Pergamon

Geochimica et Cosmochimica Acta, Vol. 61, No. 14, pp. 2909–2919, 1997

0016-7037/97 $17.00 + .00

PII S0016-7037(97)00141-5

# Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish

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(*Received November* 21, 1996; *accepted in revised form March* 20, 1997)

**Abstract**—Fish otoliths are aragonitic accretions located within the inner ear of teleost fish. The acellular nature of otoliths, along with taxon-specific shapes, chronological growth increments, and abundance in the fossil record suggest that the stable isotope chemistry of these structures may be unique recorders of environmental conditions experienced by fish in both modern and ancient water masses. To assess the factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in fish otoliths, we reared Atlantic croaker (*Micropogonias undulatus*) larvae under controlled environmental conditions. Metabolic effects apparently generated large isotopic disequilibria in the $\delta^{13}C$ values of *M. undulatus* otoliths. We found evidence of a negative regression between $\delta^{13}C_{carbonate}$-$\delta^{13}C_{water}$ ($\Delta^{13}C$) and temperature:

$$\Delta^{13}C = -1.78 - 0.18\ T°C$$

However, this relationship was aliased to a degree by a positive correlation between $\Delta^{13}C$ and somatic growth and otolith precipitation rates. Oxygen isotopes were deposited close to equilibrium with the ambient water. The relationship between temperature and the $^{18}O/^{16}O$ fractionation factor ($\alpha$) was determined empirically to be:

$$1000 \ln \alpha = 18.56(10^3\ T\ K^{-1}) - 32.54$$

The fractionation factor was not affected by either otolith precipitation or fish growth rates. Reconstruction of water temperature histories should, therefore, be possible from the $\delta^{18}O$ values of *M. undulatus* otoliths with a precision of 1°C, providing the $\delta^{18}O$ of the ambient water can be estimated. 

## 1. INTRODUCTION

Stable isotope chemistry of biogenic calcium carbonate records valuable information on environmental conditions experienced by aquatic organisms. Carbon isotope ratios from skeletons and shells of a number of marine invertebrates have, for instance, been used to trace palaeo-ocean circulation patterns (Oppo et al., 1995), record anthropogenic $CO_2$ input to surface oceans (Beveridge and Shackleton, 1994; Böhm et al., 1996), and to determine irradiance levels in the upper ocean via symbiont photosynthetic activity (Spero and Williams, 1988; Wellington and Dunbar, 1995). Oxygen isotopes from invertebrate skeletons have been used to reconstruct temperature and salinity regimes of modern and ancient oceans (e.g., Guilderson et al., 1994).

Similar information may also be available from biogenic carbonates found in marine vertebrates. Fish otoliths (ear stones) show considerable promise in this regard because, unlike bone, they are acellular and, therefore, unlikely to be metabolically reworked after deposition (Campana and Neilson, 1985). Otoliths are accretionary structures located within the inner ear of teleost fish, composed primarily of aragonite deposited on a proteinaceous matrix. Periodic deposition of growth increments within otoliths has permitted routine ageing by fish biologists for many years (Jones, 1986; Beamish and McFarlane, 1987). More recently, attention has focused on interpreting information recorded by the stable isotope and trace element chemistry of otoliths (Mulcahy et al., 1979; Kalish, 1991a; Fowler et al., 1995). By combining the chronological record of growth increments with otolith microchemistry at similar spatial resolutions, it is possible to retrospectively determine the physical and chemical characteristics of the water in which a fish has resided (Thorrold et al., 1997). Teleost fish may be particularly useful environmental recorders as the group has radiated into almost all aquatic ecosystems. Otoliths are also common in the fossil record, with samples from a number of Recent percoid families found in nonconsolidated sediments from the late Cretaceous to the present (Nolf, 1994). As shapes are generally taxon-specific, otoliths may be unique recorders of palaeo-ecological information on individual fish and palaeo-climatic data on the aquatic environments in which they lived (Smith and Patterson, 1994).

Interpretation of carbon and oxygen isotope records in fish otoliths requires an understanding of the dynamics of isotope fractionation in otolith aragonite. In particular, the potential for isotopic disequilibria between ambient waters and otoliths needs to be evaluated. Isotopic disequilibria is common in most biogenic carbonates (e.g., Swart, 1983) and is considered a result of either kinetic or metabolic effects. Kinetic effects may result from discrimination against heavier carbon and oxygen isotopes during the hydration and hydroxylation of $CO_2$ (McConnaughey, 1989a). Alternatively, lighter isotopes may be differentially transported to the crystal face due to higher diffusivities of $HCO_3^-$ species

Table 1. Summary of mean temperatures ($T$), $\delta^{13}C_{DIC}$ (PDB) and $\delta^{18}O_w$ (SMOW) of the water in individual rearing tanks maintained throughout the course of the experiment, along with mean standard length (SL) and otolith weight (OW) information from fish used in the otolith analysis. All means ± 1$\sigma$.

| | 18°C | | 20.5°C | | 22.5°C | | 25°C | |
|---|---|---|---|---|---|---|---|---|
| | Tank 1 | Tank 2 | Tank 1 | Tank 2 | Tank 1 | Tank 2 | Tank 1 | Tank 2 |
| $T$—°C | 18.2 ± 0.8 | 18.3 ± 0.9 | 20.3 ± 0.6 | 20.6 ± 1.1 | 22.3 ± 0.8 | 22.0 ± 1.1 | 24.9 ± 0.5 | 25.0 ± 0.4 |
| $\delta^{13}C_{DIC}$ | −0.5 ± 2.1 | −1.3 ± 2.9 | −0.6 ± 1.9 | −1.8 ± 2.7 | −0.4 ± 2.2 | −2.0 ± 2.6 | −0.5 ± 1.9 | −1.0 ± 2.2 |
| $\delta^{18}O_w$ | 0.34 ± 0.3 | 0.48 ± 0.3 | 0.50 ± 0.4 | 0.4 ± 0.5 | 0.62 ± 0.2 | 0.72 ± 0.6 | 0.49 ± 0.3 | 0.65 ± 0.3 |
| SL—mm | 22.2 ± 3.4 | 25.1 ± 3 | 26.0 ± 1.2 | 28.4 ± 1.5 | 38.3 ± 4.5 | 33.8 ± 1.5 | 24.2 ± 0.8 | 25.7 ± 1.4 |
| OW—mg | 0.9 ± 0.8 | 0.7 ± 0.4 | 1.0 ± 0.2 | 1.4 ± 0.3 | 2.9 ± 1.0 | 2.1 ± 0.8 | 1.1 ± 0.1 | 1.2 ± 0.1 |

incorporating $^{12}C$ or $^{16}O$ (Kalish, 1991b). However, as long as the species-specific offset from isotopic equilibrium is known and remains constant over the temperature range of interest, environmental information may still be accurately interpreted from skeletal records (e.g., Wellington and Dunbar, 1995; Leder et al., 1996).

Isotopic disequilibria due to metabolic effects, principally in carbon isotopes, are more problematic than kinetic processes. Isotopic disequilibria in this instance are generated by the incorporation of metabolically-derived carbon into the aragonite. Metabolic carbon is depleted in $^{13}C$ compared to dissolved inorganic carbon (DIC) in most ocean water. Metabolic effects in biogenic carbonates are, therefore, typically manifested as $\delta^{13}C$ values considerably more depleted than equilibrium values without similar depletions in $\delta^{18}O$ (McConnaughey, 1989b). Although the presence of significant metabolically-derived carbon limits the use of carbon isotopes in biogenic carbonates as a proxy of $\delta^{13}C_{DIC}$, carbonate $\delta^{13}C$ in such instances may record information on physiological processes regulating the rate of metabolic carbon uptake (Spero and Williams, 1988).

