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Environmental information stored in otoliths: insights from stable isotopes

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Abstract The present study compares the stable oxygen- and carbon-isotope ratios (^{18}O:^{16}O; ^{13}C:^{12}C) in the otoliths of Atlantic cod, *Gadus morhua*, with those expected at equilibrium with seawater. Otoliths from juveniles reared for a 3 mo period under controlled conditions indicate that otoliths are formed in isotopic disequilibrium with seawater. This is probably due to positive metabolic fractionating of the heavier isotopes. This "vital effect" remains constant over the temperature range studied here (9 to 16°C) but may differ among other species. Our data indicate that the concentration of ^{18}O in calcium carbonate is inversely related to temperature and is described as $\partial^{18}O_{ar} - \partial_w = 3.79 - 0.200(T\,°C)$. The ^{13}C:^{12}C ratios of otoliths and body tissues are related to the carbon ratio in the food source, although we found that the ^{13}C concentration is considerably higher in the otoliths relative to the body tissues and the diet.

Introduction

Limited information exists on the life-history dynamics of many fishes. Significantly lacking is sufficient knowledge of environmental and nutritional influences. We have been working to advance techniques to obtain knowledge of the ambient temperature conditions and dietary histories experienced by individual fish from the ^{18}O:^{16}O and ^{13}C:^{12}C concentration ratios within their otoliths.

From the otolith structure, the regular rate of increment deposition makes if feasible to accurately determine the ages of fish, measure growth, resolve feeding success and obtain mortality rates (e.g. Brothers et al. 1976; Radtke and Dean 1982; Campana and Neilson 1985; Jones 1986). Additional information about the life histories of fish can be found in the stable isotope composition of otoliths. Oxygen and carbon occur in several isotopic forms. In addition to the common forms ^{16}O and ^{12}C, the stable isotopes ^{18}O and ^{23}C occur at low but significant levels in both organic and inorganic systems. In carbonates, isotope ratios (^{18}O:^{16}O and ^{13}C:^{12}C) reflect environmental conditions at the time of deposition (McCrea 1950; Emiliani 1955, 1966; Savin 1977; Emiliani et al. 1978; Killingely and Berger 1979; Killingely 1980). The extrapolation from the stable isotope ratios to the conditions present during formation requires a substrate with a well-defined chemical composition from which exchangeable oxygen and carbon have been excluded. Hard structures of relatively long-lived organisms with marked skeletal growth bands are among the most useful for the analysis of past environmental changes using stable isotopes. Otoliths from teleost fishes are such structures (Devereux 1967; Kalish 1991a, b; Iacumin et al. 1992). The major object of the present study is to advance the usefulness of analyses of the oxygen- and stable carbon-isotope composition of otoliths as tools to determine temperature and dietary histories of individual fish.

The oxygen-isotope ratio (^{18}O:^{16}O) of calcium carbonate is dependent upon the temperature and isotopic composition of the seawater from which the carbonate was precipitated (Urey 1947; Epstein et al. 1953). Previous studies on stable oxygen-isotope ratios in fishes have attempted to resolve temperature histories from otolith analyses (Devereux 1967; Degens et al. 1968;

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Mulcahy et al. 1979; Radtke et al. 1987; Kalish 1991a, b; Iacumin et al. 1992). These studies hypothesized that oxygen is deposited in isotopic equilibrium with the surrounding water. The possibility exists, however, that metabolic CO_2 may be utilized in otolith formation instead of bicarbonate ions from the surrounding water (Radtke 1984). The calcification and isotopic composition of fish otoliths have not been sufficiently scrutinized under controlled conditions to determine if non-equilibrium fractionation of stable isotopes occurs due to biological processes. Through this study, we hope to determine whether the otoliths of juvenile Atlantic cod (*Gadus morhua*) are formed in isotopic equilibrium with the surrounding seawater and, if the isotopes are fractionated relative to seawater, establish the fractionation equation for $^{18}O{:}^{16}O$ as a function of temperature.

The carbon-isotope ratios ($^{13}C{:}^{12}C$) may reflect differences in nutritional sources. There is a strong correlation between the carbon-isotope composition of an organism and that of its food supply (DeNiro and Epstein 1979; Teeri and Schoeller 1979; Thomas 1993). The isotopic composition of the food supply depends first upon the carbon-fixing pathway of the primary producers, and then upon possible subsequent fractionation at higher trophic levels. Plants can be separated into three photosynthetic categories: C_3, C_4 and CAM (crassulacean acid metabolism). All three categories are depleted in ^{13}C (relative to the Pee Dee Belemnite PDB) standard. C_3 plants are the most depleted, CAM plants are intermediate and C4 plants are the least depleted (Calvin and Benson 1948; Smith and Epstein 1971; Bender et al. 1973). By measuring the $^{13}C{:}^{12}C$ signatures recorded in fish otoliths, it should be possible to follow the carbon flow from primary producers to fish. In the present study, we measured the effect of two different diets on the stable carbon-isotope signatures in *Gadus morhua* otoliths, and characterize, in controlled laboratory experiments, the relationship between the $^{13}C{:}^{12}C$ ratios in the otoliths, body tissues and diet.

Materials and methods

Experimental conditions

Otoliths were dissected from cod (*Gadus morhua*) maintained at controlled temperatures and on standardized diets. From the Austevoll Aquaculture Station, Norway, we obtained 900 juvenile cod (age = 60 d, mean total length = 6.1 ± 1 cm), and reared them in eight 500-liter tanks (maintained at 9°, 12°, 14°, and 16°C) at the Flødevigen Marine Research Station (Arendal, Norway). Temperatures were controlled by mixing water warmed to 25°C with ambient, filtered, flow-through seawater (34.6 ± 0.3% S) obtained from a depth of 100 m in the fjord next to the laboratory.

