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Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua* L.)

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Analysis of stable oxygen isotopes in otoliths is a promising technique for estimating the ambient temperature experienced by fish, but consistent equations relating temperature and fractionation of stable oxygen isotopes in otoliths among different fish species are lacking. Juvenile cod were reared at constant temperatures from 6 to 20 °C and the sagittal otoliths were analysed for oxygen isotope values. We determined that temperature-dependent fractionation of oxygen isotopes in the otoliths was close to that reported for inorganic aragonite at low temperatures, but there were deviations from oxygen isotope fractionation equations for otoliths of other species. The linear relationship between oxygen isotope value in the cod otoliths and temperature was determined to be: $1000\,\mathrm{Ln}\,\alpha = 16.75(10^3\,\mathrm{TK}^{-1}) - 27.09$. Temperature estimates with 1 °C precision at the 95% probability level require a sample size of ≥ 5 otoliths. Only an insignificant amount of the variance in the data was due to variance between left and right otolith, and due to repeated measurements of otolith subsamples. This study confirms that stable isotope values of cod otoliths can give precise and accurate estimates of the ambient temperature experienced by fish.

Keywords: cod, otolith, oxygen isotopes, precision, temperature.

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Introduction

Proper management of fish stocks relies partly on information about the spatial and temporal distribution of the stocks and the environmental conditions they experience. Temperature is a key environmental factor since it directly influences somatic growth rate (e.g. Brett, 1979). An estimation of the ambient temperature history experienced by the fish is also important for migration studies and stock separation purposes because different water masses often have characteristic temperatures (Furevik, 2001). The distribution of stable isotopes in biogenic carbonates such as otoliths has been widely used to estimate paleotemperatures (Patterson, 1999; Ivany *et al.*, 2000; Carpenter *et al.*, 2003). Analysis of stable isotopes in otoliths is also considered a promising tool for fisheries management (Campana, 1999). Varying proportions of the stable ^{18}O–^{16}O isotopes ($\delta^{18}O$) in otoliths are dependent upon the temperature and isotope value of the water that the fish live in at the time of precipitation. Thermodynamic relationships result in otoliths containing more of the lighter ^{16}O isotope at higher temperatures. The temperature history experienced by fish can therefore be deduced from the otoliths if the $\delta^{18}O$ of the water and the temperature-dependent fractionation of oxygen isotopes during otolith formation are known (Patterson *et al.*, 1993).

Several studies have estimated the temperature-dependent fractionation of oxygen isotopes in otoliths and concluded that the isotopes are deposited at near equilibrium with the ambient water. However, a consistent equation relating temperature and oxygen isotope fractionation in otoliths of different fish species remains elusive. Patterson *et al.* (1993) analysed otoliths of several freshwater species and found the temperature-dependent increase in $\delta^{18}O$ to be similar to the values based on aragonitic foraminifera *Hoeglundina elegans* (Grossman and Ku, 1986), but the otoliths had somewhat lower $\delta^{18}O$ values. A relationship based on otoliths of the Atlantic croaker (*Micropogonias undulatus*) (Thorrold *et al.*, 1997) had the same temperature-dependent $\delta^{18}O$ slope but a higher intercept value than the relationship presented by Patterson *et al.* (1993). The temperature-dependent $\delta^{18}O$ increase of Australian salmon

(*Arripis trutta*) otoliths was different from the relationships obtained by the aforementioned authors (Kalish, 1991a). The differences in intercepts between these studies are substantial and result in a discrepancy of 4.4 °C of estimated temperature between the relationships of Patterson *et al.* (1993) and Thorrold *et al.* (1997). Analytical imprecision due to estimated temperatures and $\delta^{18}O$ values of water in most of the studies is likely responsible for the observed differences. However, species-specific oxygen fractionation may also play a role such that there is a need to establish the relationship for other fish species. Two papers have addressed the temperature-dependent fractionation of stable isotope composition in cod otoliths. Radtke *et al.* (1996) analysed otoliths of cod reared at temperatures from 9 to 16 °C but the validity of the results has been questioned due to erroneous calculations (Radtke *et al.*, 1998). Gao (2003) also presented data on temperature-dependent fractionation of stable isotope composition in cod otoliths, but it was based on field data with limited environmental information and a narrow temperature range. In this study we present a relationship of the stable oxygen isotope composition of otoliths of juvenile cod reared at constant temperatures from 6 to 20 °C. Although one key parameter, the $\delta^{18}O$ values of water, was not measured but estimated through a isotope mixing line, the large number of otoliths analysed and a large temperature range resulted in a robust design that also allowed estimation of the precision of the temperature estimate.