A number of workers have been presented data on the carbon and oxygen composition of marine fish otoliths (e.g., Degens et al., 1969; Mulcahy et al., 1979; Radtke, 1984a,b; Nelson et al., 1989; Kalish, 1991a,b; Iacumin et al., 1992; Gauldie et al., 1994). Unfortunately the potential for significant migrations through water masses of varying physical and chemical characteristics has obscured interpretation of the isotopic information recorded by otoliths from field collections. Laboratory studies on isotopic fractionation in otoliths have invariably been confounded by a failure to constrain the oxygen isotopic composition of the water, which may vary considerably due to differential evaporation of $H_2{}^{16}O$. Patterson et al. (1993) quantified the oxygen isotopic composition of both otolith aragonite and the ambient water in which the fish resided by judicious choice of otoliths from

Table 2. Water sample data taken from each of the rearing tanks every week during the experiment and analyzed for $\delta^{13}C_{DIC}$ (‰, PDB) and $\delta^{13}O_w$ (‰, SMOW).

| | | 18°C | | 20.5°C | | 22.5°C | | 25°C | |
|---|---|---|---|---|---|---|---|---|---|
| | | Tank 1 | Tank 2 | Tank 1 | Tank 2 | Tank 1 | Tank 2 | Tank 1 | Tank 2 |
| 5 Nov | $\delta^{13}C_{DIC}$ | −4.5 | −1.97 | −2.89 | −3.5 | −4.74 | −2.61 | −2.85 | −2.4 |
| | $\delta^{18}O_w$ | 0.89 | | | −0.15 | 0.44 | 0.09 | 0.52 | |
| 12 Nov | $\delta^{13}C_{DIC}$ | | | | | | | | |
| | $\delta^{18}O_w$ | 0.01 | | −0.16 | 0.8 | 0.43 | 0.39 | | 0.63 |
| 12 Nov | $\delta^{13}C_{DIC}$ | | | | | | | | |
| | $\delta^{18}O_w$ | 0.24 | | 0.89 | −0.29 | | | 0.41 | 0.93 |
| 28 Nov | $\delta^{13}C_{DIC}$ | | | | | | | 0.083 | 0.27 |
| | $\delta^{18}O_w$ | 0.26 | 0.06 | 0.46 | 1.46 | | 0.66 | 0.06 | 0.39 |
| 3 Dec | $\delta^{13}C_{DIC}$ | | | | | | | 0.60 | 0.4 |
| | $\delta^{18}O_w$ | 0.52 | | 0.44 | | | | 0.32 | 0.6 |
| 10 Dec | $\delta^{13}C_{DIC}$ | −1.72 | −4.68 | 0.99 | 0.14 | 0.3 | 0.87 | 0.27 | −0.715 |
| | $\delta^{18}O_w$ | 0.32 | | 1.04 | 0.61 | −0.113 | | 0.74 | 0.48 |
| 19 Dec | $\delta^{13}C_{DIC}$ | | | | | 0.64 | | 0.15 | −1.38 |
| | $\delta^{18}O_w$ | 0.56 | 0.93 | 0.24 | | | 0.59 | 0.28 | 0.68 |
| 24 Dec | $\delta^{13}C_{DIC}$ | | | | 0.43 | 0.55 | | 0.39 | −1.26 |
| | $\delta^{18}O_w$ | 0.26 | 0.46 | | | | 0.63 | 0.49 | 0.9 |
| 30 Dec | $\delta^{13}C_{DIC}$ | | | | | 0.77 | | | −1.51 |
| | $\delta^{18}O_w$ | 0.34 | | | | | | 0.77 | 0.62 |
| 6 Jan | $\delta^{13}C_{DIC}$ | | | | | 0.84 | | 2.23 | 0.01 |
| | $\delta^{18}O_w$ | | | | | | | 0.65 | 0.9 |
| 13 Jan | $\delta^{13}C_{DIC}$ | | | | | | | 0.78 | 0.44 |
| | $\delta^{18}O_w$ | 0.01 | 0.45 | | 0.24 | 1.0 | 0.81 | 1.07 | 1.06 |
| 20 Jan | $\delta^{13}C_{DIC}$ | 0.1 | 0.52 | 0.84 | 0.28 | 0.83 | 0.38 | | 1.41 |
| | $\delta^{18}O_w$ | 0.34 | 0.37 | 0.34 | 0.11 | | 1.87 | | −0.03 |

various stenothermic and eurythermic fish species residing in a number of lake systems. This remains the only study to quantify the temperature dependence of oxygen isotope fractionation in fish otoliths from either marine or freshwater environments. However, the relationship between oxygen isotope fractionation and temperature has yet to be determined for any individual fish species, and the influence of intraspecimen and interspecimen variability on the precision of retrospective estimates of temperature is unknown.

The objectives of the present study were to determine the influence of environmental conditions on carbon and oxygen isotope fractionation in fish otoliths. To accurately quantify environmental exposure, fish were reared from eggs under controlled experimental conditions. The $\delta^{13}C$ and $\delta^{18}O$ values of the ambient waters were monitored throughout the period of otolith deposition, providing a powerful test of the degree to which carbon and oxygen isotopes are deposited in equilibrium with ambient seawater. Significantly different growth rates, within and among experimental treatments, allowed an examination of the effect of both fish growth and otolith precipitation rates on the $\delta^{13}C$ and $\delta^{18}O$ values of otolith aragonite.

## 2. EXPERIMENTAL METHODS

### 2.1. Larval Rearing

Larval and juvenile Atlantic croaker, *Micropogonias undulatus*, used in the experiment were reared at the National Marine Fisheries Laboratory, Beaufort, North Carolina from 5 November 1995 through 24 January 1996. Adult brood stock were induced to spawn by injecting human chorionic gonadotropin (HCG) and manipulating ambient temperatures. Eggs were placed in a common tank (22C, 33‰) for 24 h before stocking. One-day-old larvae were randomly stocked into eight 100 L rearing tanks, which had been randomly assigned one of four temperature regimes (18°C, 20.5°C, 22.5°C, and 25°C). Tanks were supplied with water in a flow-through system (water exchange rate 80–150 L · day$^{-1}$) from the same source (nominal salinity 30‰) and maintained at assigned temperatures with aquarium heaters (Table 1). Larvae were fed both enriched and unenriched rotifers and *Artemia* on alternate days.

### 2.2. Otolith Analyses

Teleost fish have three pairs of otoliths, the sagittae, lapilli, and asterisci. Sagittae are typically the largest of the three pairs and were the only otoliths analyzed in this study. Otoliths were dissected from the fish onto a glass slide, cleaned ultrasonically for 5 min in Milli-Q water and then air-dried under a class 100 positive flow fume hood. After drying, otoliths were weighed (to the nearest 10 $\mu$g) and sealed in sterile polyethylene containers for transport to the mass spectrometry lab. A single, randomly selected, sagitta from each fish was used for analysis. Both left and right otoliths were also analyzed from sixteen fish (four sagittal pairs from each of the four treatments) to assess intra-fish variability.

Otoliths were processed by an automated carbonate device (common acid bath @ 90°C) attached to a Finnigan-MAT 251 gas ratio mass spectrometer. Data were corrected for the usual isobaric interferences using the method of Craig (1957) modified for a triple collector mass spectrometer and are expressed relative to PDB. External precision (calculated from replicate analyses of an internal laboratory calcite standard) was 0.02‰ for $\delta^{13}C$ and 0.03‰ for $\delta^{18}O$.

