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OXYGEN AND CARBON STABLE ISOTOPE ANALYSIS OF THE OTOLITHS OF ATLANTIC COD (GADUS MORHUA L.)

OXYGEN AND CARBON STABLE ISOTOPE ANALYSIS OF THE OTOLITHS OF ATLANTIC COD (GADUS MORHUA L.)

by

David R. Browne

A Thesis

Submitted to the Department of Geology

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ABSTRACT

The purpose of this study was to apply oxygen and carbon stable isotope microsampling techniques to the seasonal banding of the otoliths of the Atlantic cod, Gadus morhua, in order to determine their potential use in reconstructing the environmental conditions experienced by cod. A reconstruction of the changes in environmental temperature is seen as particularly applicable to the determination of cod migration routes based on the known temperature profile of the North Atlantic. Transverse thin sections of six otoliths from cod taken off the coast of Atlantic Canada were prepared using standard methods. Material was sampled from each semi-annual band of the otoliths and run on a Finnigan MAT 251 ratio mass spectrometer to determine $\delta^{18}O$ and $\delta^{13}C$.

The results for $\delta^{18}O$ indicated that the otoliths had an approximate initial value of either 1.4‰ or 1.0‰ for sample material taken from the nucleus. The $\delta^{18}O$ signal was characterized by oscillating values in which sample material from hyaline bands corresponded predominantly with the troughs and sample material from the opaque bands corresponded with the peaks. The average range of $\delta^{18}O$ was found to be 0.87‰ corresponding to a temperature range of 3.6°C which was within the expected 3 to 4°C seasonal average temperature shift experienced by cod. Seasonal cycling was apparent in three of the otoliths, with semi-annual values alternating between high opaque bands and low hyaline bands.

It was concluded that seasonal temperature changes due to migration from offshore to inshore waters are recorded in the $\delta^{18}O$ signal and that future sampling should attempt to resolve several samples within each seasonal band in order to resolve the migratory changes in

temperature on an sub-annual basis. It is also suggested that experiments be carried out to determine the species specific $\delta^{18}O$ versus temperature relationship for cod to make accurate interpretation of the data possible.

The $\delta^{13}C$ signal was found to be characterized by an increasing logarithmic trend in $\delta^{13}C$. The $\delta^{13}C$ signal was observed to increase over the first three years of growth and to level off at age 4 and fluctuate at a value close to 0‰. The otoliths of cod were found to contain a high proportion of inorganic carbon with a minimum $\delta^{13}C$ value of -4.53‰ and a maximum of 0.23‰.

It was suggested that the initial increase in $\delta^{13}C$ was a product of a combination of factors affecting metabolic rate and therefore, indirectly, the amount of metabolically derived carbon circulating in the blood. It was concluded that further research into the growth and development of cod was necessary in order to reach a comprehensive understanding of the biological processes responsible for the observed trends in $\delta^{13}C$.

$\delta^{18}O$ was plotted against $\delta^{13}C$ for samples from growth bands of age four or greater and found to have a positive correlation with a slope of 0.269 (S.E. 0.049) and an r-squared of 0.537, $P < 0.0001$ when the results for otolith 176 were excluded due an anomalous trend in that data set. Two hypotheses were suggested to account for the observed positive correlation, a metabolic/temperature effect, and a depth/temperature effect.

It was concluded that, with further research into the controlling factors behind the fluctuations in $\delta^{13}C$, the $\delta^{13}C$ signal may provide a second source of information with regards to changes in habitat and environmental characteristics over the life time of the cod. It is suggested that a study be carried out to determine the amount of metabolic carbon incorporated into the otolith in order to clarify the observed trends.

ACKNOWLEDGEMENTS

I would like to thank Dr. H.P. Schwarcz for providing the conceptual basis for this thesis and for having faith in my abilities to carry out independent research. Without his knowledge, guidance, and inspiration I would not have been able to complete the project. His constant excitement over the directions the research was taking throughout the year inspired me to probe deeper into the problems being identified and to come to a better understanding of the topic.

I would also like to thank Dr. M.J. Risk who provided ideas and contacts during the beginning stages of my work and valuable critique of the initial draft.

The project would not have been possible without the cooperation of both Steve Campana at the Department of Fisheries and Oceans in Halifax, who provided the otolith samples for the research, and Dr. K.C. Lohmann of the University of Michighan who provided facilities for microsampling and mass spectrometric analysis of the otoliths.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE

LIST OF TABLES

TABLE

LIST OF PLATES

PLATE

Chapter 1 - Introduction

1.1 Introduction

Stable oxygen and carbon isotope analysis of calcium carbonates has been widely used to determine paleoenvironmental characteristics of a variety of fossil organisms. Oxygen and carbon isotopes differ in their physical characteristics and correspondingly act as indicators of separate aspects of the paleoenvironment of an organism. Oxygen isotope analysis has been applied to a variety of carbonate species for paleotemperature estimation. The fractionation of isotopes of oxygen during carbonate precipitation favours the more stable ^{18}O bond at lower temperatures. This results in carbonate precipitated in cooler temperatures having a higher $^{18}O/^{16}O$ ($\delta^{18}O$) ratio. The temperature versus $\delta^{18}O$ curve has been calibrated for a variety of organisms and used to estimate former climatic conditions (Wefer and Berger, 1991). Carbon isotopes, on the other hand, show less temperature effect (Grossman and Ku, 1986) and are used as indicators of paleodiet and paleoecology in terrestrial and marine organisms. Fractionation of carbon isotopes results from the process of photosynthesis which is characterized by an enzymatic preference for carbon-12. Photosynthetic organisms are highly enriched in carbon-12 and have a characteristic negative $\delta^{13}C$ value.

1.2 Otolith Structure and Growth

Organisms with rapid growth of accretionary carbonate structures offer potential for seasonal measurement of temperature fluctuations and seasonal changes in diet based on the oxygen and carbon stable isotopic composition of the carbonate. Otoliths are carbonate accretionary structures in fish ears characterized by daily growth increments, seasonal growth

banding, and no resorption. Most fish possess three pairs of otoliths, the lapilli, the asterisci, and the sagittae. The sagittae are in almost all cases the largest structure and have received the most attention in past research (Campana, 1985). For the purposes of this paper, the word otolith will refer exclusively to the sagittal otolith. Otoliths are secreted in the saccula of the fish located immediately posterior to the brain. A diagram of both the location and morphology of the otolith is provided in figure 1.1. The process of otolith calcification remains poorly understood, but is similar to that of mollusc shell secretion involving a two step process of protein layer secretion followed by carbonate precipitation onto the protein matrix (Pannella, 1980). In a study of 25 different species of fish, Degens et al. (1969) found that otolith calcium carbonate consisted exclusively of aragonite with a protein content which varied between 0.25% and 10.5% of total otolith material. In temperate fishes, seasonal differences in temperature and growth result in annual changes in the ratio of protein to calcium carbonate in the otolith. The current theory of seasonal band differentiation is based on this change in the protein to calcium carbonate ratio. Periods of slow growth or cold temperatures are thought to correspond to low calcification rates of short dense aragonite crystals containing a high percentage of protein. The high protein content bands are translucent when viewed under transmitted light and are commonly referred to as hyaline bands. Similarly, periods of fast growth result in high calcification rates of long, thick aragonite crystals with a lower percentage of protein resulting in the formation of an opaque band (Blacker, 1969 and 1974). In temperate fish, the main factor affecting otolith growth is temperature and major growth bands are formed based on the seasonal temperature changes (Pannella, 1980). By counting the number of hyaline, or winter, bands visible in thin section,

otoliths can be used to determine the age of a fish.

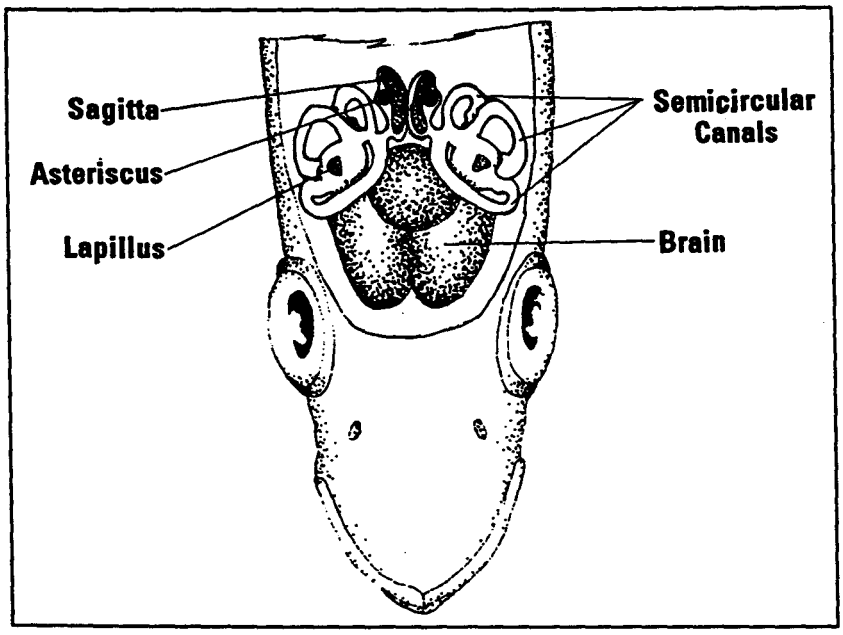
1.3 Past Research

Until recently, the focus of otolith research has been primarily on the use of otoliths in age determination, despite the fact that in 1969 Degens et al. suggested that the isotopic composition of otolith growth bands could act as records of fish life environmental conditions. Research on the potential use of otoliths as environmental records has focused on the analysis of carbon and oxygen isotope ratios as well as the analysis of the elemental composition of otoliths (Radtke, 1992). Most recently, a paper by Kalish (1991a) compiled the results of all previous otolith studies and concluded that both $\delta^{13}C$ and $\delta^{18}O$ are linearly related to temperature and that there exists a positive linear correlation between $\delta^{18}O$ and $\delta^{13}C$ which remains poorly understood (see figure 1.2). A previous study of cod otoliths by Radtke (1984) found $\delta^{18}O$ to be related to temperature and $\delta^{13}C$ to be related to the nutritional and metabolic history of the fish. Results of previous studies have been limited by the large sample size necessary for accurate stable isotope analysis and has required the use of whole otoliths (Kalish 1991a). Otoliths are between 0.5 and 30 mm in length. Seasonal growth bands in thin section are approximately .05 to .3 mm in width which makes accurate sampling difficult. The maximum mass of sample material attainable from one seasonal band is on average 0.1 mg which has been below the limit for accurate detection of mass spectrometers until fairly recently. With recent advances in microsampling techniques and mass spectrometry technology, it has become possible to take samples accurately to a resolution of 10 microns and to obtain accurate isotopic measurements on samples with masses as little as 0.01 mg (Patterson, 1993). This has made

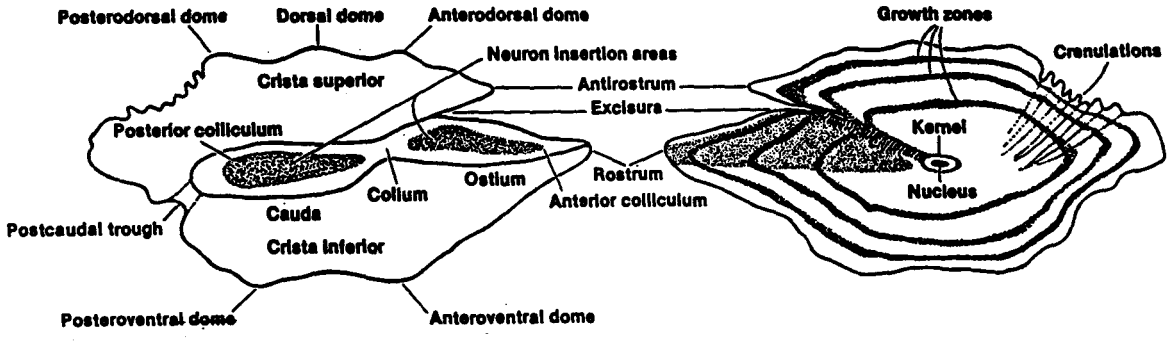
isotopic analysis of the sub-annual growth bands possible for the first time, opening up the potential for the determination of migration patterns from the analysis of otolith growth bands.

1.4 Atlantic Cod

Cod are an economically important species whose population has been decimated over the past 10 years. They are a migratory marine species found in the north Atlantic and belong to the group Paracanthopterygii and the family Gadidae. Relatively little is known about the migratory patterns of cod. This lack of knowledge posses a major problem when attempting to set appropriate fishing limits based on estimated population densities. It is known that cod spawn in the winter in deep offshore waters at a depth of approximately 300 meters with an average temperature of 2-2.5 degrees celsius. They migrate to warm, shallow inshore waters in the summer experiencing temperatures as high as 18°C. The diet of cod is a mixture of macrobenthos and fish. Annual migrations are thought to be partially determined by the migration of the cod's major food source, capelin, from offshore to inshore environments. The otoliths of cod are characterized by clear semi-annual banding and are large in size relative to many other species of fish making them ideal for isotopic analysis.



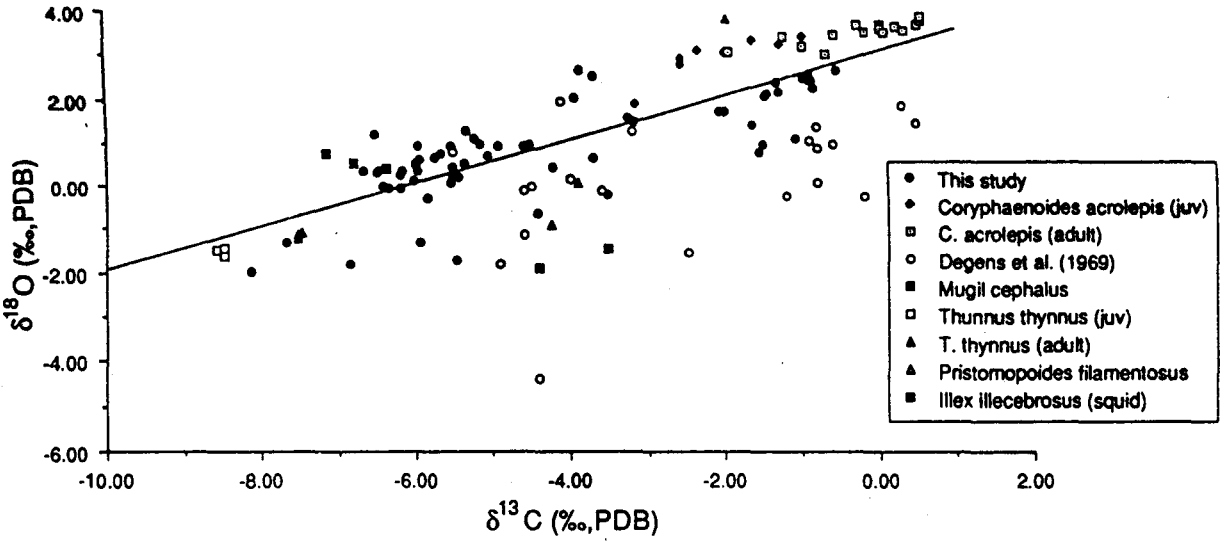
(from Stevenson and Campana, 1992)

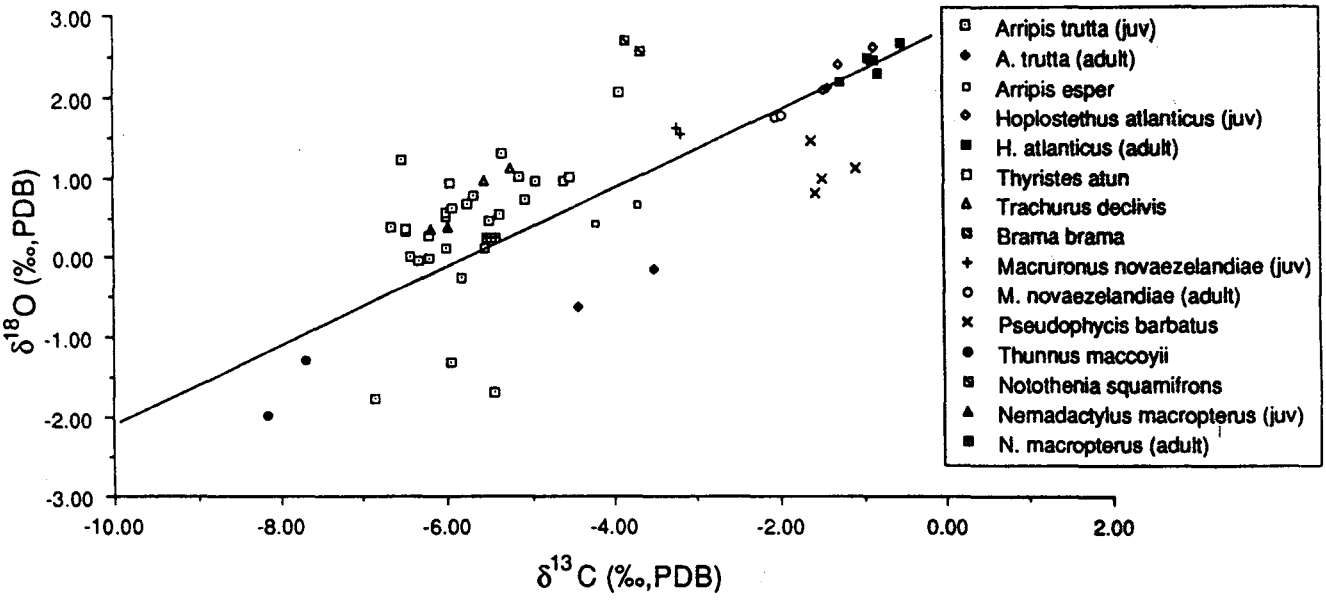


(from Pannella, 1980)

Figure 1.1 Location and morphology of the otolith.

