Calibration of the carbonate 'clumped isotope' paleothermometer for otoliths

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Abstract

Paleothermometry is an essential tool for understanding past changes in climate. The 'carbonate clumped isotope thermometer' is a temperature proxy related to ordering of ^{13}C and ^{18}O in the carbonate lattice (based on measurements of $^{13}C^{18}O^{16}O$ in CO_2 produced by acid digestion of carbonate). This thermometer has been previously calibrated for inorganic calcite and aragonitic corals [Ghosh P., Adkins J., Affek H., Balta B., Guo W. F., Schauble E. A., Schrag D., and Eiler J. M. (2006) C-13–O-18 bonds in carbonate minerals: a new kind of paleothermometer. *Geochim. Cosmochim. Acta* **70** (6), 1439–1456]. Here we determine the relationship between growth temperatures of aragonitic fish otoliths and abundances of $^{13}C^{18}O^{16}O$ produced by acid digestion of those otoliths. Our calibration is based on analyses of otoliths from six species from four genera of modern fish sampled from a latitudinal transect of the Atlantic Ocean between 54° S and 65° N, plus one species from the tropical western Pacific. The temperatures at which fish otoliths precipitated were estimated by the mean temperature in the waters in which they lived, averaged over their estimated lifetimes. Estimated growth temperatures of our samples vary between 2 and 25 °C. Our results show that the abundance of $^{13}C^{18}O^{16}O$ in CO_2 produced by acid digestion of fish otolith aragonite is a function of growth temperature, following the relationship: $\Delta_{47} = \frac{0.0568 \times 10^6}{T^2} - 0.0045$, where Δ_{47} is the enrichment, in per mil, of $^{13}C^{18}O^{16}O$ in CO_2 relative to the amount expected for a stochastic (random) distribution of isotopes among all CO_2 isotopologues, and T is the temperature in Kelvin. This relationship closely approaches that previously documented for inorganic calcite and aragonitic coral (Ghosh et al., 2006).
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1. INTRODUCTION

Inorganic calcites exhibit an inverse correlation between their growth temperatures and the proportion of their ^{13}C and ^{18}O atoms that form bonds with each other as part of the same carbonate ion. This isotope effect is the basis of the 'carbonate clumped isotope thermometer' (Ghosh et al., 2006; Schauble et al., 2006). An important feature of this thermometer is that it involves a homogeneous equilibrium (reaction among components of a single phase), and therefore can rigorously constrain the temperature of carbonate growth based on the isotopic composition of carbonate alone, independent of the isotopic composition of the water from which it grew or other phases with which it co-existed. Therefore, the carbonate clumped isotope thermometer can potentially be applied to parts of the geological record where the isotopic composition of water is unknown.

Natural coral aragonite precipitated at known temperatures generally conforms to the calibration of this thermometer based on inorganic calcite, suggesting the thermometer might be applicable to diverse biogenic and abiogenic carbonate minerals (see Ghosh et al., 2006). However, Ghosh

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et al. (2006), also found evidence for an anomalous enrichment in $^{13}C-^{18}O$ bonds in winter growth bands of Red Sea Porites coral, suggesting the thermometer might suffer from vital effects and require independent calibration for carbonate of different origins. Here we present a calibration of the carbonate clumped isotope thermometer for the otoliths of bony fishes.

Otoliths (ear stones) are aragonitic structures used for balance and/or hearing in all teleost fishes. Because otoliths are acellular and metabolically inert, elements or compounds accreted onto their growing surfaces are retained. Otoliths grow continuously from before the time of hatching to the time of death; in principle then, an otolith can contain a complete record of both the temperature and composition of the water in which the host fish lived, and these variables can be reconstructed using spatially resolved isotopic analyses (Patterson et al., 1993; Campana, 1999). Otoliths are accreted within a gel-filled endolymph and thus are isolated from direct exposure to the external water. This, along with low ratios of surface area to volume and a relatively large size (mm to cm), makes them less vulnerable to post-depositional isotopic and structural modification than many other types of biogenic carbonate.

Previous studies of the oxygen isotopic compositions of fish otoliths (i.e., their $\delta^{18}O$ values) indicate that they grow in oxygen-isotope exchange equilibrium with the water in which the host fish lives (Kalish, 1991; Patterson et al., 1993; Thorrold et al., 1997). Patterson et al. (1993) analyzed otoliths of several freshwater species and found a correlation between growth temperature, otolith $\delta^{18}O$ values and water $\delta^{18}O$ values, all of which were estimated by environmental measurements. Experimental studies in which water composition and temperature were more directly constrained report similar slopes to Patterson et al.'s correlation between growth temperature and otolith $\delta^{18}O$, but with different intercepts—perhaps because estimates of growth temperatures and/or water $\delta^{18}O$ for otoliths collected from wild fish contain unrecognized, systematic errors (Thorrold et al., 1997; Radtke et al., 1998; Elsdon and Gillanders, 2002; Høie et al., 2004).