### 2.3. Water Analyses

A common, temperature/salinity controlled, water source was used for all tanks in both experiments. However, the $\delta^{13}C_{DIC}$ of the tank water may be different from that of the source water, as respiration in a limited volume of water can overwhelm $CO_2$ exchange between the atmosphere and the water. It has also been well established that differential evaporation of $H_2{}^{16}O$ can modify the oxygen isotope composition of water masses. Therefore, water samples were collected weekly from each tank during the experiment for $\delta^{13}C$ and $\delta^{18}O$ analysis. Immediately after collection samples were filtered through a 0.45 $\mu$m filter and fixed with $HgCl_2$. The $\delta^{13}C_{DIC}$ values of the tank waters were determined by acidifying a 5 cm$^3$ aliquot with $H_3PO_4$ and extracting the evolved $CO_2$ gas through a trap to remove water. The resultant gas was then analyzed using a Finnigan-MAT 251 mass spectrometer and $\delta^{13}C$ values reported relative to PDB with an analytical error of approximately 0.1‰. Water samples were analyzed for $\delta^{18}O$ by equilibrating 1 cm$^3$ of sample with $CO_2$, following the method described by Epstein and Mayeda (1953). Data were corrected for isobaric interferences and expressed relative to SMOW. Although we did not analyze samples from all tanks in each week, we ensured that the samples analyzed spanned the entire experiment. The $\delta^{13}C$ values of water in all the tanks were depleted at the beginning of the experiment compared to the end (Table 2). This was presumably a result of a combination of lower flow-through conditions at the start of the experiment that were necessary to ensure adequate survival during early larval life and the higher numbers of

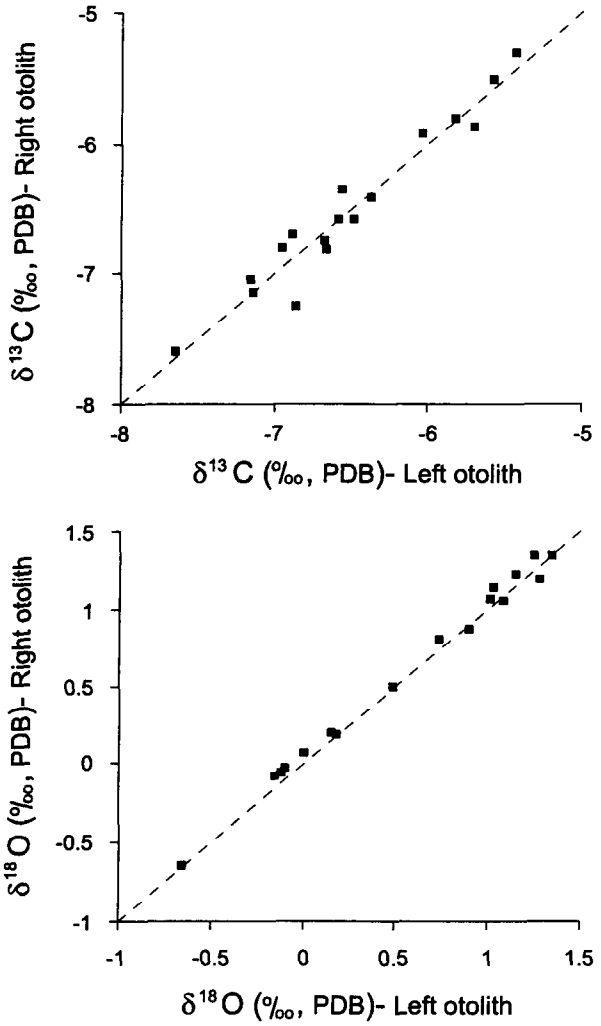


Fig. 1. $\delta^{13}C$ (‰, PDB; top) and $\delta^{18}O$ (‰, PDB; bottom) plots of left and right otoliths from *Micropogonias undulatus* juveniles raised in the laboratory under controlled environmental conditions. Dashed line shows line of 1:1 correspondence.

Table 3. Summary of $\delta^{13}C$ and $\delta^{18}O$ data from otoliths of juvenile *Micropogonias undulatus*, along with calculated carbon ($\Delta^{13}C$) and oxygen ($10^3 \ln \alpha$) isotope fractionation factors.

| T (°C) | $\delta^{13}C$ | $\delta^{18}O$ | $\Delta^{13}C$ | $10^3 \ln \alpha$ | T (°C) | $\delta^{13}C$ | $\delta^{18}O$ | $\Delta^{13}C$ | $10^3 \ln \alpha$ |
|---|---|---|---|---|---|---|---|---|---|
| 18.2 | −5.81 | 1.04 | −4.00 | 31.15 | 20.6 | 6.36 | 0.21 | −5.34 | 30.25 |
| 18.2 | −7.03 | 1.32 | −5.23 | 31.42 | 20.6 | −5.81 | 0.40 | −4.79 | 30.44 |
| 18.2 | −5.80 | 1.43 | −3.99 | 31.53 | 20.6 | −6.64 | 0.19 | −5.62 | 30.23 |
| 18.2 | −7.41 | 1.02 | −5.60 | 31.12 | 22.3 | −5.03 | 0.50 | −3.69 | 30.18 |
| 18.2 | −6.46 | 1.36 | −4.65 | 31.46 | 22.3 | −4.57 | 0.43 | −3.23 | 30.25 |
| 18.2 | −7.59 | 1.24 | −5.78 | 31.34 | 22.3 | −5.6 | 0.49 | −4.26 | 30.31 |
| 18.2 | −7.36 | 1.33 | −5.56 | 31.43 | 22.3 | −4.93 | 0.46 | −3.59 | 30.28 |
| 18.2 | −6.68 | 1.17 | −4.87 | 31.27 | 22.0 | −8.19 | 1.08 | −7.79 | 30.80 |
| 18.2 | −7.20 | 1.30 | −5.40 | 31.40 | 22.0 | −5.41 | 1.03 | −5.01 | 30.75 |
| 18.2 | −7.62 | 1.30 | −5.82 | 31.40 | 22.0 | −5.68 | 1.10 | −5.28 | 30.82 |
| 18.3 | −6.84 | 1.27 | −4.80 | 31.23 | 22.0 | −6.39 | 1.0 | −5.99 | 30.72 |
| 18.3 | −7.19 | 1.18 | −5.14 | 31.14 | 22.0 | −5.62 | 1.01 | −5.22 | 30.73 |
| 18.3 | −6.83 | 1.19 | −4.78 | 31.15 | 22.0 | −6.04 | 0.89 | −5.64 | 30.62 |
| 18.3 | −5.93 | 1.23 | −3.89 | 31.19 | 22.0 | −5.62 | 0.76 | −5.22 | 30.49 |
| 18.3 | −6.98 | 1.17 | −4.93 | 31.13 | 22.0 | −5.98 | 0.92 | −5.58 | 30.64 |
| 18.3 | −7.29 | 1.13 | −5.25 | 31.09 | 22.0 | −5.46 | 0.81 | −5.06 | 30.53 |
| 18.3 | −5.91 | 1.32 | −3.87 | 31.28 | 24.9 | −6.61 | −0.66 | −6.10 | 29.30 |
| 18.3 | −7.15 | 0.98 | −5.10 | 30.93 | 24.9 | −6.84 | −0.64 | −6.34 | 29.32 |
| 18.3 | −5.55 | 1.27 | −3.51 | 31.23 | 24.9 | −6.61 | −0.06 | −6.11 | 29.89 |
| 18.3 | −6.93 | 1.17 | −4.89 | 31.12 | 24.9 | −6.60 | −0.03 | −6.09 | 29.93 |
| 20.3 | −6.34 | 0.91 | −5.99 | 30.84 | 24.9 | −6.49 | 0.00 | −5.99 | 29.96 |
| 20.3 | −6.12 | 0.90 | −5.77 | 30.83 | 24.9 | −6.37 | −0.58 | −5.87 | 29.38 |
| 20.3 | −6.28 | 0.44 | −5.94 | 30.37 | 24.9 | −6.79 | −0.11 | −6.29 | 29.85 |
| 20.3 | −6.87 | 0.76 | −6.52 | 30.70 | 24.9 | −7.14 | −0.11 | −6.63 | 29.85 |
| 20.3 | −6.48 | 0.38 | −6.13 | 30.31 | 24.9 | −6.66 | −0.08 | −6.15 | 29.88 |
| 20.3 | −6.01 | 0.51 | −5.66 | 30.45 | 24.9 | −6.48 | −0.03 | −5.97 | 29.93 |
| 20.3 | −6.70 | 0.32 | −6.35 | 30.25 | 25.0 | −6.33 | −0.01 | −5.77 | 29.82 |
| 20.3 | −6.34 | 0.92 | −5.99 | 30.85 | 25.0 | −6.52 | 0.00 | −5.95 | 29.82 |
| 20.3 | −6.30 | 0.93 | −5.95 | 30.87 | 25.0 | −7.04 | −0.62 | −6.47 | 29.20 |
| 20.3 | −6.68 | 0.80 | −6.34 | 30.74 | 25.0 | −6.83 | 0.06 | −6.26 | 29.88 |
| 20.6 | −6.54 | 0.16 | −5.52 | 30.21 | 25.0 | −7.13 | −0.14 | −6.56 | 29.68 |
| 20.6 | −6.78 | 0.20 | −5.76 | 30.24 | 25.0 | −6.17 | 0.00 | −5.60 | 29.83 |
| 20.6 | −5.92 | 0.32 | −4.90 | 30.36 | 25.0 | −7.02 | −0.02 | −6.45 | 29.80 |
| 20.6 | −6.26 | 0.75 | −5.23 | 30.79 | 25.0 | −6.56 | −0.09 | −5.99 | 29.73 |
| 20.6 | −5.59 | 0.55 | −4.57 | 30.59 | 25.0 | −6.90 | −0.05 | −6.32 | 29.77 |
| 20.6 | −6.11 | 0.70 | −5.08 | 30.74 | 25.0 | −6.56 | 0.02 | −5.99 | 29.84 |
| 20.6 | −5.97 | 0.26 | −4.94 | 30.30 | | | | | |