Figure 1.2: Plot of $\delta^{18}O$ versus $\delta^{13}C$ for the aragonitic otoliths of 35 fish species and the aragonitic statoliths of one squid species (from Kalish, 1991a).


4.00
2.00
0.00
-2.00
-4.00
-6.00
δ18O (‰,PDB)
-10.00
-8.00
-6.00
-4.00
-2.00
0.00
2.00
δ13C (‰,PDB)
This study
Coryphaenoides acrolepis (juv)
C. acrolepis (adult)
Degens et al. (1969)
Mugil cephalus
Thunnus thynnus (juv)
T. thynnus (adult)
Pristomopoides filamentosus
Illex illecebrosus (squid)


3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
δ18O (‰,PDB)
-10.00
-8.00
-6.00
-4.00
-2.00
0.00
2.00
δ13C (‰,PDB)
Arripis trutta (juv)
A. trutta (adult)
Arripis esper
Hoplostethus atlanticus (juv)
H. atlanticus (adult)
Thyristes atun
Trachurus declivis
Brama brama
Macruronus novaezelandiae (juv)
M. novaezelandiae (adult)
Pseudophycis barbatus
Thunnus maccoyii
Notothenia squamifrons
Nemadactylus macropterus (juv)
N. macropterus (adult)

Chapter 2 - Purpose

The purpose of this project was to apply oxygen and carbon stable isotope microsampling techniques to the seasonal banding of Atlantic cod (Gadus morhua) otoliths to determine the potential for their use in reconstructing cod life environmental conditions. The ultimate goal of this research is to develop an accurate tool for reconstructing the life history of cod with regards to migration, feeding and growth in order to take an historical look at migration and spawning patterns among cod populations in the North Atlantic. This work represents the first time microsampling techniques have been applied to a migratory marine fish. It is also the first attempt to look at $\delta^{18}O$ and $\delta^{13}C$ on a sub-annual basis in a migratory marine species.

Chapter 3 - Procedure

3.1 Sample Origin and Identification

The left and right otolith from six cod originating from an unknown stock off the coast of Atlantic Canada and of unknown date of collection were supplied by Steve Campana of the Department of Fisheries and Oceans in Halifax, Nova Scotia. Each pair of otoliths had been cleaned and placed in a paper envelope with the length and weight of the fish indicated on the front. The right otolith of each pair was sectioned, prepared, and sampled as outlined below.

Table 3.1 is a summary of the known characteristics of the six cod from which the right otolith was analysed. Weights and lengths for each age differed substantially from average values found by Beacham (1982) for stock 4T.

Table 3.1: Weight, length, age and sample identification number for the six cod sampled.

OTOLITH	WEIGHT (g)	LENGTH (cm)	AGE (yrs.)
169	1300	54	6
173	2150	61	6
175	2000	63	7
174	1900	62	8
172	1800	60	8
176	2200	66	10

A second set of 15 pairs of otoliths from stock 4Vn with no length, weight or date of collection data were also supplied. These samples had been sectioned transversely through the

nucleus to a thickness of 1 mm and mounted in a strip of black epoxy with paired otoliths placed side by side.

3.2 Otolith Thin Section Preparation

The method of sample preparation was based on those described by Stevenson and Campana (1992), and Casselman (1987). Otoliths were cleaned with distilled water and dried overnight in an oven at 60°C. Whole otoliths were embedded in polyester resin. This was done by filling aluminum dishes with polyester resin and placing the otolith in the resin with the sulcus facing down. Care was taken to orient the otolith in the polyester resin such that later transverse sectioning was facilitated. The polyester resin was allowed to harden overnight in a 60°C oven and the aluminum dish was peeled off. Excess polyester was removed from the otolith using a high speed diamond saw to form a rectangular polyester block with an otolith embedded in the centre. The blocks were shaped on the high speed diamond saw to allow for mounting in the bracket of the Isomet, slow speed saw. A prepared polyester block had sides planar to the intersection of the anterior-posterior axis and the dorsal-ventral axis such that subsequent bracket mounting oriented the otolith for a transverse cut.

The otolith was sectioned using a slow speed Isomet saw set at 70% maximum rpm using a 300 μm thick high concentration diamond wafering blade with 75g of weight on the swing arm. The polyester block was mounted in the bracket of the isomet saw such that the blade was parallel to both the dorsal-ventral axis and the lateral axis and perpendicular to the anterior posterior axis. Care was taken in orienting the otolith correctly in the bracket of the isomet saw. This process was aided by drawing cross hairs on the epoxy block along the axes locating the

nucleus at their intersection. A 400-500 μm thick section was taken from the nucleus of the otolith by two successive cuts. The nucleus of the otolith was landmarked by the collum, a depression in the sulcus, located between the two points of neuron attachment. Sections were taken from the centre of the collum.

The thin section was inspected under a binocular dissecting microscope to determine the best side for polishing and sampling. This was generally the side with the least structural damage and the greatest portion of the nucleus still present. Once the best side to be polished had been selected, the opposite side was polished on 600 and 1000 grit paper and mounted on a petrographic slide with cyanoacrylate glue (Crazy glue). The exposed otolith surface was then polished successively with 600, 1000 and 2000 grit paper. Final polishing was done by hand using 0.3μm alumina paste on a polishing disk. The surface of the otolith was brought to a high lustre with little or no scratches. The final thickness of the otolith thin section was not less than 250 μm.

3.3 Microsampling technique

Prepared thin sections were sampled on a computer controlled microsampler located in the Geology Department at the University of Michighan. The device consists of a stationary drill with a custom tapered bit mounted above a stepper motor-controlled x-y-z micro positioning stage. The movement of the micro positioning stage was controlled manually through a computer using the arrow keys of the keyboard to step the stage in an x, y, or z direction by increments of 10 μm or greater. The otolith and drill bit are viewed through a binocular microscope mounted above the micro positioning stage. Prepared slides were mounted on the micro

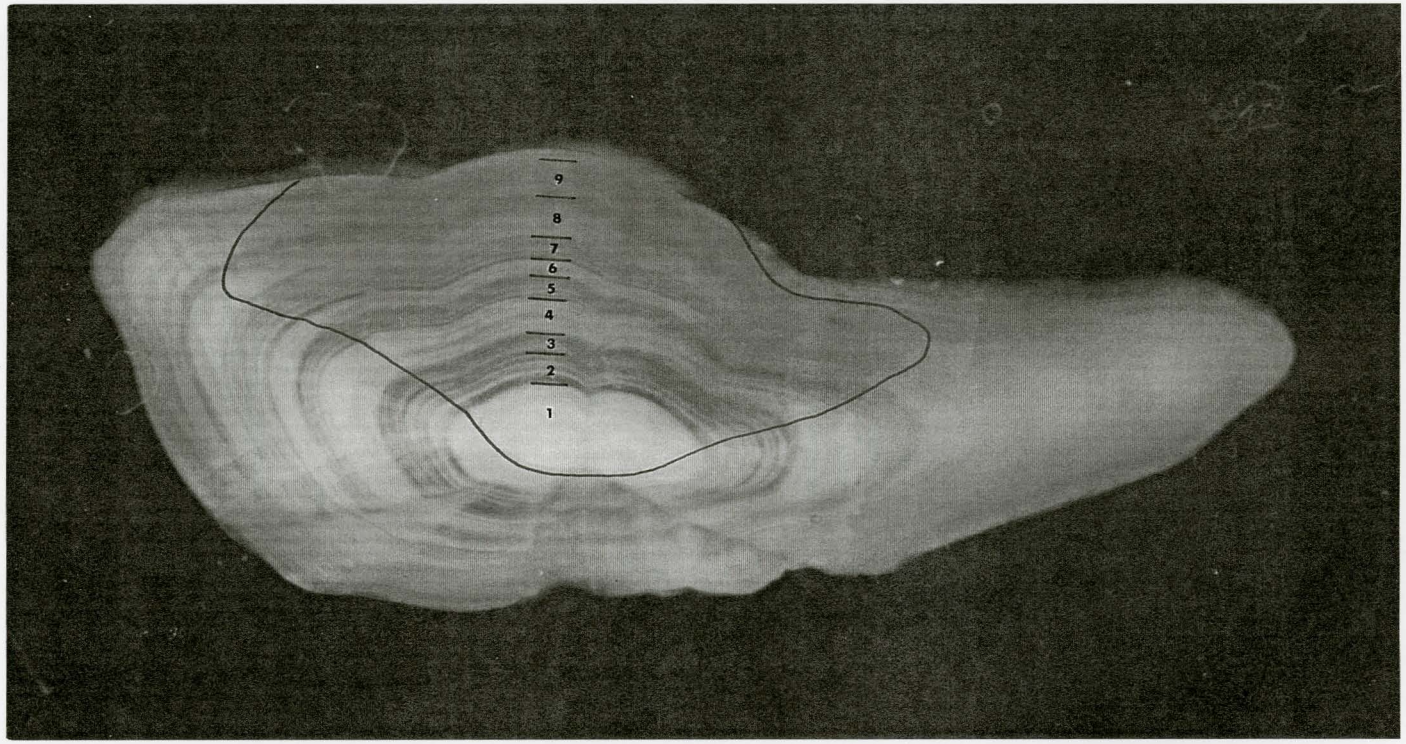
positioning stage with model glue. The nucleus of the otolith was positioned below the drill and the stage was raised to a drilling depth of 150-200 μm. Where possible, samples were taken from successive major hyaline and opaque bands moving outwards from the nucleus using the drill bit as a router. Samples typically represented a spatial resolution of 50-100 μm in width and a total mass of 20-50 micrograms. Once sufficient material from a growth band had been drilled, the material was collected from the surface of the otolith with a scalpel and modified syringe needle and placed in a labelled brass sample container. The drill bit, otolith surface, scalpel, and syringe needle were cleaned with a blast of high pressure air between samples to prevent contamination. Before taking a sample from the subsequent band, a thin band of material was routed off the edge of the band and blown away with high pressure air to ensure that each sample was discrete and uncontaminated by prior growth band material. The location of each sample was recorded on a hand drawn diagram of the otolith growth banding. Most samples consisted of material from different portions a hyaline or opaque band. Some samples were mixtures of hyaline and opaque material due to either unclear banding or thin banding which was not resolvable with manual operation of the apparatus.

An enlarged photographic image of each sampled otolith was produced using the otolith slide as a negative. The photographs were sent to Steve Campana of the Department of Fisheries and Oceans for interpretation of the age and seasonal banding. Recorded sample locations were assigned a specific age and band type (hyaline or opaque) based on the interpreted photographs. Sample locations for each otolith are found in plates 1 through 6.

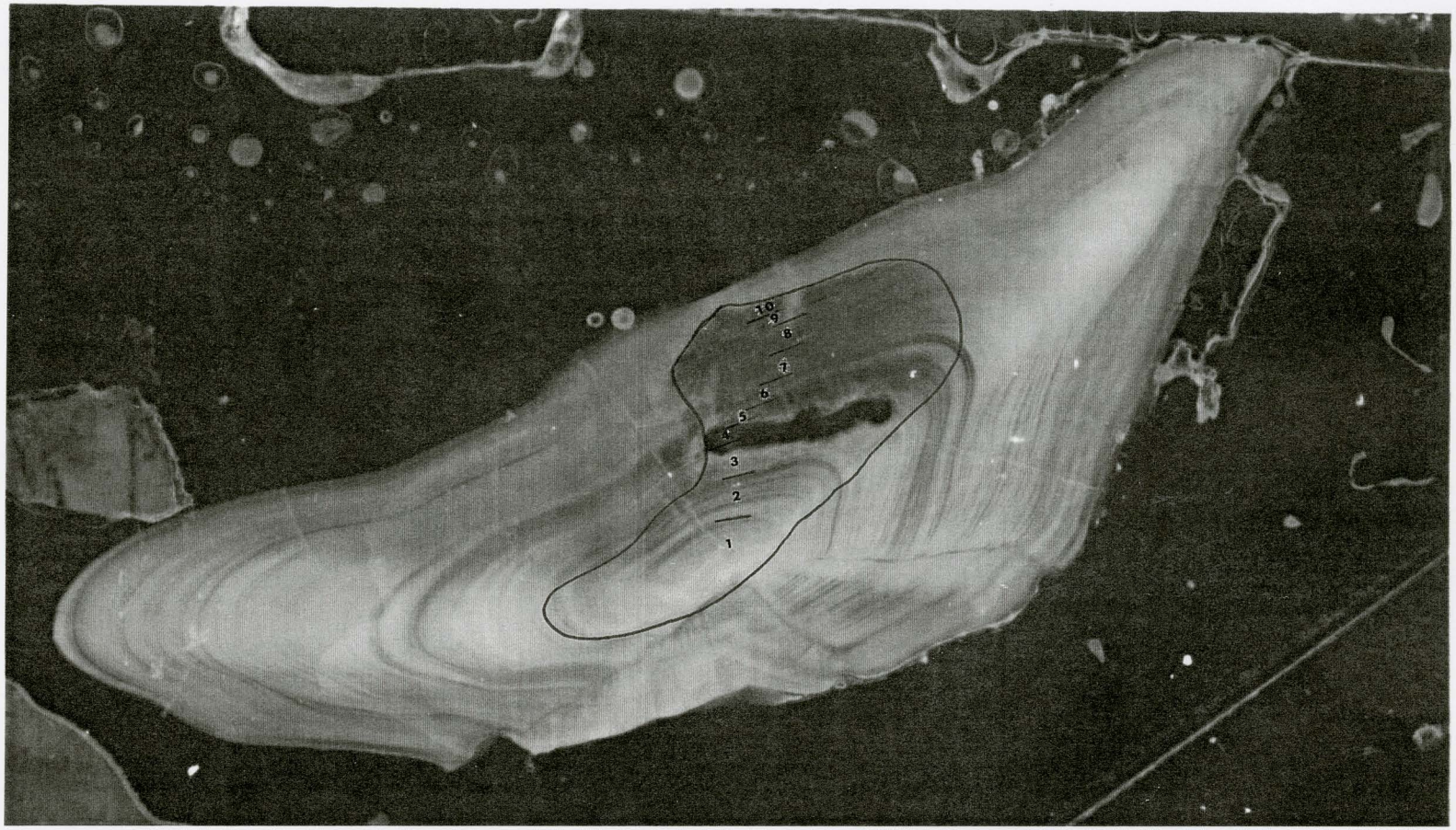
3.4 Mass Spectrometry Methodology

Sample carbonate powder recovered from the otoliths was roasted at 200°C *in vacuo* to remove volatile organic contaminants and individually reacted at 73°C with 4 drops of anhydrous phosphoric acid in a Finnigan "Kiel" carbonate preparation system coupled directly to the inlet of a Finnigan MAT 251 ratio mass spectrometer. Isotopic enrichments were corrected for acid fractionation and ^{17}O contribution and reported in per mil relative to the PDB standard. Precision was better than $\pm$ 0.06‰ for $\delta^{13}C$ with a mean error of $\pm$ 0.022 and better than $\pm$ 0.08‰ for $\delta^{18}O$ with a mean error of $\pm$ 0.037. A reproduction of the results obtained from the stable isotope laboratory of Dr. K.C. Lohmann at the University of Michighan can be found in Appendix A.

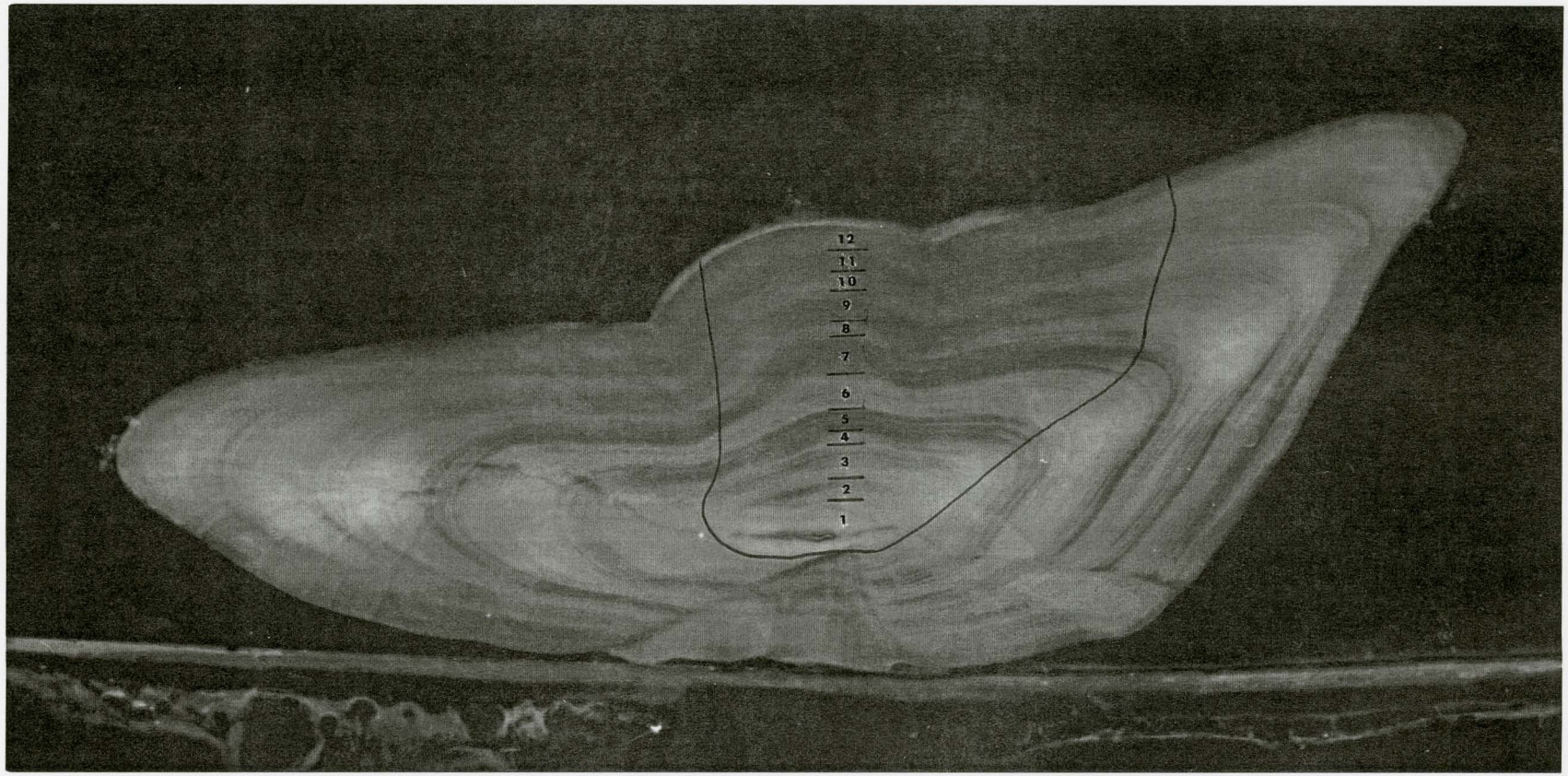
Plates 1 to 6: Location of samples. Numbers indicate the sample number and the band from which the sample was taken. The solid line indicates the edge of the sampling area. The scale for all six plates is 1cm equals 0.4 mm.