Our objective is to determine the relationship between growth temperatures of aragonitic fish otoliths and their proportions of $^{13}C-^{18}O$ bonds, as measured by the Δ_{47} value (defined below) of CO_2 produced by phosphoric acid digestion of those otoliths (Ghosh et al., 2006). We focus on fish otolith samples collected from environments where growth temperature is established by in situ measurements or can be estimated from the known habitats of the host fish.

2. SAMPLES

We obtained otolith samples from two separate archives: the collection of the Bedford Institute of Oceanography, Dartmouth (BIO) and the Fitch Otolith Collection in the Section of Fishes at the Natural History Museum of Los Angeles County (LACM). In all cases, we focus on the sagitta, usually the largest of the three otoliths present in the ears of bony fishes. Specimens were selected from eight different geographic locations spanning a range in tempera-

ture from 2 to 25 °C (Table 1 and Fig. 1). Our sample suite includes seven different species from four genera.

The samples taken from the BIO collection include: Greenland halibut (Reinhardtius hippoglossoides) from the eastern coast of Labrador in eastern Canada, Atlantic cod (Gadus morhua) from the southern Gulf of St. Lawrence in eastern Canada and from Georges Bank in the northwest Atlantic, black drum (Pogonias cromis) from off the coast of Virginia, USA, and two species of snapper (Lutjanus synagris and Lutjanus analis) from the southeastern USA. All are common teleost fishes with well-developed otoliths. The Greenland halibut and cod otoliths were collected during Canadian fish surveys where routine measurements of bottom-water temperature were available. The black drum and snapper from the BIO collection do not have directly measured host water temperatures, and so their environmental temperatures are estimated based on surface water temperatures for the areas where they were collected. In all cases, we analyzed whole otoliths, which integrate the lifetime temperature variability experienced by the fish. Therefore, we averaged temperature estimates over seasonal variations in the waters in which they grew, and, when appropriate, over temperature changes associated with migration. We estimate that calculated average growth temperatures have a precision of ± 2 °C. However, it is important to note that fish move in the water column and migrate substantial distances, and so there is always a potential for error in estimated growth temperatures.

We examined two specimens from the LACM collection: a cod icefish (Patagonotothen ramsayi, sample LACM 0-1 from this study; originally Catalog No. 11070-4 in the LACM collections) comes from the southernmost Patagonian shelf, east of the tip of South America, at 124 m depth and has an estimated growth temperature of 5 ± 2 °C. The second specimen is a tropical snapper (Lutjanus malabaricus, LACM 0-2 in our suite; Catalog No. 26509 in the LACM collections) and comes from near Bangkok, Thailand; the species is a shallow dweller and has an estimated average growth temperature of 25 ± 2 °C.

3. METHODS

All samples were cleaned with H_2O_2 for 12 h to remove organic matter. Samples were then weighed, crushed into fine powder using an agate mortar and pestle and stored in glass vials. Aliquots of sample powder weighing ca. 8 mg were digested in anhydrous phosphoric acid following the methods McCrea (1950), as modified by Swart et al. (1991). Product CO_2 was recovered in a vacuum apparatus, purified and analyzed for its isotopic composition (including abundances of mass-47 isotopologues) using methods described by Ghosh et al. (2006).

Abundances of mass-47 isotopologues of CO_2 are reported using Δ_{47} values, as defined as in Eiler and Schauble (2004), Wang et al. (2004) and Ghosh et al. (2006). Briefly, the Δ_{47} value is defined as the difference in per mil between the measured 47/44 ratio ('R^{47}') of the sample and 47/44 ratio expected for that sample if its stable carbon and oxygen isotopes were randomly distributed among all isotopologues—a case referred to as the

Table 1
Stable isotopic composition of otolith aragonite with growth temperature and isotopic composition of sea water