larvae initially stocked into each tank, creating a greater disequilibrium between respiration-derived $CO_2$ in the tanks and the atmospheric $CO_2$. Therefore, the $\delta^{13}C_{DIC}$ value for each tank was calculated by only averaging values from specific dates at the beginning (5 November), middle (12 December), and end of the experiment (20 January). Values for $\delta^{18}O$ showed no indication of any trend with time, and, therefore, the $\delta^{18}O$ for each tank was calculated as the mean value across all sampling dates (see Table 2).

## 3. RESULTS AND DISCUSSION

### 3.1. Intra-Specimen Variability

The paired nature of fish otoliths allowed a unique opportunity to assess intra-fish variability in the isotopic composition of otolith aragonite because intraspecimen variations combine with instrument error to determine the analytical error associated with $\delta^{13}C$ and $\delta^{18}O$ measurements of otoliths. The two previous studies that examined intra-fish variation provided conflicting results. Kalish (1991a) noted large differences between paired left and right otoliths, with standard deviations of 0.3‰ for $\delta^{13}C$ and 0.4‰ (deleting one outlier) to 0.8‰ (including all data) for $\delta^{18}O$. This corresponds to temperature uncertainties of up to 3°C if $\delta^{18}O$ is used to calculate ambient temperatures. In contrast, Iacumin et al. (1992) found no differences in $\delta^{13}C$ and $\delta^{18}O$ between any of fifty pairs of left and right otoliths analyzed, within the bounds of the reported instrument error (±0.05‰, 1$\sigma$). We also could detect little difference between left and right otoliths for either $\delta^{13}C$ or $\delta^{18}O$ (Fig. 1). Analytical errors, including both intraspecimen and instrument error, were ±0.15‰ (1$\sigma$) for $\delta^{13}C$ and 0.045‰ (1$\sigma$) for $\delta^{18}O$ in our study. While the differences between paired otoliths noted by Kalish (1991a) should not be overlooked, our data support the Iacumin et al. (1992) conclusion that intraspecimen variability is a minor source of error when interpreting $\delta^{13}C$ and $\delta^{18}O$ values in fish otoliths.

### 3.2. Carbon Fractionation

The $\delta^{13}C$ values of the lab-reared juvenile *M. undulatus* otoliths ranged from −8.19 to −4.57‰, with a mean of −6.44‰ (Table 3). The $\delta^{13}C$ data were then corrected for variations in $\delta^{13}C_{DIC}$ of individual tanks by subtracting the $\delta^{13}C$ of the water from the $\delta^{13}C$ of the otolith ($\Delta^{13}C$). No relation was found between temperature and $\Delta^{13}C$ if all tanks were included in the analysis (Fig. 2 MS = 0.97, $F_{1,6}$ = 1.36, $p$ = 0.29, $r^2$ = 0.19). However, one of the tanks

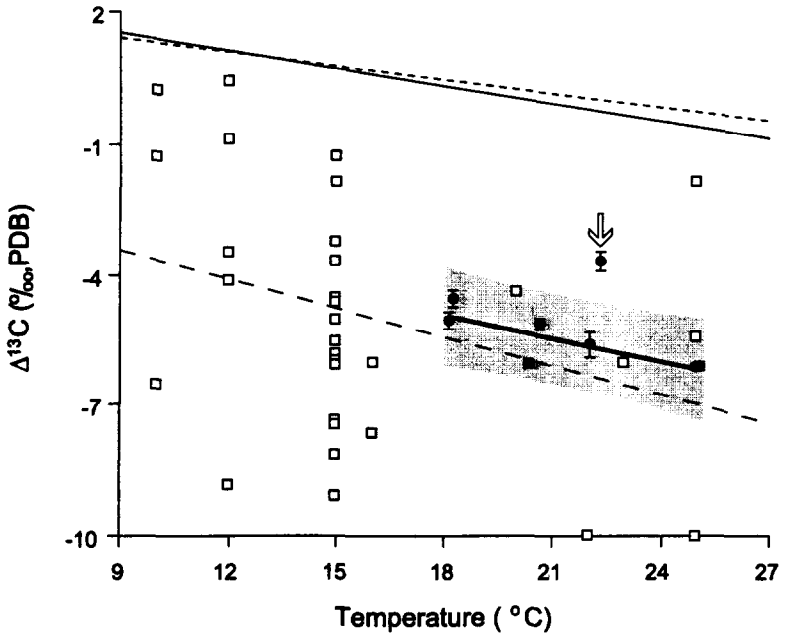


Fig. 2. Relationship between mean otolith $\Delta^{13}C$ composition (1 standard error) and temperature from otoliths of *Micropogonias undulatus* juveniles raised in the lab under controlled temperature regimes (●; thick solid line), along with an estimated relationship from a number of marine fish species (Kalish, 1991a; □, long dash line), marine mollusc shells (Grossman and Ku, 1986; thin solid line) and the foraminifera *Hoeglundina elegans* (Grossman and Ku, 1986; short dashed line). Shaded area represents 95% confidence intervals around the least-squares regression line calculated without the data point marked with an arrow.

