjacent opaque and translucent zones were small compared with the life-long trend of each otolith. The distribution of differences $\Delta_{\text{O-T}} = \delta^{13}\text{C}(\text{opaque}) - \delta^{13}\text{C}(\text{translucent})$ for a random subsample of 48 pairs is approximately normal around a value of $0.26 \pm 0.39\text{‰}$ (mean \pm SD) (Fig. 2a). Typical trends of $\delta^{13}\text{C}_{\text{oto}}$ for opaque and translucent bands parallel each other (Fig. 2a and 2b); although some otoliths display consistent ^{13}C enrichment of opaque zones with respect to translucent zones, in others the interzonal differences are quasirandom. These do not repre-

sent the maximum interzonal differences 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 interzonal differences, annual and secular variation are present as averages of adjacent translucent and opaque zones.

Every otolith measured from the 4Vs stock displayed a similar trend of $\delta^{13}\text{C}$ with age over the first 4–6 years of growth; typical examples are shown in Figs. 3a–3d. In each otolith, $\delta^{13}\text{C}$ increased through life, starting at an initial value of between -5 and -1.5‰ . The rate of yearly increase in $\delta^{13}\text{C}$ gradually diminished as the cod matured, and the majority of the otoliths (36 of 61) reached a maximum value near 0‰ at age 4–6 years. For those fish that exhibited a maximum, the pattern of subsequent $\delta^{13}\text{C}$ values differed between fish, either remaining nearly constant or decreasing steadily until the date of capture. Three otoliths showed a continuously rising record of $\delta^{13}\text{C}_{\text{oto}}$ up to the time of their capture, even after 8 years of growth. Two of these individuals had exceptionally high initial $\delta^{13}\text{C}_{\text{oto}}$ values (greater than -1.5‰), which may be significant in explaining the continuously rising $\delta^{13}\text{C}_{\text{oto}}$ signal of these fish. In Fig. 4 we have combined the data for all otoliths to show the similarity in the life-long trends of $\delta^{13}\text{C}$ through the period of sampling. The figure shows contours of $\delta^{13}\text{C}_{\text{oto}}$ versus year in which a sampled zone was deposited (y axis) and the year in the life

Fig. 4. Polynomial regression surface of $\delta^{13}\text{C}$ for 61 cod otoliths collected from the eastern Scotian Shelf between 1988 and 1994. Since all annuli within each otolith were analyzed, $\delta^{13}\text{C}$ values refer to the year and age of otolith annulus formation. Analyses of successive annuli extracted from one individual would lie along a path inclined 45° to the x and y axes (arrow).



of the cod that the sample was deposited (x axis). At each grid point on the contoured surface we plot the average $\delta^{13}\text{C}_{\text{oto}}$ of all otolith zones of a given age (ranging from 1 to 10 years) that were deposited in a given calendar year (1980–1992). The individual grid points incorporate data from between one and four analyses. A small part of the errors in positioning of these points along the x axis arises from the difficulty in milling out samples that precisely correspond to a given year; this introduces an error in age of less than 1 year. Note that the trajectory through the life of a single individual cod is traced by a path at 45° to the x and y axes (see figure).

