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^{13}C and ^{18}O isotopic disequilibria in fish otoliths: metabolic and kinetic effects

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ABSTRACT: Distribution of oxygen and carbon stable isotopes in fish otoliths from a wide range of species was investigated to determine if the isotopes were deposited in equilibrium with ambient seawater and, the potential utility of isotope data to studies of fish biology. Comparison of $\delta^{18}O$ and $\delta^{13}C$ data from otoliths with estimates of equilibrium aragonite indicate that ^{18}O, but not ^{13}C, was deposited near equilibrium and this confirms the utility of otolith oxygen isotopes as an indicator of ambient temperature. The departure of $\delta^{13}C$ from equilibrium was considered in light of several current theories on ^{13}C isotope disequilibrium. There was a strong correlation between $\delta^{13}C$ and temperature and, concomitantly, a strong relationship between $\delta^{18}O$ and $\delta^{13}C$. Evidence is presented to support the importance of metabolic effects in mediating ^{13}C disequilibria and a strong relationship between metabolic rate (VO_2) and $\delta^{13}C$ is hypothesized. Fishes with low metabolic rates or those living at low temperatures had ^{13}C deposited near to equilibrium, whereas fishes living at higher temperatures showed extreme depletion in otolith ^{13}C. Trends were also evident in $\delta^{13}C$ values in juveniles and adults of a species with the juveniles, presumably with a higher metabolic rate, being more depleted in ^{13}C than the adults. The study shows that both oxygen and carbon stable isotopes have great potential in studies of fish biology and are worthy of further investigation.

INTRODUCTION

The oxygen and carbon stable isotopic composition of marine biogenic carbonates can provide information on the biology of marine organisms and insight into mechanisms of calcification. This principle has been widely applied to studies of marine invertebrates and has only received minor attention in relation to fishes. Mulcahy et al. (1979), Radtke (1984a,b), Radtke et al. (1987) and Kalish (1991) have shown the potential applications of fish otolith oxygen isotope data to studies of fish ecology and physiology. Environmental temperature information derived from oxygen isotope data may be useful in studies of migration and distribution. They may also help researchers locate life history stages that have eluded capture, but are important in studies of basic biology and stock assessment of fish species. Carbon isotope data may provide information on metabolic rates and diet, both important in understanding the productivity of a species and its position in an ecosystem. The use of stable isotope data to gain insight into these problems would be particularly valuable to studies of both deep-sea and large pelagic fishes, where the ability to sample populations and carry out investigations with live individuals is limited. Despite the possible benefits of oxygen and carbon isotope data derived from fish otoliths, the suitability of these data to studies of most marine fish species is still uncertain, particularly when the range of potential isotopic fractionation effects is considered.

During precipitation of calcium carbonate from solution thermodynamic relationships exert the primary influence on the partitioning, or fractionation, of oxygen and carbon isotopes (O'Neil et al. 1969). In this study, fractionation refers to the partitioning of carbon and oxygen isotopes between dissolved inorganic carbon (DIC) and aragonite, and between seawater oxygen and aragonite, respectively. When thermodynamic relationships are the only factors affecting the fractionation of carbon and oxygen isotopes during precipitation of $CaCO_3$ from solution, the isotopes are precipitated in equilibrium. Precipitation of biological carbonates, however, frequently results in the deposition of both carbon and oxygen isotopes out of predicted

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equilibrium. Non-equilibrium deposition of oxygen and carbon isotopes in biogenic carbonates can be attributed to 'metabolic' or 'kinetic' isotope effects. In biological systems these effects are generally grouped as the potential mechanisms of biological fractionation. Metabolic effects result from changes in $\delta^{13}C$ and $\delta^{18}O$ of CO_2 and HCO_3^- in the region of the calcium carbonate precipitate, due to biological processes such as respiration or photosynthesis. Kinetic effects result from the discrimination against heavy carbon and oxygen isotopes during diffusion of the carbonate isotopic species to the crystal surface (Turner 1982, Grossman 1984b) or, during hydration and hydroxylation of CO_2, which results in HCO_3^- (McConnaughey 1989a, b). Many researchers have not differentiated between metabolic and kinetic effects and have simply referred to biological fractionation or vital effects (Weber & Woodhead 1970, Land et al. 1977, Kahn 1979, Kahn & Williams 1981). Deviations from equilibrium deposition of oxygen and carbon isotopes have been attributed to the relative contributions of seawater versus metabolically derived carbon and oxygen, with metabolically derived carbon and oxygen being depleted in the heavier isotopes. Using this assumption it becomes possible to estimate the relative contributions of metabolic carbon and seawater derived carbon to the makeup of biogenic carbonates (Williams et al. 1977, Tanaka et al. 1986, Kalish 1991).

This study considers the theoretical and empirical basis for the estimation of isotopic equilibrium in calcium carbonate and applies this information to isotopic data collected on fish otoliths from a wide range of species. These data are critical in determining the utility of isotopic data to studies of a fish's environment and physiology. Data collected from different fish species living at a wide range of temperatures are used to investigate the utility of oxygen isotope thermometry to studies of fish biology and carbon isotope data is considered in light of its potential as a source of information on metabolic processes and somatic and otolith growth. Finally, the relationship between oxygen and carbon isotopes collected from a wide range of species is investigated with respect to current theories on isotopic fractionation and in relation to physical and biological processes.

DETERMINATION OF EQUILIBRIUM FRACTIONATION IN ARAGONITE

Before discussing, in detail, the basis for isotopic disequilibria in fish otoliths it is important to consider the problems encountered in estimating carbon and oxygen isotopic fractionation in biogenic carbonates, particularly aragonite. Similar points, in relation to corals, were considered by McConnaughey (1989a). Epstein et al. (1953) estimated oxygen isotope fractionation in biogenic calcite by determining isotopic ratios in molluscs collected from environments of known temperature and from shell material grown in laboratories under controlled temperature conditions. They obtained results which are in close agreement with determinations of oxygen isotope fractionation in inorganically precipitated calcite (O'Neil et al. 1969). The close agreement between these studies of inorganic and biogenic calcite indicated that the biogenic carbonate of the mollusc shells was formed in isotopic equilibrium with the seawater environment and that there was no biological fractionation of oxygen isotopes.

The fractionation of ^{18}O in inorganic aragonites has only been estimated at a single temperature (25 °C) (Tarutani et al. 1969). Tarutani et al. found that the aragonite polymorph of calcium carbonate was enriched in ^{18}O by 0.6‰ relative to calcite at 25 °C. Several relationships describing the temperature dependent fractionation of oxygen isotopes in biogenic aragonite have been determined since the study of Tarutani et al. (1969):

$$\delta_{ar} - \delta_w = 3.05 - 0.220\ (T°C) \quad (1)$$

from Horibe & Oba (1972), based on shells of the mollusc *Anadara* sp.;

$$\delta_{ar} - \delta_w = (1.39 + 3.84) - [(0.038 + 0.233)\ (T°C)] \quad (2)$$

from Sommer & Rye (1978) and Rye & Sommer (1980), based on the fractionation between aragonite/calcite foraminifera shell pairs, their data actually show the fractionation between aragonite and calcite and these results must be combined with data on calcite fractionation (e.g. Epstein et al. 1953) as has been done in Eq. (2);

$$\delta_{ar} - \delta_w = 4.42 - 0.219\ (T°C) \quad (3)$$

from Grossman (1982), based on the Foraminifera *Hoeglundina elegans*;

$$\delta_{ar} - \delta_w = 4.70 - 0.228\ (T°C) \quad (4)$$

from Grossman & Ku (1986), based on the Foraminifera *H. elegans*;

$$\delta_{ar} - \delta_w = 4.65 - 0.213\ (T°C) \quad (5)$$

from Grossman & Ku (1986), based on a series of coeval molluscs;

$$\delta_{ar} - \delta_w = 5.10 - 0.268\ (T°C) \quad (6)$$

from Dunbar & Wefer (1984), based on the Foraminifera *H. elegans*;

$$\delta_{ar} - \delta_w = 4.82 - 0.226\ (T°C) \quad (7)$$

from Aharon & Chappell (1983), based on tridacnid clams;

$$\delta_{ar} - \delta_w = 6.69 - 0.326\ (T°C) \quad (8)$$

from Kalish (1991), based on the otoliths of the percoid teleost *Arripis trutta*.

