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Effect of somatic and otolith growth rate on stable isotopic composition of early juvenile cod (*Gadus morhua* L) otoliths

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Abstract

The relative amounts of the stable isotopes of carbon and oxygen in fish otoliths can be used to reveal the environmental history experienced by the fish. This requires that the relative amounts of the isotopes are deposited in equilibrium with the surrounding environment, or that the offset from this equilibrium is known and can be quantified. It is known that carbon isotopes in biogenic carbonates are a mixture of carbon from the seawater and metabolically derived carbon, but the effect of the somatic growth rate of the fish is still unclear. The possible effect of otolith growth rate and fractionation of both carbon and oxygen isotopes are also not established. We carried out a controlled laboratory experiment where we reared cod (*Gadus morhua* L.) larvae and early juveniles at two temperatures (6 and 10 °C) and generated different growth rates within each temperature by manipulation of prey levels. The otoliths of the resulting fish were analysed for carbon and oxygen isotopes. We found no effect of otolith precipitation rate on fractionation of either carbon or oxygen isotopes. However, there was a depletion of ^{13}C in the otoliths of fish with elevated metabolism. The proportion of metabolically derived carbon in the otoliths was estimated to be 28–32%. Our results suggest that measurements of oxygen isotopes in otoliths can be a reliable tool to estimate ambient temperature since the oxygen isotopes seem to be deposited in the otoliths independently of kinetic and metabolic effects. Fractionation of carbon isotopes in otoliths on the other hand can give valuable insight into metabolism and feeding pattern of fish.

Keywords: *Gadus morhua*; Growth; Metabolism; Otolith; Stable isotopes; Temperature

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1. Introduction

The time-keeping property of fish otoliths makes them an important tool for fisheries management. Growth increments in the otoliths make estimation of age possible, and the environmental history of the fish can be deduced by analysis of the chemical composition of the otoliths (Campana, 1999). The otoliths are mainly made up of $CaCO_3$ with periodic inclusion of proteins (5–8%) (Campana, 1999). Measurement of the stable isotopes of two of the main components, carbon and oxygen, is useful in ecological research. The relation of the two stable oxygen isotopes ^{16}O and ^{18}O ($\delta^{18}O$) can be used to estimate previous ambient temperature history since the deposition of the isotopes onto otoliths is temperature dependent. This has been used when studying fish migration and temperature preference (Northcote et al., 1992; Weidman and Millner, 2000; Blamart et al., 2002), stock structure (Newman et al., 2000; Begg and Weidman, 2001; Gao et al., 2001), and reconstruction of ambient temperature history and seasonality in both ancient (Patterson et al., 1993; Ivany et al., 2000) and recent time (Kalish, 1991a; Radtke et al., 1996, 1998; Thorrold et al., 1997). Carbon has two stable isotopes, ^{12}C and ^{13}C, but the relative amount of them ($\delta^{13}C$) in inorganic carbonates is not temperature dependent (Romanek et al., 1992). Carbon isotopic composition in otoliths can be used in studies of fish food supply, metabolic rate and position in the web chain (Schwarcz et al., 1998; Gao et al., 2001).

When the isotopes deposited into the biogenic carbonates are in disequilibria with the surrounding seawater, i.e. the fractionation of the isotopes does not follow the expected fractionation of inorganic carbonate, the reason is believed to be caused by metabolic and/or kinetic effects. Metabolic effects are the inclusion of metabolically derived isotopes and are common for carbon isotopes in biogenic carbonates (McConnaughey, 1989; McConnaughey et al., 1997). An example of metabolic inclusion of carbon isotopes in biogenic carbonate is a feeding experiment with prey of different $\delta^{13}C$ values where foraminifera incorporated 8–15% of the metabolically derived carbon in their skeleton (Spero and Lea, 1996). Respiration adds ^{13}C depleted carbon to the internal dissolved inorganic carbon (DIC) pool that the biogenic carbonate is formed from (McConnaughey et al., 1997). The $\delta^{13}C$ of HCO_3^- from metabolism is often highly negative since it is only enriched by 0–1‰ compared to the fish diet, which has $\delta^{13}C$ values from −19‰ to −24‰ (McConnaughey and McRoy, 1979; Peterson and Fry, 1987; Fry, 1988). The amounts of carbon from metabolic sources in otoliths have been estimated to constitute 20–30% (Kalish, 1991a; Weidman and Millner, 2000). Schwarcz et al. (1998) presented a model where the concentration of the ^{13}C isotope in otoliths is a function of carbon from the seawater and metabolically derived carbon. The often observed increase in $\delta^{13}C$ values of the otoliths by fish age can then be explained by the decreasing metabolism by age (size) (Edwards et al., 1972; Brett and Groves, 1979), which means less incorporation of the carbon of metabolic origin. A shift to higher tropic level by age, which is common for cod (Pálsson, 1994), will also have the same effect since there is a general enrichment of ^{13}C by 1‰ per tropic level (Peterson and Fry, 1987; Fry, 1988). The positive correlation between temperature and $\delta^{13}C$ in biogenic carbonates

is probably linked to temperature by a positive correlation with metabolic rate (Kalish, 1991b). In a controlled laboratory study Thorrold et al. (1997) examined the effect of metabolism of fractionation of carbon and oxygen isotopes on the otoliths of Atlantic croaker (*Micropogonias undulatus*). They found an enrichment of ^{13}C at higher somatic growth rate which is opposite that expected from the models of McConnaughey et al. (1997) and Schwarcz et al. (1998). Radtke et al. (1996, 1998) found depletion of ^{13}C in cod otoliths by increased temperature in fish with one diet while no effect of temperature was found for cod given another diet.