Sample description	$\delta^{13}C_{PDB}$	$\delta^{18}O_{PDB}$	Δ_{47}	Growth temperature (°C)	$\delta^{18}O_{sea\ water\ (SMOW)}$[a]	$\delta^{13}C_{\Sigma DIC\ sea\ water\ \#}$[b]
LACM O-1	−3.44	2.79	0.77	5 ± 2	−0.10	1.2
Patagonotothen ramsayi	−3.44	2.80	0.71			
54°39′S, 57°9′W, 124 m depth	−3.44	2.82	0.69			
Average	−3.44	2.80	0.725			
Standard error	0.002	0.010	0.022			
LACM O-2	−5.08	−3.18	0.70	25 ± 3	0.24	1.8
Lutjanus malabarius	−5.09	−3.19	0.64			
13°45′N–100°30′E	−5.08	−3.19	0.61			
	−5.08	−3.18	0.61			
	−5.08	−3.18	0.64			
	−5.08	−3.19	0.66			
Average	−5.08	−3.19	0.642			
Standard error	0.002	0.002	0.013			
BIO-3	−1.05	3.39	0.76	2 ± 2	−1.49	1.7
Reinhardtius hippoglossoides	−1.04	3.42	0.76			
65°N–60°W	−1.04	3.41	0.74			
	−1.04	3.41	0.75			
	−1.04	3.42	0.80			
Average	−1.04	3.41	0.761			
Standard error	0.003	0.006	0.010			
BIO-4	−3.14	−2.57	0.62	20 ± 2	0.45	1.8
Lutjanus analis	−3.14	−2.56	0.60			
26°N–89°W	−3.14	−2.56	0.64			
	−3.13	−2.56	0.73			
Average	−3.14	−2.56	0.647			
Standard error	0.003	0.002	0.029			
BIO-5	−1.21	0.54	0.66	7 ± 2	−1.19	1.0
Gadus morhua	−1.20	0.55	0.68			
42°N–66°W	−1.21	0.55	0.72			
Average	−1.21	0.55	0.687			
Standard error	0.001	0.003	0.018			
BIO-5 (repeat)	−0.61	2.22	0.68	7 ± 2	−1.19	1.0
Gadus morhua	−0.61	2.21	0.72			
42°N–66°W	−0.62	2.22	0.72			
	−0.61	2.22	0.70			
	−0.61	2.21	0.76			
	−0.61	2.22	0.68			
Average	−0.61	2.21	0.710			
Standard error	0.003	0.006	0.012			
BIO-5 (repeat)	0.06	2.12	0.72	7 ± 2	−1.19	1.0
Gadus morhua	0.05	2.20	0.75			
42°N–66°W	0.07	2.22	0.66			
	0.06	2.20	0.73			
Average	0.06	2.19	0.715			
Standard error	0.003	0.02	0.02			
BIO-6						
Pogonias cromis	−4.13	−2.65	0.67	15 ± 2	−1.26	1.6
37°N–75°W	−4.13	−2.65	0.72			

Table 1 (*continued*)

Sample description	$\delta^{13}C_{PDB}$	$\delta^{18}O_{PDB}$	Δ_{47}	Growth temperature (°C)	$\delta^{18}O_{\text{sea water (SMOW)}}$[a]	$\delta^{13}C_{\Sigma DIC \text{ sea water \#}}$[b]
Average	−4.13	−2.65	0.693			
Standard error	0.000	0.002	0.025			
BIO-7	−3.93	−0.58	0.63	25 ± 2	1.90	2.1
Lutjanus synagris	−3.93	−0.58	0.62			
23°N–75°W	−3.93	−0.57	0.62			
	−3.93	−0.57	0.65			
Average	−3.93	−0.58	0.629			
Standard error	0.001	0.003	0.006			
BIO-7						
Lutjanus synagris	−3.02	−0.63	0.67	25 ± 2	1.90	2.1
23°N–75°W	−3.02	−0.64	0.66			
Average	−3.02	−0.63	0.668			
Standard error	0.002	0.002	0.003			
BIO-7	−4.93	−2.60	0.67	25 ± 2	1.90	2.1
Lutjanus synagris	−4.93	−2.60	0.70			
23°N–75°W	−4.93	−2.60	0.59			
Average	−4.93	−2.60	0.653			
Standard error	0.002	0.001	0.053			
BIO-8	−0.77	1.77	0.76	3 ± 2	−1.47	1.4
Gadus morhua	−0.77	1.77	0.76			
49°N–60°W	−0.77	1.76	0.70			
Average	−0.77	1.77	0.739			
Standard error	0.001	0.003	0.021			

[a] Values for $\delta^{18}O$ of sea water at specific location are derived from data givem in Schmidt et al. (1999) (http://data.giss.nasa.gov/o18data/).
[b] Values for $\delta^{13}C$ DIC of sea water at specific location are taken from the closest station (with in 5° lat /long grid) given in Kroopnick (1980).

stochastic distribution. All Δ_{47} values reported here were standardized by comparison with CO_2 of similar bulk isotopic composition (generally within 1‰ in R^{47}) that had been heated to 1000 °C to drive it to the stochastic distribution. A small correction for non-linearity of R^{47} measurements was applied to account for differences in R^{47} between sample and heated gas standard. This correction was made based on analyses of heated gases having a range of bulk isotopic compositions.