Materials and methods

Otoliths were obtained from juvenile cod reared in laboratory in 1991 (Exp. I) and 1995 (Exp. II). The juveniles originated from a local broodstock of coastal cod and were collected from the juvenile production pond Parisvatnet. The pond is a natural seawater enclosure situated northwest of Bergen at 60°38′N, 4°49′E. It has a maximum depth of 9 m, a surface area of 50 000 m^2, and a volume of 270 000 m^3 (Blom *et al.*, 1994). About 4000 juvenile cod (weight range 1.2–1.9 g) in each experiment were transferred to the experimental facilities at the Department of Fisheries and Marine Biology, University of Bergen. The fish were transferred to 500-l tanks at stocking densities of 50 and 100 fish per tank in Exp. I and II, respectively. Subsamples of 20–22 fish from each temperature group in each experiment (subpopulation) were analysed (Table 1).

Fish were collected from the pond on 10 June in Exp. I and on 4 July in Exp. II, and were acclimated to the rearing temperatures at a rate of 2 °C d^{-1}. In Exp. I the fish were reared at three different mean temperatures (±standard deviation: s.d.), 6.2±0.2, 10.1±0.3, and 13.9±0.6 °C, and in Exp. II at five different mean temperatures, 12.0±0.1, 14.0±0.2, 16.0±0.1, 18.0±0.2, and 20.0±0.1 °C. The seawater was supplied from 90-m depth and the desired temperature was obtained by mixing cold (4 °C) and warm (22 °C) aerated water. Water flow through the tanks ranged from 8.1 to 23.4 l min^{-1} and from 7.0 to 10.6 l min^{-1} in Exp. I and II, respectively. Continuous light was used in Exp. I and a simulated natural light regime was used for the latitude of Bergen (60°25′N) in Exp. II. Commercial dry diet was offered in excess in both experiments. In Exp. I, 15 fish from each temperature group were sampled 7 d after a constant temperature was reached. The otoliths of these fish serve as a baseline for otolith material deposited before the laboratory experiment. The experiment lasted 56 d. In Exp. II, 23 baseline fish were sampled 1 d after the fish were transferred from the pond to the laboratory, which was the day the fish were distributed to the respective tanks. The constant temperature conditions were then reached after 0, 1, 2, 3, and 4 d for the 12, 14, 16, 18, and 20 °C groups, respectively. The experiment was terminated 65 d after the transfer from the pond. A detailed description of the rearing conditions, temperature-dependent somatic growth, and survival is presented in Otterlei (2000).

Daily weight-specific somatic and otolith growth rates were calculated as

$$G_0 = (e^g - 1) \times 100 \quad (1)$$

where

$$g = \left(\frac{\ln W_2 - \ln W_1}{t_2 - t_1} \right) \quad (2)$$

and W is the average weight at days t_1 and t_2.

The fish were measured for length and weight at the end of the experiment (only Exp. II) and stored at −35 °C. Left and right sagittae were removed from the juvenile cod during the summer of 2000. The otoliths were cleaned ultrasonically in distilled water and weighed to the nearest microgram using a Sartorius Micro M3P balance after being dried at 60 °C for 24 h. The otoliths were completely homogenized by a glass rod just prior to isotope measurement. A subsample of 85–100 μg of the otolith powder was used for analysis. The otoliths were measured for oxygen isotope composition by a Finnigan MAT 251 mass spectrometer. Precision of the measurements was 0.07‰ for $\delta^{18}O$ (s.d. for standard), corresponding to 0.3 °C when using the relationship for inorganic calcite (Kim and O'Neil, 1997). Isotope values are reported in δ notation relative to the Vienna Pee Dee belemnite (VPDB) standard by the International Atomic Energy Agency, Vienna:

$$\delta = \left(\frac{R_{sample} - R_{standard}}{R_{standard}} \right) \times 1000(‰) \quad (3)$$

where R is the ratio of ^{18}O:^{16}O in the sample or standard. Positive values thus indicate enrichment of the heavy isotope.

A mass balance relationship was used to calculate the isotope composition of new otolith material in the period the fish were held in the laboratory (Kalish, 1991a):

Table 1. Mean wet weight (g) at termination of the experiment for the population, for the baseline fish, and for the subpopulation used for otolith analysis. Numbers in parenthesis are 1 s.d. of the mean. The fish were measured alive, except for those marked with * that were measured just prior to otolith removal. G_0 is for the last eight weeks of the experiment. Mean individual otolith weight and $\delta^{18}O$ for each group are also given.