The variable δ_{ar} is the $\delta^{18}O$ of the carbonate sample and δ_w is the $\delta^{18}O$ of CO_2 gas equilibrated with a water sample from which the carbonate was precipitated.

Lines representing the isotopic temperature scales for each of the above relationships and the data from Tarutani et al. (1969) are plotted in Fig. 1. Although these relationships are based on different organisms from far removed higher taxa, all but one are in agreement, on average, to within about 1.5‰ and, with the ^{18}O fractionation determined by Tarutani et al. (1969) at 25 °C. The oxygen isotope fractionation equation determined by Horibe & Oba (1972) shows a significant depletion in ^{18}O relative to the other calibrations and, furthermore, indicates that aragonite is depleted in ^{18}O relative to calcite. This result disagrees with theoretical determinations of relative isotopic fractionation in calcite and aragonite and inorganic precipitate studies (Tarutani et al. 1969). Horibe & Oba's (1972) data, obtained from the mollusc *Anadara* sp., are probably the result of disequilibrium fractionation due to biological effects (Grossman & Ku 1986).

Equations for equilibrium ^{13}C fractionation in biogenic aragonite include:

$$\delta^{13}C_{ar} - \delta^{13}C_{DIC} = 12.40 - \frac{2980}{(T°K)} \quad (9)$$

from Grossman (1984a), based on *H. elegans*;

$$\delta^{13}C_{ar} - \delta^{13}C_{DIC} = 2.40 - 0.108\ (T°C) \quad (10)$$

from Grossman & Ku (1986), based on *H. elegans*; and

$$\delta^{13}C_{ar} - \delta^{13}C_{DIC} = 2.66 - 0.131\ (T°C) \quad (11)$$

from Grossman & Ku (1986), based on a series of coeval molluscs.

The variable $\delta^{13}C_{ar}$ is the $\delta^{13}C$ of the aragonite sample and $\delta^{13}C_{DIC}$ is the $\delta^{13}C$ of dissolved inorganic carbon (DIC) in the water where precipitation occurred. Eqs. (10) and (11) are in close agreement, whereas Eq. (9) results in ^{13}C fractionations enriched in the heavier isotope relative to Eqs. (10) and (11). Eq. (9) indicates that aragonite enrichment in ^{13}C increases with increasing temperature, while Eqs. (10) and (11) predict the opposite effect. The effect of temperature on carbon isotope fractionation in carbonates, based on the above relationships, is both small and uncertain.

MATERIALS AND METHODS

Otolith material used in this study was obtained during 1986 and 1987 from fish caught in Storm Bay, Tasmania and along the continental slope of Tasmania by line fishing or trawling. Antarctic fish were collected by trawl in the Southern Ocean off Heard Island in July 1987. Otoliths were extracted, the adhering otolith capsule removed and the otoliths were cleaned ultrasonically in deionised water, oven dried at 50 °C and stored in glass vials. Where isotope data is reported from both the juveniles and adults of a species, inner and outer layers of the otoliths were isolated with a dental drill.

Otoliths were roasted in a vacuum at 370 °C for 1 h and then reacted with 100% phosphoric acid at 24 °C for 24 h under vacuum. The CO_2 resulting from the reaction with phosphoric acid was purified and any non-condensables removed by a series of 3 freezing/transfer steps. The purified CO_2 samples were analysed on a VG Micromass 602C mass spectrometer. All values are reported in standard δ notation relative to the PDB-1 standard (Epstein et al. 1953):

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \quad (12)$$

where R = mass ratio (46/44 for oxygen and 45/44 for carbon) of the sample or standard. Isotopic ratios were

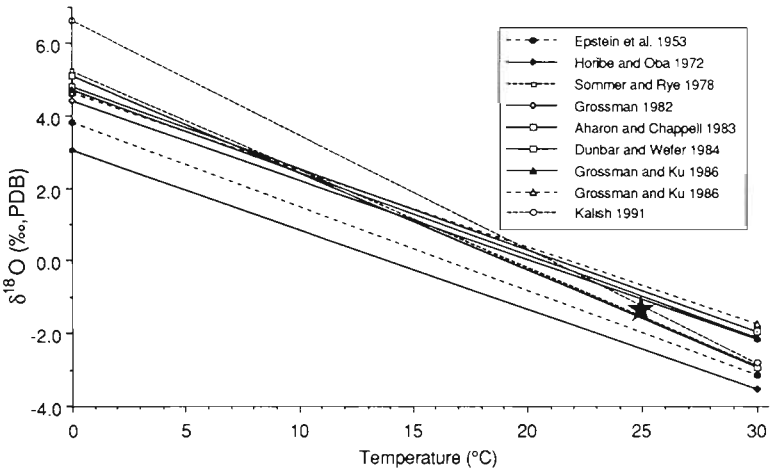

Fig. 1. Relationships describing the temperature dependent fractionation of oxygen isotopes in biogenic aragonite. One line, Epstein et al. (1953) is based on biogenic calcite. The star shows the oxygen isotope fractionation datum measured in an inorganic system by Tarutani et al. (1969). Details of these relationships appear in the text

related to the PDB standard through analysis of the Biggenden calcite standard (BCS) which had been calibrated to the PDB standard via the international standards NBS-19 (National Bureau of Standards) (Craig 1957) and TKL (Te Kuiti Limestone) (Blattner & Hulston 1978). Analytical precision of the reported measurements is ± 0.03‰ (1 SD) or better.