To evaluate the possibility that the ^{13}C concentration might be influenced by diet, the cod were reared in two groups at each temperature: one fed a commercial cod-chow diet, and the other a *Spirulina* spp. diet depleted in ^{13}C (1 part *Spirulina* spp., 5 parts wheat flour, and 5 parts soy meal by weight). Food was given in dry prepared form; it was prepared in monthly batches, and replicate or triplicate samples were analyzed to measure the ^{13}C concentration. Experiments were run for 3 mo, at which point the fish were sampled. Fish length was measured to determine growth and its impact on the isotopic fractionation of stable oxygen and carbon isotopes.

To reduce cannibalism which is prevalent under rearing conditions, individuals that became large enough to eat smaller individuals were continuously culled. Only fish (≃ 5%) in the cod-chow treatments became large enough to be culled; consequently, the size ranges have been biased in these culled groups. Culled fish were not included in subsequent analyses.

Measurement of stable isotope concentrations

Otoliths

To gain access to the otoliths, the brain tissues were removed, exposing the sagitta and the semi-circular canals in the lateral floor of the cranial cavity. The membranous labyrinth containing the otoliths was removed, immersed in water, and dissected to free the otoliths from their chambers. The otoliths were labeled as to the respective side of the head from which they came, soaked in 5.2% aqueous solution of sodium hypochloride for 2 min to remove surface organic matter, and then rinsed thoroughly with deionized water and dried at 60°C for 24 h.

A small dental drill with a bit size of < 0.5 mm was used to remove otolith material for isotopic analysis. Subsamples of carbonate were tested by x-ray diffraction to check for mineralogical conversion to calcite (Aharon 1991). The method utilized for carbonate extraction results in minimal heat generation and all x-ray diffraction analyses demonstrate monominerlic aragonite composition. Samples were taken from the edge of the sagitta, which represented < 1 mo otolith growth during the rearing period. Each powdered sample, weighing ≃ 0.1 mg, was roasted in vacuo for 1 h at 380°C. Samples were analyzed according to standard methods. (Williams et al. 1977) and were reacted in vacuo with concentrated phosphoric acid at 50°C. The resulting CO_2 was cleaned in a double-trap system using normal cryogenic techniques, and advanced to the mass spectrometer [see Showers and Margolis (1985) and Showers and Bevis (1988) for additional techniques on analyses].

The isotopic differences between the derived sample CO_2 and the PDB standard (a belemnite from the Cretaceous Pee Dee formation) were determined with a semi-automatic mass spectrometer (Finnegan Matt). All values are reported in standard ∂ notation, where:

$$\partial^{18}O(‰) = \left[\frac{^{18}O{:}^{16}O_{sample}}{^{18}O{:}^{16}O_{PDB}} - 1\right] 10^3; \quad (1)$$

$$\partial^{13}C(‰) = \left[\frac{^{13}C{:}^{12}C_{sample}}{^{13}C{:}^{12}C_{PDB}} - 1\right] 10^3. \quad (2)$$

Enrichment with the more heavy isotope relative to the PDB standard results in positive values of ∂. Conversely, if relatively more of the lighter isotope is present, values are more negative.

Triplicate analyses of ten individuals for each temperature were performed. Analytical reproducibility for individual runs was better than ± 0.05‰ for both $\partial^{18}O$ and $\partial^{13}C$. The overall reproducibility with respect to the laboratory working standard expressed in PDB was ≃ 0.15‰.

Data were corrected for sample sizes below 1.85 μ*M* (which occurred in ≃ 10% of our samples). It appears that the static-trap design that we used for small otolith samples fractionates small samples at a maximum of 0.28‰ for $\partial^{18}O$ and 0.22‰ for $\partial^{13}O$. This effect is not seen at > 2 μ*M* of CO_2. We believe it is a problem with CO_2 vapor pressure in the traps, and that the variation in the temperature of the alcohol slush (− 130 to − 85°C) causes the fractionation. We have

now changed to a 75°C reaction temperature and variable temperature traps that maintain a constant temperature (from $-196°$ to $-75°$C) to avoid this problem. To construct the correction formulas we ran NBS-20 standard at various sample sizes. To construct the correction formulas we ran NBS-20 standard at various sample sizes. The correction equations are applied to samples of < 1.85 μM, and are as follows:

$$\partial^{13}C_{corrected} = \partial^{13}C_{observed} + 0.12\,(1.85 - \mu M_{sample}, \quad (3)$$

$$\partial^{18}O_{corrected} = \partial^{18}O_{observed} + 0.15\,(1.85 - \mu M_{sample}. \quad (4)$$

The standard deviation on the measurement of the μM_{sample} was 0.44 μM, and the standard deviation for the isotopic values from the corrected regression line was: $\partial^{13}C - 0.05‰$ and $\partial^{18}O - 0.08‰$. This puts these small-volume samples within our counting statistics.

State-of-the-art analytical techniques require only small sample sizes for accurate isotopic measurements. This increased sensitivity allows spatial resolution of isotope ratios in individual otoliths. In theory, with these new techniques isotope ratios could be determined for an individual otolith at various ages, thus establishing thermal (^{18}O:^{16}O) and nutritional (^{13}C:^{12}C) histories for an individual fish.