maintained at 22.5 °C was a clear outlier, characterized by large fish size and otolith weight (Table 1). When this tank was removed, the relationship between temperature and $\Delta^{13}C$ became significant:

$$\Delta^{13}C = -1.78 - 0.18\ T°C \quad (1)$$

$$(MS = 1.50, F_{1,5} = 10.5, p = 0.023, r^2 = 0.68)$$

This relationship is similar to that determined by Kalish (1991a) from otoliths of a number of marine fish species (Fig. 2);

$$\Delta^{13}C = -1.44 - 0.22\ T°C \quad (2)$$

$$(MS = 51.26, F_{1,34} = 7.52, p = 0.01, r^2 = 0.18)$$

Both slopes (ANCOVA, MS = 0.096, $F_{1,39} = 0.02$, $p = 0.90$) and intercepts (ANCOVA, MS = 1.81, $F_{1,40} = 0.31$, $p = 0.58$) generated from the two relationships are not significantly different. Although individual species in the Kalish (1991a) study showed considerable variability around the regression line (Fig. 2), these otoliths came from adult and juvenile fish collected in the field. Temperature and $\delta^{13}C_{DIC}$ exposure histories were, therefore, based on distribution patterns of the species and averaged over the life of the fish. The similarity of the relationship between $\Delta^{13}C$ and temperature in the studies is, then, impressive given the lack of environmental constraints in the data reported by Kalish (1991a). Grossman and Ku (1986) reported relationships between $\Delta^{13}C$ and temperature for *Hoeglundina elegans* (Fig. 2);

$$\Delta^{13}C = 2.40 - 0.11\ T°C \quad (3)$$

and several related mollusc species (Fig. 2);

$$\Delta^{13}C = 2.66 - 0.13\ T°C \quad (4)$$

The proximal cause of the relationship between $\Delta^{13}C$ and temperature in biogenic carbonates remains unknown. However, the lack of a significant relationship between temperature and $\delta^{13}C$ fractionation in inorganic aragonite precipitates suggests that these results were generated by biological effects rather than a physical temperature dependence in $\delta^{13}C$ (Romanek et al., 1992). The observation that the slope of the relationship between temperature and $\Delta^{13}C$ from the fish otoliths was almost twice that of both the *H. elegans* and mollusc data is, therefore, not surprising given that very different biological factors are presumably controlling this relation among such diverse taxonomic groups.

Otoliths from the lab-reared fish were depleted in $\delta^{13}C$ by approximately 5‰ compared to inorganic aragonite precipitated at 25°C (Romanek et al., 1992) and by a similar amount from both the *H. elegans* and mollusc data in Grossman and Ku (1986). Possible factors contributing to the $\delta^{13}C$ depletion in fish otolith aragonite include either kinetic effects or incorporation of metabolic carbon. Kinetic effects would be implicated if $\delta^{13}C$ values were correlated with similar depletions in $\delta^{18}O$, as enzymes are thought to discriminate against heavier isotopes of both carbon and oxygen (McConnaughey, 1989a). We found a significant positive correlation between $\Delta^{13}C$ and $\delta^{18}O$ ($r = 0.46$, $p_{2,71} < 0.001$; Fig. 3). Kinetic effects are normally, however, characterized

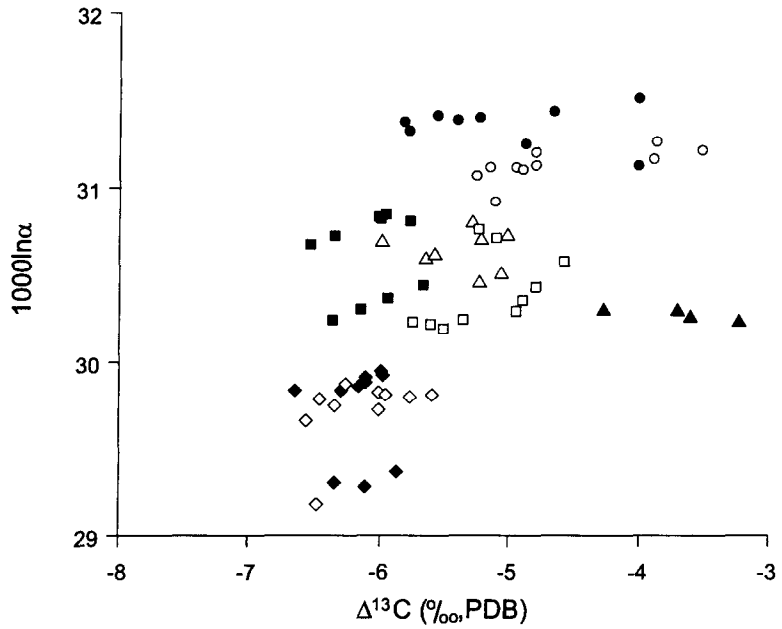


Fig. 3. Plot of carbon ($\Delta^{13}C$) vs. oxygen (1000 ln $\alpha$) fractionation factors from otoliths of *Micropogonias undulatus* juveniles raised under controlled temperature conditions at 18°C (●), 20.5°C (■), 22.5°C (▲) and 25°C (◆). Filled and open symbols represent replicate tanks within each treatment.

by simultaneous departures from isotopic equilibrium in both carbon and oxygen isotopes, with a convergence of both isotopes towards equilibrium deposition (McConnaughey, 1989a). Although oxygen isotopes in fish otoliths are generally considered to be deposited in equilibrium (Patterson et al., 1993; see next section), our data demonstrate that otolith aragonite is strongly depleted in $\delta^{13}C$. This suggests that a significant correlation between $\delta^{13}C$ and $\delta^{18}O$ may not necessarily indicate a kinetic mechanism for isotope disequilibrium in fish otoliths.

Metabolic carbon is severely depleted in $\delta^{13}C$ ($\approx -20‰$) and is a logical source of the $^{13}C$-depleted carbon in the otolith that could not have come from DIC in the water. In order to generate a correlation between $\delta^{13}C$ and $\delta^{18}O$ in otoliths in the absence of kinetic effects, the incorporation rate of metabolic carbon must be related to, although not necessarily causally correlated with, temperature. Kalish (1991a) also found a positive correlation between $\delta^{13}C$ and $\delta^{18}O$ in marine fish otoliths. Kalish (1991a) argued that $\delta^{13}C$ was linked to temperature by a positive correlation with metabolic rate, which in poikilotherms such as fish will be largely controlled by temperature. While our data is, then, generally consistent with the Kalish (1991a) hypothesis, we note that a convincing mechanism that would generate increased deposition of metabolic carbon in the otolith at higher metabolic rates has yet to be proposed.

### 3.3. Oxygen Fractionation

The $\delta^{18}O$ values in the juvenile *M. undulatus* otoliths ranged from −0.66 to 1.43‰, with a mean value of 0.58‰ (Table 3). We found a significant relationship between temperature and the fractionation factor, $\alpha$ (Fig. 4);

$$1000 \ln \alpha = 18.57(10^3\, T\, K^{-1}) - 32.54 \quad (5)$$

$$(MS = 2.22,\ F = 66.31,\ p < 0.0002,\ r^2 = 0.92)$$

where

$$\alpha = \delta_c + 1000/\delta_w + 1000 \quad (6)$$

This relationship can also be expressed in terms of $\delta_c - \delta_w$,

$$\delta_c - \delta_w = 4.64 - 0.21.\ T\ °C \quad (7)$$

$$(MS = 2.21,\ F = 64.12,\ p < 0.0002,\ r^2 = 0.91)$$

where $\delta_c - \delta_w$ is reported relative to PDB.

The relationship between temperature and $\alpha$ is similar, although not identical, to that determined for inorganically precipitated aragonite (Fig. 4), using the equation generated for inorganically precipitated calcite by O'Neil et al. (1969),

$$1000 \ln \alpha = 2.78(10^6 T\, K^{-2}) - 3.39 \quad (8)$$

and assuming a 0.6‰ enrichment in aragonite compared to calcite from 9 to 25°C (Tarutani et al., 1969). The relationship between temperature and $\alpha$ in this study is very close to that found by Grossman and Ku (1986) for *H. elegans* and several molluscs (Fig. 4),

$$1000 \ln \alpha = 18.07(10^3\, T\, K^{-1}) - 31.08 \quad (9)$$

which were considered to be deposited in isotopic equilibrium with ambient water. Taken together, these data support the conclusion that oxygen isotopes in otolith aragonite are deposited in equilibrium with seawater and that the oxygen isotope composition of otoliths may accurately record thermal histories of individual *M. undulatus* larvae and juveniles.