The external precision of individual mass spectrometric measurements of Δ_{47} is typically 0.03‰, consistent with shot-noise limits for the ion-beam intensities and analytical durations of our measurements. Most samples were measured 3–4 times, such that the standard error of their Δ_{47} values is typically in the range 0.01–0.02‰. Replicate analyses of some samples were performed to determine the total external reproducibility, including any errors that might accompany acid digestion and gas purification. Note, however, that this measure of reproducibility can be influenced by real variations in Δ_{47} among different splits of the same sample and therefore is a maximum measurement of external precision. These replicate analyses yield standard deviations in the range 0.015–0.025‰ (Table 1).

We routinely monitored R^{48} and R^{49} values of all sample gases and rejected analyses that showed evidence for significant contamination at either mass (because it is possible that such contaminants might also be accompanied by an isobar at mass 47; Eiler and Schauble, 2004). Two out of a total of 14 analyses were rejected for this reason, and in both cases could be attributed either to a recognized leak in the acid reaction vessel or an error in GC cleaning procedure (see Eiler and Schauble, 2004; Affek and Eiler, 2006 and Ghosh et al., 2006 for further discussion of such analytical artifacts).

4. RESULTS

Table 1 summarizes results of isotopic analyses of all otolith samples investigated in this study. A 0.13‰ range in Δ_{47} was observed among otolith samples (Table 1; Fig. 2) having an estimated range in growth temperature of 23 ± 2 °C. The highest Δ_{47} value of 0.76‰ was observed in Greenland halibut (BIO-3, *Reinhardtius hippoglossoides*), a cold water (est. 2 ± 2 °C) fish from Greenland. The lowest Δ_{47} value of 0.63‰ was observed in snapper (BIO-7, *Lutjanus synagris*), a tropical (23°N,75°W) fish that grew at an estimated
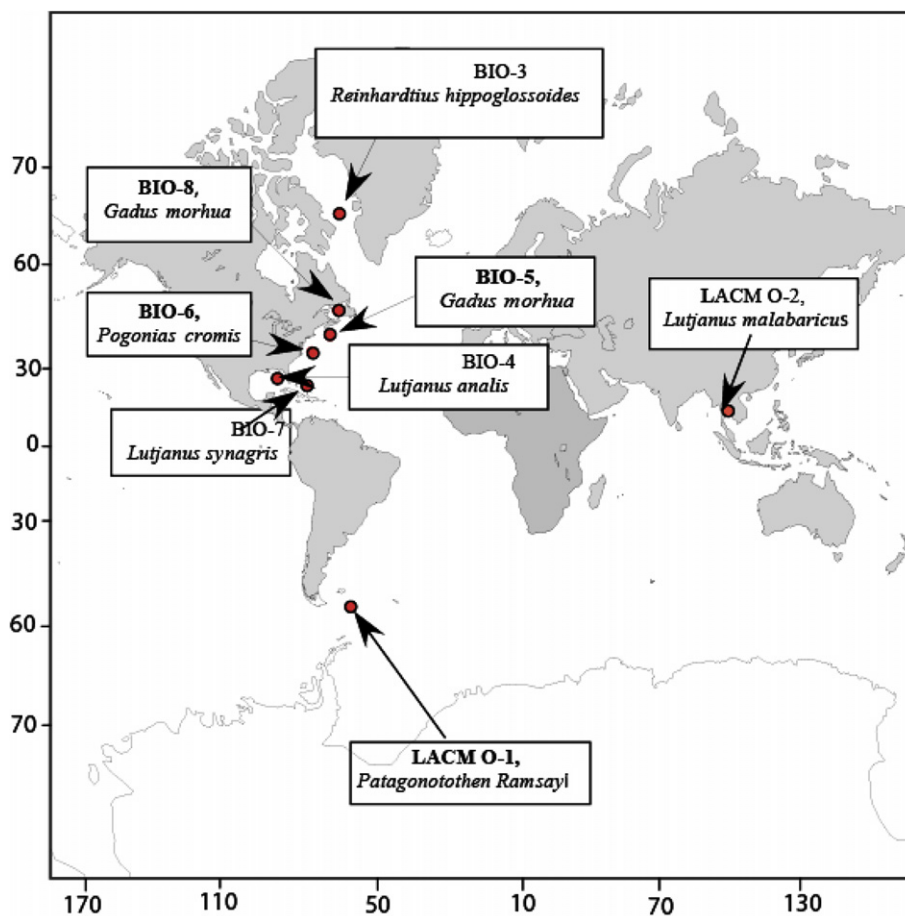
Fig. 1. Locations of samples examined in this study, with sample numbers and species identifications corresponding to the entries in Table 1.
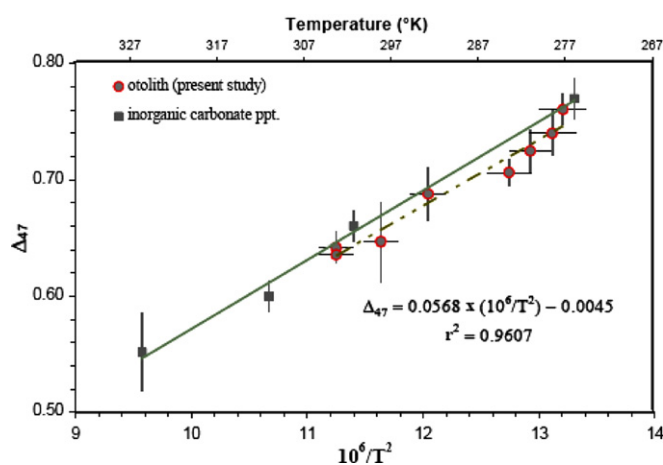