Tissues

Stable-isotope ratios from organic carbon in tissues were determined using a method in which the tissues were combusted to CO_2 and analyzed by mass spectrometry as described by Parker et al. (1972). Body tissues were removed from the fish upon sacrifice by separating the flesh from the bone and the scale integument. The tissue, taken from the lateral portion of the body, was dried and stored at $-20°$C. Approximately 10 mg dry weight of fish tissue was pulverized to a fine powder. The samples were then oxidized according to the procedures of Stump and Frazer (1973). Organic material was combusted at 900°C in the presence of copper and copper oxide. After combustion, CO_2 was separated from the water vapor and collected by a process of differential freezing as described in the preceding subsection.

Water

Water samples were taken by the procedure of Tan et al. (1973) to determine the stable isotopic composition of the water. Samples were preserved at the time of collection with saturated $HgCl_2$ solution (> 1 part $HgCl_2$ per 250 parts seawater) and stored, refrigerated, in glass bottles with polyseal caps. Three samples were analyzed for each temperature; one at the beginning, one at the middle, and one at the end of the experiment. The ^{18}O:^{16}O ratio averages and the standard deviations were calculated from the three samples for each temperature.

The $\partial^{18}O$-H_2O samples were analyzed by the CO_2-equilibration technique (Kroopnick and Craig 1972). This technique equilibrates a small amount of CO_2 gas with a larger amount of H_2O liquid. The oxygen ratio is approximately 1:300, so the $\partial^{18}O$ of the CO_2 that is measured is in equilibrium with the H_2O at the temperature at which the samples are equilibrated. The water-bath temperature is therefore very important and must be held constant throughout the equilibration period. The length of time required for the equilibration depends upon salinity, with freshwater samples requiring less time than saltwater. The present samples were equilibrated for 12 h at 50° C.

Carbon, hydrogen and nitrogen ratios

In living organisms, lipids possess lower ^{13}C:^{12}C ratios than protein and carbohydrates; thus, organisms with high fat reserves tend to be isotopically light compared to lean organisms. To make comparisons possible between individuals of different diets and possibly fat content, lipid normalization was calculated according to McConnaughey and McRoy (1979).

CHN ratios were measured with a precision of > 0.1 using a gas chromatograph CHN-analyzer. A CHN ratio of < 4 can be regarded as normal with higher ratios showing fatter individuals and lower ratios leaner individuals. In this manner, through the use of CHN analysis we are able to discover which isotopic ratios in body tissues are related to lipid content.

Microscopy techniques

To view a small sub-sample of otoliths with scanning electron microscopy (SEM), thin sections of sagittae were prepared. Each sagitta was embedded in epoxy resin and a longitudinal section was cut through the center of the otolith. A 400 µm-thick section was then taken from the middle of the otolith, ground flat, and mounted on an SEM stub. The section was then ground with 600 grit-sand paper until the growth rings in the core area became visible, and was then polished with 0.3 µm alumina polish. The polished surfaces were decalcified with 8% EDTA (disodium ethylene diamine tetraacetate) at pH 8 (pH adjusted with NaOH) for 5 min. The finished thin sections were examined by SEM.

Results

Otolith morphology

SEM examination of juvenile *Gadus morhua* sagittal otoliths revealed distinct mineral crystals in a protein matrix (Fig. 1). The crystals radiated from the core region, originating from multiple primordia. Rhythmic microstructural patterns of protein deposition, initiated from the core region, produced conspicuous increments which could be enumerated.

The cod increased their weight by $\simeq 70$ to 90 g during the confinement period. A broad outer zone was present in every otolith which corresponded to this growth period (Fig. 1a). These broad outer zones were carefully sampled to measure their stable isotope ratios.

Isotopic composition

The mean water temperatures and isotope ratios during the experimental period are given in Table 1. Isotopic values for the seawater (w) varied with temperature and were in agreement with Fairbanks' (1982) data relating salinity (s)(‰) to $\partial^{18}O_w$, where $\partial^{18}O_w = 0.258(s) - 9.14$. The salinity in the present experiments averaged $34.6 \pm 0.3‰$. The water was predominantly in equilibrium with temperature conditions. For the *Spirulina* spp. food preparation the mean $\partial^{13}C$ was -25.516, and for the cod-chow preparation -18.765.

The oxygen and carbon isotope results are given in standard notation as parts per thousand (‰) enrichment or depletion in ^{18}O or ^{13}C relative to the PDB

standard in Table 2. The experimental results were analyzed using ANOVA (Table 3).

Definite ^{18}O enrichments relative to ambient seawater conditions are evident in the portion of the otolith deposited during confinement (Fig. 2, Table 3). Diet made little difference to $\partial^{18}O$, showing a significant influence only at 9°C where it differed by only 0.3039‰, cod chow being more depleted (Table 3). Temperature had a notable and significant influence upon the ^{18}O concentration within the otoliths. The $\partial^{18}O$ values decreased as temperature increased. There is statistically no difference (Student's t-test, $P > 0.05$) in the slopes of Lines A, B and C in Fig. 2, and all show an inverse temperature relationship that makes it possible to estimate the environmental temperature from the $\partial^{18}O$ values of otolith aragonite.