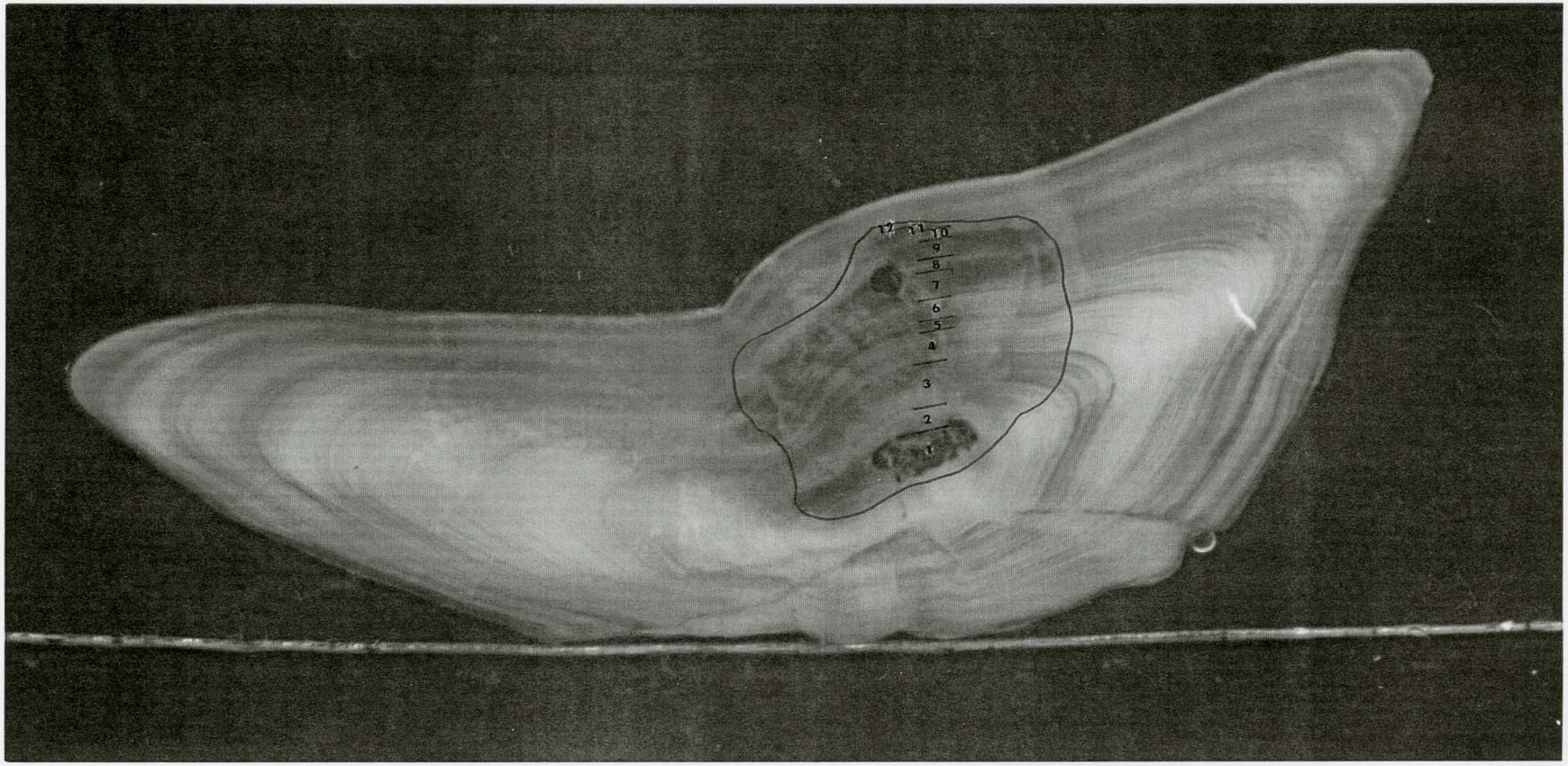
Otolith 169



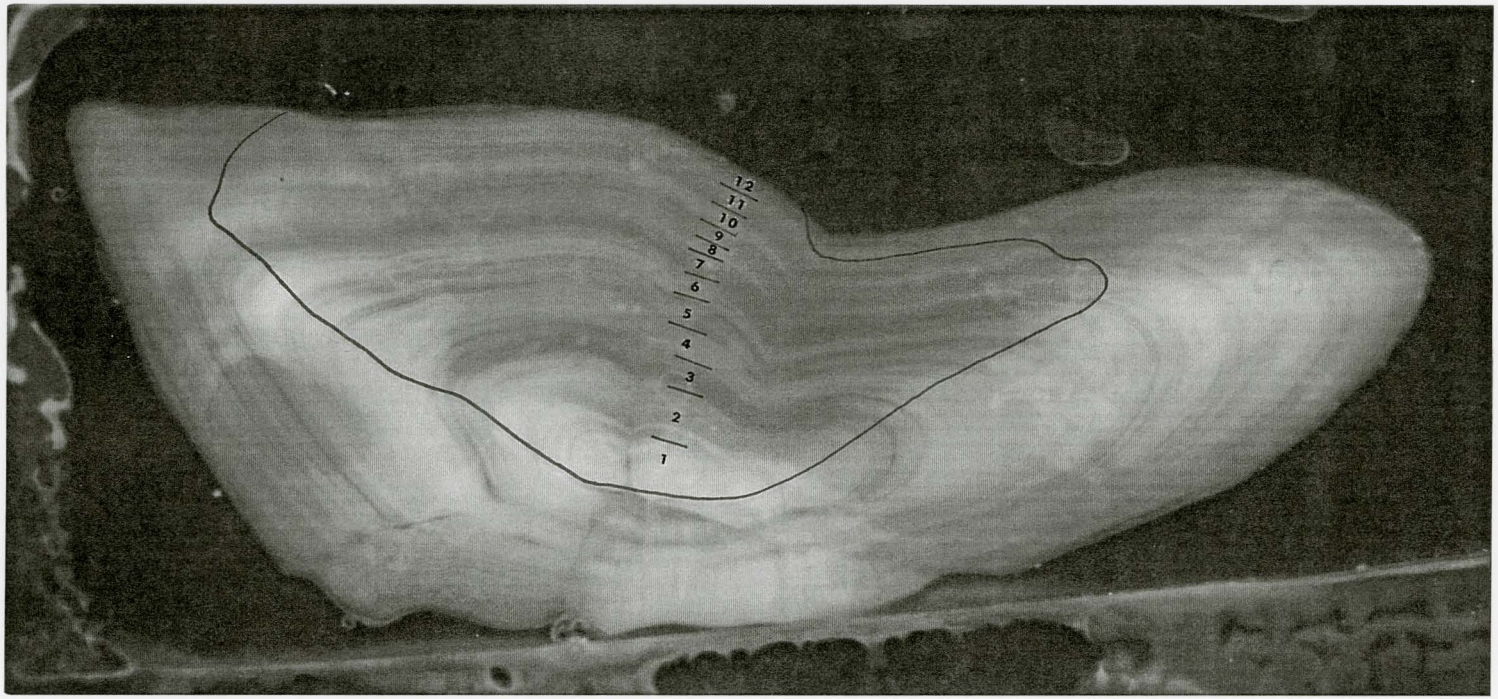
Otolith 172



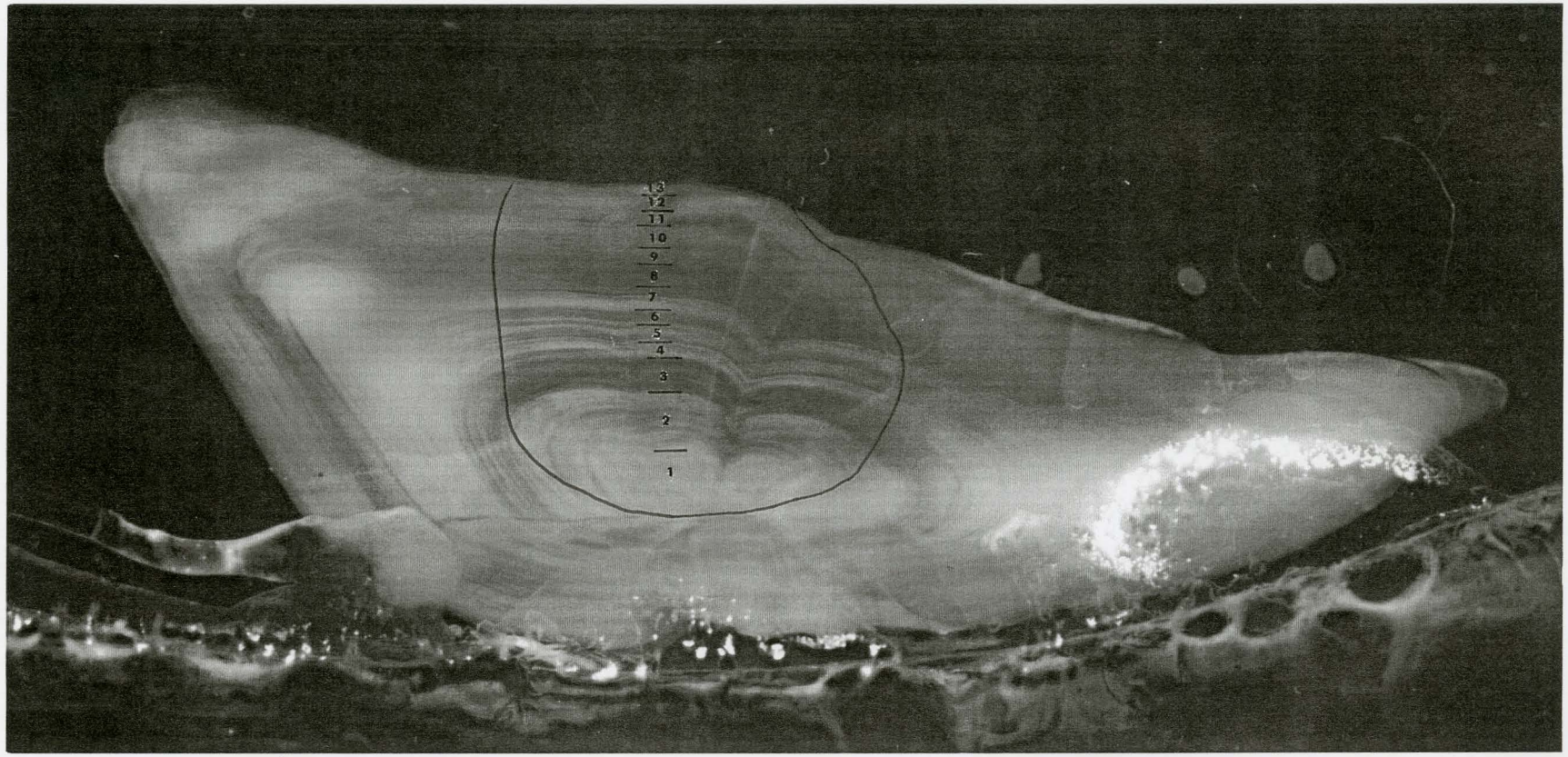
Otolith 173



Otolith 174



Otolith 175



Otolith 176

Chapter 4 - Results

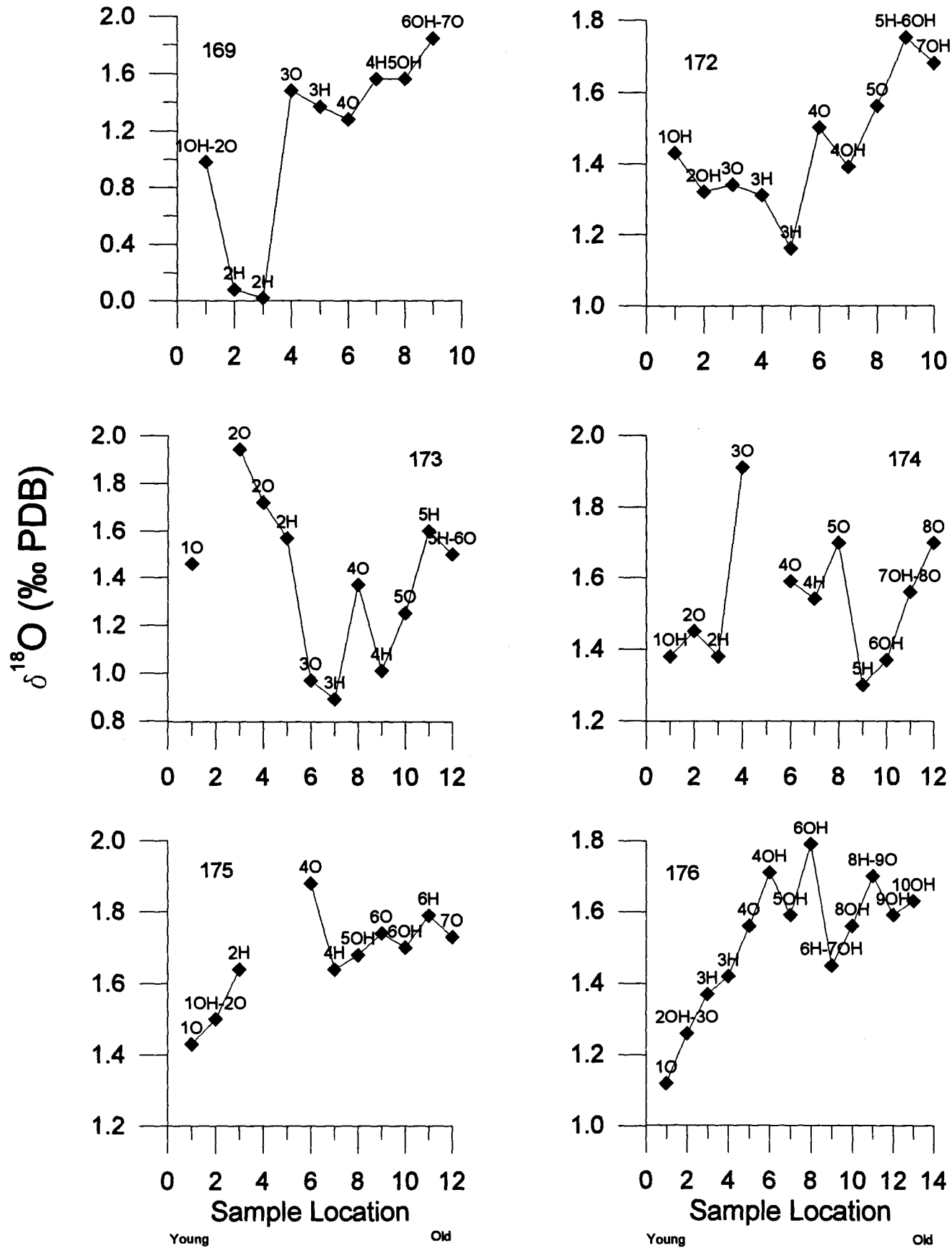
4.1 Oxygen Isotope Results

The graphs in figure 4.1 show the fluctuations in $\delta^{18}O$ between sample locations moving outwards from the nucleus of the otolith. Peaks in the $\delta^{18}O$ signal correspond predominantly to opaque bands while the troughs correspond to hyaline bands. For the entire data set, the average value for exclusively hyaline or opaque samples was found to be 1.45‰ for opaque and 1.37‰ for hyaline. Combined opaque and hyaline samples tend to occur towards the outer edge of the otolith as growth band width tends to decrease with age. The graphs indicate a common initial value of approximately 1.4‰ for otoliths 172, 173, 174, and 175. Otoliths 169 and 176 are characterized by a lower initial value of approximately 1.0‰. Seasonal cycling in $\delta^{18}O$ values, indicated by a high $\delta^{18}O$ opaque value followed by a low $\delta^{18}O$ hyaline value in the same year, is evident in three of the six otoliths. Cycling is apparent, to a limited degree, between samples 2 and 8 in otolith 172, between samples 4 and 10 in otolith 173, and in otolith 174. An interpolation of the $\delta^{18}O$ graph of otolith 174 is shown in figure 4.2. Interpolated points are based on splitting combined opaque and hyaline data points by taking the mixed point as the average value and adding plus or minus one standard deviation. This graph indicates a clear seasonal cycle with opaque bands corresponding to the peaks and hyaline bands corresponding to the troughs.