Fig. 2. Relationship between estimated environmental temperatures at which sampled fishes lived and Δ_{47} values of CO_2 produced by phosphoric acid digestion of fish otoliths (plotted as filled circles). Data for inorganic calcite (plotted as filled squares) grown in the laboratory under controlled conditions (Ghosh et al., 2006) are shown for comparison.

temperature of 25 ± 2 °C. Samples of cod (*Gadus morhua*; BIO-5 and BIO-8) collected from the eastern coast of the Canada at estimated water temperatures of 7 ± 2 and 3 ± 2 °C yielded Δ_{47} values of 0.707 ± 0.015 (3)‰ and 0.74‰, respectively (see Table 1). A sample of ice fish

(*Patagonotothen ramsayi*) from Patagonian Shelf lived at an estimated water temperature of 5 ± 2 °C and has a Δ_{47} value of 0.72‰. The black drum (BIO-6, *Pogonias cromis*) from the eastern coast of the US off Virginia lived at an estimated water temperature of 15 ± 2 °C (Richards, 1973) and

has a Δ_{47} value of 0.69‰. Finally, the four samples of snapper (LACM O-2 and three samples of BIO-7 *Lutjanus* sp.), all from relatively warm tropical waters (est. 25 ± 2 or 3 °C), have Δ_{47} values ranging between 0.67‰ and 0.63‰.

Table 1 also reports the $\delta^{18}O_{V\text{-}PDB}$ values of otoliths analyzed in this study, along with presumed growth temperatures and the $\delta^{18}O_{V\text{-}SMOW}$ values of water and $\delta^{13}C_{V\text{-}PDB}$ values of total dissolved inorganic carbon (DIC) of the sea waters in which they grew (from Kroopnick, 1980 and Schmidt et al., 1999). This database combines oxygen isotope measurements from the Atlantic GEOSECS expedition (Kroopnick, 1980) and various other sources. The $\delta^{18}O$ value of ocean water in which each sampled fish grew was calculated as the average for all surface water values recorded within $\pm 1°$ latitude and longitude of the sample location. The only relevant database for $\delta^{13}C$ of DIC of ocean water comes from the analyses of water samples collected during the GEOSEC expedition (Kroopnick, 1980); it covers 70°N to 60°S latitudes, close to the continental margin in the Atlantic Ocean. We estimated the $\delta^{13}C$ of DIC of ocean water as the average for surface water values recorded within $\pm 3°$ Latitude and Longitude of our sampling locations. For the sample originating from the equatorial Pacific, the $\delta^{13}C$ of DIC is taken from the Kroopnick (1987) data repository.