Fig. 1 *Gadus morhua.* Scanning electron micrograph of cod sagitta (**a**) and closeup of increments in peripheral edges which demonstrated a daily periodicity and increase of width with temperature (**b**). Combination of daily increments and stable isotopic analyses increases information storage-capacity of fish otoliths

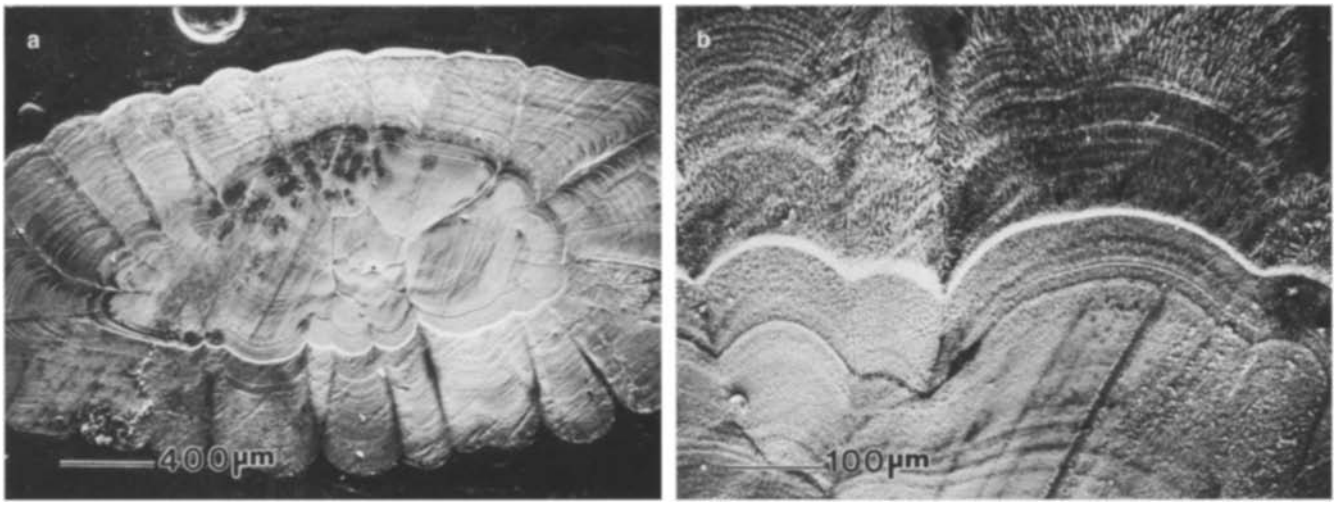

Table 1 *Gadus morhua.* Mean water temperatures and isotopic composition of water and food during experimental period. Isotopic compositions are given in standard ∂ notations as parts per thousand (‰) enrichment or depletion in ^{18}O or ^{13}C relative to Pee Dee Belemnite standard

Tank No.	T (°C)	Food source	Water composition (‰)	
			$\partial^{18}O$	$\partial^{13}C$
1	16.084 ± 0.31	Cod chow	− 0.713	− 39.813
2	15.701 ± 0.90	*Spirulina* spp.	− 0.551	− 39.941
3	13.997 ± 0.87	Cod chow	0.114	− 39.839
4	13.636 ± 0.81	*Spirulina* spp.	0.102	− 39.766
5	11.914 ± 0.91	*Spirulina* spp.	0.651	− 39.906
6	11.729 ± 0.67	Cod chow	0.488	− 39.753
7	9.018 ± 1.41	*Spirulina* spp.	0.891	− 39.575
8	8.696 ± 1.41	Cod chow	0.868	− 39.888
Diet composition:		Cod chow preparation:		− 18.765
		Spirulina spp. preparation:		− 25.516

Table 2 *Gadus morhua* Mean oxygen- and carbon-isotope composition for each treatment group, given in standard ∂ notation as parts per thousand (‰) enrichment or depletion in ^{18}O or ^{13}C relative to PDB standard (mean values of triplicate analyses of 10 individuals from each treatment ± 1SD)

	16° C	14° C	12° C	9° C
Cod chow				
$^{18}O_{otolith}$	0.38 ± 0.45	1.19 ± 0.18	1.47 ± 0.49	− 1.84 ± 0.29
$^{13}C_{otolith}$	− 3.5336 ± 0.4177	− 3.7799 ± 0.4446	− 3.3818 ± 0.4671	− 3.5597 ± 0.4923
$^{13}C_{tissue}$	− 18.6734 ± 0.1112	− 18.9258 ± 0.0730	− 18.8731 ± 0.0772	− 19.3271 ± 0.8221
Spirulina spp.				
$^{18}O_{otolith}$	0.53 ± 0.62	1.11 ± 0.57	1.65 ± 0.61	1.97 ± 0.37
$^{13}C_{otolith}$	− 6.0641 ± 0.2235	− 5.5179 ± 0.6663	− 5.1133 ± 0.7475	− 4.9079 ± 0.3898
$^{13}C_{tissue}$	− 22.752 ± 0.1289	− 21.8155 ± 0.1370	− 22.0513 ± 0.1742	− 21.8202 ± 0.1362

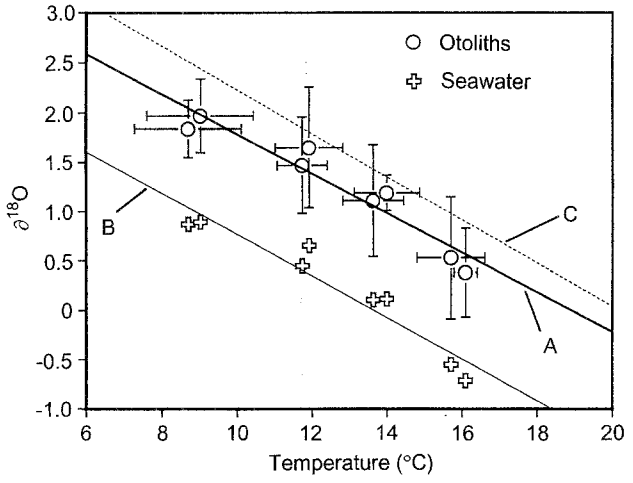

Fig. 2 *Gadus morhua.* Relationship between $\partial^{18}O$ in otolith aragonite and temperature in reared cod [*Line A* $\partial^{18}O$ values of cod otoliths, $\partial_{ar} = 3.793 - 0.200(T°C)$, $R^2 = 0.917$ (values are $\pm$ 1 SD); *Line B and associated data points* $\partial^{18}O$ values of seawater from rearing experiments (values are $\pm$ 1SD); *Line C* aragonite relationship between temperature and $\partial^{18}O$ by Grossman (1982)]