Two other studies have attempted to determine the temper-

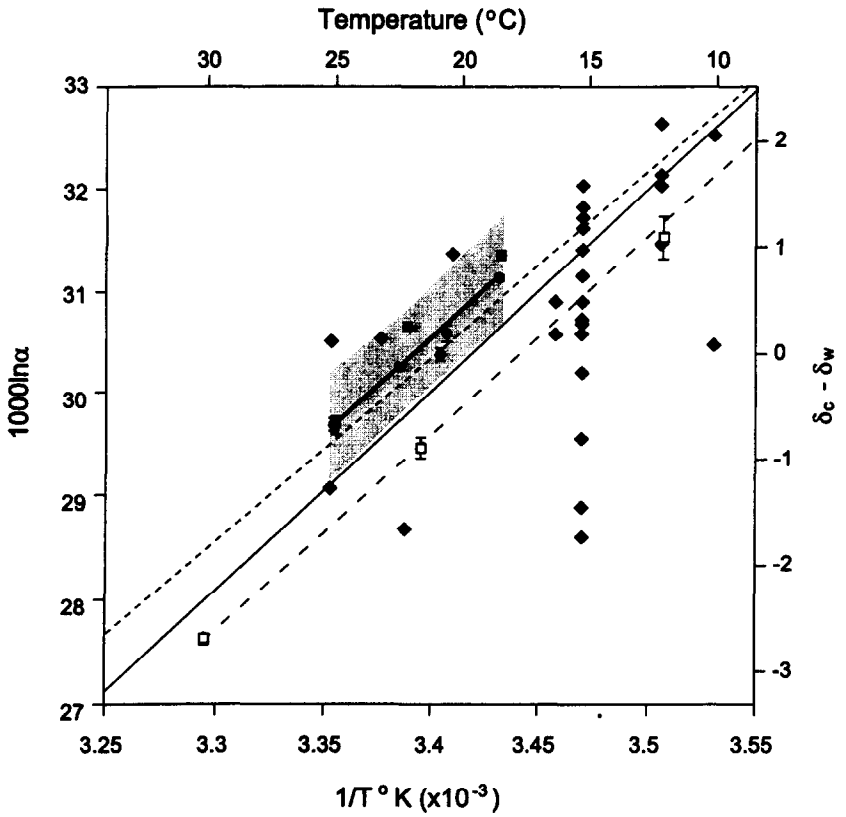


Fig. 4. Relationship between the mean oxygen isotope fractionation factor (1000 ln $\alpha$, SE) and temperature from otoliths of *Micropogonias undulatus* juveniles raised under controlled temperature regimes (●, thick solid line), along with an estimated relationship for inorganic aragonite (O'Neil et al., 1969; thin solid line), marine mollusc shells (Grossman and Ku, 1986; short dash line), otoliths from freshwater fishes (Patterson et al., 1993; (□, long dash line), and otoliths from a number of marine fish species at temperatures >10°C ( ♦; summarized in Patterson et al., 1993). Shaded area indicates 95% confidence intervals around the least-squares regression line.

ature dependence of the oxygen isotope fraction factor for fish otoliths. Patterson et al. (1993) found the following relationship,

$$1000 \ln \alpha = 18.56(10^3 T\,\mathrm{K}^{-1}) - 33.49 \quad (10)$$

from an empirical study of a number of freshwater fish species and also concluded that oxygen isotopes were deposited in isotopic equilibrium with the ambient water. The slope of the line from the Patterson et al. (1993) data is statistically indistinguishable from Eqn. 1 (ANCOVA, MS = 0.0009, $F_{1,10} = 0.03$, $p = 0.87$). However, the intercept is significantly different (ANCOVA, MS = 3.07, $F_{1,11} = 116.93$, $p = 0.0001$). We cannot explain the observed differences in intercepts between our data and that of Patterson et al. (1993). It would be somewhat surprising if the variation in intercept reflected differences between marine and freshwater taxa, as both studies apparently adjusted the isotope fractionation factor for the $\delta^{18}O$ values of the ambient water. Whatever the cause, our data question the applicability of a single relationship between temperature and oxygen isotope fractionation in the otoliths of fish from freshwater and marine systems.

Kalish (1991b) derived the following relationship between temperature and $\delta_c - \delta_w$ from juvenile Australian salmon held in the laboratory for approximately two months at known temperatures:

$$\delta_c - \delta_w = 6.69 - 0.33T°C \quad (11)$$

Both slopes and intercepts differ from Eqn. 3. However, the biological significance of these differences are questionable given that Kalish estimated $\delta_w$ from a salinity-$\delta_w$ relationship for the South Pacific Ocean. Patterson et al. (1993) suggested the variations in the isotopic composition of larval rearing tanks may also have caused the apparent nonequilibrium deposition of $\delta^{18}O$ in larval Atlantic cod and mullet otoliths (Radtke, 1984a,b).

It would be circular to attempt to estimate the precision of temperature estimates from our equation using the same dataset that generated the relationship. However temperatures can be estimated from published relationships for other taxa in which it was concluded that oxygen isotope deposition was in equilibrium with ambient waters. The equation generated by Grossman and Ku (1986) for foraminifera slightly underestimated actual temperatures experienced by the lab-reared *M. undulatus* ($\mathrm{mean}_{(\mathrm{observed - predicted})} = 1.0°C$), but with reasonable precision ($\sigma_{(\mathrm{observed - predicted})} = 1.18°C$). The Grossman and Ku (1986) equation for molluscs gave both accurate