5. DISCUSSION

5.1. Relationship between temperature and Δ_{47}

Taken together, our results (Table 1) define a strong positive correlation ($r^2 = 0.94$) between Δ_{47} and T^{-2}, where T is the estimated growth temperature, in Kelvin (Fig. 2), with a mean trend given by:

$$\Delta_{47} = \frac{0.0568 \times 10^6}{T^2} - 0.0045$$

The deviation of the data from this trend averages 0.008‰, comparable to our nominal analytical precision of ca. 0.01–0.02‰. The slope of this trend is statistically indistinguishable from that previously obtained for inorganic calcites (Ghosh et al., 2006; filled circles in Fig. 2), but the trend is clearly offset to slightly lower Δ_{47} (by -0.016‰, on average) at any given temperature within the calibration range. If not accounted for, this offset would correspond to an average temperature error of 2.6 °C (between 25 and 2 °C). We do not believe this discrepancy can be attributed to differences in standardization of the Δ_{47} scale between these two studies because measurements of carbonate standards revealed no such secular drift in the Δ_{47} reference frame. The discrepancy might reflect differences between
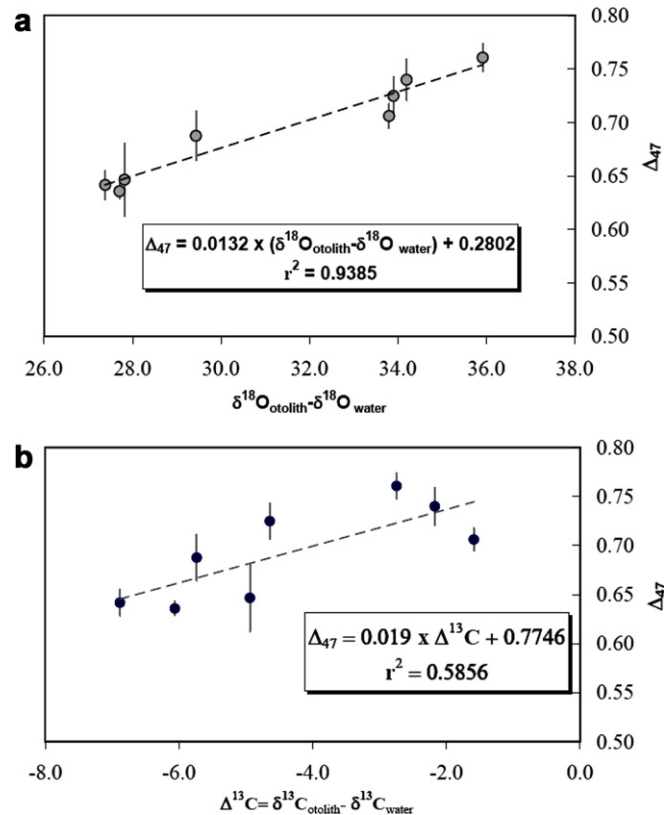

Fig. 3. (a) Relationship between Δ_{47} and $\Delta^{18}O$, which is defined as the difference, $\delta^{18}O_{\text{otolith}} - \delta^{18}O_{\text{water}}$ (in SMOW scale). The estimated $\delta^{18}O$ of water in which sampled fishes grew are given in Table 1 and are obtained from Schmidt et al. (1999). (b) Relationship between Δ_{47} and $\Delta^{13}C$, which is defined as the difference, $\delta^{13}C_{\text{otolith}} - \delta^{13}C_{\text{DIC}}$ (in PDBscale). The estimated $\delta^{13}C$ of DIC of water in which sampled fishes grew are given in Table 1 and are obtained from Kroopnick (1980).
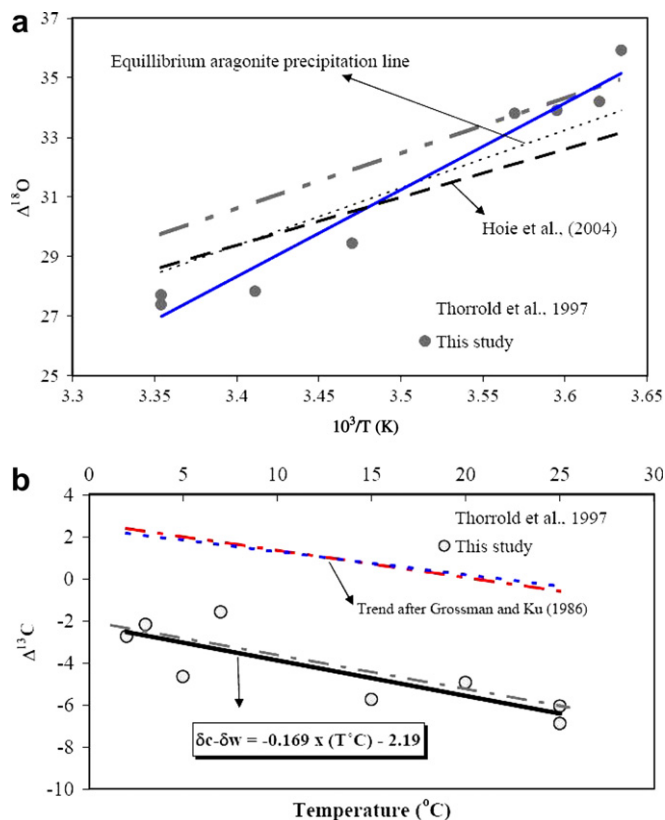
Fig. 4. (a) Relationship between environmental temperature and $\Delta^{18}O$ found by this study (grey circles). Also shown here for comparison are results from Thorrold et al. (1997), Høie et al. (2004) and equilibrium aragonite precipitation line established based on Kim and O'Neil (1997) and Tarutani et al. (1969). (b) Relationship between environmental temperature and $\Delta^{13}C$ found by this study (grey circles). Also shown here for comparison are results from Thorrold et al. (1997), and trend established based on study of $\Delta^{13}C$ and growth temperatures for foraminifera and mollusk species Grossman and Ku (1986).