Table 3 *Gadus morhua.* Comparison of stable isotope ratios among four temperature groups and two feeding regimes using one-way ANOVAs. (*T* temperature; *NS* $P > 0.05$; *$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$)

Constant	Independent	(*df*)	*F*
Dependent variable = $\partial^{18}O_{otolith}$			
Cod chow	*T*	(3)	39.69***
Spirulina spp.	*T*	(3)	8.27**
T, 16°C	Food	(1)	3.87^{NS}
14°C	Food	(1)	0.06^{NS}
12°C	Food	(1)	0.13^{NS}
9°C	Food	(1)	5.05*
Dependent variable = $\partial^{13}C_{otolith}$			
Cod chow	*T*	(3)	1.23^{NS}
Spirulina spp.	*T*	(3)	7.13**
T, 16°C	Food	(1)	276.10***
14°C	Food	(1)	43.61***
12°C	Food	(1)	39.51***
9°C	Food	(1)	42.11***
Dependent variable = $\partial^{13}C_{tissue}$			
Cod chow	*T*	(3)	2.45^{NS}
Spirulina spp.	*T*	(3)	43.53***
T, 16°C	Food	(1)	2869.75***
14°C	Food	(1)	1674.25***
12°C	Food	(1)	2149.59***
9°C	Food	(1)	44.18***
Dependent variable = standard length			
Cod chow	*T*	(3)	2.15^{NS}
Spirulina spp.	*T*	(3)	7.69**
T, 16°C	Food	(1)	283.50***
14°C	Food	(1)	148.11***
12°C	Food	(1)	132.62***
9°C	Food	(1)	66.95***

^{13}C was more enriched in both otoliths and body tissues relative to the surrounding seawater, and was more enriched in the otoliths than the body tissues, although isotope ratios in otoliths

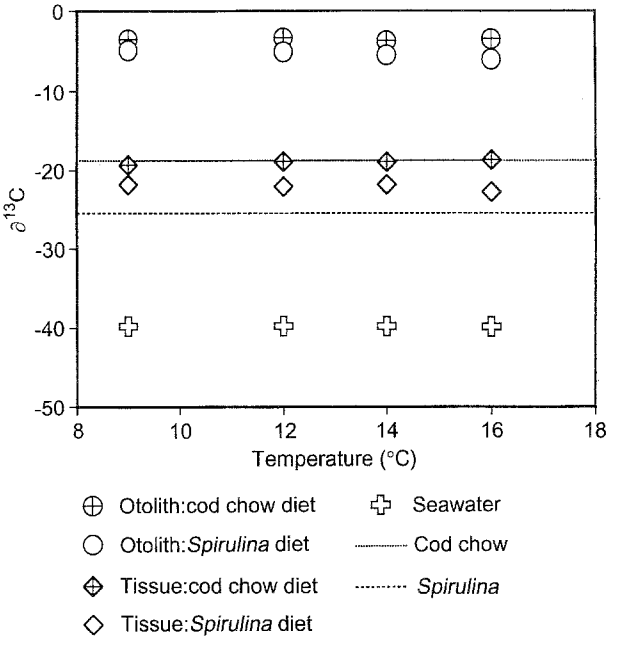

Fig. 3 *Gadus morhua.* Relationship between $\partial^{13}C$ and temperature in cod reared under varying temperature regimes. Cod fed diets differing in $\partial^{13}C$ content reflected these differences in their tissues. This translated into shift in $\partial^{13}C$ values in otoliths, but these values differed from those in body tissues. A small change was found between temperature and incorporation of carbon C^{13} among cod fed diet of *Spirulina* spp. There was slight difference in growth rate, with fish reared at colder temperatures ($\simeq$ 9 to 10°C) having higher growth rate than those reared at warmer temperatures of $\simeq$ 16° C

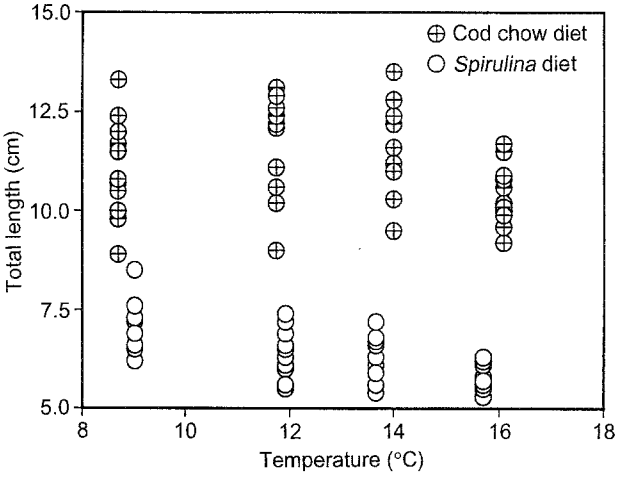

Fig. 4 *Gadus morhua.* Size distribution of cod reared in each individual tank as a function of maintenance temperature and diet

and body tissues were clearly related (Fig. 3, Table 2).