		Population			Subpopulation			
Exp.	Group	Weight (g)	n	G_0 (% d^{-1})	Weight (g)	Otolith weight (mg)	Otolith $\delta^{18}O$	n
I	6°C	4.91 (2.33)	46	1.99	4.39 (2.30)*	4.25 (1.42)	2.79 (0.31)	20
I	Baseline 6°C				1.63 (0.62)*	1.53 (0.34)	1.44 (0.11)	15
I	10°C	9.74 (4.72)	50	2.99	8.71 (3.93)*	6.70 (1.86)	1.56 (0.20)	20
I	Baseline 10°C				1.60 (0.34)*	1.65 (0.26)	1.21 (0.11)	15
I	14°C	18.08 (7.72)	50	3.83	17.69 (7.42)*	10.62 (2.33)	0.69 (0.14)	20
I	Baseline 14°C				1.73 (0.56)*	1.84 (0.37)	0.83 (0.18)	13
II	12°C	21.71 (5.82)	196	3.61	20.80 (5.03)	11.06 (1.78)	1.09 (0.10)	22
II	14°C	23.94 (6.77)	196	3.65	24.21 (5.90)	11.97 (1.65)	0.64 (0.10)	21
II	16°C	23.90 (6.56)	193	3.54	26.73 (7.80)	13.38 (2.72)	0.22 (0.09)	20
II	18°C	18.62 (5.54)	187	3.07	20.65 (7.13)	11.63 (2.24)	−0.18 (0.09)	20
II	20°C	5.70 (1.75)	148	1.16	5.95 (1.54)	7.18 (1.17)	−0.52 (0.11)	20
II	Baseline	1.89 (0.47)	1 000		1.72 (0.51)*	1.79 (0.32)	−0.06 (<0.01)	23

*Measured in summer 2000 after storage in freezer.

$$\delta^{18}O_{total} = \delta^{18}O_{pre-exp}\left(\frac{M_{bc}}{M_{ac}}\right) + \delta^{18}O_{exp}\left(\frac{M_{ac} - M_{bc}}{M_{ac}}\right) \quad (4)$$

$\delta^{18}O_{total}$, $\delta^{18}O_{pre\text{-}exp}$, and $\delta^{18}O_{exp}$ refer to isotope ratios of the whole otoliths, of the otolith material prior to the experiment, and of the otolith material deposited after onset of the experiment, respectively. M_{bc} and M_{ac}, respectively, refer to the mean otolith mass prior to the onset of the experiment (the baseline fish) and otolith mass after termination of the experiment.

Since the baseline fish in Exp. II were sampled before constant temperature was reached in all the tanks, the otolith growth and isotope value during the temperature increase were corrected for by applying an expanded mass balance relationship. The average difference of estimated otolith $\delta^{18}O$ values after using the expanded mass balance relationship of otoliths of fish reared at 20°C compared with the measured otolith $\delta^{18}O$ values was 0.04‰, corresponding to 0.22°C when estimating temperature by using the relationship for inorganic calcite (Kim and O'Neil, 1997).

The oxygen isotope fractionation is expressed as 1000 Ln α where

$$\alpha = \frac{\delta^{18}O_{otolith} + 1000}{\delta^{18}O_{water} + 1000} \quad (5)$$

The conversion equation of the Vienna standard mean ocean water (VSMOW) standard (for water samples) vs. the VPDB standard (for carbonate samples) of Coplen *et al.* (1983) was used:

$$\delta^{18}O_{(VSMOW)} = 1.03091\ \delta^{18}O_{(VPDB)} + 30.91 \quad (6)$$

When expressing the relationship in terms of $\delta_C - \delta_W$ where δ_C represents the oxygen isotope composition of the otoliths and δ_W represents the oxygen isotope composition of the water, the conversion factor of $\delta^{18}O_{VSMOW}$ vs. δ_W of Friedman and O'Neill (1977) was used:

$$\delta_W = 0.99978(\delta^{18}O_{VSMOW}) - 0.22 \quad (7)$$

$\delta^{18}O$ values of water were not determined at the time when the experiment was conducted, but were estimated using an oxygen isotope mixing line from the North Sea (Israelson and Buchardt, 1991). The mean salinity of 34.7 and 33.1 PSU in Exp. I and II gave $\delta^{18}O$ values of 0.18 and −0.23‰, respectively. Water samples from the same inlet at 90-m depth were measured in spring 1999 and the oxygen isotope value was found to be 0.0‰ vs. the VSMOW standard. The same value was obtained when estimating it from the oxygen isotope mixing line from the North Sea.

The size of 95% prediction limit when estimating temperature from a 1000 Ln α value (estimating x from y) was calculated by

$$\overline{x} + \frac{b_{yx}(y_i - \overline{y})}{D} \pm H \quad (8)$$

where

$$H = \frac{t_{0.05[n-2]}}{D}\sqrt{MSE\left[D\left(1 + \frac{1}{n}\right) + \frac{(y_i - \overline{y})^2}{\sum x^2}\right]}$$

and

$$D = (b_{yx})^2 - (t_{0.05[n-2]})^2\left(\frac{MSE}{\sum x^2}\right)^2,$$

where MSE is the mean square error, and b_{yx} is the slope of the 1000 Ln α vs. temperature ($10^3\ TK^{-1}$, K is degree kelvin) relationship (Sokal and Rohlf, 1994).