Kinetic fractionations originate from different hydration and hydroxylation speed of CO_2 with different isotopes when it diffuses across a cell membrane. An example of kinetic effects is that coral skeleton in areas of rapid skeletal growth was more depleted in ^{18}O and ^{13}C than in the slower growing areas (McConnaughey, 1989). Kinetic fractionations are expressed when the HCO_3^-, formed from the CO_2, precipitates as $CaCO_3$ before re-establishing isotopic equilibrium with the cell DIC pool (McConnaughey et al., 1997). Kinetic effects act on both carbon and oxygen isotopes simultaneously and result in depletion of the heavier isotopes in rapid growing carbonate. McConnaughey (1989) presented a model where the extension rate of biogenic carbonates is negatively correlated (kinetic behaviour) with both $\delta^{13}C$ and $\delta^{18}O$. However, no significant effect of precipitation rate for $\delta^{13}C$ was found when producing synthetic inorganic aragonite (Romanek et al., 1992) and Thorrold et al. (1997) found a positive correlation between the amount of ^{13}C in the otoliths of Atlantic croaker and precipitation rates. How the kinetic effects act on fractionation of carbon and oxygen isotopes on fish otoliths and other biogenic carbonates is therefore still unclear.

The metabolic and kinetic effects are hard to separate in biogenic carbonates such as otoliths since increased temperature leads to both increased metabolism and growth rate causing the two mechanism to covary. There are a number of papers describing the effect of temperature on biogenic carbonates isotopic composition (Campana, 1999), but information on the influence of kinetic and metabolic effects by manipulation of fish and otolith growth rates through manipulations of prey levels is lacking. These effects have to be considered in order to use stable isotopes in otoliths to predict past environmental history. In this study we separated the two mechanisms by manipulation of larval and juvenile cod growth rates. We reared the cod at two different temperatures and two different feeding regimes, and sampled slow and fast growing fish at the same somatic size. In this manner we produced young juveniles with different otolith and somatic growth rates which also had experienced different metabolic rates. Our null-hypotheses in this study is that there are no kinetic or metabolic effects operating on the fractionation of oxygen and carbon isotopes in cod otoliths.

2. Materials and methods

An orthogonal laboratory experiment incorporating two temperatures and two growth rates was conducted during spring and summer 2000. Cod eggs were incubated in conical tanks at 6 °C and the larvae were transferred to the Department

of Fisheries and Marine Biology, University of Bergen, where the experiment was performed. The experimental design was to rear the larvae at two temperatures, 6 and 10 °C, at high (HGR) and medium (MGR) growth rates within each temperature, thereby producing cod which had experienced four different treatments (6HGR, 6MGR, 10HGR and 10MGR, where the number refers to °C). Each treatment had two parallels, resulting in eight tanks. This experiment was part of a larger experiment examining oxygen consumption of cod larvae reared at different environmental conditions (unpublished data). Two days after hatching, 2000 larvae were distributed to each of the eight square tanks of 500 l. Natural photoperiod for the time of the year and latitude were maintained through the experiment by a computer programme Lysstyr 2.0 (Hansen, 1990). Temperature in the tanks was recorded daily and oxygen content was recorded weekly. Salinity was measured five times during the 86-day experiment. The larvae were fed live natural zooplankton, mainly nauplii and copepods, once a day (see Otterlei et al. (1999) for further details). Growth rate was manipulated by offering the larvae different concentrations of prey items. In order to ensure sufficient survival through the early larval phase, the HGR and MGR groups were offered nominal prey densities of 2000 and 750/l, respectively in the start of the experiment. This was reduced to 1000 and 250 prey per litre by age 14 days. By feeding fish larvae in relation to prey per litre, the available prey per larvae will increase by age due to two reasons. First, mortality will reduce the number of larvae so the number of prey available per larvae increases, and second, the searching and feeding efficiency of larvae increase by age/size of the larvae. To maintain a reduction in growth for the MGR groups, the food level was further reduced to 125 and 100 prey per litre at 10 °C at age 57 and 63 days after hatching. In the 6MGR group the prey densities were only reduced to 200 prey per litre at age 80 days. Further reductions were not necessary at 6 °C due to lower larval mortality than at 10 °C. In this manner we were able to reduce the growth for the MGR groups, while the HGR groups grew well throughout the entire experiment. Some individuals developed cannibalistic behaviour in the MGR groups after initiation of metamorphosis. These fish were removed and are not included in the analysis.

Young juveniles of 18–21 mm standard length were used for isotopic analysis of otoliths. The cod in the HGR groups reached this length at age 51 and 70 days at 10 and 6 °C, respectively, while the smallest cod from the MGR groups were sampled at age 69 and 86 days at 10 and 6 °C, respectively. Dry weight (DW) of each individual fish was measured after being dried at 60 °C for 24 h.

Both left and right sagittae were removed from each fish. The otoliths were cleaned in an ultrasonic cleaner, dried at 60 °C for 24 h, and weighed to the nearest microgram. Due to the small size, the left and right otoliths were pooled and weighed together. Otoliths of a total of 95 cod were measured for isotopic composition. Left and right sagittae of the largest cod ($n = 14$) were analysed separately due to the setting of the mass spectrometer, and the results are presented as a mean of two measurements. Where isotopic values for both left and right otoliths were analysed, the relationship between the isotopic values of left minus right otolith versus fish DW had both slope and intercept indistinguishable from zero (linear regression, $P>0.05$). Hence the isotopic composition of the left and right otoliths is not significantly different.