calcite and aragonite in the isotopic fractionation that accompanies acid digestion, a vital effect that inhibits $^{13}C–^{18}O$ 'clumping' in otoliths relative to that predicted for equilibrium precipitation, or a systematic error in our estimates of otolith growth temperatures. It should be possible to rule out an acid digestion fractionation by exploring the dependence of the difference in Δ_{47} between inorganic calcite and otoliths on the temperature of acid digestion, and it should be possible to examine the role of differences in equilibrium isotopic clumping between calcite and aragonite through inorganic precipitation experiments. One could evaluate the possibility of systematic errors in estimated otolith growth temperature by analyzing otoliths from cultured fishes grown at known temperature, or use of fish from very well constrained natural growth environments. If all the above causes can be disproved, a vital effect would be implicated.

5.2. Relationship between $\delta^{18}O$ vs. Δ_{47}

Several experimental studies have reported strong correlations between the temperature of water in which fishes live and the difference in $\delta^{18}O$ between otoliths and water (Thorrold et al., 1997; Radtke et al., 1998; Elsdon and Gillanders, 2002; Gao, 2003; Høie et al., 2004). These studies

reported temperature-fractionation relationships with slopes that are statistically indistinguishable both from each other and from the relationship for inorganic aragonite (Kim and O'Neil, 1997)—although with intercepts that differ significantly from one another in some cases.

Our data, combined with estimates of the $\delta^{18}O$ of water in which sampled fishes grew (Table 1), document a correlation between Δ_{47} of otoliths and the difference, $\delta^{18}O_{otolith} - \delta^{18}O_{water}$ (hereafter referred to as '$\Delta^{18}O$'). This trend is illustrated in Fig. 3a and can be described by the linear relationship, $\Delta_{47} = 0.0132 * \Delta^{18}O + 0.28$ ($r^2 = 0.94$). The most obvious interpretation of this trend is that it reflects the influence of growth temperature on both Δ_{47} and the carbonate-water oxygen isotope fractionation. The correlation between estimated growth temperature and oxygen isotope fractionation between otoliths and host waters defined by our data suggests that a 1 °C temperature change corresponds to a change of ca. 0.28‰ in $\Delta^{18}O$ (Fig. 4a). This compares with a proportionality of 0.33‰ per °C estimated by Thorrold et al. (1997) and 0.20‰ per °C measured by Høie et al. (2004) (Fig. 4a). While the slope defined by our data is intermediate between these two estimates, the $\Delta^{18}O$ value we observe at any given temperature agrees more closely with the previous observations in Høie et al. (2004). The Thorrold et al. (1997)

calibration was based on measurements of cultured otoliths grown at temperatures varying between 18.2 and 25 °C (Fig. 4a). On the other hand, Høie et al. (2004) measured isotopic compositions of otoliths of juvenile cod reared over a wider range of temperatures (6–20 °C; Fig. 4a). It is possible that the better agreement of our results with those of Høie et al. reflects the fact that these two studies examined a similar range in temperature. Fig. 4a also shows the trend in $\Delta^{18}O$ vs temperature for inorganic aragonite (dashed line; calculated based on data for inorganic calcite from Kim and O'Neil, 1997, plus an estimated aragonite–calcite fractionation of 0.6 based on results of Tarutani et al., 1969). The temperature-dependent relationship obtained in this study (and that of Høie et al., to which it is similar) closely resembles that expected for inorganic aragonite.