To test for the magnitude of variance of intra- and inter-specimen differences (between fish and between left and

right otolith), a nested ANOVA (mixed model) was performed where fish number was nested as a random effect within temperature groups. The sum of squares of error then contains the component variance between left and right otolith (in addition to the unexplained variance). In order to have a balanced design, 19 fish in each temperature group were selected randomly to be included in the analysis. Because fish were reared at 14 °C in both Exp. I and II, two nested ANOVAs were performed including one of the 14 °C group each time. Otoliths of eight fish in each of the 12, 16, and 20 °C temperature groups were measured once again several months later to test for variance between otolith subsamples. The data were analysed by a nested ANOVA (mixed model) where fish number was nested in temperature groups and otoliths (left and right) was nested in temperature groups and fish numbers. Fish number and otoliths were set as random effects and temperature groups were set as a fixed effect. The sum of squares of error then contains the component variance between subsamples (in addition to the unexplained variance).

Results

Somatic growth

Growth rate of all fish in the tanks (population) was significantly influenced by temperature with increasing mean weight from 6 through 14 °C in Exp. I. Final weight of the population in Exp. II increased at temperatures from 10 to 16 °C but decreased at higher temperatures (Table 1). The fish analysed in this experiment (the subpopulation) showed the same pattern in weight as the mean of all fish (population) (Table 1). The otolith weight was also clearly influenced by temperature with increasingly larger otoliths at 6–14 °C in Exp. I. As with dry weight in Exp. II, the otolith weight of fish reared at different temperatures had a bell-shaped relationship, with the highest otolith weight at 16 °C.

Isotope composition of the otoliths

The mean $\delta^{18}O$ values (±s.d.) of the baseline otoliths were 1.44(±0.11), 1.21(±1.11), and 0.83(±0.18) in the 6, 10, and 14 °C groups in Exp. I, respectively, and −0.06(±0.05) in Exp. II (Table 1). All values were significantly different from each other (ANOVA, $p<0.001$). The relationship between 1000 Ln α (mean values of left and right otolith) vs. temperature was best described by a second order polynomial equation (±95% confidence limit range):

$$1000\,\mathrm{Ln}\,\alpha = -180.00(\pm 153.72)\,(10^3\,\mathrm{TK}^{-1}) + 28.15(\pm 21.99)\,(10^3\,\mathrm{TK}^{-1})^2 + 316.62(\pm 268.55),\quad r^2 = 0.95,\ n = 163 \qquad (9)$$

Although the second order term was highly significant ($p<0.001$), the maximum deviation of estimated temperature between the second order polynomial equation and a linear equation was minor; 0.6 and 0.7 °C at the lowest and highest temperatures, respectively. In order to be able to compare our results with others that have reported linear relationships between temperature and oxygen isotope fractionation, we chose to use the linear equations in the following.

A linear regression through the data of temperature and oxygen isotope fractionation yields the relationship:

$$1000\,\mathrm{Ln}\,\alpha = 16.75(\pm 1.33)\,(10^3\,\mathrm{TK}^{-1}) - 27.09(\pm 4.62),\quad r^2 = 0.94,\ n = 163 \qquad (10)$$

(Figure 1), or presented as $\delta_C - \delta_W$ for a given °C change:

$$\delta_C - \delta_W = 3.90(\pm 0.24) - 0.20(\pm 0.019)\,(T\,^\circ C),\quad r^2 = 0.94,\ n = 163 \qquad (11)$$

The mean otolith 1000 Ln α at 14 °C was significantly different between Exp. I and II (t-test, $p<0.001$), and the separate slopes of the relationship for Exp. I and II were significantly different ($MS = 0.94$, $F_{1,159} = 38.17$, $p<0.001$).

Figure 1 shows the temperature-dependent fractionation of oxygen isotopes in otoliths found in this and other studies, and the equations of the relationships are given in Table 2. The slope of Equation (10) is significantly different from the slope of the relationship found by Patterson *et al.* (1993) ($MS = 0.96$, $F_{1,208} = 16.95$, $p<0.001$). The slope of the relationship for inorganic aragonite (calcite enriched 0.6‰) (Kim and O'Neil, 1997) was not different from that found in this study ($MS = 0.17$, $F_{1,168} = 3.87$, $p > 0.05$), but the two relations had different intercepts ($MS = 1.04$, $F_{1,169} = 22.90$, $p<0.001$). By comparing Equation (10) with the relationship presented by Thorrold *et al.* (1997) we found a marginally significant difference between the slopes ($MS = 0.21$, $F_{1,232} = 4.25$, $p = 0.04$). Radtke *et al.* (1996, 1998) did not present the individual data of oxygen isotope composition of cod otoliths, so a comparison with our data by analysis of covariance was not possible. However, both the intercept and the slope of the equation of Radtke *et al.* (1996, 1998) were within the 95% confidence limits of Equation (11), so we concluded that there was no significant difference between those two equations. Gao (2003) also presented an equation of the temperature-dependent fractionation of oxygen isotopes in cod otoliths based on field data, but the isotope value of the water was not related to the VPDB scale. A direct comparison with the aforementioned equations was therefore not possible. However, we calculated the water temperature from oxygen isotope ratio of the otoliths using the $\delta^{18}O_W$ values related to the VSMOW scale as input in the equation given by Gao (2003). The deviations of estimated temperature using Equation (11) and the equation presented by Gao (2003) varied from 0.20 to −0.25 °C at 6 and 20 °C, respectively.