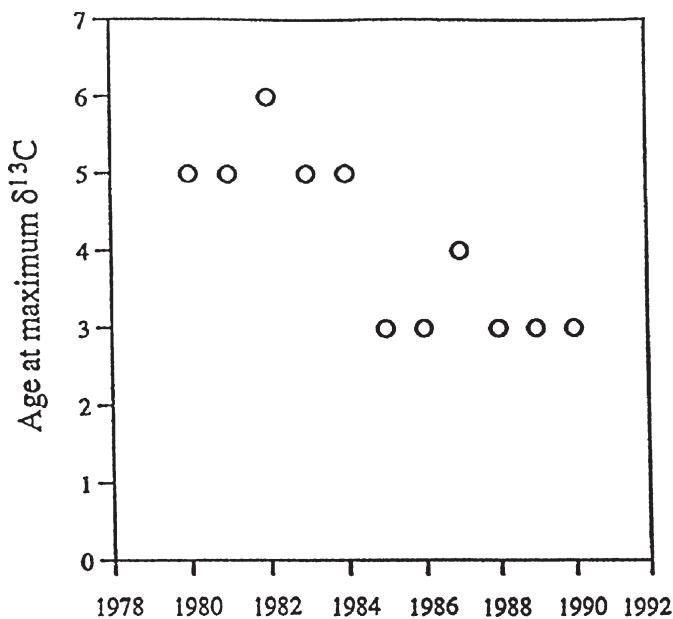
The contours show that a general trend of exponentially rising $\delta^{13}\text{C}$ values was prevalent in the first 4–6 years of the entire population and over the entire period of sampling (except for fish hatched after 1987, which had not yet lived long enough to reach a maximum). One can trace a general “ridge” of maximum values across the diagram starting about age 6 for the oldest fish sampled and drifting to a slightly lower age with increasing year of sample. The height of the maximum clearly decreases toward the top of the diagram. The decrease in the age at maximum $\delta^{13}\text{C}_{\text{oto}}$ is shown more clearly in Fig. 5; the age at maximum $\delta^{13}\text{C}_{\text{oto}}$ is seen to have decreased from about 5 years before 1984 to about 3 years after 1985. Figure 6 shows the average maxi-

mum $\delta^{13}\text{C}$ for those cod that reached a maximum in a given year versus the year in which the maximum occurred. That is, this graph shows the peak value in the plots of $\delta^{13}\text{C}_{\text{oto}}$ versus age for each of the cod that showed such a peak. The graph shows that these peak values decreased significantly (by about 1‰) between 1984 and 1993. Note that the position of the “crest” in Fig. 4 differs slightly from the ages and $\delta^{13}\text{C}_{\text{oto}}$ values in Figs. 5 and 6; this is because data for all the otoliths are shown in Fig. 4, whereas the other figures include only fish whose $\delta^{13}\text{C}_{\text{oto}}$ displayed a definite maximum, rather than a plateau. Inclusion of those fish that showed a plateau rather than a definite maximum would not have significantly altered the form of Figs. 5 and 6.

Discussion

The changes in $\delta^{13}\text{C}_{\text{oto}}$ described above appear to fall into two categories: (i) life-long changes in $\delta^{13}\text{C}_{\text{oto}}$ of individual cod, which appear to be virtually independent of the year in which the cod was hatched (that is, of the cohort to which it belonged), and (ii) longer term trends in $\delta^{13}\text{C}_{\text{oto}}$ seen in all cod of a given age living at a given time, which would therefore appear to be driven by changes in the environment and (or) by long-term changes in the behaviour of the fish.

Fig. 5. Age at which maximum $\delta^{13}\text{C}$ was reached in a series of cod which were hatched between 1980 and 1990 as a function of year in which maximum occurs.



Year-independent, life-cycle changes: variation in $\delta^{13}\text{C}$ of immature cod (0–5 years old)

Variation of $\delta^{13}\text{C}$ in cod otoliths is strikingly uniform during the first 6 years of growth in all the fish studied. The pattern of variation is almost independent of the years in which growth occurred, suggesting that it is a result of persistent characteristics of the organism, presumably a blend of genetically controlled physiological changes acting together with life-long changes in living environment and (or) food preferences of the cod. The persistent, repetitive character of this variation suggests that it is not a consequence of changing regional environment. In general, $\delta^{13}\text{C}$ starts at initial values between -5 and $-2.5\text{\textperthousand}$ and rises steeply, gradually levelling off to a value near 0\textperthousand after age 4–6 years. All of the 4Vs otoliths that we studied exhibited this trend of increasing $\delta^{13}\text{C}$ values, although in the last year's samples the steepness of the rise is less. This trend with age is similar to that shown by Mulcahy et al. (1979) for *Coryphaenoides* from the Pacific and may be a widespread characteristic of marine fish.