Values for seawater $\delta^{18}O$ (δ_w) and $\delta^{13}C$ ($\delta^{13}C_{DIC}$) for each of the species investigated was approximated on the basis of data available in the literature (Epstein & Mayeda 1953, Craig & Gordon 1965, Kroopnick et al. 1972, Kroopnick 1974a, b, 1980, Kroopnick & Craig 1976, Williams et al. 1977, Grossman 1984a, b). Isotopic equilibrium for aragonite ($\delta^{18}O_{eq}$ and $\delta^{13}C_{eq}$) in each of the environments was calculated using Eq. (4) for oxygen and Eqs. (9) and (10) for carbon. The oxygen isotope versus temperature relationship from Grossman & Ku (1986) was used, rather than the relationship between *Arripis trutta* otoliths and temperature (Kalish 1991) to avoid any potential bias in the estimation of isotopic equilibrium. The resulting values are shown in Table 1, which also presents the mean isotope data measured in otoliths ($\delta^{18}O_m$ and $\delta^{13}C_m$) from each species considered in this study. For example, for shallow-dwelling fishes from southeast Australian waters (e.g. *Arripis trutta*) the isotopic composition of arago-

Table 1. Summary of isotopic composition of fish otoliths, water, and dissolved inorganic carbon for species used in Figs. 2 to 7 and data used to estimate deviations from equilibrium isotopic fractionation. Values are means based on the number of replicates at each species. Full species names appear in Figs. 2 & 3. Isotopic values are reported in ‰ notation relative to the PDB standard. Headings are defined in the 'Materials and Methods' section

Species[a]	Depth (m)	Temp. (°C)	$\delta^{18}O_m$	δ_w	$\delta^{18}O_{eq}$[h]	$\Delta^{18}O_{eq}$	$\delta^{13}C_m$	$\delta^{13}C_{DIC}$	$\delta^{13}C_{eq}$[i]	$\delta^{13}C_{eq}$[j]	$\Delta^{13}C_{eq}$[i]	$\Delta^{13}C_{eq}$[j]
C. acrolepis (23)[b]	991	4	3.37	−0.25	3.53	−0.16	−0.84	0	1.24	1.57	−2.09	−2.41
Ariomma[c]	200	15	0.80	−0.30	0.98	−0.18	−5.50	0	2.05	0.78	−7.55	−6.28
Nomeus[c]	200	15	−0.10	−0.30	0.98	−1.08	−4.60	0	2.05	0.78	−6.65	−5.38
Cubiceps[c]	200	15	0.00	−0.30	0.98	−0.98	−4.50	0	2.05	0.78	−6.55	−5.28
Pampus[c]	50	15	0.20	−0.30	1.25	−1.05	−4.00	1	3.05	1.78	−7.05	−5.78
Peprilus[c]	50	15	−1.10	−0.30	1.25	−2.35	−4.60	1	3.05	1.78	−7.65	−6.38
Stromateus[c]	50	15	0.90	−0.30	1.25	−0.35	−0.80	1	3.05	1.78	−3.85	−2.58
Psenopsis[c]	200	15	1.10	−0.30	0.98	0.12	−0.90	0	2.05	0.78	−2.95	−1.68
Seriolella[c]	200	15	1.00	−0.30	0.98	0.02	−0.60	0	2.05	0.78	−2.65	−1.38
Schedophilus[c]	200	15	−0.10	−0.30	0.98	−1.08	−3.60	0	2.05	0.78	−5.65	−4.38
Centrolophus[c]	200	15	1.30	−0.30	0.98	0.32	−3.20	0	2.05	0.78	−5.25	−3.98
Hyperglyphe[c]	200	10	−0.20	−0.30	2.12	−2.32	−1.20	0	1.87	1.32	−3.07	−2.52
Stenotomus[c]	50	15	−1.80	−0.03	1.25	−3.05	−4.90	1	3.05	1.78	−7.95	−6.68
Roccus[c]	50	25	−4.40	−0.03	−1.03	−3.37	−4.40	1	3.40	0.70	−7.80	−5.10
Centropristes[c]	50	25	0.10	−0.03	−1.03	1.13	−0.80	1	3.40	0.70	−4.20	−1.50
Prionotus[c]	50	15	−0.20	−0.03	1.25	−1.45	−0.20	1	3.05	1.78	−3.25	−1.98
Merluccius[c]	150	12	1.40	−0.20	1.76	−0.36	−0.80	0	1.99	1.15	−2.79	−1.95
Melanogrammus[c]	150	12	1.50	−0.20	1.76	−0.26	0.50	0	1.99	1.15	−1.49	−0.65
Gadus[c]	150	10	1.90	−0.20	2.22	−0.32	0.30	0	1.92	1.37	−1.62	−1.07
Ceratoscopelus[c]	250	12	2.00	−0.20	1.76	0.24	−4.10	0	1.94	1.10	−6.04	−5.20
Osmerus[c]	10	15	−1.50	−0.03	1.25	−2.75	−2.50	2	4.05	2.78	−6.55	−5.28
M. cephalus (2)[d]	0	23	−1.65	−1.80	−2.35	0.70	−3.96	2	4.34	1.92	−8.30	−5.88
T. thynnus (6)[e]	50	25	−1.32	−0.03	−1.03	−0.29	−8.03	2	4.40	1.70	−12.43	−9.73
P. filamentosus (3)[f]	30	20	1.01	0.05	0.19	0.82	−3.36	1	3.23	1.24	−6.59	−4.60
I. illecebrosus (3)[g]	20	12	0.57	−0.03	2.04	−1.47	−6.77	2	3.73	2.95	−10.50	−9.72
A. trutta (25)	3	16	0.25	0.05	1.10	−0.85	−5.61	2	4.09	2.67	−9.70	−8.28
H. atlanticus (10)	1040	4	2.39	−0.25	3.59	−1.19	−1.04	0	1.23	1.59	−2.27	−2.63
T. atun (4)	10	15	0.80	0.05	1.33	−0.53	−5.32	2	4.05	2.78	−9.37	−8.10
N. macropterus (4)	10	15	0.38	0.05	1.33	−0.96	−6.07	2	4.05	2.78	−10.12	−8.85
T. declivis (2)	10	15	1.05	0.05	1.33	−0.28	−5.39	2	4.05	2.78	−9.44	−8.17
B. brama (2)	100	10	0.35	0.05	2.47	−2.13	−6.48	0	1.87	1.32	−8.35	−7.80
M. novaezelandiae (4)	400	8	1.71	0.05	2.93	−1.23	−2.61	0	1.77	1.51	−4.38	−4.12
P. barbatus (4)	10	12	1.10	0.05	2.01	−0.91	−1.44	2	3.94	3.10	−5.38	−4.54
T. maccoyii (2)	50	22	−1.63	0.05	−0.27	−1.36	−7.92	2	4.30	2.02	−12.22	−9.94
A. esper (2)	10	16	0.56	0 05	1.10	−0.54	−3.97	2	4.09	2.67	−8.06	−6.64
N. squamifrons (2)	10	1	2.65	0.05	4.52	−1.87	−3.75	1	2.92	3.69	−6.67	−7.44

[a] Number of replicates in parentheses
[b] Mulcahy et al. (1979); [c] Degens et al. (1969); [d] Radtke (1984a); [e] Radtke et al. (1987); [f] Radtke (1987); [g] Radtke (1983)
[h] Using Grossman & Ku (1986)
[i] Using Grossman (1984a)
[j] Using Grossman & Ku (1986)

nite precipitated in equilibrium ($\delta^{18}O_{eq}$ and $\delta^{13}C_{eq}$) with Tasman Sea surface water varying from 10 to 20 °C was estimated as follows. The value for $\delta^{13}C_{DIC}$ was estimated to be 2.0‰, based on depth and apparent oxygen utilization (AOU) (Williams et al. 1977). Seawater $\delta^{18}O$ (δ_w) was estimated to be 0.086‰ for Tasman Sea surface water (using data from the South Pacific Ocean from Craig & Gordon 1965). Using Eqs. (9), (10) and (11), $\delta^{13}C_{eq}$ of otolith aragonite is estimated to range from 2.04 to 4.23‰ and, $\delta^{18}O_{eq}$ ranges from 0.13 to 2.32‰ based on Eq. (4). The departures from isotopic equilibrium ($\Delta^{18}O_{eq} = \delta^{18}O_m - \delta^{18}O_{eq}$ and $\Delta^{13}C_{eq} = \delta^{13}C_m - \delta^{13}C_{eq}$) also appear in Table 1.