A summary of the mean, standard deviation and range of $\delta^{18}O$ values for each otolith is presented in table 4.1. The means vary between 1.13 and 1.67 with a mean of 1.46 for the entire data set. The standard deviation in $\delta^{18}O$ varied between .66 and .13 with a standard deviation

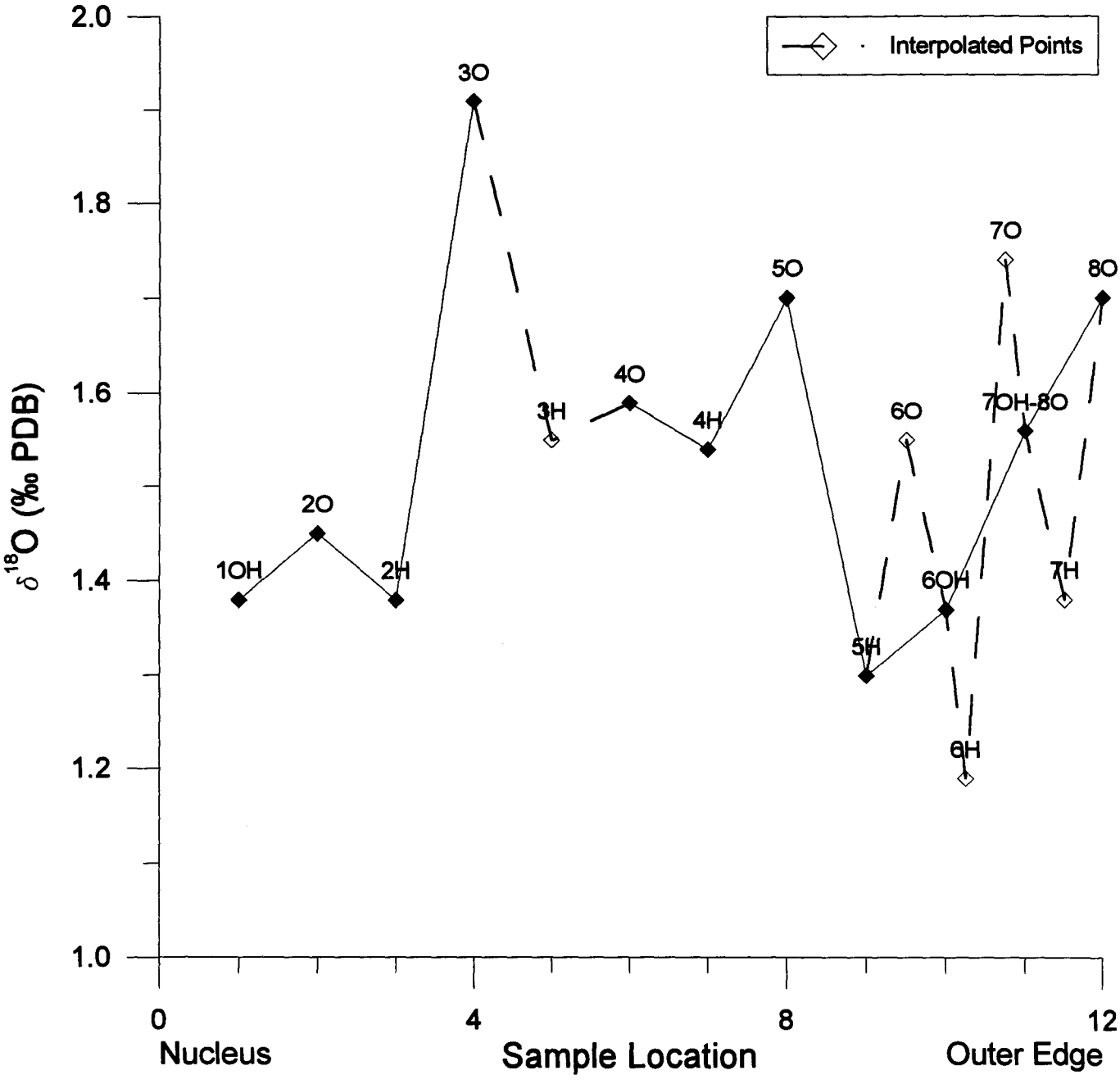
of .34 for the entire data set. The difference between the maximum and minimum value for the entire data set was 1.92. The range for each otolith varied between 1.82 for otolith 169 and .45 for otolith 175 with an average range of .87.

Figure 4.1: Plots of $\delta^{18}O$ versus sample location for the six otoliths sampled



Point Labels:
Numbers indicate the age of the growth band
H indicates a Hyaline band
O indicates an Opaque band

Figure 4.2: Plot of $\delta^{18}O$ versus sample location showing interpolated seasonal cycling in otolith 174



Point Labels:
Numbers indicate the age of the growth band
H indicates a Hyaline band
O indicates an Opaque band

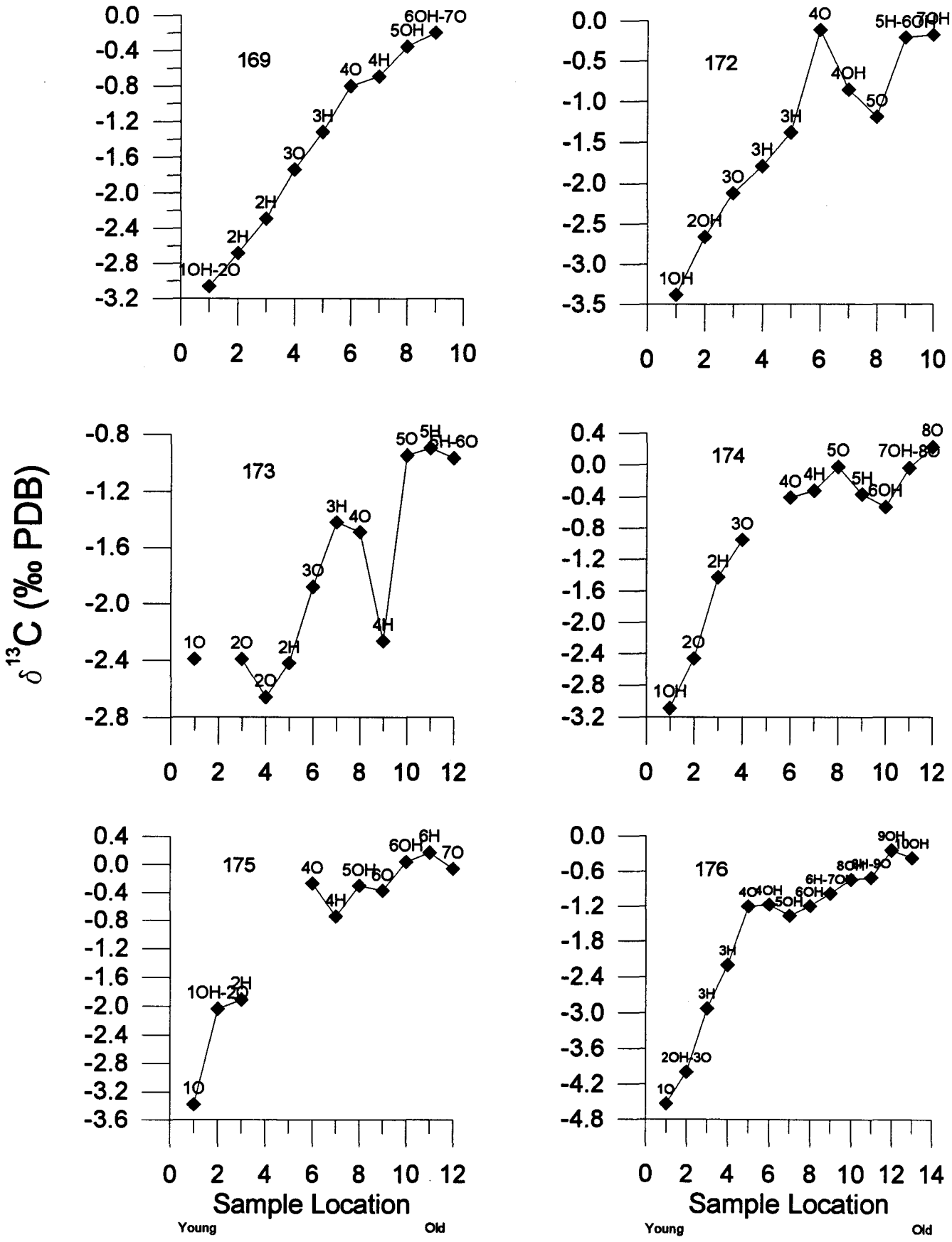
Table 4.1: Mean, standard deviation, range and number of valid samples for $\delta^{18}O$ by otolith

Otolith	Mean	Std. Dev.	Range	Sample #
169	1.13	.66	1.82	9
172	1.44	.18	.59	10
173	1.39	.33	1.05	11
174	1.53	.18	.61	11
175	1.67	.13	.45	10
176	1.52	.19	.67	13
total	1.46	.34	1.92	64
average	1.45	.28	.87	10.67

4.2 Carbon Isotope Results

The graphs in figure 4.3 show the trends in $\delta^{13}C$ moving outwards from the nucleus of each otolith. The graphs show a characteristic increase from an initial negative $\delta^{13}C$ value to a value close to zero with a decrease in the rate of change occurring at approximately age 4. The values from the initial three years of growth appear almost linear. Values from year four and above tend to level off and fluctuate near 0‰. Minimum values, corresponding to the initial sample in all otoliths except for otolith 173, range between -4.53 for otolith 176 and -2.66 for otolith 173. Maximum values range between -.90 for otolith 173 and .23 for otolith 174. The mean, standard deviation, range and valid sample number are summarized in table 4.2. The means vary between -1.66 and -.85 with an average value of -1.34. Standard deviations vary between .67 and 1.36 with a standard deviation of 1.12 for the entire data set. The range varied between 1.76 and 4.29 with an average range of 3.18 and an overall range of 4.76. The graphs in figure 4.4 show the results of a logarithmic curve fit to the $\delta^{13}C$ data for each otolith. A summary of each regression is presented in table 4.3. The $\delta^{13}C$ data from five of the six otoliths showed a strong logarithmic correlation with $r^2 > 0.88$ and $P < 0.001$. Otolith 173 showed a lower logarithmic correlation with $r^2 = .503$ and $P>0.015$.

Figure 4.3: Plots of $\delta^{13}C$ versus sample location for the six otoliths sampled



Point Labels:
Number indicates the age of the growth band
H indicates a Hyaline band
O indicates an Opaque band

Figure 4.4: Plots of the logarithmic curve fit for $\delta^{13}C$ versus sample location for each otolith.

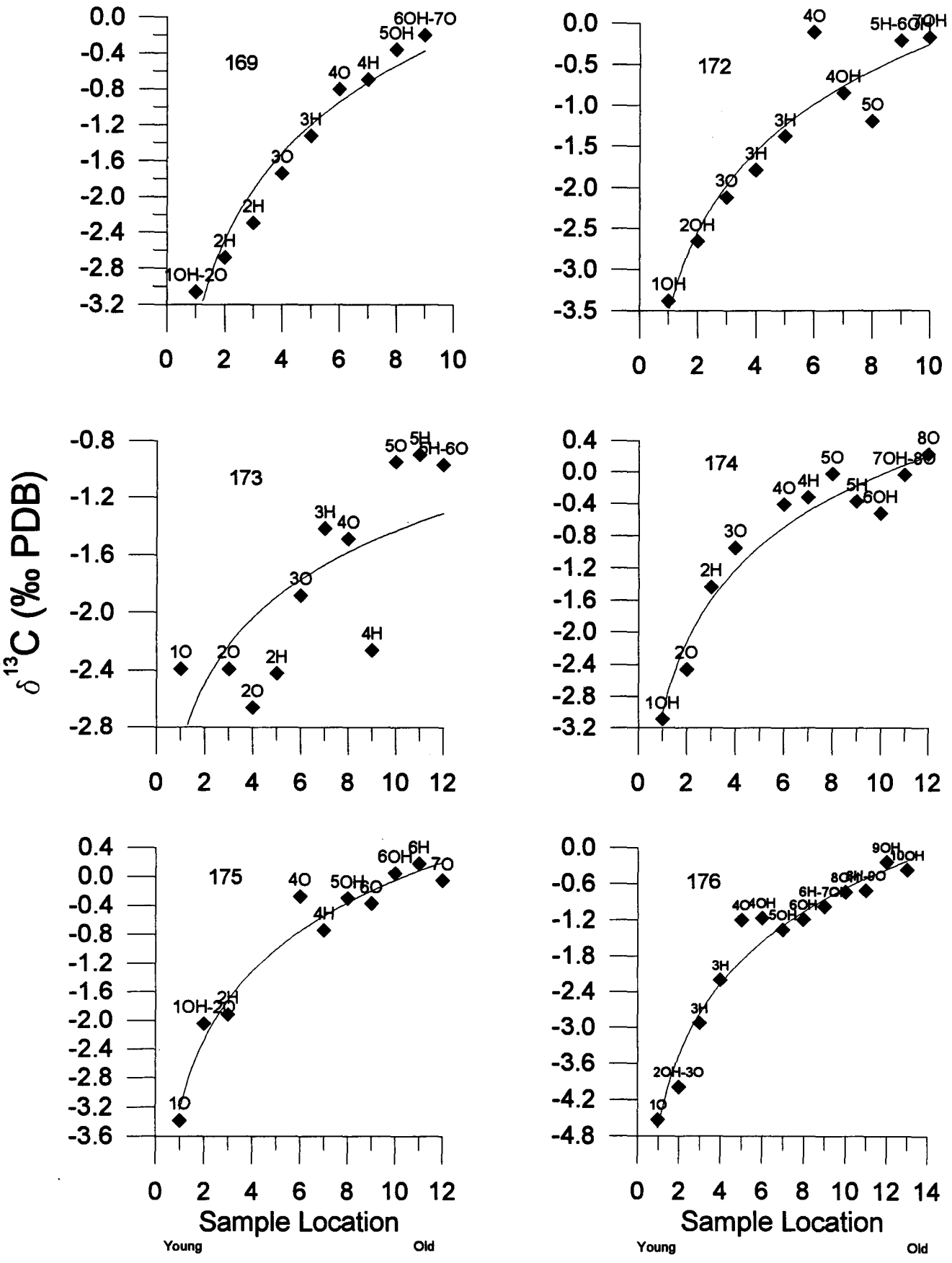

169
172
173
174
175
176
δ13C (‰ PDB)
Sample Location
Young
Old
Point Labels:
Number indicates the age of the growth band
H indicates a Hyaline band
O indicates an Opaque band

Table 4.2: Mean, standard deviation, range and valid sample number for $\delta^{13}C$ by otolith

Otolith	Mean	Std. Dev.	Range	Sample #
169	-1.46	1.04	2.86	9
172	-1.39	1.11	3.27	10
173	-1.79	.67	1.76	11
174	-.85	1.06	3.32	11
175	-.88	1.17	3.56	10
176	-1.66	1.36	4.29	13
total	-1.35	1.12	4.76	64
average	-1.34	1.07	3.18	10.67

Table 4.3: Summary of the logarithmic regression of $\delta^{13}C$ versus sample location by otolith.

Otolith	n	r^2	P	Equation
169	9	0.944	<0.001	Y=1.40539*log(X) - 3.45906
172	10	0.881	<0.001	Y=1.42287*log(X) - 3.53516
173	11	0.503	>0.015	Y=0.656208*log(X) - 2.94463
174	11	0.937	<0.001	Y=1.2979*log(X) -3.02023
175	10	0.959	<0.001	Y=1.38136*log(X) - 3.23014
176	13	0.953	<0.001	Y=1.73438*log(X) - 4.67031

4.3 Correlation Between $\delta^{18}O$ and $\delta^{13}C$

The graphs in figure 4.5 show the results of the linear regression of $\delta^{18}O$ versus $\delta^{13}C$ for samples taken from growth bands 4 and above for each otolith. Otoliths 169, 172, 173, 174, and 175 gave a positive correlation while otolith 176 showed a negative correlation. Three of the otoliths had an r-squared value greater than 0.5. These were otoliths 169, 173, and 174 with r-squared values of 0.766, 0.671, and 0.562 respectively. The remaining three otoliths have low r-squared values of 0.224, 0.157, and 0.0136 for otoliths 172, 175, and 176 respectively. The graph in figure 4.6 shows the result of a linear regression of $\delta^{18}O$ versus $\delta^{13}C$ for samples taken from growth bands of age 4 and above for the entire data set. The correlation is positive with an r-squared value of 0.321, $n = 35$ and $P < 0.001$. The equation of the line was calculated to be $Y = 0.192483*X + 1.69174$ with a standard error for the slope of 0.047354 and for the intercept 0.037649. A second linear regression excluding the data from otolith 176 due to the anomalous negative correlation of the data of that sample is shown in figure 4.7. Based on the data from otoliths 169, 172, 173, 174, and 175, the regression of $\delta^{18}O$ versus $\delta^{13}C$ was found to have a positive correlation with an r-squared of 0.537, $n = 26$ and $P < 0.0001$. The equation of the line was calculated to be $Y = 0.269064*X + 1.69861$ with a standard error for the slope of 0.048988 and for the intercept 0.035983.

Figure 4.5: Linear regressions of $\delta^{18}O$ versus $\delta^{13}C$ for samples from growth bands age 4 and older for each otolith.