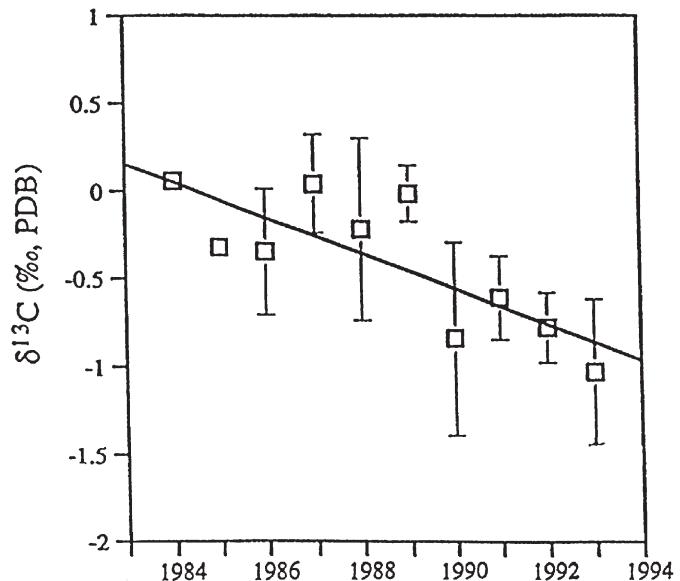
To understand possible causes of this trend of rising $\delta^{13}\text{C}$ with age, we must consider the factors that determine the carbon isotopic composition of otolith aragonite. We assume that otolithic aragonite forms in isotopic equilibrium with DIC, which is dominantly HCO_3^- . The isotopic fractionation between aragonite and HCO_3^- varies only slightly with temperature, T ($^{\circ}\text{C}$) (Grossman and Ku 1986):

$$(1) \quad \delta^{13}\text{C}_{\text{oto}} - \delta^{13}\text{C}(\text{HCO}_3^-) = -0.131T + 2.66$$

where $\delta^{13}\text{C}(\text{HCO}_3^-)$ refers to DIC in the endolymph; the coefficient (-0.13) represents the temperature dependence of the isotopic fractionation between aragonite and DIC; it is so small that we can neglect its effect on $\delta^{13}\text{C}_{\text{oto}}$. Assuming an average temperature of 5°C , we get

$$(2) \quad \delta^{13}\text{C}_{\text{oto}} \approx \delta^{13}\text{C}(\text{HCO}_3^-) + 2.0$$

Fig. 6. Plot of the average maximum $\delta^{13}\text{C}_{\text{oto}}$ of cod for versus the year in which the maximum occurred. Error bars are 1 SD. Line is the least squares regression through points: $\delta^{13}\text{C} = -0.101Y + 201.4$, $r^2 = 0.618$, where Y is the year.



Since there is little metabolic activity in the endolymph itself, its $\delta^{13}\text{C}(\text{HCO}_3^-)$ should be the same as that of blood HCO_3^- and, therefore, would reflect the result of mixing of the two major sources of blood bicarbonate: (i) metabolic generation of oxidized carbon and (ii) uptake of inorganic carbon from seawater through the gills:

$$(3) \quad \delta^{13}\text{C}(\text{HCO}_3^-) = M \delta_d + (1 - M) \delta_{sw}$$

where M is the fraction of metabolic carbon in the blood HCO_3^- . Therefore:

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

Now let us consider how each of the terms in this equation contributes to the life-long variation in $\delta^{13}\text{C}_{\text{oto}}$.

δ_{sw} : As a result of photosynthetic activity of phytoplankton, $\delta^{13}\text{C}$ of marine DIC decreases steeply in the upper few hundred meters of the sea, from near-surface values of about 2\textperthousand , to slightly negative values at depths of about 1 km (Kroopnick 1980). δ_{sw} also varies seasonally in response to changes in sunlight (Kroopnick 1974). In the upper 300 m of the water column, δ_{sw} would average to about $1 \pm 1\text{\textperthousand}$ but moving to greater living depth, either seasonally or on a life-long time scale, would cause increase in δ_{sw} and therefore in $\delta^{13}\text{C}_{\text{oto}}$.

δ_d : Like other marine fish, their diet changes from lower to higher trophic-level organisms within the lifetime of the cod. 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. $\delta^{13}\text{C}$ of the flesh of marine organisms increases with trophic level, by slightly

less than 1‰ for each trophic level (Peterson and Fry 1987). We might therefore expect δ_d to increase by as much as 3‰ through the first 5 years of life; this is a large fraction of the total observed shift.