Diet had a pronounced and significant effect on carbon-isotope ratios in the otoliths and body tissues (Table 3). There was a definite downward shift of $\partial^{13}C$ in those fish fed a diet depleted in ^{13}C (Fig. 3, Table 2). Hence the carbon values in otoliths may constitute a record of the food that a fish has consumed, as the

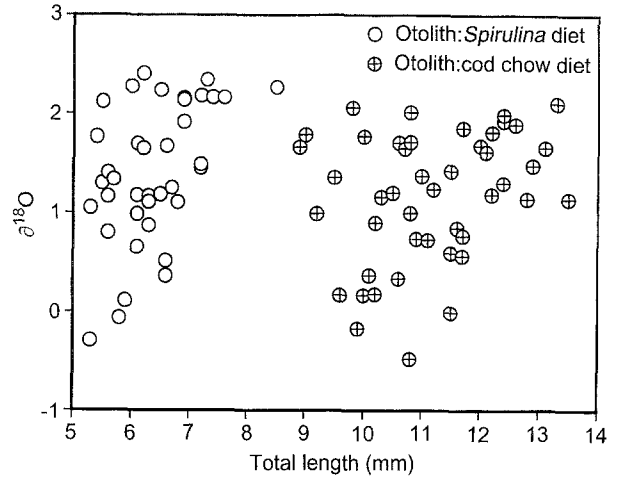

Fig. 5 *Gadus morhua.* $\partial^{18}O$ contents of otolith carbonate as a function of total length of juvenile cod

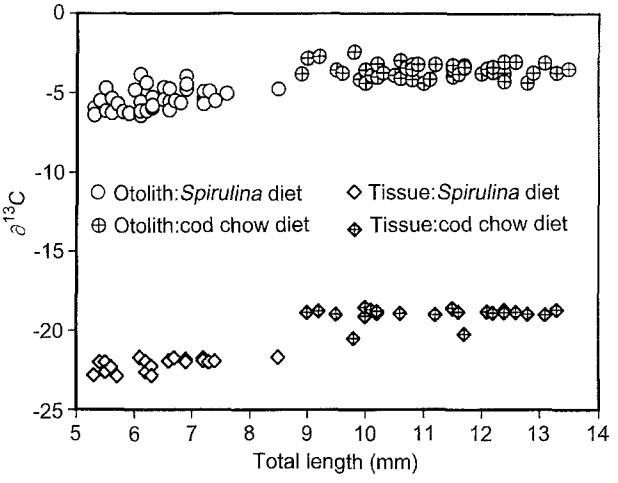

Fig. 6 *Gadus morhua.* $\partial^{13}C$ values of otolith carbonate and body tissue as a function of total length of juvenile cod

carbon may be a result of CO_2 produced by the fish itself. Temperature had a significant effect only on those fish fed a *Spirulina* spp. diet. ^{13}C concentrations steadily decreased relative to the PDB standard as temperature increased. There was a 1.16‰ decrease in the $\partial^{13}C$ of otolith carbon and a 0.93‰ decrease in the $\partial^{13}C$ of tissue carbon at 9°C compared to 16°C. This decrease is evidence of metabolic isotope fractionation. Consequently, otoliths may be of value in producing a life-history model of the fish's previous past.

Growth rates

Although weight and body length demonstrated dietary differences (Fig. 4), stable isotope ratios do not appear to be related to fish growth-rates (Figs. 5 and 6). Among fish reared upon a cod-chow diet there were no significant correlations between total lengths at the end of the experiment and isotope ratios ($\partial^{13}C_{otolith}$: $R^2 = 0.005$, $F = 0.223$, $P = 0.639$; $\partial^{13}C_{tissue}$: $R^2 = 0.016$, $F = 0.350$, $P = 0.560$; $\partial^{18}O_{otolith}$: $R^2 = 0.076$, $F = 3.564$, $P = 0.066$). Among fish reared on a *Spirulina* spp. diet, there were significant correlations between final total lengths and isotope ratios but the correlations were weak ($\partial^{13}C_{otolith}$: $R^2 = 0.184$, $F = 8.098$, $P = 0.007$; $\partial^{13}C_{tissue}$: $R^2 = 0.356$, $F = 9.938$, $P = 0.006$; $\partial^{18}O_{otolith}$: $R^2 = 0.228$, $F = 10.564$, $P = 0.024$).

Cod reared at the colder temperature, 10°C, showed a higher growth rate than those reared at 16°C. This, however, had little or no effect on the incorporation of ^{18}O and ^{13}C. Comparisons of growth rate and stable isotope composition did not reveal any significant relationship when tested within temperature groups (ANOVA, $P > 0.05$). The carbon in the otoliths shows a positive fractionation from that found in body tissues, suggesting that the carbon utilized for otolith aragonite has a metabolic input

Carbon, hydrogen and nitrogen

CHN data showed no difference between the different treatments. The lipid content of fish reared under differing feeding regimes did not vary.

Discussion

It is often difficult and impractical, due to sampling limitations, bias, and expense, to empirically correlate the distribution and abundance of fish to environmental factors. The value of stable-isotope studies to fishery biology and ecology has been demonstrated in recent studies (Radtke et al. 1987; Kalish 1991a, b; Iacumin et al. 1992; Northcote et al. 1992). Previous stable-isotope studies of otoliths have indicated the possibility of retrospectively resolving both the trophic status and the hydrographic regime experienced by an individual fish (Devereux 1967; Degens et al. 1968; Mulcahy et al. 1979). The oxygen-isotope composition of carbonate has been demonstrated to be temperature-dependent (Williams et al. 1981; Jones et al. 1983; Krantz et al. 1987), while the carbon-isotope composition has been demonstrated to reflect the carbon-isotope composition of the diet (DeNiro and Epstein 1979; Teeri and Schoeller 1979; Thomas 1993).