Water samples from each tank were collected weekly and fixed with $HgCl_2$. All isotopic values are reported to standards by the International Atomic Energy Agency, Vienna. $\delta^{13}C$ and $\delta^{18}O$ of the otoliths and $\delta^{13}C$ of the water DIC are reported in standard δ notation relative to the Vienna Pee Dee belemnite (VPDB) standard, and the $\delta^{18}O$ of the water samples ($\delta^{18}O_{water}$) is expressed relative to the Vienna standard mean oceanic water (VSMOW) standard:

$$\delta = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 1000 \ (‰) \quad (1)$$

where R is the ratio $^{18}O/^{16}O$ or $^{13}C/^{12}C$ in the sample or standard. The otoliths were measured for oxygen isotopic composition by a Finnigan MAT 252 mass spectrometer. Precision of the measurements was 0.07‰ for $\delta^{18}O$ and 0.06‰ for $\delta^{13}C$ (standard deviation of repeated measurements of the standard). The water samples were equilibrated with CO_2 at 20 °C in an automated Finnigan preparation line, and measured for $\delta^{18}O$ with a Finnigan DELTA-E mass spectrometer with an analytical precision of 0.1‰. The isotopic composition of carbon in the water samples was measured by equilibrating the DIC with CO_2, and the samples were then analysed by continuous flow using an ANCA-GSL and Geo 20-20 IRMS. The carbon isotopic composition of plankton samples was measured five times during the experiment by a Europa Scientific ANCA-GSL elemental analyser. The isotopic composition of the otoliths and oxygen isotopic composition of the water samples was measured at the Laboratory of Geological Mass Spectrometry at the University of Bergen, while the carbon isotopic composition of the water and plankton was measured by Iso-Analytical, Cheshire, UK. Nine measurements of $\delta^{13}C$ (all belonging to the 6HGR group) were not included due to analytical problems with the mass spectrometer. The $\delta^{13}C$ values of the otoliths minus the $\delta^{13}C$ values of the DIC in the water are expressed as $\Delta^{13}C$. When expressing the relationship in terms of $\delta_C - \delta_W$ where δ_C represents $\delta^{18}O$ of the otoliths and δ_W represents $\delta^{18}O$ of the water, the conversion factor of $\delta^{18}O_{SMOW}$ versus δ_W of Friedman and O'Neil (1977) was used:

$$\delta_W = 0.99978(\delta^{18}O_{SMOW}) - 0.22 \quad (2)$$

There are also other conversion factors suggested (Bemis et al., 1998) but we chose this one since it is the most commonly used for oxygen isotope studies on otoliths (e.g. Kalish, 1991a).

To enable calculation of a growth rate for otoliths we wanted to measure the otolith weight at the start of the experiment. However, otolith weight of the larvae at day 2 could not be measured since the otoliths were too small to be handled. Instead, we estimated the weight by calculating the volume of sagittal otoliths of seven larvae. The volume was estimated by embedding the otoliths in a fluorescent dye (alizarine) and measuring the area at 1 μm depth interval by a confocal microscopy. Aragonite has a density of 2.95 g cm^{-3}. However, about 5–8% of the otolith consists of protein with a density of about 1 g cm^{-3}. The density of the otolith was therefore set to 2.6 g cm^{-3}.

Daily weight specific somatic and otolith growth rate was calculated as

$$G_0 = (e^g - 1) \times 100 \tag{3}$$

where

$$g = \left(\frac{\ln W_2 - \ln W_1}{t_2 - t_1}\right) \tag{4}$$

and W is weight at days t_1 and t_2.

Otolith growth rate differences between temperature groups are given as Q_{10} values where

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}} \tag{5}$$

and R_2 and R_1 are the growth rate for temperatures T_2 and T_1, respectively.

The amount of metabolically derived carbon in the otoliths was estimated using the equation given by McConnaughey et al. (1997):

$$R(\delta^{13}C_{resp}) + (1 - R)(\delta^{13}C_{env}) = \delta^{13}C_c - \Delta \tag{6}$$

where R is the fraction of respired carbon in the otolith; $\delta^{13}C_{resp}$ and $\delta^{13}C_{env}$ are the isotopic composition of HCO_3^- derived from respiration and seawater, respectively; $\delta^{13}C_c$ is the isotopic composition of the otolith; and Δ is the ^{13}C fractionation between tissue HCO_3^- and the otolith which is assumed to be the equilibrium value for $\varepsilon_{aragonite-HCO_3^-}$, which is estimated to be 2.7 by Romanek et al. (1992). $\delta^{13}C_{resp}$ was not measured but is assumed to be the value of the food enriched by 1 ‰ since the $\delta^{13}C$ of bicarbonate from metabolism is enriched by 0–1 ‰ compared to the fish diet (McConnaughey and McRoy, 1979; Peterson and Fry, 1987; Fry, 1988).

All statistical analyses and data presentations were carried out with Statistica 5.5 for Windows. A significance level of 0.05 was used on all tests. Homogeneity of variances of the data was tested by Levene's test. The DW-specific increase in otolith weight at the two temperatures and feeding level was analysed with analysis of covariance (ANCOVA) where DW was set as covariate. The effect of temperature and feeding level on oxygen and carbon isotopic composition of the otolith minus the isotopic composition of the water ($\delta_C - \delta_W$ and $\Delta^{13}C$) was analysed by two-way analysis of variance (ANOVA) where temperature and feeding level were fixed effects. The parallel tanks were nested in temperature and feeding level to reveal possible differences between parallel tanks. Post hoc comparisons of differences between the mean values were conducted by Newman–Keuls test.