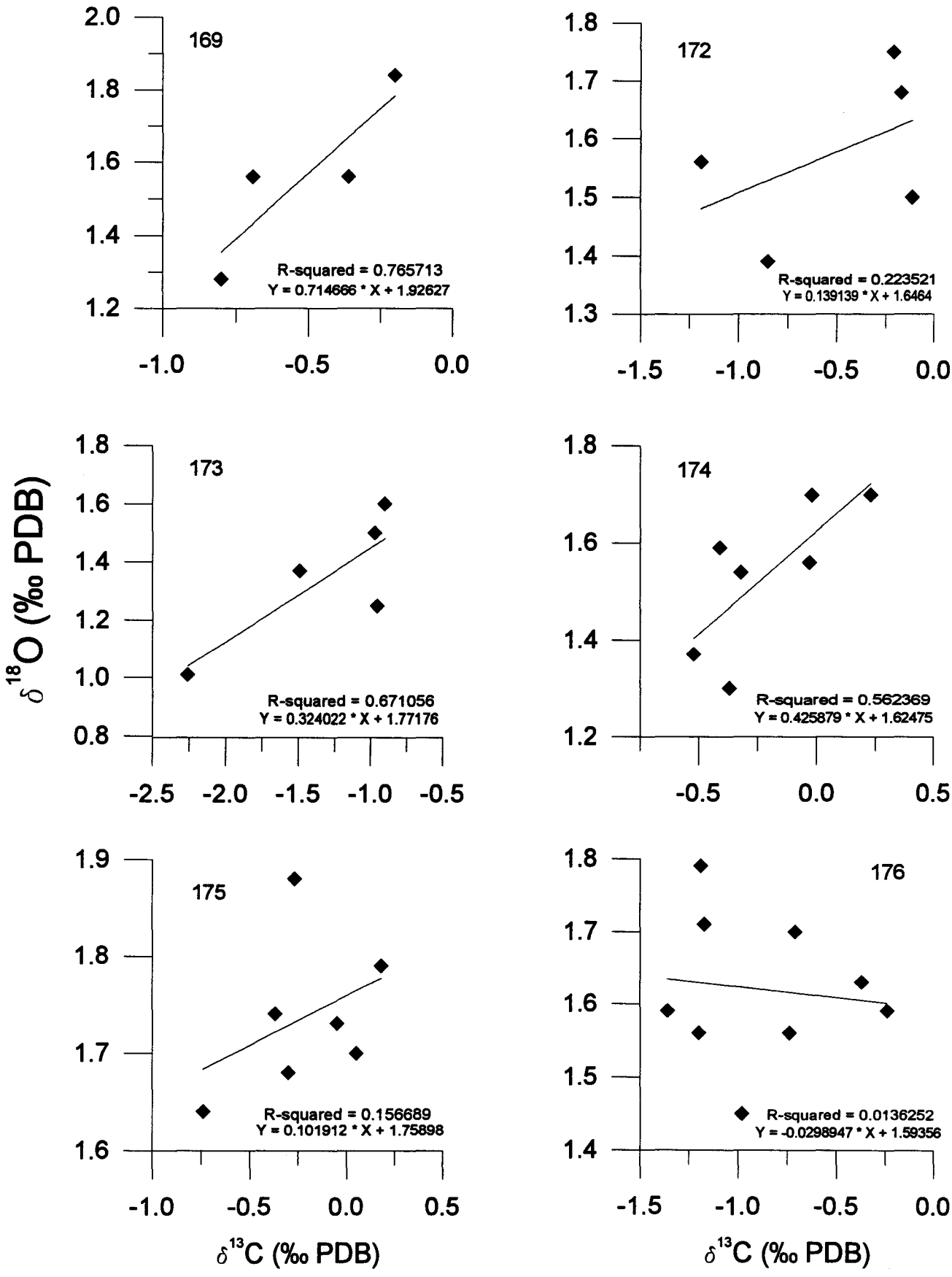

169
R-squared = 0.765713
Y = 0.714666 * X + 1.92627
172
R-squared = 0.223521
Y = 0.139139 * X + 1.6464
173
R-squared = 0.671056
Y = 0.324022 * X + 1.77176
174
R-squared = 0.562369
Y = 0.425879 * X + 1.62475
175
R-squared = 0.156689
Y = 0.101912 * X + 1.75898
176
R-squared = 0.0136252
Y = -0.0298947 * X + 1.59356
δ18O (‰ PDB)
δ13C (‰ PDB)
δ13C (‰ PDB)

Figure 4.6: Linear regression of $\delta^{18}O$ versus $\delta^{13}C$ for samples from growth bands age 4 and older for the entire data set

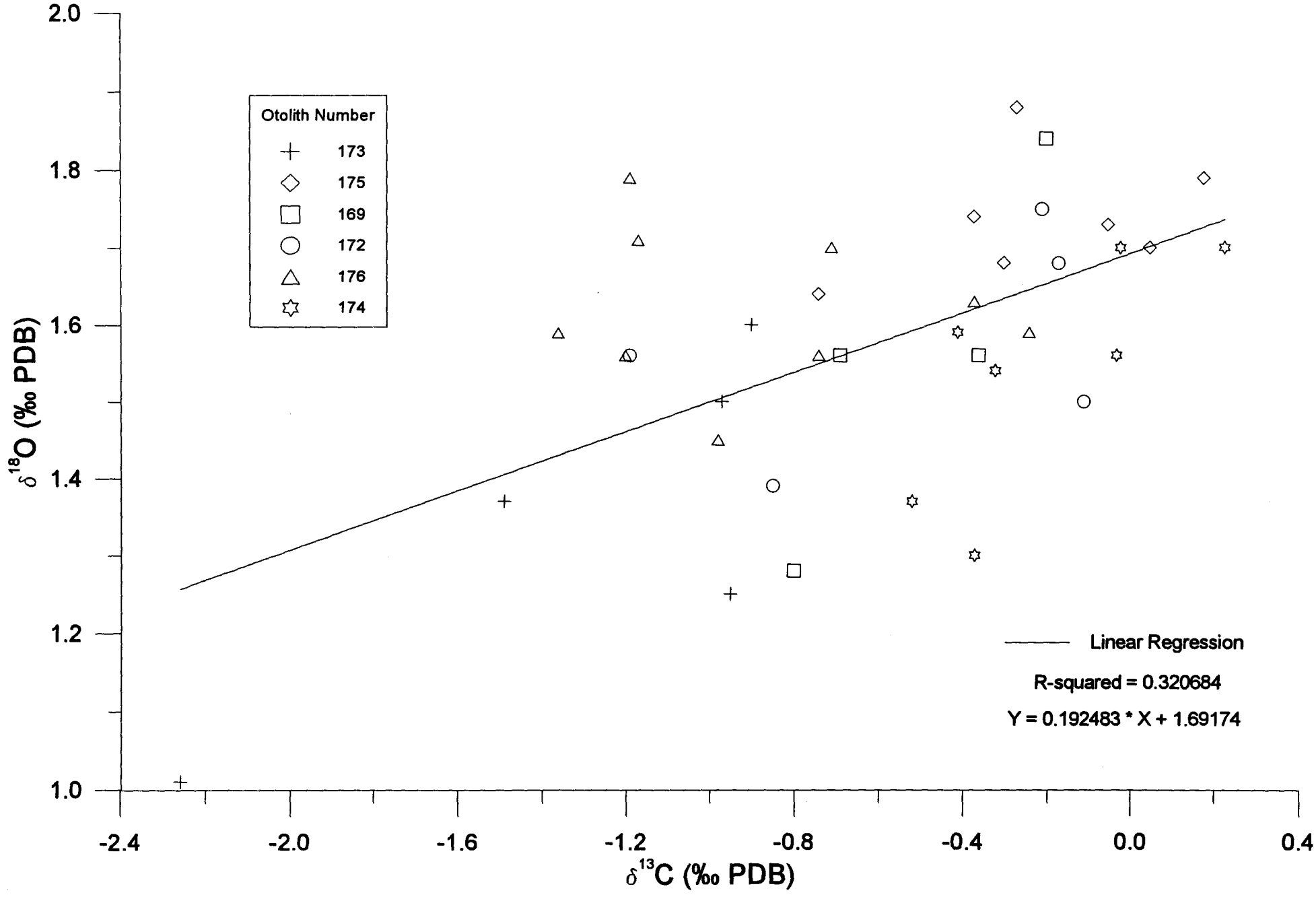
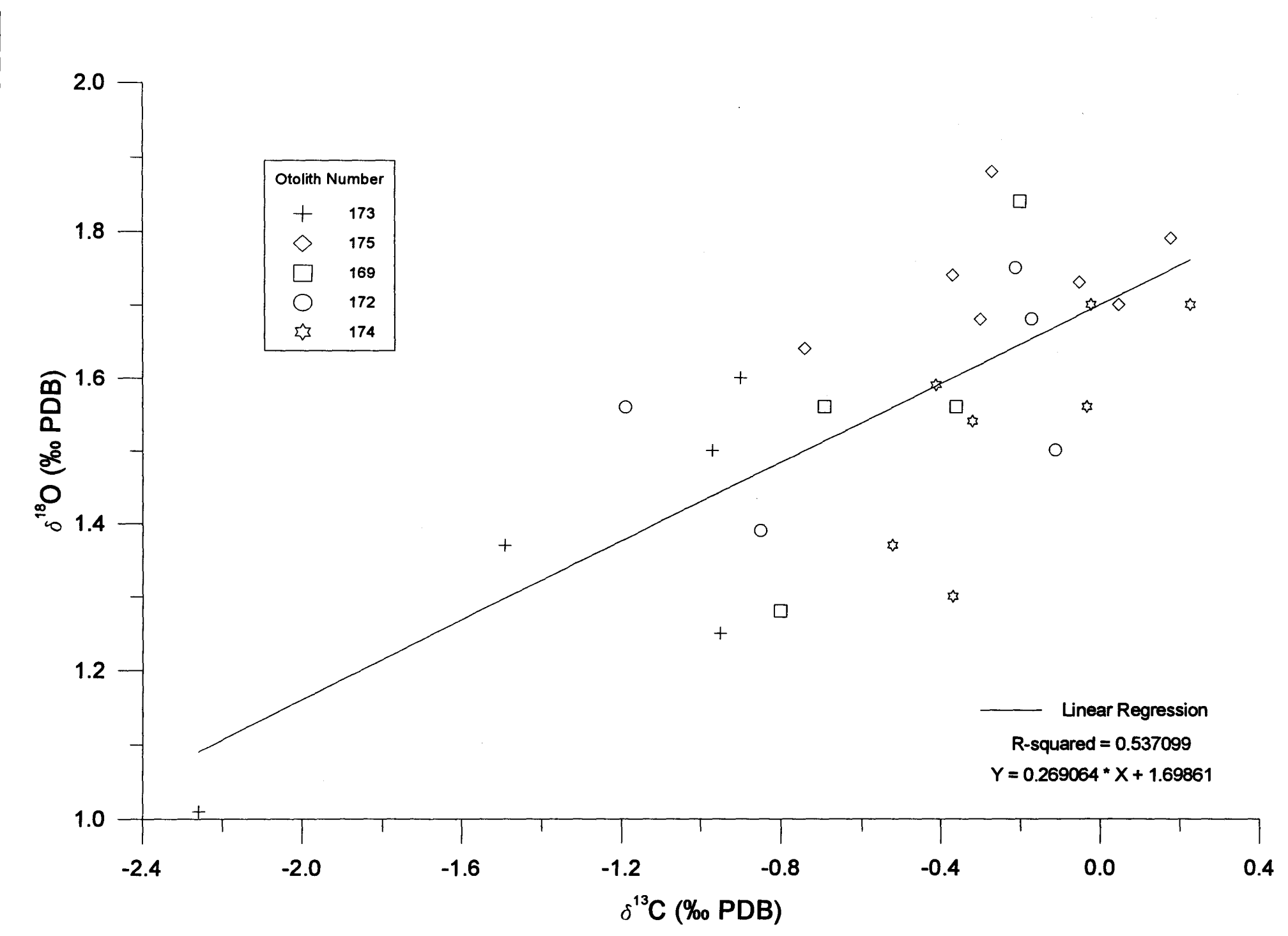

Otolith Number
173
175
169
172
176
174
Linear Regression
R-squared = 0.320684
Y = 0.192483 * X + 1.69174
δ18O (‰ PDB)
δ13C (‰ PDB)
2.0
1.8
1.6
1.4
1.2
1.0
-2.4
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4

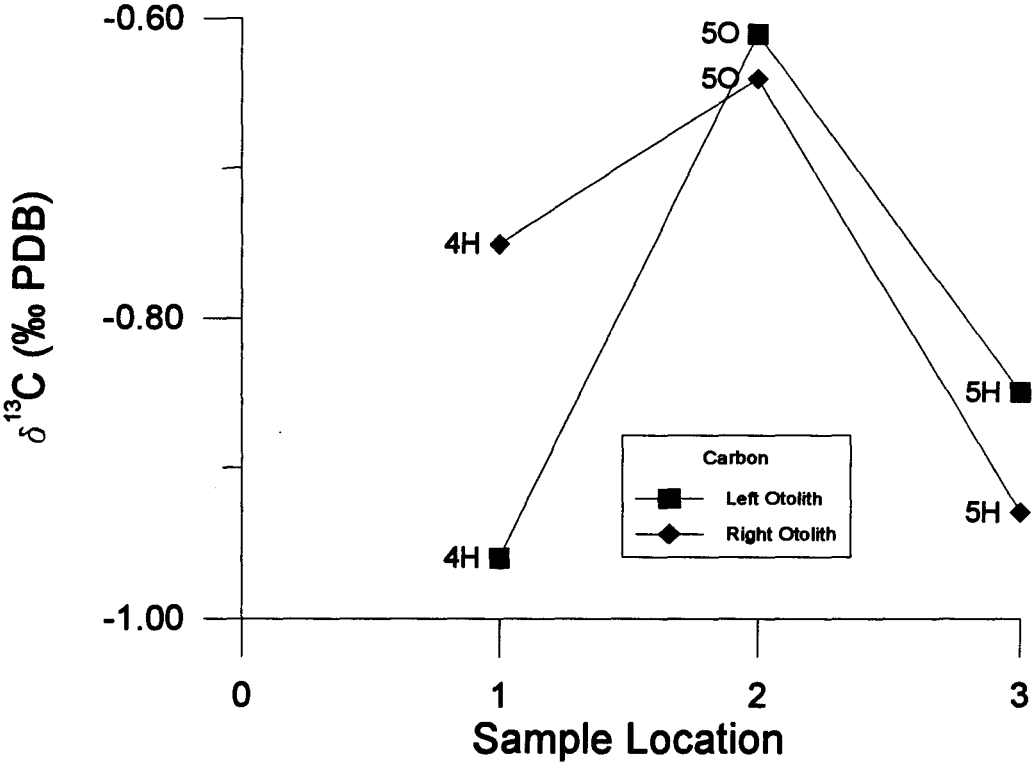
Figure 4.7: Linear regression of $\delta^{18}O$ versus $\delta^{13}C$ for samples from growth bands age 4 and older, data from otolith 176 excluded.

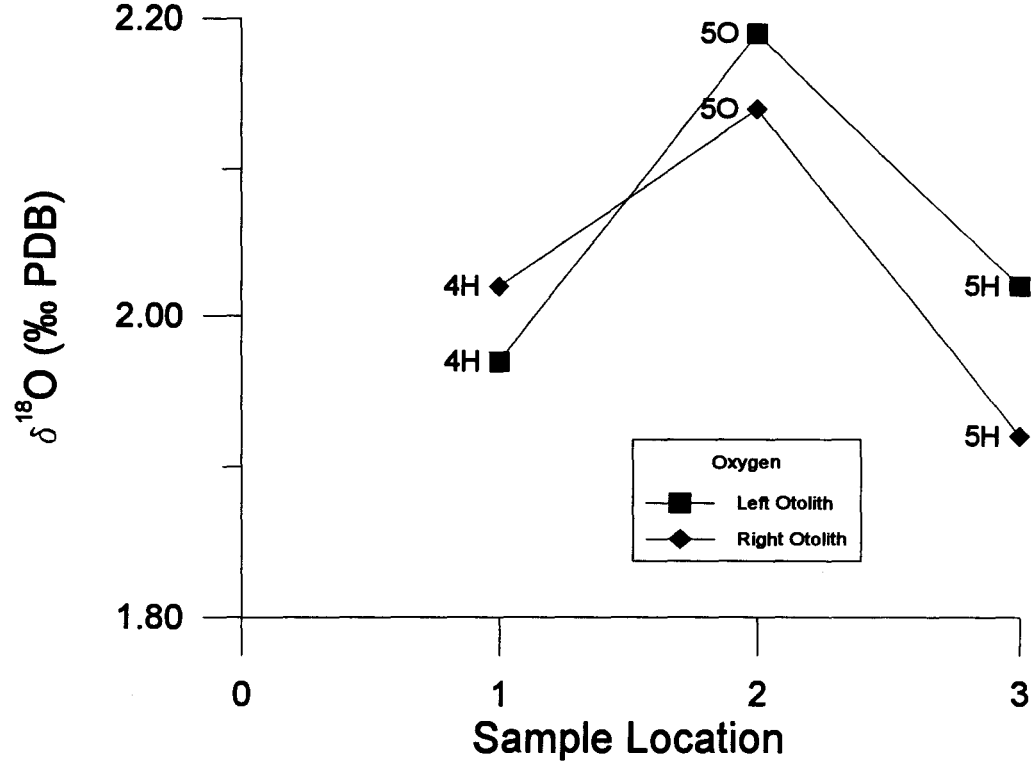

Otolith Number
173
175
169
172
174
2.0
1.8
1.6
1.4
1.2
1.0
δ18O (‰ PDB)
-2.4
-2.0
-1.6
-1.2
-0.8
-0.4
0.0
0.4
δ13C (‰ PDB)
Linear Regression
R-squared = 0.537099
Y = 0.269064 * X + 1.69861

4.4 Comparison of Left and Right Otolith

The graphs in figure 4.8 show a strong degree of similarity between samples taken from the left and right otolith of the same fish. As in the previous samples, opaque samples correspond with the peak and hyaline samples correspond with the troughs. The mean, standard deviation and range are summarized in table 4.4. The $\delta^{18}O$ values show a difference between the means of left and right otoliths of only 0.03‰. The samples show identical ranges between left and right samples for $\delta^{18}O$ with a value of 0.22‰. The $\delta^{13}C$ values indicate a 0.04‰ difference in the means. The range of the $\delta^{13}C$ values varies from 0.35‰ in the left otolith to 0.29‰ in the right otolith for a difference of 0.06‰. The result of the linear regression of $\delta^{18}O$ versus $\delta^{13}C$ gave an r-squared value of .88608, $P < 0.001$ (figure 4.9) indicating a strong linear relationship between $\delta^{18}O$ and $\delta^{13}C$.