The oxygen-isotope ratio of mollusc-shell calcium carbonate has been demonstrated to be contingent upon the isotopic composition of the seawater (∂_w) from which it was precipitated (Urey 1947; Urey et al. 1951; Epstein et al. 1953; Romanek et al. 1987). Similarly, the oxygen-isotope ratios of fish otoliths have been postulated to be in equilibrium with the surrounding water (Devereux 1967; Degens et al. 1968; Mulcahy et al. 1979). However, data for the cod (*Gadus morhua*) otoliths examined in the present study (Fig. 2, Line A)

Table 4 Equations describing equilibrium fractionation of stable oxygen isotopes in biogenic aragonite (*ar*) and calcite (*ca*) as a function of temperature (∂_w correction for water)

Fractionation of oxygen	Material	Source
$\partial^{18}O_{ar} - \partial_w = 3.05 - 0.220\,(T\,^\circ C)$	molluscs	Horibe and Oba (1972)
$\partial^{18}O_{ar} - \partial_w = 4.42 - 0.219\,(T\,^\circ C)$	foraminiferans	Grossman (1982)
$\partial^{18}O_{ar} - \partial_w = 4.70 - 0.228\,(T\,^\circ C)$	foraminiferans	Grossman and Ku (1986)
$\partial^{18}O_{ar} - \partial_w = 4.65 - 0.213\,(T\,^\circ C)$	molluscs	Grossman and Ku (1986)
$\partial^{18}O_{ar} - \partial_w = 3.79 - 0.200\,(T\,^\circ C)$	*Gadus morhua*	Present study
$\partial^{18}O_{ca} - \partial_w = 3.93 - 0.229\,(T\,^\circ C)$	molluscs (calcite)	Epstein et al. (1953)[a]

[a] Linear approximation (with temperature range of 0 to 30°C) of published equation: $(T\,^\circ C) = 16.5 - 4.3\,(\partial^{18}O_{calcite} - \partial_w) + 0.14(\partial^{18}O_{calcite} - \partial_w)^2$

indicate that ^{18}O was not deposited in isotopic equilibrium (Fig. 2, Line B), as ^{18}O was enriched in the otoliths relative to the surrounding seawater. This disequilibrium was constant over the temperature range examined in this study; therefore, an equation relating oxygen-isotope ratios to temperature can be empirically derived from Fig. 2.

The results of the present study show that the ^{18}O concentration in cod otoliths is inversely related to temperature (Fig. 2, Line A). Previous studies (Devereux 1967; Degens et al. 1968; Mulcahy et al. 1979) applied the calcite fractionation equation given by Epstein et al. (1953) to estimate temperature histories from otoliths, even though the otoliths were composed of aragonite (Irie 1955; Carlström 1963; Degens et al. 1969). Data from aragonitic benthic deep-sea Foraminifera (Grossman 1982; Dunbar and Wefer 1984; Grossman and Ku 1986) and inorganically precipitated aragonite (Tarutani et al. 1969) support a temperature equation for aragonite that is 0.6‰ enriched with ^{18}O in comparison to calcite. Only recently have equations describing the oxygen-isotope fractionation in biogenic aragonite (*ar*) as a function of temperature been available (Table 4; see also Kalish 1991a). These equations are very similar to that in the present study for cod: $\partial^{18}O_{ar} = 3.793 - 0.200\,(T\,^\circ C)$. As an example, Line C in Fig. 2 was calculated from Grossman's (1982) isotopic temperature equation for the foraminiferan *Hoeglundina elegans*. The lines of $\partial^{18}O$ versus temperature for seawater, otoliths and the foraminiferan are parallel; the slopes of the three lines are not significantly different (Student's *t*-test, $P > 0.05$).

The similarity of the slopes is not surprising, since cod are poikilotherms and their metabolic rates are temperature-dependent. However, the fact that the intercepts for inorganically precipitated aragonite and otolith aragonite are different indicates that the stable oxygen isotopes in cod otoliths were deposited in disequilibrium with seawater. McConnaughey (1989a, b) identified two patterns of isotopic disequilibrium in biogenic carbonates, "kinetic" and "metabolic". Kinetic effects result in depletions of both ^{18}O and ^{13}C relative to equilibrium during CO_2 hydration and hydroxylation. The processes observed here are clearly not simple kinetic effects, since both isotopes are enriched, not depleted, relative to seawater and the food source. The observed disequilibrium is largely due to a "vital", or metabolic, effect, and may be attributable to physiological isotope-fractionation during catabolism or anabolism which alters the $\partial^{13}C$ of the fluid within the endolymphatic sac from which otolith material precipitates. It can be presumed that the CO_2 used in otolith deposition probably originated from the tricarboxylic acid cycle (Lehninger 1975). Because the metabolic effects on oxygen deposition may be species-specific, it may be necessary to determine the particular isotopic-fractionation equations for the otoliths of each species individually, as a function of temperature.

The carbon isotopes ^{12}C and ^{13}C generally occur in proportions of 98.89 and 1.11%, respectively (Hoefs 1980), and form identical chemical compounds. Yet, due to the difference in mass, a kinetic variation in reactions results in the enrichment of the heavier isotope in the end-products of photosynthetic and metabolic reactions (van der Merwe 1982). Since the pioneering studies of Wickman (1952) and Craig (1954), it has been established that different groups of plants display different ratios of ^{13}C:^{12}C in their organic material. Plants, which are the ultimate source of most organic carbon, can be separated into three photosynthetic categories: C_3, C_4 and CAM (crassulacean acid metabolism). C_3 plants have $\partial^{13}C$ values that range from -24 to -34‰ and use the Calvin cycle for CO_2 fixation (Calvin and Benson 1948). C_4 plants have $\partial^{13}C$ values that range from -6 to -19‰ (Smith and Epstein 1971) and use the C_4 dicarboxylic acid pathway for the assimilation of CO_2 (Hatch and Slack 1966). CAM plants have $\partial^{13}C$ values of -14 to -34‰ that fall within the range of C_3 and C_4 plants (Bender et al. 1973). Marine planktonic algae display a range of $\partial^{13}C$ values from -16 to -36‰, with species-specific values (Wong and Sackett 1978; Haines and Montague 1979), while blue-green algae display $\partial^{13}C$ values as high as -5‰ (Pardue et al. 1976).