When estimating the isotopic composition of aragonite precipitated in equilibrium for Atlantic bluefin tuna *Thunnus thynnus* and southern bluefin tuna *Thunnus maccoyii* otoliths, an approximation of these fishes' body temperature was used rather than the ambient water temperature. This was done because tuna maintain muscle, eye and brain temperatures significantly above ambient temperatures (Stevens & Fry 1971, Linthicum & Carey 1972, Carey & Lawson 1973).

RESULTS

Plots of $\delta^{18}O$ as a function of $\delta^{13}C$ for the sagittal otoliths of 35 marine fish species and the statoliths of one squid species appear in Figs. 2 & 3; the mean values are tabulated by species in Table 1. Data are broken down into 2 graphs (Figs. 2 & 3) so that it is possible to distinguish which data are attributable to a particular species. The bulk of the data are from isotopic measurements made in this study with the remainder of the data being obtained from the literature. Despite the fact that the data are from numerous species encompassing a wide range of higher taxa, collected in different oceans, at different depths and, that the samples were analysed by several different laboratories, there is still a strong correlation between $\delta^{13}C$ and $\delta^{18}O$. The least squares regression line for all data is $\delta^{18}O = 2.73 + (0.45)\delta^{13}C$ ($r^2 = 0.50$, $p < 0.001$) and, for data collected in this study only, it is $\delta^{18}O = 2.64 + (0.41)\delta^{13}C$ ($r^2 = 0.58$, $p < 0.001$). The majority of the $\delta^{18}O$ and $\delta^{13}C$ data from the various fish species fall close to the regression line, with the greatest scatter attributable to the data of Degens et al.(1969) who only made a single isotopic measurement for each species. Data on mullet *Mugil cephalus* from Radtke (1984a) also fall relatively far from the regression line, perhaps due to the fact that these data are from fish reared at a salinity of 32‰. In general, it must be assumed that intercalibration through the international standard PDB is satisfactory and that these deviations are real. The significance of the relative distribution of the various species along the regression line will be considered further in the discussion.

Plots of carbon isotopes against temperature show that otolith carbonate becomes more depleted in ^{13}C with increasing temperature (Fig. 4). Although the data show a negative correlation with temperature ($r^2 = 0.50$, $p < 0.001$) as described by Grossman & Ku (1986) (Eqs. 10 & 11), the slope of the otolith carbon data versus temperature is approximately 2 times what they observed for a single species of foraminifera and several related mollusc species. If deviations from equilibrium precipitation ($\Delta^{13}C_{eq}$) are plotted against temperature (Fig. 5) an even stronger negative correlation ($r^2 = 0.62$, $p < 0.001$) results. This appears to indicate that changes in $\delta^{13}C$ with temperature are probably due to biological fractionation of isotopes and are not the result of the influence of $\delta^{13}C_{DIC}$. To draw this conclusion, it must be assumed that errors in the estimation of both $\delta^{13}C_{DIC}$ and $\delta^{13}C_{eq}$ are insignificant relative to biologically mediated fractionation effects. The $\delta^{13}C_{DIC}$ values were obtained from the literature and, although every effort was made to obtain values representative of the sample locations in question, these values are only estimates. Given the range of

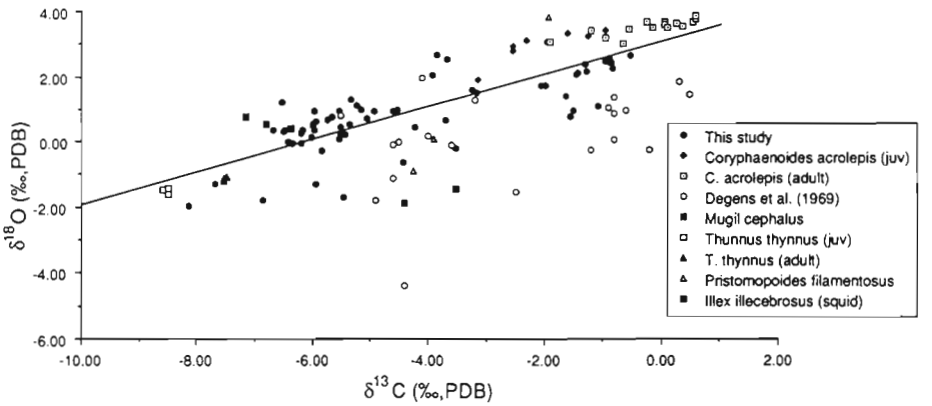

Fig. 2. Plot of $\delta^{18}O$ versus $\delta^{13}C$ for the aragonitic otoliths of 35 fish species and the aragonitic statoliths of one squid species. Data identified by species in this graph are from literature. Data sources indicated in Table 1. For comparison data collected in this study are also plotted on the graph and these data are identified to species in Fig. 3

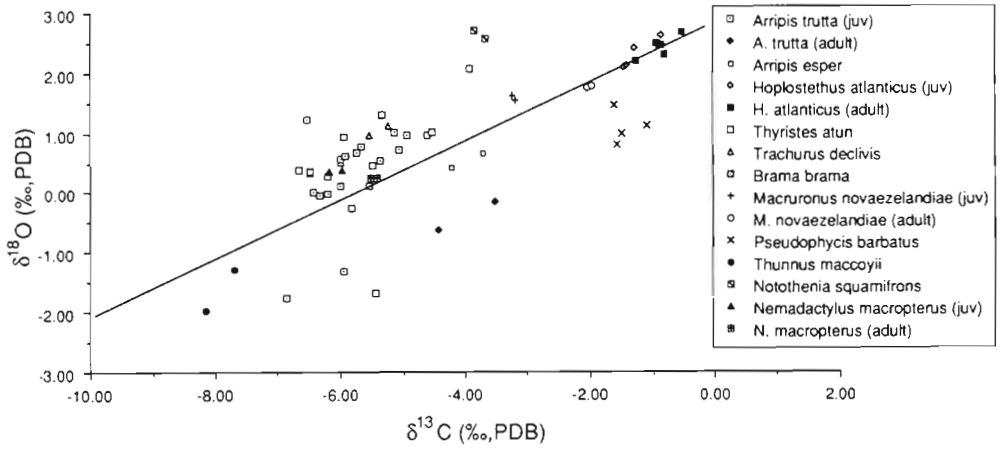

Fig. 3. Plot of $\delta^{18}O$ versus $\delta^{13}C$ for the aragonitic otoliths of 11 fish species. For some species data have been collected from both juveniles and adults; this is indicated in the inset; all data were collected in this study

$\delta^{13}C_{DIC}$ values measured in the ocean, however, potential errors in the estimation of $\delta^{13}C_{DIC}$ would be small compared with the deviations from $\delta^{13}C_{eq}$ measured in fish otoliths. Some studies have shown a small temperature effect on the precipitation of carbon isotopes in foraminifera (Williams et al. 1977, Grossman 1984b, Grossman & Ku 1986) and molluscs (Mook & Vogel 1968, Fritz & Poplawski 1974), however, as for the estimation of $\delta^{13}C_{DIC}$, the magnitude of this effect would not be significant in view of the large deviations from equilibrium in the fish otolith data.