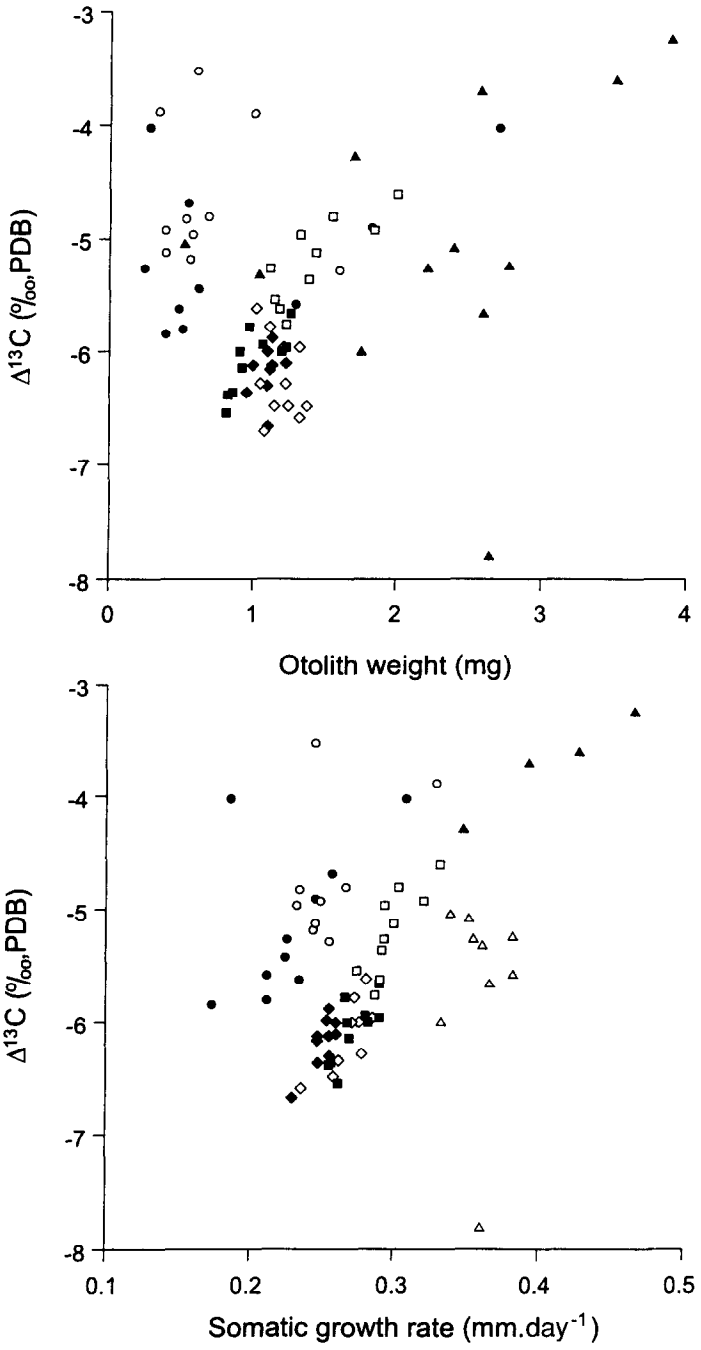


Fig. 5. Relationships between somatic growth (top) and otolith weight (bottom) and $\delta^{13}C$ in the otoliths of lab-reared *Micropogonias undulatus* juveniles raised at 18°C (●), 20.5°C (■), 22.5°C (▲) and 25°C (♦). Filled and open symbols represent replicate tanks within each treatment.

and precise estimates of temperatures in the rearing tanks ($mean_{(observed - predicted)} = 0.11°C$, $\sigma_{(observed - predicted)} = 1.19°C$). The Patterson et al. (1993) relationship from freshwater fish otoliths (Eqn. 5) consistently underestimated temperature histories ($mean_{(observed - predicted)} = 4.4°C$), although again precision was reasonable ($\sigma_{(observed - predicted)} = 1.16°C$). As temperature estimates using the empirical relationship we describe here will be more accurate than the three equations used above, reconstruction of temperature history should be possible with *M. undulatus* otoliths with errors of less than 1°C.

Most palaeoclimatic studies that have estimated past temperature histories from $\delta^{18}O$ values of skeletal material have assumed that the $\delta^{18}O$ of the water has remained unchanged throughout the period of interest, allowing changes in the $\delta^{18}O$ of the aragonite or calcite to be unambiguously inter-

Table 4. Correlations and associated probabilities of $\Delta^{13}C$ and 1000 ln $\alpha$ against otolith precipitation rate and somatic growth in otoliths of lab-reared *Micropogonias undulatus* juveniles from each of 8 experimental tanks maintained under controlled environmental conditions. Bold $r$ values significant at $\alpha = 0.05$.

| | | 18°C | | 20.5°C | | 22.5°C | | 25°C | |
|---|---|---|---|---|---|---|---|---|---|
| | | Tank 1 ($n$ = 10) | Tank 2 ($n$ = 10) | Tank 1 ($n$ = 10) | Tank 2 ($n$ = 10) | Tank 1 ($n$ = 4) | Tank 2 ($n$ = 9) | Tank 1 ($n$ = 10) | Tank 2 ($n$ = 10) |
| $\Delta^{13}C$ | | | | | | | | | |
| Otolith weight | $r$ | 0.44 | −0.15 | **0.78** | **0.81** | **0.96** | −0.33 | 0.35 | **0.65** |
| | $p$ | 0.20 | 0.67 | 0.01 | 0.005 | 0.04 | 0.39 | 0.32 | 0.043 |
| Somatic growth | $r$ | 0.49 | 0.59 | **0.73** | **0.81** | **0.98** | 0.01 | **0.81** | **0.79** |
| | $p$ | 0.15 | 0.08 | 0.02 | 0.004 | 0.02 | 0.98 | 0.004 | 0.006 |
| 1000 ln $\alpha$ | | | | | | | | | |
| Otolith weight | $r$ | 0.47 | −0.09 | 0.18 | 0.21 | −0.85 | −0.39 | **0.71** | 0.60 |
| | $p$ | 0.24 | 0.81 | 0.62 | 0.55 | 0.15 | 0.30 | 0.02 | 0.07 |
| Somatic growth | $r$ | 0.42 | 0.43 | 0.14 | 0.41 | −0.89 | −0.01 | 0.13 | 0.43 |
| | $p$ | 0.22 | 0.21 | 0.69 | 0.24 | 0.11 | 0.97 | 0.71 | 0.22 |

preted as a change in temperature. Although the validity of such an assumption is open to question, this problem is exacerbated in a species such as *M. undulatus* where seasonal migrations from offshore to near-coastal areas encompass waters with varying $\delta^{18}O$ values. It may be necessary to develop another chemical tracer in the otolith that correlates with changes in $\delta^{18}O_{seawater}$ that the fish are likely to experience. Both Sr and Ba show patterns across juvenile *M. undulatus* otoliths that are consistent with movement from offshore waters to estuarine nursery areas in the Mid and South Atlantic Bights (Thorrold et al., 1997) and would, therefore, seem likely to be correlated with changes in $\delta^{18}O_{seawater}$ in these areas.

### 3.4. Growth Effects on Isotopic Fractionation

The possible effects of precipitation rate on carbon and oxygen fractionation in biogenic carbonates remain a source of considerable debate. Early work on corals suggested there was no influence of growth rate on carbon and oxygen isotopic composition (Weber and Woodhead, 1970; Erez, 1978). These conclusions have largely been replaced by a model whereby both $\delta^{13}C$ and $\delta^{18}O$ are considered to be negatively correlated with extension rate (McConnaughey, 1989b). Under this model, slow-growing structures will be closer to isotopic equilibrium than faster-growing structures (e.g., McConnaughey, 1989b; Carpenter and Lohmann, 1995; de Villiers et al., 1995). Given that at least $\delta^{13}C$ fractionation appears independent of precipitation rate in synthetic aragonite (Turner, 1982; Romanek et al., 1992), these effects have been attributed to biological processes during $CaCO_3$ deposition (Swart, 1983; McConnaughey, 1989b). However, a recent study was unable to find any relationship between $\delta^{13}C$ and $\delta^{18}O$ values and extension rate in coral skeletons (Leder et al, 1996; Swart et al., 1996), and the generality of the McConnaughey (1989b) model of kinetic disequilibrium in biogenic carbonates remains debatable.

We found a positive, but nonsignificant, correlation between otolith precipitation rates and $\Delta^{13}C$ ($r$ = 0.22, $p_{2,71}$ = 0.06) and a positive, significant, correlation between somatic growth and $\Delta^{13}C$ ($r$ = 0.37, $p_{2,71}$ = 0.001; Fig. 5). The correlation between somatic growth and $\Delta^{13}C$ argues that metabolic, rather than kinetic, processes are generating $\delta^{13}C$ disequilibrium in fish otoliths. Furthermore, while kinetic isotope effects during $CO_2$ hydration and hydroxylation are postulated to lead to more negative $\delta^{13}C$ values at high precipitation rates, we found a small positive correlation between otolith precipitation rates and $\Delta^{13}C$. Consideration of otoliths from each tank separately, where individual fish was exposed to the same temperature and $\delta^{13}C_{DIC}$ regimes, strengthened these conclusions. Four of the tanks showed significant positive relationships otolith precipitation rate and $\Delta^{13}C$, with $r$ values ranging from 0.65 to 0.96 (Table 4). Two tanks showed negative correlations between otolith precipitation rate and $\Delta^{13}C$, although neither was significant. Correlations between somatic growth and $\Delta^{13}C$ were even stronger. Five of the tanks showed significant positive correlations between somatic growth and $\Delta^{13}C$ (Table 4), and there were no negative correlations recorded from any tank.