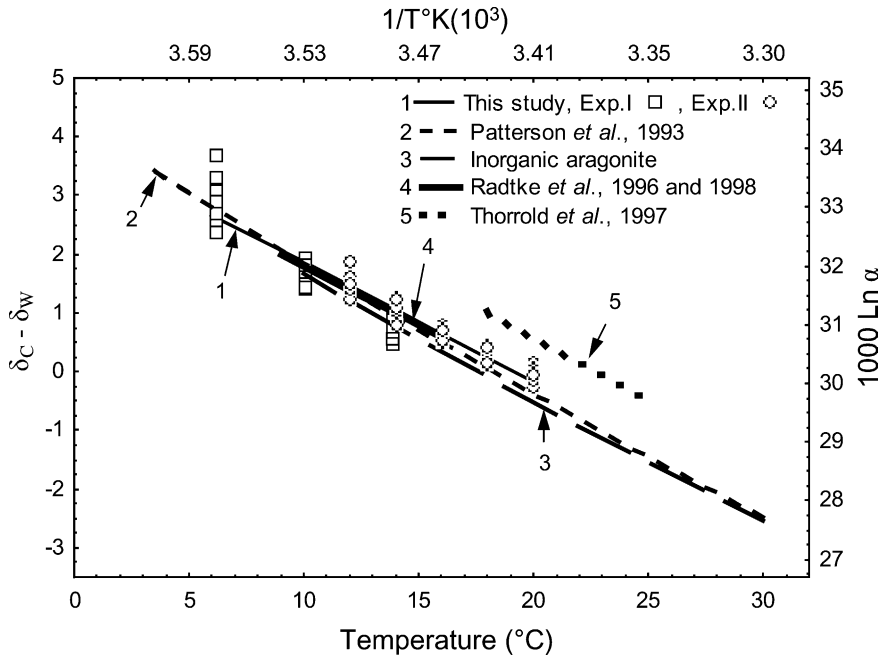

Figure 1. Relationship between temperature and fractionation of oxygen isotopes in otoliths. Mean values of left and right otolith in this study are given. The relationship for inorganic calcite (Kim and O'Neil, 1997) enriched 0.6‰ (aragonite) is also shown.

Precision of temperature estimate

By using data from Exp. I ($n = 60$) and II ($n = 103$) separately, the size of 95% prediction limits of a single observation of 1000 Ln α corresponds to ± 1.6 and ± 1.0 °C, respectively, and sample sizes of 13 and 5 are required to obtain a 95% prediction limit size better than ± 0.5 °C. The size of the 95% prediction limit of a single observation of 1000 Ln α corresponds to ± 2.1 °C when using the combined data of Exp. I and II (Equation (10)). These results must be viewed in light of the measurement error of the mass spectrometer that constitutes 95% prediction limits of 0.59 and 0.18 °C at 1 and 10 measurements, respectively. The size of the prediction limits varied for a given 1000 Ln α value, but the variation in the temperature range in this study was negligible (< 0.01 °C).

Sources of variance in the otolith material

When the variance of the otolith material is attributed to the different levels (nested ANOVA, Table 3), it is abundantly evident that the majority of the variance (97.6%) is caused by the temperature treatment. The inter-fish differences and difference between left and right otolith (plus the unexplained variance) constituted 1.5 and 0.9% of the total variance, respectively. We obtained the same results with either the Exp. I or II 14 °C groups included in the analysis. Generally, the same pattern was seen when additional measurements on the same material were also included for three of the temperature groups (Table 4). More than 95% of the overall variance was attributed to the temperature treatment. When selected otoliths were re-sampled after several months, the additional measurements contributed little to the overall variance in the data (only an additional

Table 2. Temperature-dependent fractionation of oxygen isotopes in otoliths estimated from laboratory experiments. The equation of inorganic aragonite is also given. Values in parenthesis are 95% confidence limits of the respective parameters.