Otolith oxygen isotopes become more depleted in the heavy isotope (^{18}O) at higher temperatures (Fig. 6), similar to the trend for carbon isotopes. Least squares linear regression shows that the relationship between oxygen isotopes and temperature for all otolith data is $\delta^{18}O_m = 3.58 - 0.196$ (T°C), ($r^2 = 0.69$, $p < 0.001$). This line is in reasonable agreement with inorganic precipitate data from Tarutani et al. (1969) and relationships derived from other studies of biogenic aragonites shown in Fig. 1. It must be remembered, however, that $\delta^{18}O_m$ values for individual fish otolith data points represent a mean value accumulated over the life of a fish, which, in this study, might range from some fraction of 1 yr to, perhaps, over 20 yr. Furthermore, the estimates of temperature are based on current knowledge on the distribution of each species over the fish's lifetime, and, of course, have not been measured. Under these circumstances, the strong correlation appears to provide good evidence for a relationship between the $\delta^{18}O$ measured in fish otoliths and temperature. These points also apply to the carbon isotope data above, but it is clear that differences between the carbon data presented here and data appearing in the literature

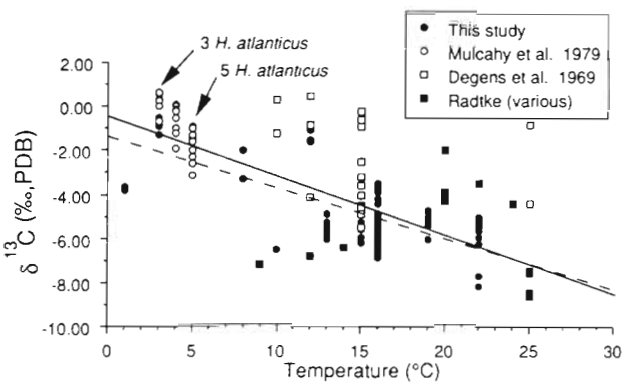

Fig. 4. Relationship between $\delta^{13}C$ and ambient water temperature. Solid line based on linear regression for all data points; dashed line, only on data collected in this study. Eight data points from *Hoplostethus atlanticus* are obscured and their location is indicated in the figure

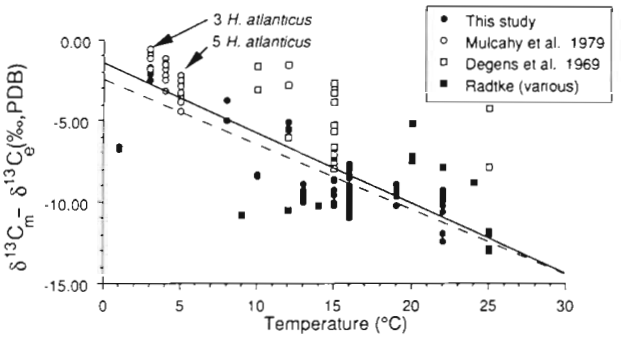

Fig. 5. Carbon isotope enrichment between otolith aragonite and estimated equilibrium aragonite as a function of temperature. Data used to estimate equilibrium appear in Table 1 Solid line based on linear regression for all data points; dashed line, only on data collected in this study. Eight data points from *Hoplostethus atlanticus* are obscured and their location is indicated in the figure

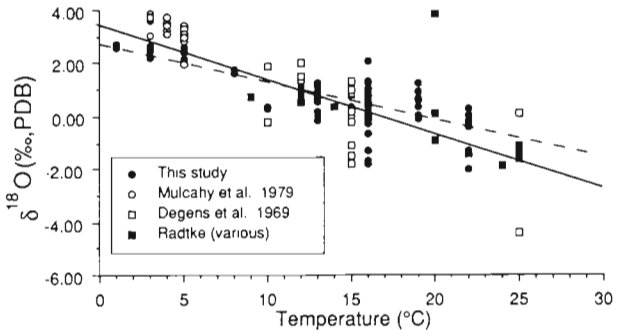

Fig. 6. Relationship between $\delta^{18}O$ and ambient water temperature. Solid line based on linear regression for all data points; dashed line, only on data collected in this study

(Grossman 1984a, Grossman & Ku 1986) are more extreme than differences between oxygen isotopes measured in otoliths and literature values for other biogenic carbonates.

A plot of the differences between $\delta^{18}O_m$ and $\delta^{18}O_{eq}$ for all data (Fig. 7) shows that deviations from oxygen isotopic equilibrium ($\Delta^{18}O_{eq}$) are not related to temperature ($r^2 = 0.03$, $p > 0.08$) and the mean deviation from equilibrium fractionation approaches zero. Consideration of data from this study alone (Fig. 7) indicates that there may be evidence for a positive correlation between $\Delta^{18}O_{eq}$ and temperature ($r^2 = 0.27$, $p < 0.01$) with relatively large depletions in the heavier oxygen isotope occurring at the lower temperatures. Closer examination of the data, however, indicates that this relationship is largely the result of a few data points at the extreme high and low temperatures. Furthermore, there was no evidence of a linear relationship among these data ($F = 1.23$, $df = 1,59$, $p > 0.25$).

DISCUSSION

Use of $\delta^{18}O$ data as an environmental thermometer

Data collected from a wide range of fish species provide convincing evidence for a relationship between $\delta^{18}O$ and temperature. Furthermore, the precipitation of oxygen isotopes in the aragonite of fish otoliths appears to be in equilibrium with seawater $\delta^{18}O$. Similar conclusions were made in Kalish (1991), however, more precise determinations from further laboratory experiments carried out under controlled temperature conditions are needed to conclusively determine the equilibrium deposition of otolith oxygen isotopes.

Values of $\delta^{18}O$ from fish otoliths can be used to investigate the temperature of environments experienced during the fish's life. The $\delta^{18}O$ data obtained from the otoliths of *Thunnus* sp., however, confirms that the otoliths of these fish are maintained at temperatures above ambient and, presumably, at temperatures similar to the brain and body. Therefore, they cannot be used as recorders of environmental temperatures as suggested by Radtke & Morales-Nin (1989). At present, the utility of $\delta^{18}O$ data in the determination of environmental temperatures is restricted primarily by the sample size requirements of stable isotope mass spectrometers. Developments in laser ablation mass spectrometry and other, microanalytical related methods may make it possible to investigate variations in $\delta^{18}O$ over small portions of an otolith.

Relationship between $\delta^{13}C$ and $\delta^{18}O$

The interpretation of the relationship between $\delta^{13}C$ and $\delta^{18}O$ (Fig. 8) has been open to debate since Keith & Weber (1965) first suggested that the correlation might be due to calcification processes incorporating carbon and oxygen compounds originating from 2 isotopically different sources or, the incorporation of specific portions of oxygen and carbon compounds that displayed a correlation between $\delta^{13}C$ and $\delta^{18}O$. In those corals that contain symbiotic algae (hermatypic corals) the correlation between $\delta^{13}C$ and $\delta^{18}O$ is not always evident because only carbon is fractionated during photosynthesis (Swart 1983). Furthermore, the combined effect of respiration, which depletes both oxygen and carbon heavy isotopes and, photosynthesis results in there often being no apparent relationship between $\delta^{13}C$ and $\delta^{18}O$ in hermatypic corals (Weber & Woodhead 1970, Goreau 1977, Swart & Coleman 1980, Swart 1983). Most researchers have concluded that the correlation between carbon and oxygen isotopes in ahermatypic corals and other organisms is the result of the incorporation of metabolically derived CO_2 in the biogenic carbonate (Keith & Weber 1965, Weber & Woodhead 1970, Vinot-Bertouille & Duplessy 1973, Land et al. 1977, Kahn 1979, Kahn & Williams 1981, Williams et al. 1981a, b, Grossman 1984b).