5.3. Relationship between $\delta^{13}C$ and Δ_{47}

Kalish (1991) and Thorrold et al. (1997) found correlations between growth temperature and the $\Delta^{13}C$ value of fish otoliths, where $\Delta^{13}C$ is the difference in $\delta^{13}C$ between the otolith and the dissolved inorganic carbon (DIC) pool in the water in which its host fish grew (i.e., $\Delta^{13}C = \delta^{13}C_{DIC} - \delta^{13}C_{otolith}$, where both are relative to the PDB standard). By combining our measurements of otoliths with estimates of the $\delta^{13}C$ values of DIC in waters from which our samples were collected (Kroopnick, 1980; Table 1), We find a scattered but statistically significant correlation between Δ_{47} and $\Delta^{13}C$ (Fig. 3b). We also find a correlation between estimated growth temperature and $\delta^{13}C$ for the otoliths we examined (Fig. 4b), with mean trend defined by the equation: $\Delta^{13}C = -0.169 \times (T\,°C) - 2.19$. Despite potential complexities in the C budgets of fish otoliths (e.g., due to variations in stress and metabolic rate; Wurster et al., 2005), it is noteworthy that this trend is similar to those determined by Kalish (1991) and Thorrold et al. (1997). Grossman and Ku (1986) reported relationships between $\Delta^{13}C$ and growth temperatures for foraminifera and mollusk species. These trends are similar in slope to those we and previous workers have found for otoliths, but with different, generally higher, intercepts (varying by up to 4.4‰; Fig. 4b).

Carbon isotopic fractionation between inorganic aragonite and bicarbonate is approximately independent of temperature between 10 and 40 °C, based on measurement of carbon isotopic ratios in DIC and aragonite precipitates (Romanek et al., 1992). Thus, the relationship between $\Delta^{13}C$ and growth temperature observed in biogenic carbonates (Fig. 4b) is not an equilibrium phenomenon, and could instead reflect a metabolic vital effect (Schwarcz et al., 1998; Campana, 1999; Wurster et al., 2005). The relatively low-$\delta^{13}C$ values of otoliths may reflect large (est. 10–30%) contributions of carbon from metabolic sources, i.e., because the $\delta^{13}C$ value of DIC in the fish body water reflects mixing between DIC from environmental water and DIC produced by hydration of CO_2 from metabolic sources (Kalish, 1991; Gauldie, 1993; Thorrold et al., 1997; Schwarcz et al., 1998). Therefore, correlations between growth temperatures and carbon-isotopic compositions of otoliths may be related to temperature dependent changes in metabolic rate (Wurster and Patterson, 2003; Wurster et al., 2005) and perhaps could be used as a proxy for variations in metabolic rates of fishes (i.e., increases in temperature enhance metabolic rate, increasing the production of CO_2 with relatively low $\delta^{13}C$ values). However, despite the apparent simplicity of the temperature dependence in the Fig. 4b, it is important to note that the contribution of metabolic carbon also varies with the diet and age of a fish (Schwarcz et al., 1998). This phenomenon should be explored further by conducting controlled experiments in which the $\delta^{13}C$ values of environmental and body-water DIC, water temperature, and the $\delta^{13}C$ and Δ_{47} of fish otoliths are continuously monitored.

6. CONCLUSIONS

We document a relationship between the abundance of $^{13}C^{18}O^{16}O$, as measured by the Δ_{47} value, in CO_2 produced by phosphoric acid digestion of fish otoliths and the environmental temperatures of the fishes in which those otoliths grew. This relationship is similar in slope to that previously observed for inorganic calcite (Ghosh et al., 2006) but is offset to slightly lower ($\sim$0.01–0.02‰) values of Δ_{47} at any given temperature. We suggest that this relationship is suitable for paleothermometry using well-preserved aragonitic fish otoliths. Simultaneous measurements of $\delta^{18}O$ and $\delta^{13}C$ values of otoliths can be combined with this version of the "carbonate clumped isotope thermometer" to allow estimation of the $\delta^{18}O$ of water. Otoliths occur in marine and non-marine settings, and frequently preserve seasonal variations in growth conditions that can be recovered by spatially resolved sampling. Thus, the approach we suggest will allow reconstruction of seasonal and longer-term variations in temperature and the $\delta^{18}O$ of water from the analyses of fish otoliths.

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