M : The rate of metabolic production of DIC decreases with age. For example, Edwards et al. (1972) showed that oxygen consumption, Q_{O_2} , of cod varied as a function of weight (W , in g)

$$(5) \quad Q_{O_2} = 0.245W^{0.82} \text{ (mg/h)}$$

Therefore, the oxygen consumption per gram decreases as the fish increases in weight with age. Assuming that there is no simultaneous decrease in the rate of exchange of blood with seawater through the gills, this would lead to a decrease in M with age.

The combination of these effects of increasing age on the values of M , δ_d , and δ_{sw} could lead to the increase in $\delta^{13}C_{oto}$ in the first 4–5 years of the fish's life. The steep initial increase in $\delta^{13}C_{oto}$ by up to 4‰ could be due to an increase in the trophic level of the cod with age. Although we do not yet have any direct evidence of this increase in δ_d with age, this could easily be tested by studying $\delta^{13}C$ of the flesh of cod of successively increasing age.

Decrease in M , the metabolic fraction of HCO_3^- in the fish's blood would also lead to an increase in $\delta^{13}C_{oto}$, because $\delta^{13}C$ of HCO_3^- from respiration is close to that of the fish's flesh and, therefore, much lower than that of seawater. For example, 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. Recalling that the typical range of δ_{sw} ($\delta^{13}C$ of total DIC in seawater) is between 2 and 0‰, over depths of 100–300 m, we see that a decrease in M would lead to a sharp rise in $\delta^{13}C_{oto}$.

At maturity, most otoliths reach a maximum $\delta^{13}C$ value close to 0‰, after which $\delta^{13}C$ changes little or decreases slightly. The near-constant value observed in many fish suggests that blood DIC has reached a steady state with respect to diffusion and metabolic input, corresponding to M having reached a minimum with increasing age of the fish. At the minimum limiting value of $M = 0$, eq. 2 would give $\delta^{13}C_{oto} = 3\text{\textperthousand}$; clearly, the fish could never reach this state, which would require zero metabolic contribution. The maximum observed value of $\delta^{13}C_{oto}$ is about 0.8‰, which using eq. 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. If we assume that juvenile cod consume a diet that is 3‰ lighter than that of mature cod, then the lower limit to $\delta^{13}C_{oto}$, about -5‰, corresponds to $M = 0.43$. These calculations show that changes in both M and δ_d probably contribute to the increase in $\delta^{13}C_{oto}$ in the first 4–6 years of life of the cod.

Cod are known to change their living depth both seasonally and as a function of age (Clark and Green 1991) and, therefore, will experience varying δ_{sw} . However, the maximum variation to be expected is $\pm 1\text{\textperthousand}$, which is small compared with the effects of changes in δ_d and M . In general, cod move to greater depths with advancing age and, therefore,

would experience slightly lower δ_{sw} values (approaching 0‰). This would tend to lower estimates for the minimum value of M , approaching 0.07 for otolith samples with the highest $\delta^{13}C_{oto}$ values ($\approx 0\text{\textperthousand}$). At such low values of M , when the fish reaches a size such that no further changes in trophic level are likely to occur, most of the further variation in $\delta^{13}C_{oto}$ would probably be due to changes in δ_{sw} and, therefore, to changes in living depth.

Thus, in summary, these calculations show that the rise in $\delta^{13}C_{oto}$ over the first 6 years of life are probably accounted for by a combination of a progressive decrease in M , the fraction of metabolic bicarbonate in endolymph, and an increase in $\delta^{13}C$ of the average diet, δ_d , due to an increase in trophic level. The effect on $\delta^{13}C_{oto}$ of decreasing metabolic activity of the cod with increasing age was also suggested by Mulcahy et al. (1979). However, typically $\delta^{13}C_{oto}$ increases approximately linearly with age (Fig. 3), whereas the variation of M with age is nonlinear (eq. 5). Therefore, it appears that most of the early variation in $\delta^{13}C_{oto}$ is a result of change in diet, rather than declining metabolic rate. At age 4–6 years, as the cod matures, it attains its maximum trophic value and also reaches a minimum in metabolic rate per kilogram. As a result, $\delta^{13}C_{oto}$ either reaches a constant (plateau) value or slowly begins to decline. Decreases in $\delta^{13}C$ observed in some cod after age of about 6 years are most likely due to decrease in δ_{sw} as a result of increase in the living depth of the cod.