Kinetic effects, due to varying weights and, thus, diffusion rates of different isotopes, are one of the main factors affecting isotopic fractionation. Kinetic effects could result in the depletion of the heavier carbon and oxygen isotopes, rather than incorporation of metabolic CO_2 alone. For example, Turner (1982) found that calcium carbonate, specifically calcite, precipitated in an inorganic system, could be depleted in ^{13}C by 0.35 to 3.37 ‰ depending on the rate of carbonate precipitation with the magnitude of the ^{13}C depletion increasing with increased rate of precipitation. Unfortunately, he was only able to measure $\delta^{13}C$ and, thus, it is not possible to directly interpret these data with respect to the relationship between $\delta^{18}O$ and $\delta^{13}C$.

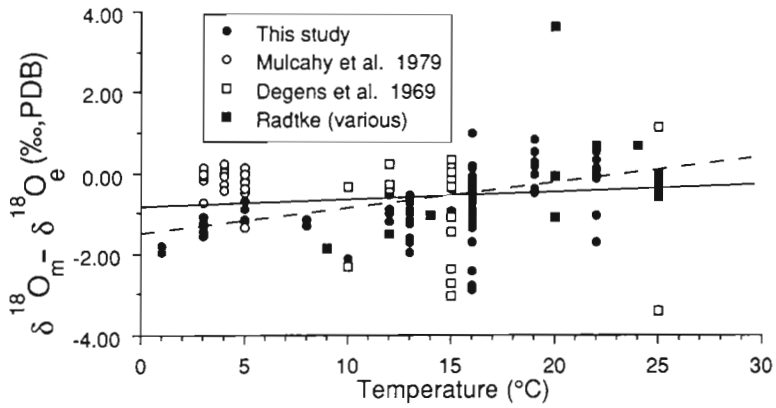

Fig. 7 Oxygen isotope enrichment between otolith aragonite and estimated equilibrium aragonite as a function of temperature. Data used to estimate equilibrium appear in Table 1. Solid line based on linear regression for all data points; dashed line, only on data collected in this study

The magnitude of the kinetic fractionations, in carbonates, due to different rates of diffusion can be approximated by estimating the ratio of diffusivities of the CO_3^{-2} isotopic species involved in carbonate precipitation. The ratio of diffusivities, D/D' is approximated by:

$$\frac{D}{D'} = \sqrt{\frac{m^L}{m^H}} \quad (13)$$

where D and D', and m^L and m^H = diffusivities and masses of light and heavy carbonate isotopic species, respectively. The ^{13}C and ^{18}O fractionation factors due to diffusion are 0.992 ($^{12}C^{16}O_3^{-2}/^{13}C^{16}O_3^{-2}$) and 0.984 ($^{12}C^{16}O_3^{-2}/^{12}C^{18}O^{16}O_2^{-2}$). The ratios of the molecular velocities during diffusion indicate that depletions of 8.0 and 16.0‰ occur in $\delta^{13}C$ and $\delta^{18}O$, which corresponds to a slope of 2.0 for the regression of $\delta^{18}O$ against $\delta^{13}C$. Grossman (1984b) rejected the kinetic hypothesis, as outlined above, on the basis that his data did not have the required slope. However, this calculation fails to consider the relative abundance of ^{13}C:^{12}C versus ^{18}O:^{16}O, where the heavy isotopes are approximately 1.1% (Nier 1950) and 0.2% (Garlick 1969) of the total carbon and oxygen, repectively. The net result is that a CO_3^{-2} molecule with ^{13}C is 5 times more likely to occur at the crystal surface than a molecule with ^{18}O, indicating net depletions of 8 and 3.2‰ for carbon and oxygen, and a slope of 0.4. The mean slope of $\delta^{18}O$ versus $\delta^{13}C$ data obtained from the literature for several higher taxa (Fig. 8) is approximately 0.37 and, for the fish otolith data the slope is 0.45, very close to the approximations based on the kinetic/diffusion hypothesis.

The significance of a different kinetic effect, during hydration and hydroxylation of CO_2, in altering $\delta^{13}C$ and $\delta^{18}O$ and causing isotopic disequilibria in biogenic carbonates, has recently been investigated by McConnaughey (1989a, b). He concluded that the linear relationship between $\delta^{18}O$ and $\delta^{13}C$ indicates the influence of a kinetic mechanism that would result in simultaneous depletions and that such a mechanism is responsible for mediating isotopic disequilibria in

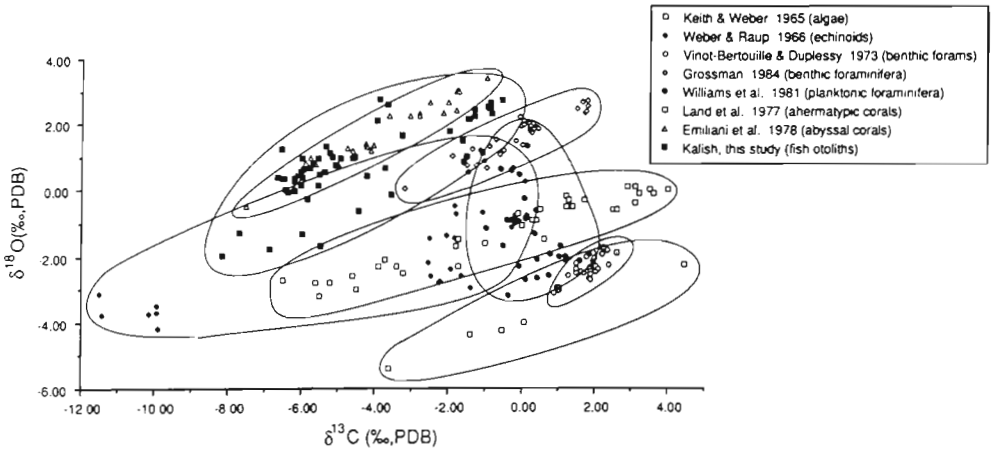

Fig. 8. Plot showing general trend in $\delta^{18}O$ versus $\delta^{13}C$ from several taxa, reported in the literature and from fish otoliths analysed in this study. Only data from the photosynthetic planktonic foraminifera (Williams et al. 1981a) deviate from the general pattern. Of the remaining groups, only the algae are photosynthetic

biogenic carbonates, particularly during rapid precipitation. McConnaughey's (1989a) data from photosynthetic and non-photosynthetic corals show extreme departures from isotopic equilibrium and he states that isotopic disequilibria increase with increased growth rate in these organisms. He does not consider the additional effects of a kinetic/diffusion effect acting at the crystal face.

Other sources present evidence that contradicts the kinetic hypothesis and provide support for the role of metabolism in influencing the carbon isotopic composition of calcium carbonate. Fritz & Poplawski (1974) cultured freshwater molluscs in water with $\delta^{13}C_{DIC}$ of −35.5, −13.1, and +5.4 ‰. Those animals cultured in aquaria with $\delta^{13}C_{DIC}$ of −35.5 ‰ had shells that were enriched in ^{13}C relative to the water, while animals cultured in water with $\delta^{13}C_{DIC}$ of −13.1 and +5.4 ‰ were depleted in ^{13}C relative to the culture media. This result implies the inclusion of carbon with an isotopic composition of between −35.5 and −13.1 ‰, presumably metabolically derived carbon. The isotopic composition of metabolically derived carbon in molluscs should fall within this range based on measurements of δ^{13} in molluscan tissue (Gearing et al. 1984). Tissue $\delta^{13}C$ is an accepted estimator of the $\delta^{13}C$ of metabolically derived CO_2 present in the body fluids.