Figure 4.8: Graphical comparison of the $\delta^{13}C$ and $\delta^{18}O$ of samples taken from identical locations on left and right otoliths of the same fish





Point Labels:
Number corresponds to age of growth band
H corresponds to Hyaline band
O corresponds to Opaque band

Figure 4.9: Linear regression of $\delta^{18}O$ versus $\delta^{13}C$ for samples taken from left and right otoliths of the same fish

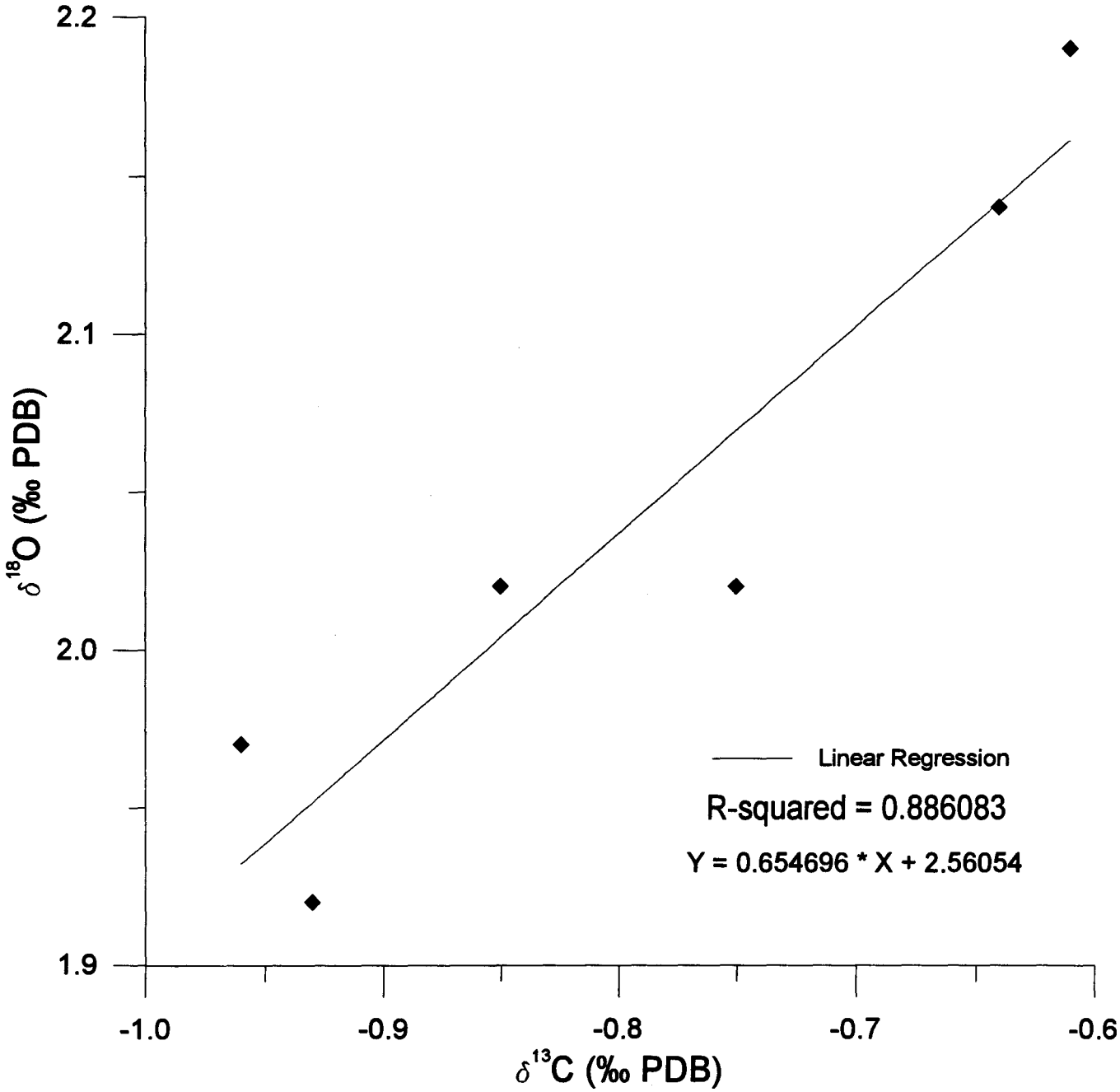

2.2
2.1
2.0
1.9
$\delta^{18}O$ (‰ PDB)
-1.0
-0.9
-0.8
-0.7
-0.6
$\delta^{13}C$ (‰ PDB)
Linear Regression
R-squared = 0.886083
Y = 0.654696 * X + 2.56054

Table 4.4: Mean, standard deviation, and range of $\delta^{13}C$ and $\delta^{18}O$ for left and right otolith samples

	Mean	Std. Dev.	Range
Left $\delta^{13}C$	-.81	.18	.35
Right $\delta^{13}C$	-.77	.15	.29
Left $\delta^{18}O$	2.06	.12	.22
Right $\delta^{18}O$	2.03	.11	.22

Chapter 5 - Discussion

5.1 Experimental Error

The major source of variation in the data set was the precision of the microsampling technique. Otolith growth bands are approximately elliptical in shape, but follow an undulating path with several folds and bulges. Manual sampling required that the operator manipulate the otolith beneath the drill bit such that the contour of the growth band was followed as closely as possible. In general it can be said that the material sampled from a particular band varied with respect to the proportion of material taken from different points along the width of the band. Blending of material from different periods of time within one band and between bands can be said to have occurred due to variations in the portion of the hyaline or opaque band sampled. This may account for the few conflicting hyaline samples which show high $\delta^{18}O$ values as well as the opaque samples which show low $\delta^{18}O$ values. Growth bands tend to decrease in width with age resulting in the most recent growth bands also being the most narrow. This accounts for the number of mixed hyaline and opaque samples taken from growth bands from years 5 and above. For the above reasons, each sample was considered to be a mixture of material from different portions of a seasonal band or combination of seasonal bands.

Samples do not correspond to an exact time period, but represent an average value for the season of band formation. Due to variations in the drill path some samples may reflect earlier or later zones of accretion within a seasonal band. This is particularly important for the interpretation of the logarithmic regression of the $\delta^{13}C$ data for each otolith as the x-axis labelled as sample location is not an exact time scale. The data tends to be compressed as the sample

location increases as time was actually increasing faster than sample location scale indicates.

The results of the comparison of samples taken from left and right otoliths of the same cod show a high degree of reproducibility of sample location and isotopic composition (figure 4.8). These samples were taken after the operator had over 20 hours of experience in the use of the microsampler. The ability to reproduce sample locations on separate otoliths indicates that the microsampling technique, despite the above criticisms, was capable of achieving a relatively high degree of precision. Due to the importance of experience in sample precision, sampling was considered to have increased in precision over time with the order of otolith sampling from first to last sampled being 173, 175, 169, 172, 176, and 174. Otolith 174 is therefore considered to be the most precisely sampled otolith.

The second source of error arises from the fact that otoliths were aged after having been sampled. This meant that at the time of sampling the exact identification of each band was unknown and sampling either overlooked or combined some bands as a result. The correlation of a specific sample to a specific age is considered accurate for otoliths with distinct banding, however, otolith 176 was characterized by particularly unclear banding making the assignment of a specific age to each sample difficult.

In order to correct for the above sources of error, it is suggested that in the future samples be taken using a digitized drill path which is an accurately known distance from the nucleus of the otolith. Drill paths should be marked on a photograph of the otolith banding which was previously interpreted for age. Aside from improving accuracy, this method would allow for $\delta^{13}C$ to be plotted against the distance of the sample from the nucleus which would result in a better

correlation with time and therefore growth.

5.2 Interpretation of $\delta^{18}O$ Signal

The common $\delta^{18}O$ value for the year 1 sample (sample location 1) is an indication of similarity in hatching and early development temperature. Spawning and hatching occur in deep offshore waters characterized by 2 to 3 degree Celsius water temperatures which is near the lower limit of the living range of cod and therefore a common initial point near the upper limit of observed $\delta^{18}O$ is expected.

The fact that opaque samples correspond with peaks in $\delta^{18}O$ or low temperatures and hyaline samples correspond with troughs in $\delta^{18}O$ or high temperatures supports the findings of Dannevig (1956) who concluded that, in cod, hyaline bands represented summer growth between the months of August and November, and opaque bands represented winter growth between the months of December and July. Subsequent studies have found the opposite relationship, reporting the formation of hyaline bands during winter months and opaque bands during summer months (Mina, 1968, Blacker, 1974). Blacker suggested that Dannevig's contradictory interpretation was due to a misinterpretation of hyaline versus opaque banding and that Dannevig had reversed the terminology. Pannella (1980) reports hyaline bands as forming during periods of cold temperatures and slow growth in agreement with Blacker. The results of this study clearly support the formation of hyaline bands during the summer as they correspond to significantly warmer temperatures in all six otoliths as well as in the paired otoliths. If the hyaline bands of the cod sampled were formed during the winter, as Pannella and Blacker suggest, then it would appear that cod experience warmer temperatures during the winter than during the summer which

runs counter to reason, but raises new questions as to the factors controlling the isotopic composition of cod otoliths. It is known that the season of band formation varies between stocks (Blacker, 1974) and as the sampled otoliths were from fish of unknown stock origin, it is difficult to comment on whether the noted discrepancy is significant or not. Future studies with cod of known stock origin will be able to further clarify the contradiction.

The average range of 0.87 corresponds to a shift of 3.6°C, while the range of 1.36 containing at least 75% of the data corresponds to a temperature shift of 5.7°C. This is a fairly low variation in temperature when one considers that cod are found in waters ranging from 19°C to -2°C (Buchanan et al., 1982). Cod, however, migrate daily from deep to shallow waters during the summer, corresponding to temperatures of 5 and 10-12 degrees respectively (Clark and Green 1991). Data collected during the month of September for Gulf of St. Lawrence cod show a mean temperature of occurrence of approximately 5 degrees with 90% of the fish occurring between 0 and 10 degrees (Tremblay, 1985). If we accept the daily migrations and take the inshore temperature as an average of the maximum and minimum temperatures we would expect a summer average temperature of approximately 5° to 6° Celsius. The deep ocean temperature environment of cod lies between 2° and 2.5°C (Rose, 1993). This suggests an expected average temperature range of 3° to 4° Celsius which corresponds with the average observed range. The maximum range of the $\delta^{18}O$ data was 1.92‰ which corresponds to a temperature range of approximately 8°C and falls within the maximum temperature range experienced by Atlantic cod which is estimated at 20°C (Buchanan et al., 1982). The observed correlation between expected migratory temperature ranges and the temperature range resulting from the semi-annually sampled

cod otoliths suggests that the otoliths contain a record of the temperature changes due to the seasonal migration. With more precise sampling of the otoliths, the seasonal shifts may be resolved and a fairly continuous record of the environmental temperature of the cod may be observed.

The expected seasonal cycling mentioned above was apparent in portions of otoliths 172 and 173 and throughout otolith 174. The best example is otolith 174 which was also the most precisely sampled. In otolith 174 the opaque zones are consistently heavy in $\delta^{18}O$ and the hyaline zones are consistently light. The interpolated graph in figure 2 shows seasonal cycling with an annual .4 per mil shift or approximately 1.7°C. Seasonal cycling is not apparent in otoliths 169, 175, and 176. This is most likely due to a low degree of precision in sampling which resulted in samples of mixed band origin as opposed to discrete seasonal samples.

From the above trends of a common initial value, the correlation of hyaline and opaque bands with summer and winter periods respectively, an observed temperature range which corresponds to the expected value, and evidence of seasonal cycling in the $\delta^{18}O$ signal of otolith 174, it is concluded that $\delta^{18}O$ has the potential to become an accurate indicator of cod environmental temperatures and indirectly of cod migration routes. The clarity of the data is limited by the quality of microsampling and it is suggested that with digitized computer controlled microsampling techniques, providing samples of 10 to 20 μg of material every 10 to 20 μm across the otolith, clear seasonal cycling in environmental temperatures will be observed as noted in Patterson 1993 (see figure 5.1).

The relationship between $\delta^{18}O$ and temperature has been shown to vary according to

species as indicated by the graph in figure 5.2 taken from Kalish (1991a). Further interpretation of the $\delta^{18}O$ signal requires the calibration of a species specific $\delta^{18}O$ versus temperature curve using otoliths from cod raised at known temperatures. This would allow for accurate reconstructions of the environmental temperature changes occurring over the life of a cod.

Figure 5.1: Intra-annual variation in oxygen isotopic composition for the third and forth years of the freshwater drum. Isotopic composition, $\delta^{18}O$ SMOW, is plotted relative to sample distance in millimetres beginning in the winter of 1949-50, from left to right (taken from Patterson, 1993).

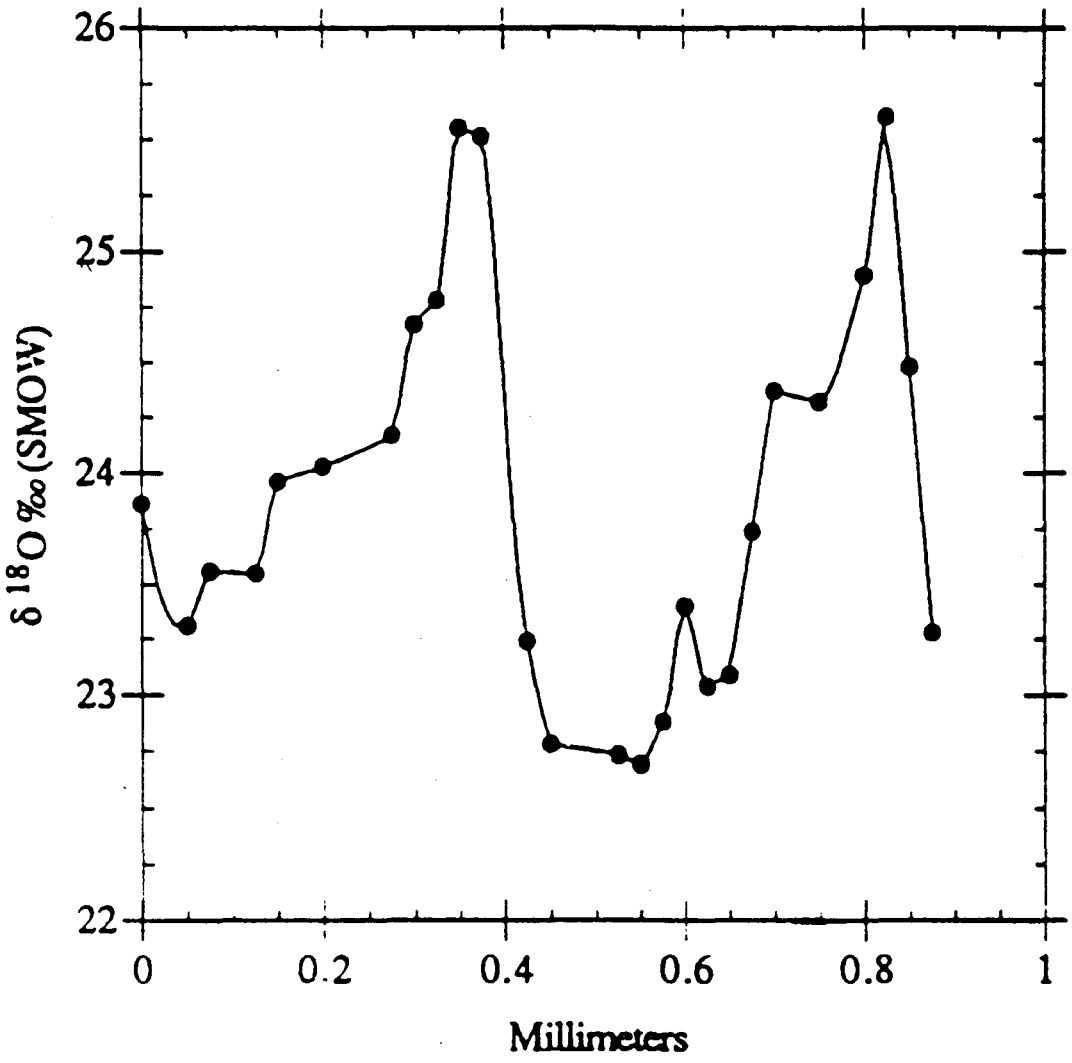
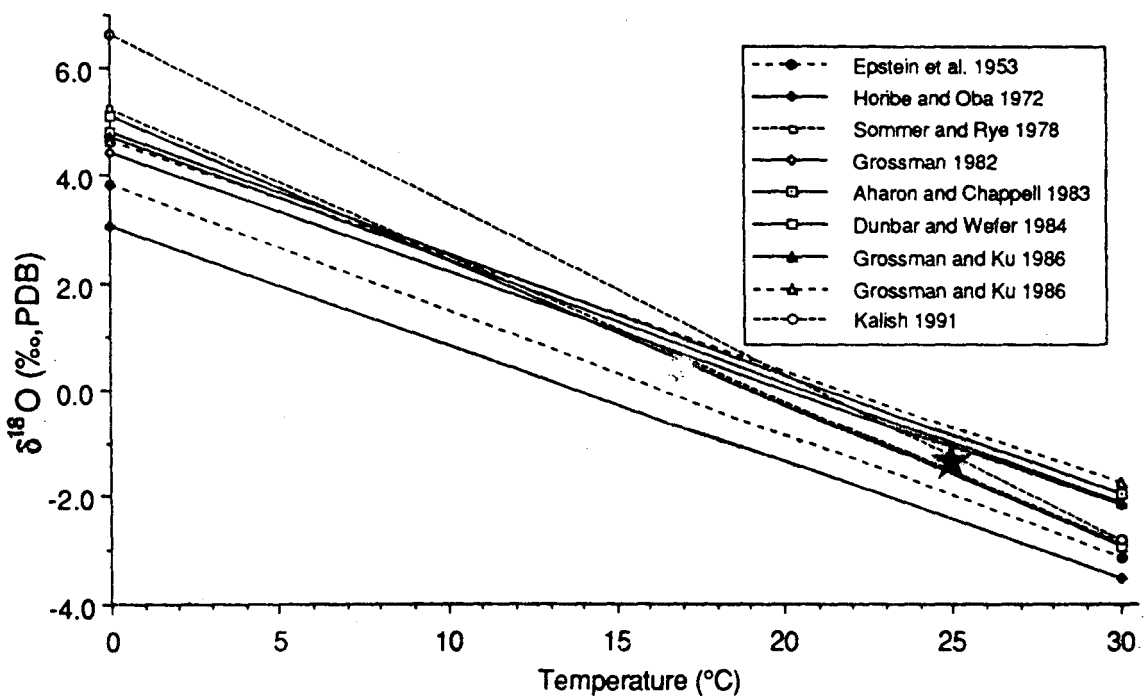

26
25
24
23
22
0
0.2
0.4
0.6
0.8
1
δ 18O ‰ (SMOW)
Millimeters

Figure 5.2: Relationships describing the temperature dependent fractionation of oxygen isotopes in biogenic aragonite. One line, Epstein et al. (1953) is based on biogenic calcite (taken from Kalish, 1991a).