The attainment of a maximum or plateau in the $\delta^{13}C$ value of otolithic aragonite between age 3 and 6 may correlate with some change in the physiological characteristics of the fish. This is close to the age at which cod typically attain sexual maturity (Lear 1993). Unfortunately, the state of sexual maturity was not recorded for the cod whose otoliths we analysed. It is nevertheless possible that stable isotopic analyses may provide a means by which to determine when maturity had been reached in a given individual or a population of fish.

Lack of interseasonal difference in $\delta^{13}C_{oto}$

There is a surprisingly small difference between $\delta^{13}C$ of opaque and translucent zones ($0.26 \pm 0.39\text{\textperthousand}$). The metabolic rate of Atlantic cod doubles with a 10°C rise in temperature (Brander 1997). We therefore expect a large increase in M , the metabolic fraction of DIC, 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 summer–winter difference in $\delta^{13}C_{oto}$ is positive, and for the majority of fish studied here, the $\delta^{13}C$ of aragonite formed 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, it is surprising 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 move to warmer deep waters in winter so as to avoid the colder surface waters (Sinclair 1992; Page et al. 1994). 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 (1998)

shows, as expected, a seasonal fluctuation in $\delta^{13}\text{C}_{\text{oto}}$ of up to 4‰, with $\delta(\text{summer}) < \delta(\text{winter})$. Secondly, there is a seasonal cycle in the gradient of $\delta^{13}\text{C}$ of DIC with depth (Kroopnick 1980). The enrichment of near-surface seawater bicarbonate in ^{13}C is a result of photosynthetic activity by plankton, which preferentially reduce $\text{H}^{12}\text{CO}_3^-$, leaving the residue enriched in ^{13}C . During the summer the $\delta^{13}\text{C}$ of DIC in seawater at a given depth will be higher than in the winter, because of lower photosynthetic activity at the lower diurnal light levels experienced in winter. Both of these effects tend to oppose the temperature-driven shift to lower $\delta^{13}\text{C}_{\text{oto}}$ values expected in the summer.

Time-dependent (secular) changes: variation of $\delta^{13}\text{C}$ in older cod (age >5 years)

The maximum $\delta^{13}\text{C}$ attained by each otolith decreased through the period 1984–1993 (Fig. 6). This decrease could be due to a number of possible causes. In principle this could be the result of an increase in the metabolic activity during this period. However, Campana et al. (1995) have shown that the growth rate of cod in 4Vs declined over this period; this is likely to have been coupled with a decrease in metabolic rate. Water temperature also affects the metabolic activity. However, the shift to lower $\delta^{13}\text{C}_{\text{oto}}$ values that we observe would correspond to an increase in M during this period, whereas the trend during this period was toward decreasing water temperatures. A decrease in $\delta^{13}\text{C}$ of the nutrient sources could also account for this shift. Local variations have been recorded in $\delta^{13}\text{C}$ of phytoplankton in the Atlantic (Fontugne and Duplessy 1981), but we have no evidence that the distribution pattern of $\delta^{13}\text{C}$ values has changed over recent times. In particular, there is no reason to expect such a shift over the past decade. The most likely cause of this downward shift in $\delta^{13}\text{C}$ is a decrease in $\delta^{13}\text{C}$ of DIC in the seawater (δ_w) to which the cod is exposed. This would happen if the cod migrated into deeper waters, as a result of the steep gradient in δ_w typically observed in the upper 1 km of the sea (Kroopnick 1980). As temperatures fell in the cold intermediate layer (Drinkwater 1996) the cod may have moved to lower depths in the warmer bottom waters of the Scotian Shelf, thus encountering waters with lower δ_w . This could also partly account for the decreasing trend in $\delta^{13}\text{C}_{\text{oto}}$ observed in individual fish.

We have suggested that the age at maximum $\delta^{13}\text{C}_{\text{oto}}$ may mark the time of sexual maturity of the cod. Fish hatched prior to 1985 reach maximum $\delta^{13}\text{C}$ at age 5–6 years, while after 1985, they reach their maximum value at an average age of 3 years (Fig. 5). The time of this change in the isotopic behaviour of the cod also marks a period of abrupt change in the population dynamics of the 4Vs stock. However, 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 there remains some uncertainty as to the significance of the shift shown in Fig. 5.

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