Author(s)	Equation	Species
This study	$\delta_C - \delta_W = 3.90(\pm 0.24) - 0.20(\pm 0.019)$ T °C 1000 Ln $\alpha = 16.75(\pm 1.33)$ $(10^3\ TK^{-1}) - 27.09(\pm 4.62)$	*Gadus morhua*
Patterson *et al.*, 1993	1000 Ln $\alpha = 18.56(\pm 0.32)$ $(10^3\ TK^{-1}) - 33.49(\pm 0.307)$	Several freshwater species
Tarutani *et al.*, 1969; Kim and O'Neil, 1997	1000 Ln $\alpha = 18.03$ $(10^3\ TK^{-1}) - 31.82$	Inorganic aragonite
Thorrold *et al.*, 1997	1000 Ln $\alpha = 18.56$ $(10^3\ TK^{-1}) - 32.54$	*Micropogonias undulatus*
Radtke *et al.*, 1996, 1998	$\delta_C - \delta_W = 3.793 - 0.200$ T °C	*Gadus morhua*

Table 3. Summary of nested ANOVA (mixed model) of 1000 Ln α where fish number is nested in temperature groups. Fish number is a random effect, and temperature is a fixed effect. The error term consists of variance between left and right otolith (and the unexplained variance). The results are based on the analysis with the 14 °C group in Exp. I included.

	Sum of squares	Mean squares	d.f.	f-ratio	p Value	Variance component (%)
Temperature	294.88	49.14	6	4135.38	<0.001	97.6
Fish	6.54	0.051	126	4.31	<0.001	1.5
Error (otolith)	1.58	0.012	133			0.9

Table 4. Summary of nested ANOVA (mixed model) of additional measurements of 1000 Ln α in three temperature groups (12, 16, and 20 °C in Exp. II) where fish number is nested in temperature groups and otoliths are nested in temperature groups and fish numbers. Fish number and otolith are random effects, and temperature is a fixed effect. The error term consists of variance between the two measurements (and the unexplained variance).

	Sum of squares	Mean squares	d.f.	f-ratio	p Value	Variance component (%)
Temperature	38.89	19.44	2	482.06	<0.001	95.2
Fish	0.85	0.040	21	4.40	<0.001	1.6
Otolith	0.22	0.011	24	0.56	0.93	0.7
Error (additional measurement)	0.78	0.016	48			2.5

2.5%). Temperature-specific values of measurement 2 minus measurement 1 of 1000 Ln α were analysed by one-way ANOVA. No differences were found between the three temperature groups ($p>0.05$). Also, 95% confidence intervals of measurement 2 minus measurement 1 of 1000 Ln α in two of the three temperature groups included zero, meaning that the additional measurements were not significantly different.

There was apparently no difference in 1000 Ln α between left and right otolith (one-way ANOVA, $p = 0.93$). The 1000 Ln α value of left minus right otolith vs. temperature relationship had both a slope and an intercept indistinguishable from zero (linear regression, $p = 0.44$ and 0.38, respectively, Figure 2). The range in the 1000 Ln α values between left and right otolith was almost 0.5‰ in some of the temperature groups. However, an estimated temperature difference using left and right 1000 Ln α of a pair corresponds to less than 0.5 °C temperature difference in the majority of the observations when using Equation (10) (Figure 3).

Discussion

The relationship between the oxygen isotope values of the cod otoliths at different temperatures found in this study is similar to the relationship Radtke *et al.* (1996, 1998) found for cod otoliths in a narrower temperature range. Our data are also in agreement with the result obtained by analyses

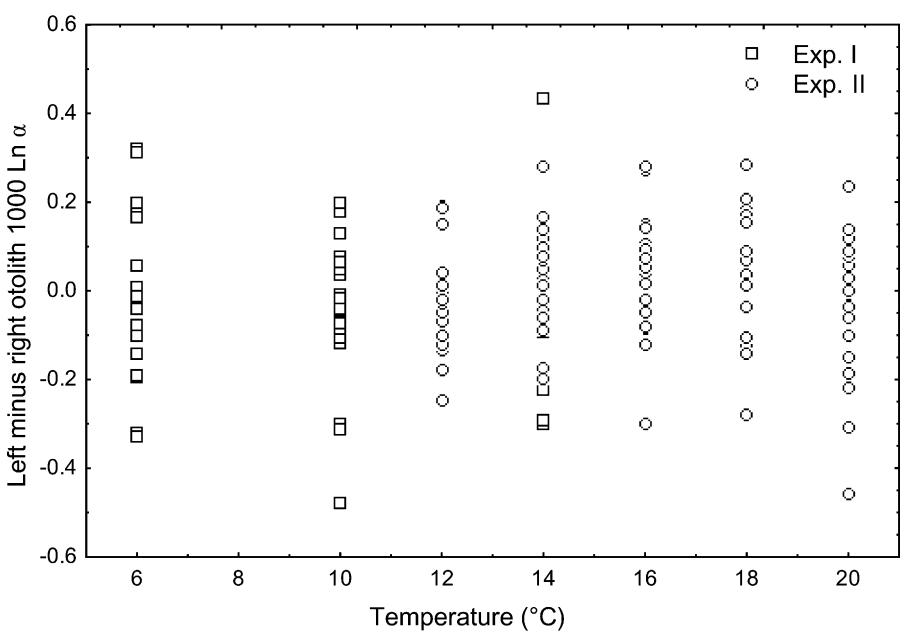

Figure 2. Left minus right otolith 1000 Ln α at different temperatures.