The carbon-isotope ratios of prey items act as labels that can be measured in the fleshy body tissues and carbonate tissues of consumers and can be used to trace carbon flow through marine ecosystems (Thayer et al. 1978; DeNiro and Epstein 1979; Fry and Parker 1979; Haines and Montague 1979; McConnaughey and McRoy 1979; Teeri and Schoeller 1979; Fry et al. 1984; Araujo-Lima et al. 1986; Tanaka et al. 1986; Bunn et al.

1989; Hesslein et al. 1991; Thomas 1993). For example, Bunn et al. (1989) have used $\partial^{13}C$ values in the tissues of co-existing juvenile brook trout, *Salvelinus fontinalis*, and juvenile Arctic charr, *S. alpinus*, to show that these species partition food resources. $\partial^{13}C$ values of adult Arctic charr confirm that these fish obtain most of their biomass carbon from feeding at sea, and then transport this carbon into freshwater systems.

Yet all organisms may not selectively consume one carbon source. This results in a multi-component system with some overlap in the ranges of isotopic values, making it difficult to accomplish a unique interpretation of any given $\partial^{13}C$ value. The utilization of another stable isotope in organic matter, ^{15}N, may make feasible a cross-referencing of individual energy sources. Nitrogen isotopes, ^{14}N and ^{15}N, exist in a proportion of 99.7 and 0.3%, respectively, in air (Junk and Svec 1958), and are also fractionated in metabolic reactions (Sweeney et al. 1978).

Unfortunately, the carbon-isotope label from the food source may be modified by subsequent metabolic fractionation in the consumer organism. Among the cod studied here, only body tissues of fish fed the cod-chow diet matched the carbon-isotope composition of the food source. Neither the otoliths nor the tissues (of cod fed *Spirulina* spp.) reflected the isotopic composition of the diet (Fig. 3). Goreau (1977) suggests that in coral, carbon is fractionated by a different mechanism than is oxygen and that carbon recirculates within the organism before deposition. Among the cod, otoliths and body tissues has originally been formed at a higher $\partial^{13}C$ value; when the fish were switched to a diet depleted in ^{13}C, both the carbonate of the otoliths and the body tissue became depleted. However, the effect of the depleted ^{13}C diet was less pronounced in the otoliths. The mechanisms by which each of these tissues is formed are radically different. The formation of otolith carbonate is ultimately an inorganic reaction whereby $CaCO^3$ is precipitated from the endolymphatic fluid. In addition to pure kinetic effects, the $\partial^{13}C$ of the otolith is controlled by only those metabolic pathways that modify the $\partial^{13}C$ of the endolymphatic fluid. The formation of body tissue on the other hand is much more complex, involving the entire metabolism of the organism. Future experimental work is needed to determine if longer culture times result in a complete turnover of body tissue carbon. This should be reflected in a closer similarity in the carbon isotopic composition of the otoliths, body tissue and the food source. Until then, however, the utility of stable-carbon isotope labels in otoliths and body tissues is limited to revealing only general dietary-history information.

Conclusion

The oxygen-isotope composition of cod (*Gadus morhua*) otoliths is not deposited in equilibrium with the surrounding seawater. However, the degree of the disequilibrium remained constant over the temperature range of this study (9 to 16°C) making possible an empirically-derived relationship between temperature and the isotopic composition of the otolith. The concentration of ^{18}O in cod otoliths is inversely related to temperature, and is described as $\partial^{18}O_{ar} - \partial_w = 3.79$ to $0.200(T\,°C)$. In theory (Urey 1947) and in practice (e.g. Epstein et al. 1953), the temperature at which calcium carbonate is formed can be determined within $\pm$ 0.5C° using stable-isotope techniques. A 1C° temperature difference will result in an $\partial^{18}O$ change of $\sim 0.22‰$. The new computer-controlled generation of ratio-mass spectrometers has an analytical precision of 0.008‰ for oxygen with large ($> 30\ \mu M$) samples, and 0.016‰ for oxygen with small (down to $0.05\ \mu M$) samples. With this increased analytical precision and these small sample capabilities, it will be possible to detect, from their otoliths, temperature changes of 0.1C° experienced by fish.

Stable carbon-isotope ratios of prey items act as labels that can be detected in the fleshy body tissues and carbonate tissues of consumers. This opens the possibility of retrospectively determining the diet of individual fish. In this study, the *Spirulina* spp. diet depleted in ^{13}C ($\partial^{13}C = -25.52$) resulted in significantly lower ^{13}C concentrations in the otoliths and body tissues of cod. However, the carbon-isotope ratios in neither the otoliths nor the body tissues were lowered so far as to match the isotopic composition of the diet. The 3 mo rearing period may have been insufficient to complete a turnover of carbon in the tissues, or there may have been significant metabolic fractionation of the dietary carbon by the cod themselves. Knowledge of the degree of subsequent metabolic fractionation in fishes will be of paramount importance to the future use of carbon-isotope ratios to reveal dietary histories in any detail.

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