3. Results

The mean $\delta^{18}O$ and $\delta^{13}C$ of the cod otoliths in the different groups ranged from 0.70 ‰ to 1.47 ‰ and −5.02 ‰ to −4.71 ‰, respectively (Table 1). The temperature

Table 1
Data of mean isotopic composition of otoliths and water, and hydrography in the tanks. Numbers in brackets are standard deviation of the mean

Group (parallel)	Food ratio	Number of cod	Termination day of the experiment	Otolith		Water					
				$\delta^{18}O$	$\delta^{13}C$	Temperature °C	Salinity PSU	$\delta^{18}O$	*n*	$\delta^{13}C$	*n*
6HGR (I)	High	12 (8)*	70	1.36 (0.09)	− 4.90 (0.20)	5.99 (0.12)	34.30 (0.14)	− 0.26 (0.21)	10	− 2.90 (0.43)	7
6HGR (II)	High	11 (6)*	70	1.40 (0.09)	− 4.81 (0.15)	6.13 (0.11)	34.14 (0.15)	− 0.33 (0.25)	10	− 3.06 (0.51)	7
6MGR (I)	Medium	16	86	1.47 (0.12)	− 4.74 (0.12)	6.05 (0.13)	34.30 (0.19)	− 0.24 (0.19)	11	− 2.89 (0.47)	8
6MGR (II)	Medium	14	86	1.41 (0.14)	− 4.76 (0.24)	6.01 (0.10)	34.26 (0.15)	− 0.28 (0.18)	11	− 2.72 (0.29)	8
10HGR (I)	High	5	51	0.70 (0.08)	− 5.02 (0.18)	10.09 (0.20)	34.24 (0.11)	− 0.23 (0.21)	8	− 2.62 (0.29)	5
10HGR (II)	High	12	51	0.71 (0.14)	− 4.71 (0.29)	10.03 (0.23)	34.26 (0.11)	− 0.24 (0.17)	8	− 2.50 (0.35)	5
10MGR (I)	Medium	6	69	0.75 (0.11)	− 4.98 (0.14)	10.08 (0.22)	34.40 (0.12)	− 0.15 (0.12)	9	− 2.40 (0.19)	6
10MGR (II)	Medium	13	69	0.80 (0.09)	− 4.93 (0.20)	9.95 (0.24)	34.32 (0.15)	− 0.18 (0.12)	9	− 2.35 (0.17)	6

Lower number of otoliths measured for $\delta^{13}C$ due to analytical problems.

Table 2
Otolith and somatic (dry weight) growth rate (% day^{-1}) from age 2 days to sampling

Group	Otolith				Dry weight			
	Mean	SD	CV%	*n*	Mean	SD	CV%	*n*
6MGR	10.8	0.35	3.23	30	6.68	0.22	3.29	30
6HGR	13.6	0.37	2.71	23	8.53	0.29	3.40	23
10MGR	15.1	0.29	1.92	19	8.75	0.26	2.97	19
10HGR	20.5	0.64	3.12	17	11.9	0.60	5.04	17

in the tanks remained relatively stable close to the predetermined level through the experiment (Table 1). The tanks were filled up with water from 160 m depth at the start of the experiment, which had $\delta^{18}O_{water}$ values close to 0‰. As the experiment proceeded the $\delta^{18}O_{water}$ became more negative which is reflected in the more negative mean $\delta^{18}O_{water}$ values of the tanks which were terminated last. This was caused by accumulation of water added together with the plankton which were pumped from 5 m depth where the water had slightly more negative $\delta^{18}O_{water}$ values. The $\delta^{13}C$ of the plankton fed to the larvae had a value of -24.0‰ in the beginning of the experiment, but thereafter showed a steady increase and ended with a value of -20.4‰ at the end of the experiment. Mean value for the entire experiment was -21.6‰.

Mean otolith weight of 2-day-old larvae ($n=7$) was estimated to be 0.0068 μg (± 0.0012). The fish in the 6HGR and 10MGR groups had very similar somatic and otolith growth rate while fish in the 10HGR group had the highest growth rates with mean value for otolith growth rate higher than 20% day^{-1} (Table 2). The otolith growth rate

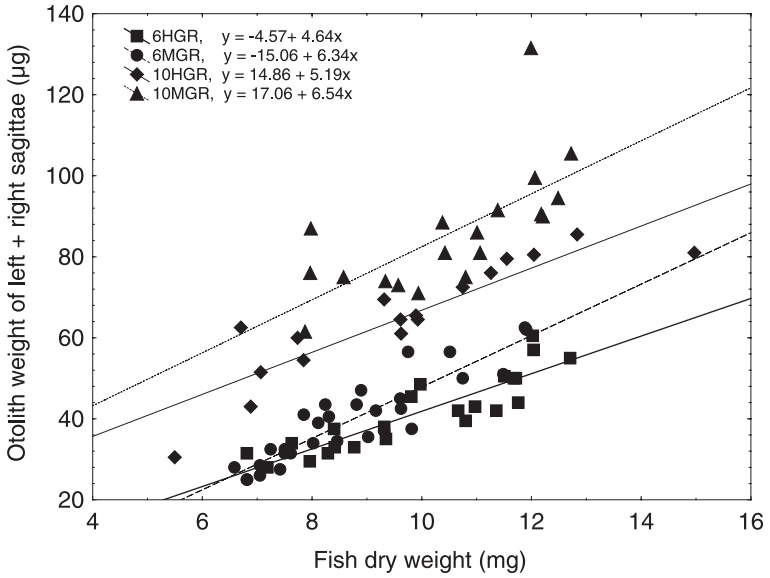

Fig. 1. Otolith weight versus fish dry weight. Regression equations for each treatment are presented.

differences between 10 and 6 °C in the HGR and MGR groups correspond to Q_{10} values of 2.8 and 2.3, respectively.