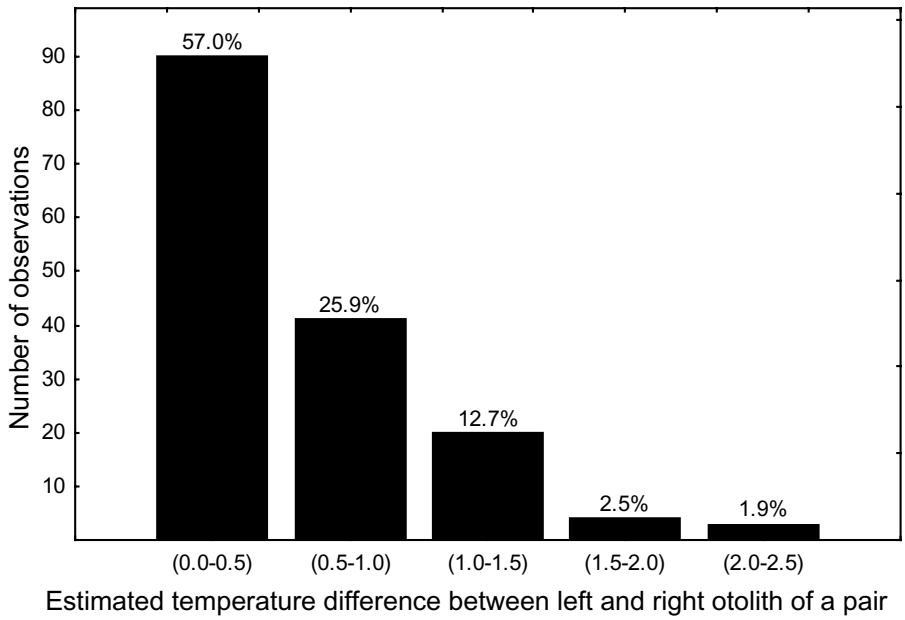

Figure 3. Estimated temperature difference between left and right otoliths of a pair when using Equation (10). The number on each bar represents the percent distribution.

of wild caught cod otoliths (Gao, 2003). Based on the consistency of these three separate studies, we conclude that the relationship between oxygen isotope values of cod otoliths at different temperatures (Equations (10) and (11)) is appropriate for the estimation of temperature from cod otolith $\delta^{18}O$ values. Although temperature estimates within 1 °C accuracy of the measured temperature were obtained at the lower temperature range when using the equations of inorganic aragonite enriched 0.6‰ (Tarutani *et al.*, 1969; Kim and O'Neil, 1997) and the one presented by Patterson *et al.* (1993), other relationships produced temperature estimates with low accuracy compared to the measured water temperatures. It is therefore necessary to validate the temperature-dependent fractionation of oxygen isotopes in the otoliths for a species if the ambient temperature experienced by fish is to be estimated through otolith oxygen isotope value. The search for a common relationship describing stable oxygen isotope fractionation by temperature for all fish species may not be realistic. Although kinetic and metabolic effects do not seem to operate on $\delta^{18}O$ of otoliths (Thorrold *et al.*, 1997; Høie *et al.*, 2003), species-specific fractionation of oxygen isotopes can still not be excluded. Physiological adaptations to environmental conditions other than temperature could possibly influence the isotope value of otoliths. Reduced salinities led to lower $\delta^{18}O$ values of black bream (*Acanthopagrus butcheri*) otoliths (Eldson and Gillanders, 2002), but this is almost certainly caused by lower $\delta^{18}O$ values of the water at lower salinities. The reasons for the disparity between the different studies therefore remain largely unknown. The failure to measure all environmental parameters or instrumental artefacts can at least partly explain some of the variation. One other potential factor can be the crystalline morphs of otoliths that usually consist of aragonite (Degens *et al.*, 1969), but can be replaced by vaterite and calcite (Strong *et al.*, 1986; Gauldie, 1993; Brown and Severin, 1999). This replacement will affect the $\delta^{18}O$ values (Tarutani *et al.*, 1969). The mechanism behind these replacements in otoliths is not known, but can be genetically controlled (Brown and Severin, 1999). Future studies of oxygen isotope fractionation in otoliths should consider and test for the significance of crystal structure.