Epstein et al. 1953
Horibe and Oba 1972
Sommer and Rye 1978
Grossman 1982
Aharon and Chappell 1983
Dunbar and Wefer 1984
Grossman and Ku 1986
Grossman and Ku 1986
Kalish 1991
6.0
4.0
2.0
0.0
-2.0
-4.0
δ18O (‰,PDB)
0
5
10
15
20
25
30
Temperature (°C)

5.3 Interpretation of $\delta^{13}C$ Signal

Assuming respiration to be the primary source of CO_2 in the blood, the flesh of adult cod has an expected $\delta^{13}C$ of approximately -15‰. The value of $\delta_{s.w.DIC}{}^{13}C$ (s.w., sea water) is 0 ±2‰. The observation of a minimum $\delta^{13}C$ value of -4.53 for the carbonate of otoliths suggests that the carbon present in otoliths is a mixture of metabolically and inorganically derived carbon. The $\delta^{13}C$ signal is characterized by an increase in $\delta^{13}C$ until age 4 after which the signal appears to level off and begin fluctuating annually at a value close to 0‰ (figure 4.3). The $\delta^{13}C$ signal was found to fit a logarithmic curve suggesting a relationship between $\delta^{13}C$ and growth. The highest value observed for $\delta^{13}C$ is approximately 0.2‰. Given that the fractionation between aragonite and DIC is approximately +1‰, this corresponds to a $\delta^{13}C$ of about -0.8‰ for the endolymph of the saccula which is assumed to be in isotopic equilibrium with the blood. The heaviest $\delta^{13}C$ values the cod might encounter are about 2‰ which is considerably higher than the observed maximum for the endolymph of the cod suggesting that the blood of the cod does not quite reach isotopic equilibrium with the sea water DIC. The increasing nature of $\delta^{13}C$ over the first three years of growth has not been observed previously due to the difficulty in resolving the semi-annual bands. Differences between juvenile and adult $\delta^{13}C$ values have been noted for other species of fish. Kalish (1991a) observed that juvenile fish tend to have lower $\delta^{13}C$ values than adult fish of the same species, however the initial, almost linear, increase was unknown prior to this analysis. It is necessary, therefore, to make an initial speculation on the origin of the observed trend with regards to cod growth and development in order to suggest directions for future research.

It is possible that the logarithmic trend observed is partially a product of the method of sampling. The $\delta^{13}C$ values are plotted against sample location which is a non-uniform scale based on both distance from the nucleus and age. The result of the variable scale of the x-axis is to compress or expand $\delta^{13}C$ data points horizontally. This would affect the shape of the graph, however, due to the annual or semi-annual nature of the sampling it is unlikely that the observed logarithmic trend is simply a stretched linear relationship.

The results suggest that over the first three years of life, the fraction of inorganic over total carbon is increasing approximately linearly. Over time, however, this relationship appears to logarithmic which is characteristic of most processes which would affect the ratio of inorganic to organic carbon such as, changes in surface to volume ratio, metabolic rate, and diet, change in a non-linear fashion with age (Hoar and Randall, 1984). This suggests that the observed trend may be a result of a combination of biological processes which act in consort to produce the observed increase in $\delta^{13}C$ during juvenile years and the subsequent fluctuation around values close to isotopic equilibrium with sea water DIC in adult years.

The following is a discussion of possible biological and environmental factors which could affect the ratio of inorganic to metabolic carbon in fish including dietary change, changes in standard and routine metabolism, changes associated with sexual maturity, and vertical migrations.

1) Dietary Change

Over the first 3 years of life the diet of a cod switches from zooplankton and crustaceans to a diet consisting mainly of fish (Chaput, 1981). Kalish (1991a) suggests a dietary influence

in a study of $\delta^{13}C$ for Australian salmon in which he suggests that 30% of the otolith carbon is metabolically derived from the diet and that a 10‰ increase in $\delta^{13}C$ of the dietary source would, therefore, result in a 3‰ increase in the otolith $\delta^{13}C$. An increase of 10‰ in $\delta^{13}C$ of the dietary source of a fish is, however, unrealistic and overestimates the potential effect of a dietary change. The hypothesis of the increase in $\delta^{13}C$ being related to diet although possible, is considered a minor factor as the shift is small between food sources and could not account for the 3 to 5 ‰ shift identified in the results.

2) Metabolic Rate

Coupled with the change in diet over early life is a decrease in instantaneous metabolism and growth rate relative to body mass which occurs with age. This change in metabolic rate is a combination of a number of environmental and biological factors, including ambient temperature, energy expenditure, food availability and intake, fish size, and respiration rate. Each of theses factors are discussed in sequence with regards to the observed trend in $\delta^{13}C$.

All species of fish have an optimum temperature for max. metabolic efficiency which can vary seasonally depending on hormonal balances in the organism. It has been found that below the maximum living temperature of a fish, a 10 degree increase in temperature is equivalent to a 2.3 fold increase in metabolic rate (Hoar and Randall, 1979). The implication is that seasonal temperature changes may also involve seasonal changes in metabolic rate. It has been found that, on average, cod experience a 5°C shift in temperature between summer and winter seasons. The difference between the maximum and minimum temperature experienced in summer or winter is approximately 15-20°C (Buchanan, 1982). Temperature changes in this range could result in

substantial changes in metabolic rate resulting in increases and decreases in the amount of metabolically derived carbon present in the blood for otolith formation. The expected result would be a high abundance of metabolic carbon or low $\delta^{13}C$ in the blood during periods of high temperature or low $\delta^{18}O$. This suggests a potential correlation between $\delta^{13}C$ and $\delta^{18}O$ values. This relationship is investigated in the following section.

At different periods in the lifecycle of a cod, the organism will have different energy requirements for example, migration may require increased energy expenditure thereby increasing the metabolic rate; feeding in shallow waters during the summer with warm temperatures may increase energy expenditure due to energy used to capture and digest prey; cold temperatures may induce a type of torpor in fish greatly reducing the metabolic rate (Hoar and Randall, 1979). Therefore, fluctuations in $\delta^{13}C$ may be a result of changes in metabolic rate in response to different stages or activity experienced by the cod.

Metabolism and growth rates are strongly correlated with food intake (Brown et al., 1989). Metabolic rate may vary according to the amount of available food during a particular month or season. Therefore, food availability and feeding becomes another factor which could affect metabolic rate and the amount of metabolically derived carbon in the blood thereby altering the isotopic make up of the otolith.

There is a known relationship between the weight or size of a fish and its metabolic rate. Edwards (1972) calculated the rate of oxygen consumption Qo_2 of cod with respect to weight (W) in grams as: $Qo_2 = 0.245\ W^{0.82}$ (mg/h). The power component indicates the efficiency of conversion of food to energy and is generally between .75 and .85 in fish. A one year old cod

of 100g has Q=10.7mg/h while a 6 year old cod of 2000g has a Q=124.7mg/h. Therefore a 20 fold increase in size results in a 10 fold increase in metabolic rate. On a per gram basis, therefore, metabolic rate in $mgO_2/g/h$ can be said to decrease as a fish increases in size. That is, the 100g fish has an instantaneous rate of 0.107 $mgO_2/g/h$ while the 2000g fish has an instantaneous rate of 0.06235 $mgO_2/g/h$ which is 1.72 times less than the 100g fish. Instantaneous growth rates decrease in a similar fashion with age. Beacham (1982) found the instantaneous growth rate of 9-10 year old cod from areas 4Vn, 4Vs, 4W, and 4X to be reduced by up to one fifth of the instantaneous growth rate of cod age 1-2. The question which remains unanswered is what does a 2 fold decrease in oxygen consumption per gram or a 5 fold decrease in instantaneous growth rate correspond to in terms of a $\delta^{13}C$ shift? To answer this question it is necessary to look at the amount of CO_2 produced through respiration versus diffusion of sea water DIC across the gills in order to determine the amount of metabolic CO_2 present in the blood during different periods in the life of a cod. This is beyond the range of this thesis.

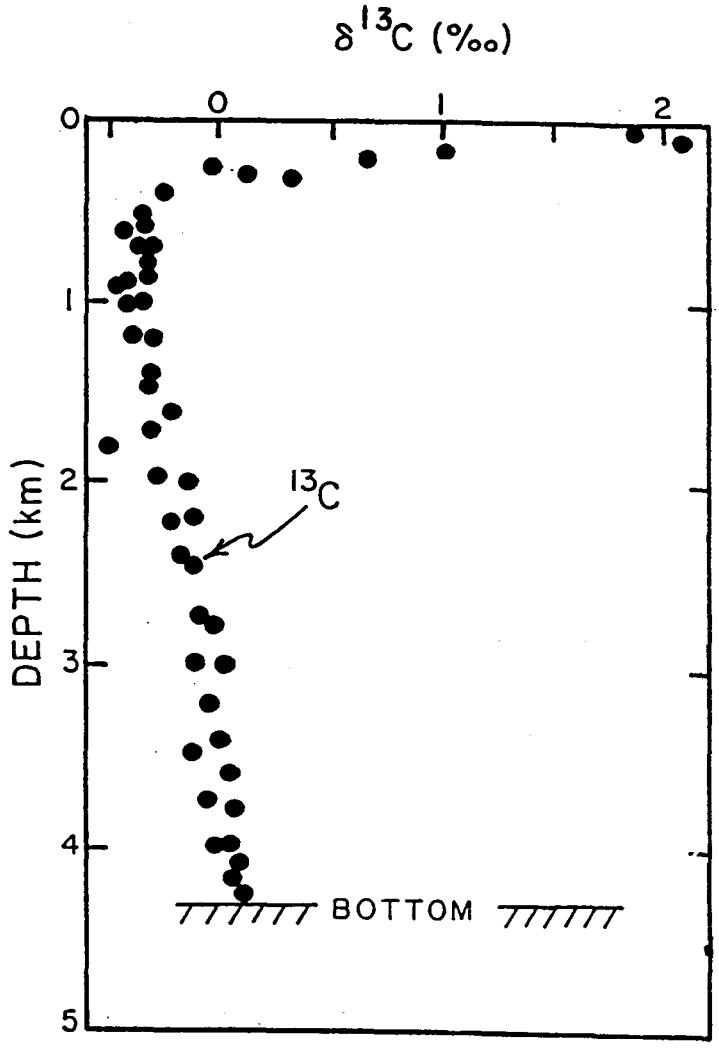
3) Biochemical Changes Associated with Sexual Maturity

Cod reach sexual maturity between the ages of 3 and 5 (Beacham, 1982, 1983, Trippel, 1992). The observed change in the trend of $\delta^{13}C$ from increasing approximately linearly to a levelling off and fluctuating signal occurs at approximately age 4 in all six otoliths sampled. This suggests that changes associated with sexual maturity or the first spawning event may be a factor affecting the $\delta^{13}C$ signal. The production of eggs and sperm and the hormonal changes involved in spawning could have an effect both on metabolism and more specifically on the biochemistry of otolith formation and CO_2 levels in the blood which varies with blood pH.

4) Vertical Migrations

Kroopnick (1974) found that $\delta^{13}C$ varied with depth in the ocean and reported a 2‰ decrease in $\delta^{13}C$ over the first 300 metres (figure 5.3). This shift in $\delta^{13}C$ may be reflected in the isotopic composition of the otolith. The observed fluctuations in $\delta^{13}C$ may be an indication of changes in the depth of occurrence of the cod. This hypothesis is contingent upon the degree to which blood CO_2 is in equilibrium with the surrounding water and the amount of metabolic CO_2 which is being incorporated into the otolith.

Figure 5.3: Plot of $\delta^{13}C$ versus depth (after Kroopnick, 1974).


δ13C (‰)
0
1
2
0
1
2
3
4
5
DEPTH (km)
13C
BOTTOM

5.4 Discussion of the Correlation Between $\delta^{18}O$ and $\delta^{13}C$

There was a strong correlation between $\delta^{13}C$ and $\delta^{18}O$ for samples taken from growth bands of age four and older. Age four was chosen as the cut off as this was the age at which the $\delta^{13}C$ values tended to level off and begin fluctuating. In the only previous research on the correlation between $\delta^{13}C$ and $\delta^{18}O$ in otoliths, Kalish (1991a) proposes two hypotheses to explain the observed positive correlation, a kinetic isotope effect, and a metabolic effect.

The kinetic isotope effect (KIE) suggested by Turner (1982) involves an increase in the magnitude of ^{13}C depletion with increased rate of calcium carbonate precipitation. This corresponds to a lower $\delta^{13}C$ value during times of high calcification. If it is assumed that high rates of calcification correspond with high growth rates, then the kinetic hypothesis would result in low $\delta^{13}C$ during periods of warm temperatures (corresponding to high growth rates) or low $\delta^{18}O$ resulting in a positive correlation between $\delta^{13}C$ and $\delta^{18}O$. This corresponds with the observed correlation shown in figure 4.5. Kalish (1991a) rejects a kinetic isotope effect as the cause of the observed trend due to the fact that oxygen is in isotopic equilibrium with sea water and not controlled by a kinetic isotope effect and therefore carbon cannot be singularly affected by the KIE while oxygen is not. The logarithmic trend in $\delta^{13}C$ also supports a rejection of this hypothesis as growth fluctuates seasonally over the first 3 years of life; however, the $\delta^{13}C$ signal does not. There is no evidence of a kinetic isotope effect over the first three years of life and it seems unlikely that the KIE suddenly starts to take effect in year 4.

The second hypothesis suggests that changes in $\delta^{13}C$ are directly related to metabolism. The $\delta^{13}C$ data provide evidence which supports a metabolic mechanism of $\delta^{13}C$ control. Over

the life of the cod as instantaneous metabolic rate decreases $\delta^{13}C$ follows a logarithmic curve suggesting a reduction in the amount of metabolically derived CO_2 with increased age. Kalish (1991a) points to evidence from several species which indicate lower $\delta^{13}C$ values in juveniles as opposed to adults. He also notes a general trend among species which indicates that pelagic high metabolism fish have the lowest $\delta^{13}C$ values and deep-sea, low metabolism fish have the highest $\delta^{13}C$ values. The observed positive correlation between $\delta^{13}C$ and $\delta^{18}O$ on a seasonal basis is explained by the metabolic hypothesis in the following manner. As temperatures cool down, metabolic rates decrease and $\delta^{13}C$ increases due to a decrease in the amount of metabolically derived CO_2 (^{12}C rich) present in the blood. The necessary assumption is that cod metabolism does slow down with decreasing temperature. This is not necessarily a valid assumption as Clark and Green (1990) found that the optimal temperature preference of juvenile (3-year-old) cod changes seasonally, decreasing over the winter to a low of 0°C and increasing over the summer to a high of 9°C. This suggests that the cod undergoes a physiological change between winter and summer which allows it to maximize growth in either season. On the other hand, Brown et al. (1989) found that it cost juvenile cod more (metabolically) to live at 8.3°C than at lower water temperatures. While activity in the cod was reduced with reduced temperature, growth did not appear to be affected. This suggests that cod are able to maintain the same growth rate at a lower metabolic rate during the winter. This translates into higher $\delta^{13}C$ values in the winter resulting in a positive correlation with the observed higher $\delta^{18}O$ values of winter. In order to clarify the relationship between $\delta^{13}C$, $\delta^{18}O$, and metabolism, more research is necessary into the temperature preference and changes in metabolic rate of cod of different ages. It is also

necessary to determine the proportion of carbon precipitated onto the otolith which is metabolically derived versus that which is inorganically derived. Methods for determining the percentage of metabolically derived carbon in calcified structures involve carbon-14 labelling of food and dissolved CO_2 and are outlined in studies performed on sea urchins (Sikes et al., 1981), and mollusc shells (Tanaka et al., 1986).