The DW-specific otolith weight (Fig. 1) was examined by a two-way ANCOVA where the effects of temperature and feeding level (high and medium) were examined and DW was set as covariate. Fish reared at the two temperatures and feeding levels had similar DW-specific increase in otolith weight (homogeneity of slopes interaction effects, $P>0.05$). The effect of feeding level on the DW-specific otolith weight was different at the two temperatures (two-way ANCOVA interaction effect, $F_{1,\ 84}=12.22$, $P<0.001$). Excluding two of the most extreme outliers (standard error of residuals of predicted otolith weight >4) did not alter the significance of the results. We therefore reduced the model and performed two one-way ANCOVAs where the effect of temperature at the two feeding levels and the effect of feeding levels within each temperature were tested separately for the DW-specific otoliths weight. Fish reared at different temperatures but the same feeding regime had the same DW-specific increase in otolith weight (homogeneity of slopes, $P>0.05$). Within each feeding level, we found a temperature effect both at MGR (ANCOVA, $F_{1,\ 46}=164.54$, $P<0.001$) and HGR (ANCOVA, $F_{1,\ 37}=205.98$, $P<0.001$), where the DW-specific otolith weight of cod reared at 10 °C was 34.5 and 24.8 μg heavier than those reared at 6 °C at MGR and HGR, respectively. Fish from the two feeding regimes had the same DW-specific increase in otolith weight at 10 °C (homogeneity of slopes, $P>0.05$), but not at 6 °C (homogeneity of slopes, $P<0.05$). Within the 10 °C group, we found an effect of feeding regimes on otolith weight (ANCOVA, $F_{1,\ 33}=23.72$, $P<0.001$), where mean otolith weight of fish from the 10MGR group was 16.0 μg heavier than that from the 10HGR group.

The $\delta^{18}O$ of the otoliths minus $\delta^{18}O$ of the water ($\delta_C-\delta_W$) was significantly influenced by temperature (with highest values at 6 °C), but not by feeding level (two-way ANOVA, Tables 3 and 4). There were no significant differences between the parallels. Plotting the $\delta_C-\delta_W$ values of the different groups against otolith growth rate (Fig. 2) clearly shows that the $\delta_C-\delta_W$ values in MGR and HGR groups within each

Table 3
Results of two-way factorial ANOVA on $\delta_C-\delta_W$ and $\Delta^{13}C$ where temperature and feeding level are variables

	SS	*df*	MS	*F*	*P*
$\delta_C-\delta_W$					
1: Temperature	10.77	1	10.77	825.04	<0.01
2: Feeding level	0.01	1	0.01	0.40	0.53
Interaction (1 × 2)	<0.01	1	<0.01	0.21	0.65
Parallel (nested in 1 and 2)	0.11	4	0.03	2.10	0.09
Error	1.06	81	0.01		
$\Delta^{13}C$					
1: Temperature	4.81	1	4.81	116.05	<0.01
2: Feeding level	0.48	1	0.48	11.45	<0.01
Interaction (1 × 2)	0.18	1	0.18	4.33	0.04
Parallel (nested in 1 and 2)	0.58	4	0.15	3.52	0.01
Error	2.99	72	0.04		

Parallel tanks are nested in temperature and feeding level.

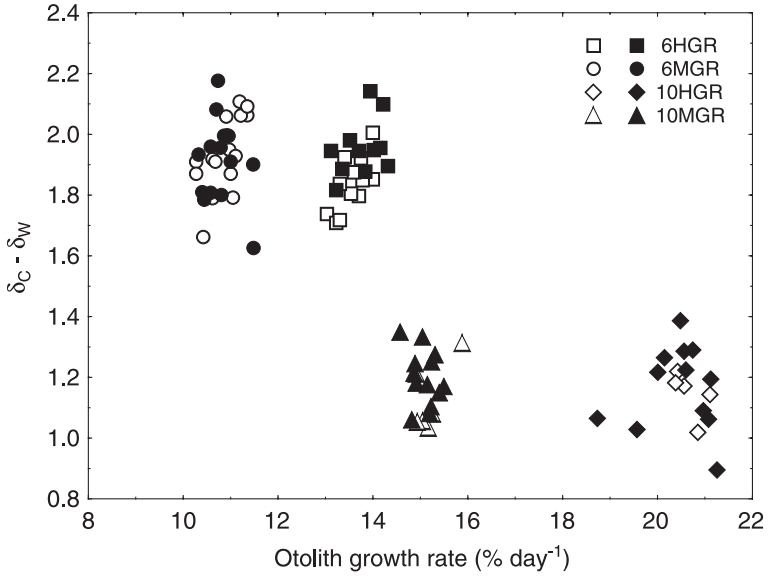

Fig. 2. Relationship between $\delta_C-\delta_W$ of juvenile cod otoliths and otolith growth rate of fish reared at different temperatures and feeding regimes. Open and closed symbols refer to replicates 1 and 2 within each group.

temperature group are similar. Within each group no significant correlation was found between $\delta_C-\delta_W$ and somatic or otolith growth rate except for a positive correlation in the 6HGR group ($P<0.05$, Table 5).

The effect of feeding level on $\delta^{13}C$ of the otoliths minus $\delta^{13}C$ of the water ($\Delta^{13}C$) was different at the two temperatures (two-way ANOVA interaction effect; Table 3). The parallels were also significantly different (Table 3), but only in the 6HGR group where there was a 14% difference between the mean values of parallels 1 and 2 ($P=0.01$). This difference was regarded to be of minor importance and the replicates were pooled for further analysis. There was no difference between the mean $\Delta^{13}C$ values of the 6HGR and 6MGR groups (two-way ANOVA, $P=0.54$). However, the otoliths of the fast growing larvae in the 10HGR group had a significant enrichment of ^{13}C compared to the slower growing otoliths of cod in the 10MGR group (two-way ANOVA, $P<0.01$, Table 4). The $\Delta^{13}C$ difference between 6HGR and 10MGR, which

Table 4
Mean $\delta_C-\delta_W$ and $\Delta^{13}C$ values for the different food levels within each temperature group

	6 °C		10 °C	
	HGR	MGR	HGR	MGR
$\delta_C-\delta_W$	1.89 a	1.92 a	1.17 b	1.16 b
$\Delta^{13}C$	− 1.89 a	− 1.94 a	− 2.27 b	− 2.58 c

Mean values followed by different letters at the same variable are significantly different ($P<0.05$). The results are from post hoc comparisons (Newman–Keuls test) of two-way factorial ANOVA where temperature and feeding level are variables.