Studies by Pearse (1970), Sikes et al. (1981), Tanaka et al. (1986) and Spero & Williams (1988, 1989) on sea urchins, corals, molluscs, barnacles and foraminifera have all shown that both metabolic carbon and DIC are incorporated into shell calcium carbonate. Pearse (1970) and Sikes et al. (1981) showed the importance of metabolic carbon by providing organisms with ^{14}C-labelled food which was subsequently detected in the shell. Tanaka et al. (1986) looked at naturally occurring levels of ^{14}C in DIC and, various food sources in a natural system and concluded that up to 85 % of the carbon in shell carbonate was derived from metabolic sources. Spero & Williams (1988, 1989) found that $\delta^{13}C$ values measured in foraminifera were not influenced by temperature, but that the values were a function of metabolism in the form of symbiont photosynthetic activity and irradiance levels.

The fish otolith isotope data presented here, when plotted with isotope data from other organisms follows a trend similar to that described by McConnaughey (1989a) (Fig. 8) and the overall distribution of the otolith isotope data is very similar to that of the ahermatypic corals (Swart 1983). However, my estimates of $\delta^{13}C$ and $\delta^{18}O$ for aragonite precipitated in isotopic equilibrium with the various fish environments represented in this data set (Table 1), indicate that only carbon isotopes are fractionated out of isotopic equilibrium. This point is illustrated by graphs of the carbon isotope enrichment between biogenic aragonite of fish otoliths and seawater dissolved inorganic carbon (Fig. 5) and oxygen isotope enrichment between the otoliths and seawater $\delta^{18}O$ (Fig. 7), both as a function of temperature. As mentioned previously, otolith $\delta^{18}O$ values do not differ significantly from predicted equilibrium values. The highly significant regression between $\delta^{13}C$ and $\delta^{18}O$ may indicate that some mechanism, perhaps related to temperature (e.g. metabolic rate) is resulting in this relationship in fish otoliths.

On the basis of the relationships described to this point it seems reasonable to draw the same conclusion as Grossman (1984b), Tanaka et al. (1986) and many others, that carbon isotopic disequilibria, in fish otoliths in this case, are due to the incorporation of metabolically derived CO_2. This further supports calculations made to estimate the relative contributions of DIC and metabolic carbon to fish otoliths as in Kalish (1991).

Variations in carbon and oxygen stable isotopes among species

There are several points relating to the carbon and oxygen isotope data that support the hypothesis that $\delta^{13}C$ values measured in the otoliths are related to the metabolic rate of the fish. The relationship between $\delta^{13}C$ and temperature is not likely to be directly due to temperature as discussed in the previous section. In the majority of studies on biogenic carbonates a significant relationship between temperature and $\delta^{13}C$ has not been found. Those studies that found $\delta^{13}C$ to be a function of temperature are not consistent, even with regard to the slope (positive or negative) of the relationship. Also, the magnitude of the temperature effect on $\delta^{13}C$ that has been found is much smaller than the apparent relationship in fish otoliths.

Several trends in the isotope data add support to the hypothesis that there is a relationship between $\delta^{13}C$ and metabolic rate. Those species that display the smallest departures from $\delta^{13}C$ equilibrium appear to have low metabolic rates, live in deep, cold waters and possess relatively large otoliths, most notably the macrourid *Coryphaenoides acrolepis* and orange roughy *Hoplostethus atlanticus*. At the opposite end of the spectrum are the fast growing, highly active, homeothermic tunas, which have, relative to body size, small otoliths. It is notable that the older macrourids are at the point most opposite from the tunas, whereas the younger fish, which presumably have a higher metabolic rate, display a greater tendency to be more depleted in ^{13}C, thus, showing that the potential relationship between $\delta^{13}C$ and temperature/metabolic rate may also be manifest within a species. A similar relationship is also evident among samples from juvenile and adult Atlantic bluefin tuna *Thunnus thynnus* (Radtke et al. 1987), Australian

salmon *Arripis trutta*, jackass morwong *Nemadactylus macropterus* and blue grenadier *Macruronus novaezelandiae*. In each of these species, ^{13}C was more depleted in the otoliths of juveniles than in the adults. The differences in both $\delta^{13}C$ and $\delta^{18}O$ between juvenile and adult orange roughy are very small and may be directly related to the biology of these deepwater fishes, which are very slow growing both as juveniles and adults (Mace et al. 1990).

The isotopic data from the otoliths of other fish species readily falls within the spectrum of metabolic rates and $\delta^{13}C$ values outlined by *Coryphaenoides acrolepis* and *Thunnus* spp. (Figs. 2 & 3). Less active species with large otoliths, such as red cod *Pseudophycis barbatus* and blue grenadier have $\delta^{13}C$ values about 4.0 ‰ depleted in ^{13}C relative to equilibrium. The $\delta^{13}C$ values measured in a wide range of pelagic species, which might be classified as moderately active and have medium sized otoliths, are clustered towards the tuna end of the range and show a depletion of ca 7.0 to 12 ‰ relative to $\delta^{13}C_{eq}$.

Differences between $\delta^{13}C$ measured in juveniles and adults of a single species are very small when compared with differences among species such as *Thunnus maccoyii, Coryphaenoides acrolepis* and *Hoplostethus atlanticus*. This may be due to the relatively small differences in metabolic rates between juveniles and adults, when compared with the metabolic rates of other species. Information on the metabolic rates of each of these species is not available but, in many cases, these rates can be inferred from data available on other species. The mean oxygen consumption rate (VO_2) for *Coryphaenoides acrolepis, Coryphaenoides armatus* and *Sebastolobus altivelis* measured at depths of more than 200 m was 2.8 ± 0.5 µl g^{-1} h^{-1} (Smith & Hessler 1974, Smith 1978, Smith & Brown 1983). Oxygen consumption rates for skipjack *Katsuwonus pelamis* and albacore *Thunnus alalunga* tuna have been measured as 505 (at 23 to 25 °C) and 212 µl g^{-1} h^{-1} (at 15 to 19 °C), respectively (Gooding et al. 1981, Graham & Laurs 1982), more than 75 times the rate measured for the 3 deepwater species. Brett & Groves (1979) calculated a mean VO_2 of 62 ± 24 µl g^{-1} h^{-1} with a range of 18 to 160 µl g^{-1} h^{-1} for 34 fish species (not including tunas or deep-sea species). Clearly, the fish represented in the isotope data cover the widest possible extremes of metabolic rates. Taking this range into consideration, it is not surprising that differences in metabolic rates within a species are not always resolved by the $\delta^{13}C$ data.