This study puts forth a third hypothesis with regards to the relationship between $\delta^{18}O$ and $\delta^{13}C$ which suggests that the $\delta^{13}C$ of the otoliths of adult cod varies with depth. The rise in $\delta^{13}C$ with age suggests that the endolymph is approaching isotopic equilibrium with seawater DIC. The subsequent levelling off of the $\delta^{13}C$ signal suggests that the cod have grown to a state at which the rate of equilibration through the gills with DIC(sw) is matched as closely as possible with the rate of endogenous production of DIC ($\delta^{13}C \approx -15‰$). It is hypothesized that in this adult state of isotopic equilibrium, the variation in $\delta^{13}C$ is due to changes in the DIC(sw) that the cod is exposed to. Cod live in both inshore and offshore environments which are characterized by a reversal in the thermal stratification of the water column. Inshore environments are stratified according to the classic limnological model with water temperatures decreasing as depth increases (Clark and Green, 1991). In the inshore environment, therefore, a negative correlation between $\delta^{13}C$ and $\delta^{18}O$ is expected if the DIC of the cod is in equilibrium with the sea water DIC. Offshore environments over the Grand Banks are characterized by an opposite trend in thermal stratification with temperatures increasing as depth increases (Rose, 1993). This relationship results in an expected positive correlation between $\delta^{13}C$ and $\delta^{18}O$ for the carbonate material of the otolith if the endolymph DIC is in equilibrium with sea water DIC. The precision of

sampling in this study did not allow for the resolution of multiple samples within a single season. It is, therefore, difficult to substantiate the hypothesis as variations in $\delta^{13}C$ within a particular season were not observed. The results of the semi-annual sampling performed in this study do not appear to support the hypothesis. Assuming that the inshore migration periods correspond to the highest temperatures (low $\delta^{18}O$), the data indicates that shallow inshore areas (troughs in $\delta^{18}O$) have a low $\delta^{13}C$ signal and deep offshore areas have a high $\delta^{13}C$ signal. This is contrary to the expected decrease in $\delta^{13}C$ with depth as described by Kroopnick (1974). Despite the observed contradiction, the depth hypothesis is put forth based on the observed increasing trend in $\delta^{13}C$ with age which suggests that, in adult cod, the carbonate of the otolith is being precipitated in an environment which is close to being in isotopic equilibrium with sea water DIC.

Chapter 6 - Conclusions

The purpose of this study was to apply oxygen and carbon stable isotope microsampling techniques to the seasonal banding in otoliths of the Atlantic cod, Gadus morhua, to determine their potential for use in reconstructing cod life environmental conditions.

The $\delta^{18}O$ analysis gave results which strongly suggested that the thermal record of seasonal migration of the cod is recorded in the $\delta^{18}O$ signal of the annual banding of the otolith. The otoliths were found to have a common initial value corresponding to the first year of growth and development of the fish. This observation was expected based on the current knowledge of early cod development and its confirmation in the results of the study strengthens the validity and accuracy of the method of analysis. Samples taken from opaque bands were found to correspond with low temperatures, and samples taken from hyaline bands were found to correspond with high temperatures. While the season of formation of a particular growth band type is to some degree stock dependant for Atlantic cod results of this study contradict the accepted regime of winter hyaline bands and summer opaque bands. Regardless of the discrepancy in the season of formation, the observed difference between hyaline and opaque bands suggests that a record of seasonal temperature variations is recorded in the otoliths. The results of the study indicated that the average range of 0.87‰ for $\delta^{18}O$, corresponding to a temperature range of approximately 3.6°C, was within the expected average seasonal temperature shift of 3 to 4°C for Atlantic cod. Finally, seasonal cycling of the $\delta^{18}O$ values was apparent in three of the otoliths with otolith 174 showing a consistent seasonal trend in $\delta^{18}O$. These results support the potential use of $\delta^{18}O$ as a paleothermometer in determining cod migration routes.

It is suggested that, in order to achieve a clear resolution of the changes in temperature future sampling be carried out using a digital microsampling device which will make it possible to take multiple samples within a single season. It is also suggested that priority be placed on calibrating a $\delta^{18}O$ versus temperature curve for cod such that the $\delta^{18}O$ values may be accurately interpreted as specific temperatures.

Results of the $\delta^{13}C$ analysis for the six cod otoliths revealed a previously unreported increasing logarithmic trend in $\delta^{13}C$ with age. The $\delta^{13}C$ signal was found to increase from an initial value of approximately -5 to -3.5‰ until age 4 at which point $\delta^{13}C$ values levelled off and began to fluctuate at a value close to 0‰. Otoliths were found to consist of a mixture of metabolically derived DIC and sea water DIC and the amount of sea water DIC present in the otolith was found to increase with age logarithmically. Four factors were suggested to account for the observed changes in the ratio of metabolic carbon to total carbon. They were: changes in diet, changes in metabolic rate due to a variety of factors, such as food availability, weight, and activity, changes associated with sexual maturity, and changes due to vertical migrations in the water column. During adult years (age 4 and older) the isotopic composition of the otoliths were found to be relatively close to that of the surrounding sea water. It was therefore suggested that, in adult cod, fluctuations in $\delta^{13}C$ are due primarily to changes in the $\delta^{13}C$ of the sea water which is correlated with depth.

The results showed a positive correlation between $\delta^{13}C$ and $\delta^{18}O$. Three hypotheses were put forward to explain the observed correlation: the kinetic isotope effect, metabolic rate effects, and a depth effect. It was concluded that the kinetic isotope effect was not applicable to cod

otoliths. Results supported the role of changes in metabolic rate as the primary factor explaining the positive correlation between $\delta^{13}C$ and $\delta^{18}O$. While the results of this study did not support the hypothesis of changes in depth of occurrence as the main factor contributing to the positive correlation between $\delta^{13}C$ and $\delta^{18}O$, the concept was put forth based on the observed trend in $\delta^{13}C$.

It is suggested that more research into the effects of changes in metabolic rate on the amount of metabolic DIC present in the blood of cod is necessary. It is also suggested that a study be carried out to determine the percentage of metabolically derived DIC present in cod otoliths using carbon-14 labelled food.

In summary, the $\delta^{18}O$ results clearly indicate that they may be used to reconstruct the temperature changes occurring in the cod's environment throughout its life time and that the accuracy of this method is limited primarily by the accuracy of the microsampling technique used to take the samples. The $\delta^{13}C$ analysis revealed a previously unseen increasing logarithmic trend in $\delta^{13}C$ with age which requires further research before hypotheses as to the dominant factors driving the observed trend can be validated. Finally, it is suggested that the positive correlation between $\delta^{13}C$ and $\delta^{18}O$ is a product of either changes in depth or metabolic rate and further research is necessary to clarify the potential role of $\delta^{13}C$ in determining changes in the environment of the cod. It is suggested that $\delta^{13}C$ may act as a record of growth and metabolism providing indications of periods of increased environmental stress in the life history of a cod based on observed abberations in the $\delta^{13}C$ signal.

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Appendix B: Results of mass spectrometric analysis as reported by the stable isotope laboratory at the University of Michighan

ID	ID	SMPL	DATE	TIME	line/#	∂13C (KIS)	±	∂18O (KIS)	±	∂13C (PDB)	∂18O (PDB)	Max P	Error? (I)
Otoloith 173	1	1-G51	8/18/94	17:24:06	A / 3	0.83	0.01	6.27	0.03	-3.09	1.46	349	
Otoloith 173	2		lost										
Otoloith 173	3	3-G51	8/18/94	23:02:25	A / 4	1.54	0.02	6.75	0.05	-2.39	1.94	552	
Otoloith 173	4	4-G51	8/18/94	23:46:32	B / 4	1.17	0.01	6.40	0.02	-2.66	1.72	305	
Otoloith 173	5	5-G51	8/19/94	0:20:14	A / 5	1.50	0.03	6.38	0.03	-2.42	1.57	677	
Otoloith 173	6	6-G51	8/19/94	0:53:39	B / 5	1.95	0.03	5.65	0.03	-1.88	0.97	204	
Otoloith 173	7	7-G51	8/19/94	1:27:58	A / 6	2.51	0.01	5.70	0.02	-1.42	0.89	579	
Otoloith 173	8	8-G51	8/19/94	2:02:09	B / 6	2.34	0.04	6.05	0.05	-1.49	1.37	435	
Otoloith 173	9	9-G51	8/19/94	2:34:55	A / 7	1.66	0.02	5.82	0.02	-2.26	1.01	567	
Otoloith 173	10	10-G51	8/19/94	3:08:22	B / 7	2.88	0.03	5.93	0.02	-0.95	1.25	208	
Otoloith 173	11	11-G51	8/19/94	3:41:49	A / 8	3.02	0.02	6.41	0.01	-0.90	1.60	259	
Otoloith 173	12	12-G51	8/19/94	4:16:40	B / 8	2.86	0.02	6.18	0.05	-0.97	1.50	546	
Otolith 175	2	13-G51	8/19/94	4:49:30	A / 9	1.59	0.05	6.58	0.08	-2.33	1.77	483	
Otolith 175	3	14-G51	8/19/94	5:22:58	B / 9	1.92	0.03	6.32	0.01	-1.91	1.64	325	
Otolith 175	4		lost										
Otolith 175	5		lost										
Otolith 175	6	17-G51	8/19/94	5:59:19	A / 11	3.65	0.01	6.69	0.03	-0.27	1.88	535	
Otolith 175	7	18-G51	8/19/94	7:40:22	B / 11	3.09	0.02	6.32	0.05	-0.74	1.64	600	
Otolith 175	8	19-G51	8/19/94	7:06:07	A / 12	3.62	0.03	6.49	0.04	-0.30	1.68	558	

ID	ID	##-G	DATE	TIME	line/#	∂13C (KIS)	±	∂18O (KIS)	±	∂13C (PDB)	∂18O (PDB)	Max P	Error? (!)
Otolith 169	sample 1	1-G77	10/12/94	12:19:30	A / 12	0.87	0.06	5.79	0.04	-3.06	0.98	403	!
Otolith 169	sample 2	2-G77	10/12/94	13:00:57	B / 12	1.15	0.01	4.76	0.04	-2.68	0.08	270	
Otolith 169	sample 3	3-G77	10/13/94	0:23:07	B / 9	1.54	0.02	4.70	0.03	-2.29	0.02	398	
Otolith 169	sample 4	4-G77	10/13/94	0:56:09	A / 10	2.18	0.02	6.28	0.03	-1.74	1.48	283	
Otolith 169	sample 5	5-G77	10/13/94	13:11:01	A / 3	2.60	0.02	6.17	0.02	-1.32	1.37	497	
Otolith 169	sample 6	6-G77	10/13/94	1:38:04	A / 11	3.13	0.04	6.09	0.04	-0.80	1.28	403	
Otolith 169	sample 7	7-G77	10/13/94	14:24:53	A / 4	3.23	0.02	6.37	0.03	-0.69	1.56	636	
Otolith 169	sample 8	8-G77	10/13/94	2:17:22	A / 12	3.56	0.02	6.37	0.08	-0.36	1.56	485	!
Otolith 169	sample 9	9-G77	10/13/94	15:39:05	A / 5	3.72	0.06	6.65	0.07	-0.20	1.84	493	!
Otolith 175	sample 1	10-G77	10/13/94	3:01:33	A / 13	1.88	0.02	6.31	0.05	-2.04	1.50	187	!
Otollth 175	sample 9	11-G77	10/13/94	16:53:00	A / 6	3.55	0.02	6.55	0.04	-0.37	1.74	300	
Otolith 175	sample 10	12-G77	10/13/94	3:45:57	A / 14	3.97	0.01	6.51	0.03	0.05	1.70	257	
Otolith 175	sample 11	13-G77	10/13/94	18:07:23	A / 7	4.10	0.03	6.60	0.02	0.18	1.79	295	
Otolith 175	sample 12	14-G77	10/13/94	4:27:20	A / 15	3.87	0.01	6.54	0.02	-0.05	1.73	273	
Otolith 172	sample 1	15-G77	10/13/94	19:21:57	A / 8	0.54	0.04	6.24	0.01	-3.38	1.43	451	
Otolith 172	sample 2	16-G77	10/13/94	5:08:55	A / 16	1.27	0.03	6.13	0.02	-2.66	1.32	537	
Otolith 172	sample 3	17-G77	10/13/94	20:38:14	A / 9	1.80	0.03	6.15	0.02	-2.12	1.34	277	
Otolith 172	sample 4	18-G77	10/13/94	5:50:11	A / 17	2.13	0.01	6.12	0.03	-1.79	1.31	348	
Otolith 172	sample 5	19-G77	10/13/94	21:55:50	A / 10	2.54	0.02	5.96	0.07	-1.38	1.16	444	!
Otolith 172	sample 6	20-G77	10/13/94	6:30:38	A / 18	3.81	0.02	6.31	0.03	-0.11	1.50	250	
Otolith 172	sample 7	RERUN	11/5/94	18:38:49	B / 21	2.98	0.03	6.07	0.03	-0.85	1.39	570	
Otolith 172	sample 8	22-G77	10/13/94	7:13:45	A / 19	2.73	0.03	6.37	0.08	-1.19	1.56	446	!
Otolith 172	sample 9	RERUN	11/5/94	19:12:30	A / 22	3.71	0.02	6.56	0.02	-0.21	1.75	283	
Otolith 172	sample 10	24-G77	10/16/94	17:31:13	A / 4	3.76	0.01	6.49	0.06	-0.17	1.68	489	!
Otolith 176	sample 1	25-G77	10/16/94	6:23:00	B / 19	-0.71	0.02	5.80	0.06	-4.53	1.12	248	!
Otolith 176	sample 2	26-G77	10/16/94	18:44:15	A / 5	-0.07	0.01	6.07	0.08	-3.99	1.26	435	!
Otolith 176	sample 3	27-G77	10/16/94	7:12:40	B / 20	0.91	0.01	6.05	0.03	-2.92	1.37	300	
Otolith 176	sample 4	28-G77	10/16/94	19:57:25	A / 6	1.72	0.04	6.23	0.06	-2.20	1.42	434	!
Otolith 176	sample 5	29-G77	10/16/94	8:01:00	B / 21	2.63	0.02	6.25	0.02	-1.20	1.56	303	
Otolith 176	sample 6	30-G77	10/16/94	21:09:49	A / 7	2.76	0.01	6.52	0.01	-1.17	1.71	397	
Otolith 176	sample 7	31-G77	10/16/94	8:51:37	B / 22	2.47	0.02	6.27	0.09	-1.36	1.59	421	!
Otolith 176	sample 8	32-G77	10/16/94	22:22:44	A / 8	2.73	0.01	6.60	0.02	-1.19	1.79	338	
Otolith 176	sample 9	33-G77	10/16/94	9:39:54	B / 23	2.85	0.01	6.14	0.03	-0.98	1.45	324	
Otolith 176	sample 10	34-G77	10/13/94	13:50:57	B / 3	3.10	0.02	6.24	0.01	-0.74	1.56	251	

ID	ID	##-G	DATE	TIME	line/#	∂13C (KIS)	±	∂18O (KIS)	±	∂13C (PDB)	∂18O (PDB)	Max P	Error? (!)
Otolith 176	sample 11	35-G77	10/13/94	15:05:41	B / 4	3.13	0.03	6.38	0.04	-0.71	1.70	282	
Otolith 176	sample 12	36-G77	10/13/94	16:19:44	B / 5	3.59	0.01	6.27	0.01	-0.24	1.59	282	
Otolith 176	sample 13	37-G77	10/13/94	17:34:06	B / 6	3.46	0.01	6.31	0.02	-0.37	1.63	360	
Otolith 174	sample 1	38-G77	10/13/94	18:48:20	B / 7	0.73	0.01	6.06	0.02	-3.09	1.38	381	
Otolith 174	sample 2	39-G77	10/15/94	0:11:28	B / 13	1.37	0.01	6.14	0.04	-2.46	1.45	310	
Otolith 174	sample 3	40-G77	10/13/94	20:05:33	B / 9	2.40	0.01	6.06	0.04	-1.43	1.38	158	

ID	ID	ID	SMPL	DATE	TIME	line/#	∂13C (KIS)	±	∂18O (KIS)	±	∂13C (PDB)	∂18O (PDB)	Max P	Error? (!)
Otolith 174	sample 4		1-S2	10/7/94	13:42:33	A / 3	2.97	0.04	6.72	0.03	-0.95	1.91	552	
Otolith 174	sample 5		2-S2	failed										
Otolith 174	sample 6		3-S2	10/7/94	14:56:42	A / 4	3.51	0.03	6.40	0.08	-0.41	1.59	495	!
Otolith 174	sample 7		4-S2	10/7/94	15:39:00	B / 4	3.51	0.02	6.22	0.04	-0.32	1.54	418	
Otolith 174	sample 8		5-S2	10/7/94	16:11:24	A / 5	3.91	0.02	6.51	0.02	-0.02	1.70	327	
Otolith 174	sample 9		6-S2	10/7/94	16:52:33	B / 5	3.47	0.02	5.98	0.04	-0.37	1.30	424	
Otolith 174	sample 10		7-S2	10/7/94	17:26:01	A / 6	3.40	0.02	6.17	0.04	-0.52	1.37	607	
Otolith 174	sample 11		8-S2	10/7/94	18:07:10	B / 6	3.81	0.02	6.24	0.03	-0.03	1.56	365	
Otolith 174	sample 12		9-S2	10/7/94	18:39:46	A / 7	4.15	0.01	6.51	0.03	0.23	1.70	366	
Paired otoliths	sample 1	left	10-S2	10/7/94	19:22:18	B / 7	2.87	0.00	6.65	0.02	-0.96	1.97	384	
Paired otoliths	sample 2	left	11-S2	10/7/94	19:56:34	A / 8	3.31	0.03	7.00	0.11	-0.61	2.19	461	!
Paired otoliths	sample 3	left		failed										
Paired otoliths	sample 4	left	13-S2	10/7/94	20:45:30	A / 9	3.07	0.01	6.83	0.07	-0.85	2.02	401	!
Paired otoliths	sample 1	right	14-S2	10/8/94	13:52:01	B / 9	3.08	0.01	6.70	0.03	-0.75	2.02	356	
Paired otoliths	sample 2	right	15-S2	10/7/94	21:24:07	A / 10	3.28	0.02	6.95	0.04	-0.64	2.14	364	
Paired otoliths	sample 3	right		failed										
Paired otoliths	sample 4	right	17-S2	10/7/94	22:05:12	A / 11	2.99	0.02	6.73	0.06	-0.93	1.92	603	!