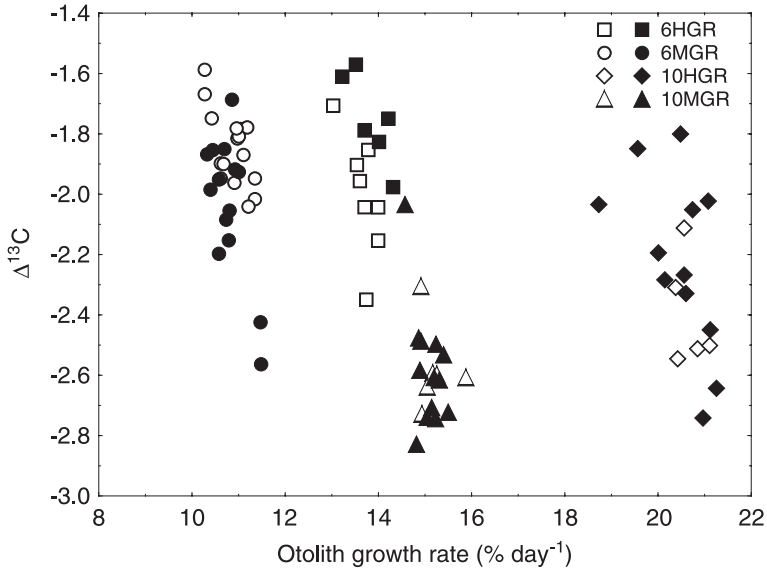

Fig. 3. Relationship between $\Delta^{13}C$ of juvenile cod otoliths and otolith growth rate of fish reared at different temperatures and feeding regimes. Open and closed symbols refer to replicates 1 and 2 within each group.

had similar somatic growth rates, was 0.69‰. This pattern is also seen when $\Delta^{13}C$ is plotted against otolith growth rate (Fig. 3). The correlations between $\Delta^{13}C$ and otolith growth rate were negative but only significant in the 6MGR and 10HGR groups (Table 5). Somatic growth rate and $\Delta^{13}C$ were negatively correlated in all four groups although not significantly in the 10MGR group. We found no significant correlation between $\Delta^{13}C$ and $\delta_C-\delta_W$ except for a positive correlation in the 10MGR group (Table 5).

The amount of carbon derived from metabolism was estimated to constitute 28% in both feeding levels at 6 °C, and 30% and 32% in the HGR10 and MGR10 groups, respectively.

Table 5
Correlation within each group of $\delta_C-\delta_W$ versus $\Delta^{13}C$, and $\delta_C-\delta_W$ and $\Delta^{13}C$ versus otolith and somatic growth rate

	6HGR	6MGR	10HGR	10MGR
$\delta_C-\delta_W$ versus $\Delta^{13}C$	−0.02	0.14	0.34	**0.47**
$\delta_C-\delta_W$ versus				
Otolith growth rate	**0.58**	0.24	−0.05	0.02
Somatic growth rate	**0.42**	0.25	−0.26	0.15
$\Delta^{13}C$ versus				
Otolith growth rate	−0.41	**−0.49**	**−0.53**	−0.39
Somatic growth rate	**−0.61**	**−0.58**	**−0.66**	−0.42

Bold R values are significant at $\alpha = 0.05$.

4. Discussion

By manipulation of temperature and prey levels we produced juvenile cod with different otolith and somatic growth rate. The DW-specific otolith growth was both dependent on temperature and feeding level. We found that metabolic effects were influencing carbon but not oxygen isotopic composition of the otolith. No effect of otolith precipitation rate was found on either the carbon or oxygen isotopes in the otoliths.

The temperature-dependent DW-specific otolith size was expected since several researchers have reported similar patterns (Mosegaard et al., 1988; Hoff and Fuiman, 1993; Wright et al., 2001; Otterlei et al., 2002). However, the magnitude of the differences, Q_{10} values of 2.3 and 2.8, was relatively large. Estimated weight increase of otoliths in Atlantic salmon (*Salmo salar* L.) reared at 5 and 15 °C yielded a mean Q_{10} value of 1.8 (Wright et al., 2001). Arctic charr (*Salvelinus alpinus* L.) reared at 8 to 15.9 °C had Q_{10} values up to 2.3 for corresponding temperature-dependent otolith weight increase (Mosegaard et al., 1988). At 10 °C we found that slow-growing fish had larger otoliths at a given length than faster-growing fish. This is also a well-known phenomenon described for several fish species (Mosegaard et al., 1988; Reznick et al., 1989; Secor and Dean, 1992). From the present experimental design we were thus able to produce similar-sized larval and early juvenile cod otoliths which had varying precipitation rates. Oxygen consumption of cod from the same experimental tank showed that larvae reared at 10 °C had higher resting metabolism than larvae reared at 6 °C ($Q_{10}=2.4$), but there was no difference between the feeding levels within the same temperature groups (unpublished data). The larger otolith size of slow-growing fish in the 10 °C group might therefore not only be related to metabolic difference caused by varying food concentrations. Wright et al. (2001) also concluded that there is no direct relationship between otolith growth and resting metabolic rate.