Several earlier studies interpreted $\delta^{18}O$ data of otoliths by using equations of temperature-dependent fractionation derived from other taxa (Kalish 1991a, b). Later studies have shown that other biogenic (non-otolith) carbonates can be deposited in disequilibria with the surrounding water (McConnaughey, 1989; Leder *et al.*, 1996). In light of this and other studies, we therefore conclude that care should be taken when choosing an equation for estimating ambient temperature through measurement of otolith oxygen isotope value. Further experiments for estimating the temperature-dependent fractionation of oxygen isotopes in otoliths for other species are therefore needed.

The relationship between stable oxygen isotopes in cod otoliths and temperature in this study was best described by a second order polynomial equation. Most other studies on biogenic carbonates have reported linear relationships between oxygen isotope ratio of the carbonate and temperature although polynomial fits also are reported

(Epstein and Mayeda, 1953; Craig, 1965; Anderson, 1990). Whether the fractionation of oxygen isotopes in cod otoliths reared at different temperatures causes a polynomial relation or if this is an effect of our methodology is not known. However, in the temperature range in our study the choice of linear or polynomial seems to be of minor importance.

The precision of temperature estimates using otoliths has earlier been questioned since Kalish (1991a) reported a need of 130 measurements (otoliths) to obtain a precision of 1 °C when using Australian salmon otoliths. The large number of samples analysed at each temperature gives us a good estimation of the precision of such analyses. The temperature estimation by use of a single $\delta^{18}O$ observation using data of Exp. II yields a 95% prediction limit size of ± 1.0 °C, although lower precision was obtained using the combined data of Exp. I and II. It is therefore possible to obtain a precise and accurate estimate of the ambient temperature experienced by fish population with relatively few samples and at low cost. This assumption is based on accurate measurements or estimates of the $\delta^{18}O$ of the surrounding water.

There was a higher variance in otolith $\delta^{18}O$ values between fish than between left and right otoliths of individuals. This is in accordance with other studies that also reported minor intra-specimen variation in oxygen isotopes (Iacumin *et al.*, 1992; Meyer-Rochow *et al.*, 1992; Thorrold *et al.*, 1997), although Kalish (1991b) reported large differences between left and right otoliths of otolith pairs. There was also insignificant variance between additional measurements of the same otolith. As a consequence of this, effort should be put in analysing one sample of an otolith of several fish instead of analysing both sagittae of fewer fish or several samples of the same otolith when designing new experiments.

Application of oxygen isotope analysis of cod otoliths

Measurements of oxygen isotope composition of otoliths have been performed on a number of fish species and have been used to gain further insight into a variety of research areas (Campana 1999). The recent development of computer controlled micromill machines allows high spatial resolution sampling in otoliths which corresponds to high temporal resolution in the fish life (Wurster *et al.*, 1999). The otolith growth follows the fish somatic growth rather closely, so the annulus size of a typical cod otolith will decrease with increasing age. With a minimum sample size of 20 µg required for the mass spectrometer, it is possible to sample 10–30 samples per annulus in otoliths of five-year-old cod (Høie, unpublished data). The northeast Arctic cod feeds in the Barents Sea, an area characterized by heterogeneous water masses with large spatial temperature differences, but with a relatively stable temperature in specific areas at depths greater than 100 m where the cod are often found. Mature cod in this area experience large, interannual temperature differences resulting from their migratory behaviour, from 6–8 °C at spawning in the Lofoten area in the spring to 0–1 °C in the feeding area near the Polar Front in the summer/autumn (Ottersen *et al.*, 1998). The ambient temperature experienced by the cod in that area during wintertime is well described (Ottersen *et al.*, 1998), but information for the rest of the year is scarce. Migratory patterns and temperature preferences of individual fish could be detected by measurements of oxygen isotopes in cod otoliths. Electronic data storage tags (DST) have revealed substantially individual differences in temperature preferences of cod in the Barents Sea (Godø and Michalsen, 2000). Information on these variations is of importance since ambient temperatures are used as input in consumption models. Although DST can provide ambient temperature data with very high temporal resolution, estimation of ambient temperature by means of oxygen isotopes in otoliths has the advantage of not relying on tagging experiments, and information on the entire lifespan of the fish can be obtained. For example, juvenile cod experience large temperature fluctuations over the first year of life, with rapidly increasing temperature until the juveniles settle to the bottom in the Barents Sea in the autumn (Sundby, 2000). The exact timing of the bottom settlement and the factors that trigger it are not clear. By combining analysis of otolith microstructure for estimating the fish age and oxygen isotope analysis of the otoliths to estimate ambient temperature, new information on this stage in the cod life history can be achieved.

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