A notable exception to the relationship between temperature/$\delta^{18}O$ and metabolism/$\delta^{13}C$ in this study is the data collected from the Antarctic fish *Notothenia squamifrons*, where the deviation from equilibrium $\delta^{13}C$ is much greater than would be expected on the basis of habitat temperature and the postulated relationship between metabolic rate and $\delta^{13}C$ (Fig. 5). The mean $\delta^{13}C$ value for *N. squamifrons* otoliths, collected in the Southern Ocean off Heard Island (mean temperature ca. 1 °C), is −3.75 ± 0.13, a value that would be expected for fishes living at a temperature of about 11.5 °C and with oxygen consumption rates between those determined for the deep-sea species (2.8 ± 0.5 µl g^{-1} h^{-1}) and the estimated mean for a range of species (62 ± 24 µl g^{-1} h^{-1}). This deviation can be explained by the fact that cold-adapted fishes have metabolic rates above what would be expected on the basis of temperature and size alone (Macdonald et al. 1987). This property is frequently attributed to metabolic cold adaptation (MCA), although the exact nature of this phenomenon is still open to debate (Macdonald et al. 1987). There are no published data on the VO_2 of *N. squamifrons*, but an estimate can be derived by calculating the mean VO_2 from oxygen consumption data (measured below 3 °C) on 10 species in the genus *Notothenia* appearing in Macdonald et al. (1987). The mean value for VO_2 for these 10 species is 43.4 ± 17.0 µl g^{-1} h^{-1}, placing these species within the range of metabolic rates and $\delta^{13}C$ values expected for fishes occurring in warmer waters. This information provides further support for the idea that variations in $\delta^{13}C$ are due to some metabolic effect and that they are not directly related to temperature.

The data presented above provide the initial framework for development of a relationship between $\delta^{13}C$ and oxygen consumption. Although few data are available, I have plotted $\delta^{13}C$ versus oxygen consumption (µl g^{-1} h^{-1}) in Fig. 9. The data for *Coryphaenoides acrolepis, Notothenia* spp. and *Thunnus* spp. are based

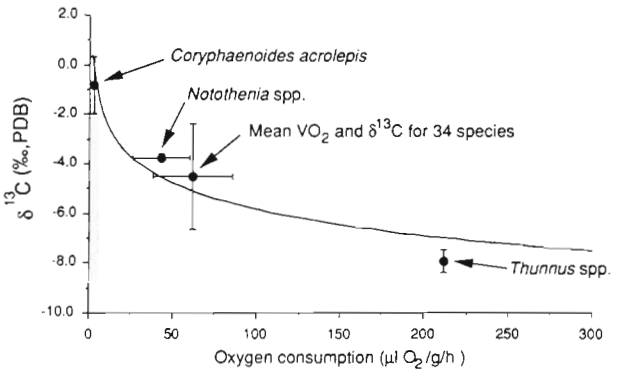

Fig. 9 Relationship between $\delta^{13}C$ and oxygen consumption (VO_2). Data points are explained in the text. There is a significant correlation between $\delta^{13}C$ and oxygen consumption (r^2 = 0.92, n = 4, $p<0.05$). Logarithmic curve is described by equation $\delta^{13}C = 1.1979 + (-3.5161)\log(VO_2)$. The logarithmic form of the relationship is similar to that representing the relationship between temperature and metabolic rate (Brett & Groves 1979)

on multiple observations of both metabolic rates and otolith isotopic composition. An additional point, representative of a mean value for 'all fish', is also plotted. This point is based on the mean oxygen consumption for 34 species (62 ± 24 µl g^{-1} h^{-1}) from Brett & Groves (1979) and the mean value for $\delta^{13}C$ obtained from isotope data collected in this study (-4.51 ± 2.15). If the data from the isotope studies of Mulcahy et al. (1979), Radtke (1984a, 1987) and Radtke et al. (1987) are included the mean value for $\delta^{13}C$ is 4.25 ± 2.32. I have chosen not to include the data from Degens et al. (1969) because they did not do replicate analyses, thus, reducing the reliability of their data. The resultant relationship (Fig. 9) is a reasonable approximation of models that relate temperature to metabolic rate (Brett & Groves 1979). The relationship between oxygen consumption and $\delta^{13}C$ is also very similar to a relationship between minimum depth of occurrence (which can be related to temperature) and oxygen consumption found by Torres et al. (1979) for migratory and non-migratory midwater fishes. On the basis of this initial result, it would be worthwhile to investigate further the significance of both intra- and interspecific variations in $\delta^{13}C$. This could be accomplished by carrying out isotopic analyses on the otoliths of those species for which oxygen consumption data are available. This exercise should be carried out over a wide range of species from different habitats and should also consider intraspecific variations, related to age and sex, in a species for which there is detailed information on oxygen consumption rates. Such research is necessary before it is possible to establish a firm relationship between metabolic rate and $\delta^{13}C$.

The relationship between $\delta^{13}C$ and temperature, the potential association with metabolic rate and the fact that, in many cases, otolith size relative to body size decreases as the otolith aragonite becomes more depleted in $\delta^{13}C$, are points not consistent with recent data on isotopic fractionation in corals (McConnaughey 1989a, b). There are several points that support this conclusion with regard to fish otoliths. Firstly, although there is a strong correlation between $\delta^{18}O$ and $\delta^{13}C$, the departures of $\delta^{18}O$ and $\delta^{13}C$ from equilibrium do not seem to be attributable to the same mechanism. The $\delta^{18}O$ values are satisfactorily explained on the basis of the temperature fractionation effects first discussed by Urey (1947). The kinetic/hydration and hydroxylation mechanism of isotope fractionation would result in simultaneous depletions in oxygen and carbon isotopes independent of temperature. Furthermore, the kinetic effect is postulated to result in greater isotopic disequilibria in those calcium carbonate structures that are growing rapidly. In the extreme case of the tuna, otolith growth is probably slow despite very rapid somatic growth, because the otoliths are relatively small. Nevertheless, the tuna otoliths are the most depleted in ^{13}C of those measured. Of course, in drawing this conclusion it is necessary to assume that a unit of otolith material, such as a daily increment (Campana & Neilson 1985) is produced over a similar time frame in all species, e. g. both tuna and orange roughy otolith daily increments are formed over a period of 12 h.

The kinetic effect of isotope fractionation, and its link to isotopic disequilibria, is based on the idea that simultaneous depletions in ^{18}O and ^{13}C occur during CO_2 hydration and hydroxylation, presumably in the presence of some catalyst such as carbonic anhydrase. In fish, it seems unnecessary to carry out the process of CO_2 hydration and hydroxylation given the very large pool of available HCO_3^-. Mugiya (1977, 1986) and Mugiya et al. (1979) investigated the roll of the enzyme carbonic anhydrase in fish otolith formation by a combination of in vivo and in vitro experiments. In a study of otolith formation in goldfish it was found that moderate doses of acetazolamide (Diamox), a carbonic anhydrase inhibitor, did not effect otolith growth, whereas large dose multiple injections reduced otolith growth by less than 17 %. He concluded that the catalyzed hydration and hydroxyalation of CO_2 was not necessary for otolith formation in goldfish (Mugiya 1977). A subsequent study on in vitro preparations of intact rainbow trout sacculi found relatively low levels of carbonic anhydrase activity, and it was concluded that, perhaps, carbonic anhydrase was not required at low rates of otolith deposition (Mugiya et al. 1979). These studies indicate that carbonic anhydrase and CO_2 may play a non-essential role in fish otolith formation. If this is so than kinetic/hydration-hydroxylation effects would not be significant in carbon and oxygen isotope disequilibria, thus, providing further evidence that the correlation between $\delta^{13}C$ and $\delta^{18}O$ for fish otolith data is not due to a kinetic isotope fractionation mechanism as proposed by McConnaughey (1989a, b) for corals. Furthermore, in light of our present knowledge on processes of otolith growth and otolith growth rates it seems unlikely that isotopic disequilibria are due to the kinetic/diffusion mechanism.

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