This study examined otoliths of similar-sized juvenile grown at different growth rates that therefore were of different age depending on the treatment (51–86 days old) at the time of sampling. Several other studies have shown a general increase in otolith $\delta^{13}C$ by age for adult cod, where the $\delta^{13}C$ values reached maximum approximately at time of sexual maturation (Schwarcz et al., 1998; Weidman and Millner, 2000; Gao et al., 2001; Begg and Weidman, 2001). However, this $\delta^{13}C$ increase by age has been attributed to varying fish diet and decreasing metabolism by age. Larval and juvenile cod in the size range from hatching to sampling in this study (5–21 mm length) undergo substantial developmental changes (Hunt von Herbing et al., 1996). Since larval length is a better measurement of ontogenetic state than age and somatic dry weight (Fuiman et al., 1998) we chose a sampling protocol based on similar fish length.

5. Metabolic effect

The data of this study confirms that somatic growth rates do not affect deposition of oxygen isotopes in otoliths. However, we found that there were differences in $\Delta^{13}C$ between 6 and 10 °C and between otoliths of fish with different growth rates at 10 °C.

Both somatic and otolith growth rates were similar for fish in the 6HGR and 10MGR, while the $\Delta^{13}C$ was 0.69‰ lower in the 10MGR group compared to the 6HGR group. The difference between these two groups must be related to metabolic effects. The estimated inclusion of metabolic carbon in the 6HGR and 10MGR groups was 28% and 32%, respectively. The significant negative correlations between $\Delta^{13}C$ and somatic growth rate within three of the four groups are also strong indications of depletion of ^{13}C in the otoliths at higher respiration. These observations are in agreement with other models on fractionation of carbon and oxygen isotopes in biogenic carbonates. The model by Schwarcz et al. (1998) predicts that increased metabolism of the fish leads to a depletion of ^{13}C in the otoliths and hence a lower $\delta^{13}C$. This model is also supported by data of various otoliths presented by Kalish (1991b). However, Thorrold et al. (1997) reported a positive correlation between both otolith growth and somatic weight versus $\Delta^{13}C$ for Atlantic croaker. We cannot give any explanations for the inconsistency of the results in the study of Thorrold et al. (1997) and our results.

The higher $\Delta^{13}C$ values found in fast growing otoliths in the 10HGR group compared to the 10MGR group are opposite of that expected if we assume that the HGR group had higher metabolic load than the MGR group. The phenomenon cannot be explained by differences in $\delta^{13}C$ of the food since the prey items offered were the same for all groups. In general, the $\delta^{13}C$ of the prey items decreased from a value of -24.0‰ at the start of the experiment to -20.4‰ at the end. The fish in the 10HGR group, which had higher $\delta^{13}C$ than expected, were sampled earlier in the experiment than the other fish. Although the resting metabolism of fish in 10HGR and 10MGR was similar, the fish might have experienced different metabolism related to behavioural differences in feeding pattern. High temperature increases the basal metabolism of fish which causes a higher demand for food, and low prey density can have increased inter-fish competition for food. Increased swimming activity when prey density was reduced (Munk, 1995) or during starvation (Skiftesvik, 1992) has been found for cod larvae, and this can increase the overall metabolism. Also, in a study of juvenile cod, restricted food availability leads to increased swimming activity and metabolism equal to 60% higher standard metabolism compared to fish fed to satiation (Bjørnsson, 1993). Since the fish were sampled at equal size, the lower $\Delta^{13}C$ of otoliths from fish in the 10MGR group compared to the 10HGR group can be a result of behavioural differences. Lower basal metabolism may have prevented this phenomenon from becoming apparent at 6 °C. In addition, the larger variance in the carbon isotopes compared to the oxygen isotopes in the otoliths in all groups can be caused by individual differences in behaviour such as swimming speed and feeding activity.

Our null-hypothesis of no metabolic effects of fractionation of isotopes in cod otoliths cannot be rejected for the oxygen isotopes but is rejected for the carbon isotopes.

6. Kinetic effects

We found no evidence of any kinetic effects on carbon and oxygen isotopes in cod otoliths. The values of $\delta_C - \delta_W$ are within the 95% confidence limit range of the

equation of inorganic calcite (enriched 0.6‰ for aragonite) found by Kim and O'Neil (1997). We therefore conclude that the fractionations of oxygen isotopes in the cod otoliths are in equilibrium with the surrounding seawater. Kinetic fractionation acts simultaneously on both carbon and oxygen isotopes and causes the lower $\delta^{13}C$ and $\delta^{18}O$ values of the $CaCO_3$ formed at higher precipitation rates (McConnaughey et al., 1997). We found no difference in $\delta_C-\delta_W$ values between slow- and fast-growing otoliths at 6 and 10 °C and in $\Delta^{13}C$ between slow- and fast-growing otoliths at 6 °C. Also, we only found a significant positive correlation between $\delta_C-\delta_W$ and $\Delta^{13}C$ in the 10MGR group, and not in the 10HGR group which had a higher otolith growth rate. The higher $\Delta^{13}C$ values in the 10HGR group than in the 10MGR group cannot be attributed to kinetic effects since fast-growing carbonates discriminate against the heavier isotope. In two studies of scleractinian corals the growth was thoroughly measured by staining with alizarine-red S (Leder et al., 1996; Swart et al., 1996). In spite of the difference in coral growth up to 6.6 mm $year^{-1}$, neither the fractionation of carbon nor of oxygen isotopes was different between the slow- and fast-growing parts. This is also in accordance with Romanek et al. (1992) who found no effect of precipitation rate on the $\delta^{13}C_{arg}-\delta^{13}C_{HCO_3^-}$ relationship for inorganic aragonite. Kim and O'Neil (1997) produced inorganic calcite at different precipitation rates, but found that it had little or no influence on the oxygen isotopic composition of the product. We found that otolith growth rate and $\Delta^{13}C$ were negatively correlated, but this was most likely caused by higher metabolism and not by kinetic effects. Kinetic effects therefore do not seem to influence fractionation of carbon and oxygen isotopes in cod otoliths. The null-hypothesis of no kinetic effect operating on the fractionation of oxygen and carbon isotopes in cod otoliths is therefore not rejected.