The observation that temperature and $\Delta^{13}C$ were negatively correlated, while somatic growth rates and $\Delta^{13}C$ were positively correlated, was largely due to fish raised in both tanks at an intermediate temperature (22.5°C) recording the fastest growth rates of any treatment (Fig. 5). Comparison of fish with more similar growth rates revealed a negative effect of temperature on $\Delta^{13}C$ that generated a 0.2‰ decrease in $\Delta^{13}C$ per °C (Eqn. 2). However, somatic growth and $\Delta^{13}C$ were positively correlated, and increased by approximately 0.6‰ per 0.1 mm · day$^{-1}$ increase in growth rate (Fig. 5). Temperature normally has a strong influence on both somatic and otolith growth in the field (e.g., Campana, 1996). Any negative relationship between temperature and $\delta^{13}C$ will, then, act to alias the effect of somatic growth on $\delta^{13}C$. However, temperature can be determined independently from the $\delta^{18}O$ of the otolith. In this instance, the residuals from an empirical model of temperature vs. $\delta^{13}C$ depletion such as Eqn. 2, in which temperature is determined independently from the $\delta^{18}O$ of the sample, may be a useful measure of fish growth and condition.

Despite significant correlations between $\Delta^{13}C$ and both otolith precipitation rates and somatic growth, we could find no evidence of a relationship between $\alpha$ and either otolith precipitation rates ($r$ = −0.17, $p_{2,71}$ = 0.15) or somatic

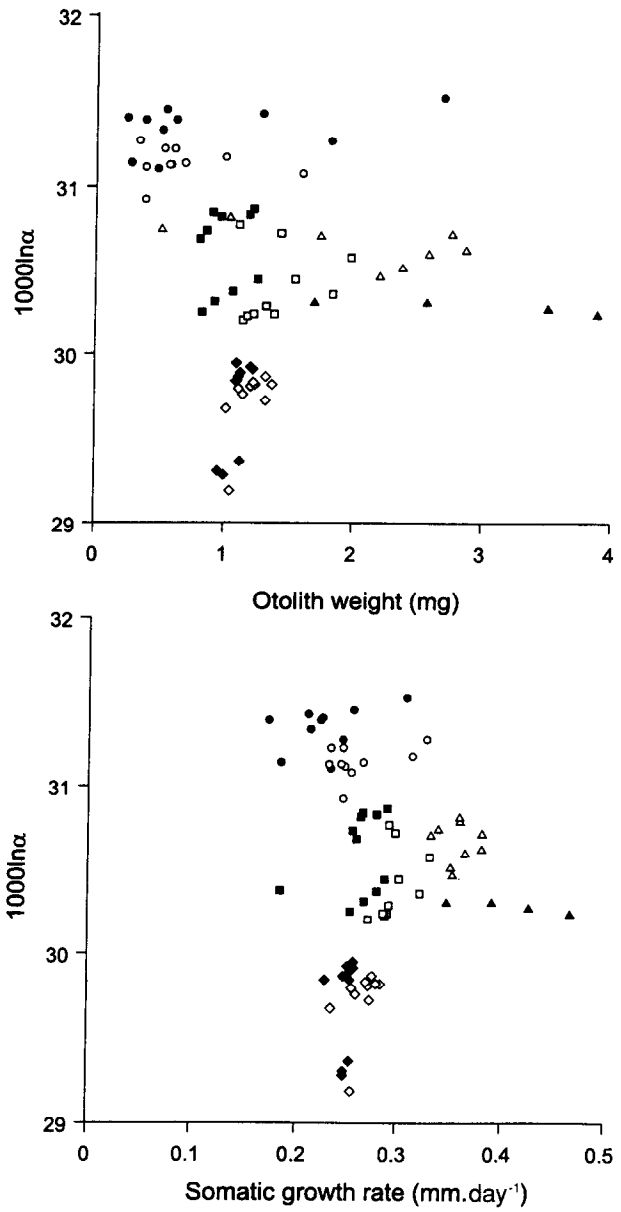


Fig. 6. Relationships between somatic growth (top) and otolith weight (bottom) and the oxygen isotope fractionation factor (1000 ln $\alpha$) in the otoliths of lab-reared *Micropogonias undulatus* juveniles raised at 18°C (●), 20.5°C (■), 22.5°C (▲) and 25°C (◆). Filled and open symbols represent replicate tanks within each treatment.

growth ($r = -0.08$, $p_{2,71} = 0.49$), either among treatments or within tanks (Fig. 6, Table 4). This is strong confirmation that oxygen isotopes are deposited in equilibrium over a wide range of otolith precipitation and somatic growth rates, and that $\alpha$ is an accurate proxy of water temperatures experienced by individual fish providing $\delta_w$ is known. Evidence from scleractinian corals has suggested that $\delta^{18}O$ values of coral skeletons are related to skeletal extension rates (Land et al., 1977; McConnaughey, 1989b; de Villiers et al., 1995). As both $\delta^{13}C$ and $\delta^{18}O$ were generally significantly depleted, it was concluded that the disequilibria were generated by kinetic effects which were stronger at higher precipitation levels. More recently, Leder et al. (1996) could find little relationship between extension rate $\delta^{18}O$ in *Montastraea annularis*. They suggested that at least some of the differences reported by other workers may have been caused by attenuation of the $\delta^{18}O$ signal due to the spatial resolution of the sample extraction method. Although the absence of significant kinetic effects in fish otoliths may make comparison with models of isotope fractionation in corals suspect, our data suggests that precipitation rates do not affect oxygen isotope composition of otolith aragonite.

## 4. SUMMARY

Juvenile *M. undulatus* otoliths are severely depleted in $\delta^{13}C$ compared to DIC. Although carbon and oxygen fractionation factors were significantly correlated, only oxygen isotopes were deposited close to equilibrium. This argues that $\delta^{13}C$ disequilibrium in fish otoliths is a result of metabolic rather than kinetic effects.

Significant positive correlations were measured between somatic growth and $\Delta^{13}C$, and, to a lesser extent, between otolith precipitation rates and $\Delta^{13}C$. This is further evidence for the presence of metabolic effects in $\delta^{13}C$ fractionation, as kinetic models predict a negative relationship between precipitation rate and both $\delta^{13}C$ or $\delta^{18}O$.

A negative relationship was found between temperature and $\Delta^{13}C$, although this appeared aliased to a degree by the positive relationship between somatic growth rate and $\Delta^{13}C$.

Oxygen isotopes were deposited approximately in equilibrium with ambient water. The relationship between temperature and the oxygen isotope fractionation factor was similar to that of *H. elegans* and several species of molluscs, although significantly different to that of two published relationships from fish otoliths.

Equilibrium deposition of $\delta^{18}O$, combined with the absence of any detectable effect of precipitation rate on oxygen isotope fractionation, confirmed that otoliths are accurate recorders of temperature ($\pm<1$°C) when the $\delta^{18}O$ of the ambient water is known.

*Acknowledgments*—This work was funded by NSF under grant number OCE-9416579 to CMJ and SEC. Larval rearing was possible due to the support of the NMFS Southeast Fisheries Center's laboratory at Beaufort, N.C. We thank John Burke and Bill Hettler for advice during the lab rearing and Gretchen Bath, Joanne Hamel, Amel Saied, and Brian Wells for technical support during the study. Comments by W. Patterson and two anonymous reviewers significantly improved an earlier draft of the manuscript.

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