Several attempts have been made to establish the relationship between $\delta_C-\delta_W$ of otoliths and temperature (Kalish, 1991a; Patterson et al., 1993; Radtke et al., 1996, 1998; Thorrold et al., 1997), but there are surprisingly large deviations between the equations. The reasons for these differences remain unclear. Our results, confirming that kinetic and metabolic effects do not influence the fractionation of the stable oxygen isotopes in cod otoliths, imply that the reasons for the differences between the various equations are probably not related to biological processes, or that there exist species-specific differences in the fractionation of the stable isotopes. However, when the relationship between $\delta_C-\delta_W$ of otoliths and temperature is established for a species, analyses of otolith $\delta^{18}O$ should be a good proxy for ambient temperature experienced by the fish regardless of fish growth rate and metabolic load.

The effect of lower $\Delta^{13}C$ values at higher metabolism confirms previous studies on fish otoliths where a general increase in $\delta^{13}C$ by age is believed to be caused by a combination of decreased metabolism and change in diet with age (Schwarcz et al., 1998; Weidman and Millner, 2000; Gao et al., 2001; Begg and Weidman, 2001). The relative magnitude of these two mechanisms is not clear and needs to be established by long time rearing experiments under controlled conditions. By considering the possible factors that can cause increasing $\delta^{13}C$ values with age in cod otoliths from one area, the Barents Sea, one might sort out the relative importance of the different factors. For cod from the Barents Sea, the otolith $\delta^{13}C$ values generally increased from a value of −4‰ in the first year of life to 0‰ at age 3–4 years (Weidman and

Millner, 2000). Lower ambient temperature by age could possibly explain some of this trend. Increasing temperature from 6 to 10 °C in this study caused the otolith $\Delta^{13}C$ to decrease by 0.4‰ to 0.6‰ in the HGR and MGR groups, respectively. However, cod in the Barents Sea experienced quite stable ambient temperature in their first 3–4 years, the temperature range is often less than 2 °C at these ages (Ottersen et al., 1998). Also, the trend is increasing temperature with age in this period which would lead to lower $\delta^{13}C$ values of the otoliths. Increased metabolism through increased ambient temperature cannot therefore explain the increased $\delta^{13}C$ of the otoliths with age.

Lower metabolic load at larger fish size is also suggested to explain the increasing $\delta^{13}C$ of the otolith at age. The weight-specific oxygen consumption of a 3-kg cod is 22% lower than for a 1-kg cod (Edwards et al., 1972). For comparison, the mass-specific routine rate of oxygen uptake for juvenile cod of 10 mg DW increased 34% when increasing temperature from 7 to 10 °C (Finn et al., 2002). Since the temperature-specific reduction in otolith $\Delta^{13}C$ in this study was 0.4–0.6‰, the reduced metabolism with age should therefore account for a lower reduction than the observed decrease in otolith $\delta^{13}C$ with age.

Changing diet is also a factor to be considered. Generally for north Atlantic cod stocks, the main prey for young cod is crustaceans but there is a shift towards fish prey with increasing age (Pálsson, 1994). The position in the web-chain for cod therefore increases by 2–3 steps through its lifetime, and there is a general increase of ^{13}C in the animals by 1‰ per tropic level (Peterson and Fry, 1987; Fry, 1988). Since 28–32% of the carbon in the otoliths is of metabolic origin, one can expect that more than 1‰ of the observed increase in otolith $\delta^{13}C$ with age is caused by change in the diet. The $\delta^{13}C$ of the seawater will obviously also influence the otolith $\delta^{13}C$ values. The $\delta^{13}C$ value of the seawater in the Barents Sea is not available. However, the cod in the area are quite stationary, the first 2 years of life inhabiting the same water masses. It is therefore unlikely that $\delta^{13}C$ of the seawater could be responsible for the large change in otolith $\delta^{13}C$. These considerations suggest that the most important reason for the decrease in otolith $\delta^{13}C$ by age found in cod from the Barents sea is a change in the diet. The decreasing metabolism with increased fish size is also of importance but probably to a lesser extent than the change in diet.

The use of information from measurements of both carbon and oxygen isotopes in otoliths can be a powerful combination. The ambient temperature experienced by the fish can be estimated with a high degree of precision and accuracy if the $\delta^{18}O$ of the water is known or can be estimated through knowledge of the salinity and oxygen isotope mixing lines. Given that the basal metabolism can be estimated through temperature estimates using the oxygen isotopes, one can possibly gain insight into which tropic level the fish inhabit and its overall metabolism through the carbon isotopic composition. With the use of computer-controlled micromill machines, sampling with high temporal resolution in the fish life can be performed (Wurster et al., 1999). However, photosynthetic activity alters the $\delta^{13}C$ content of the water both spatially, from about 2‰ near the surface to 0‰ near the bottom, and seasonally (Kroopnick, 1980). This will affect the accuracy of the estimates if detailed knowledge of $\delta^{13}C$ of the water is not known. Also, more detailed knowledge of the link between fish metabolism and

$\delta^{13}C$ of the otoliths is clearly needed before otolith $\delta^{13}C$ values can be used to gain information about the feeding pattern of fish.

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