Isotopic composition of otoliths from a benthopelagic fish, *Coryphaenoides acrolepis*, Macrouridae: Gadiformes

Otoliths
Benthopelagic fish
Coryphaenoides
^{18}O
^{13}C
Isotopes
Otolithes
Poisson benthopélagique
Coryphaenoïdes
^{18}O
^{13}C
Isotopes

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ABSTRACT

Otoliths from a living benthopelagic Macrourid, *Coryphaenoides acrolepis*, are aragonitic and monomineralic. Isotopic measurements along cross sections of individual otoliths show progressive increases in both oxygen-18 and carbon-13 with age. The oxygen isotopic values generally agree with the expected temperature range of this fish, 6 to 2°C, corresponding to a possible downward depth migration from 600 to 2 000 m as the fish ages. The carbon-13 values indicate disequilibrium precipitation of the carbon isotopes in the aragonite which is suggested to reflect a slowdown of metabolic activity with age and decreasing temperature. Results show that isotopic analysis can help elucidate the life history of deep sea fishes.

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RÉSUMÉ

Composition isotopique des otolithes d'un poisson benthopélagique *Coryphaenoides acrolepis*, Macrouridae: Gadiformes

On a démontré que les otolithes d'un Macrouridé benthopélagique vivant, *Coryphaenoides acrolepis*, sont aragonitiques et monominéralogiques. Des mesures isotopiques effectuées le long de sections transversales d'otolithes ont montré un enrichissement progressif en oxygène-18 et carbone-13 du noyau à la périphérie. Les teneurs en oxygène isotopique pourraient être reliées aux variations de température auxquelles ce poisson serait soumis, 6 à 2°C, lors d'une migration vers les profondeurs, de 600 à 2 000 m, au cours de sa vie. Les teneurs en carbone-13 montrent que les isotopes du carbone de l'aragonite ne sont pas précipités en équilibre. On suggère que ce déséquilibre reflète un ralentissement de l'activité métabolique associé à un accroissement de l'âge et une diminution de la température. Les résultats obtenus montrent que les analyses isotopiques peuvent aider à élucider l'histoire de la vie de poissons marins.

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INTRODUCTION

Following Urey (1947), the study of stable isotopes in the mineral precipitates of organisms has proved of immense value in collecting information about past environmental conditions in the ocean, and also about the life histories of the organisms. Otoliths have received rather little attention in this respect, perhaps because they are rare in sediments. Devereux (1967), and Degens *et al.* (1969) suggest that fish otoliths are formed in isotopic equilibrium with the sea water in which they live. The stable isotope composition of otoliths, therefore, could provide valuable paleoclimatic information in those areas where otoliths are preserved.

Otoliths are calcareous bodies which form in the chambers of the inner ear of bony fishes. Each of the three chambers, the ultriculus, sacculus, and lagena, contains one otolith, the lapillus, sagitta, and asteriscus, respectively. These

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organs differ in size. In general, the sagitta is the largest otolith and the lapillus is the smallest (Spoczynska, 1976). Otoliths excite the macular receptors and function in maintenance of equilibrium and perception of position (Morris, Kittleman, 1967). An otolith grows in concentric layers around a nucleus affording a means of determining the age of a fish (Pannella, 1974; Williams, Bedford, 1974). The exact mechanism of otolith growth is not well understood.

In this study, we present analyses of the Recent fish *Coryphaenoides acrolepis* (Rattail). This benthopelagic macrourid is found at depths of 600 to 2 500 m (Iwamoto, Stein, 1974). Analyses include oxygen isotope measurements, thin section, X-ray diffraction, and electron microscopy. This is the first such analysis for a macrourid fish, and the first serial isotopic analysis of single otoliths.

METHODS AND MATERIALS

The macrourids were collected at a depth of about 1 200 m, by free vehicle, 17 miles off San Diego, California, during the spring of 1973. The fifteen specimens selected for study were all female fish with a length range of 67 to 84 cm and a weight range of 934 to 2 604 g. They were preserved in a freezer until readied for analysis.

The otoliths of these fish are unusually large, which facilitates their removal. A horizontal section was cut across the top of the head exposing the brain cavity. The otolith-containing sacs were easily extracted with forceps.

Once removed, the otoliths were labelled with respect to their type and position in the brain cavity. Each otolith was soaked in a 5.2% aqueous solution of sodium hypochlorite for 2 minutes to remove surface organic matter. The samples were rinsed thoroughly with deionized water and dried at 80°C before being weighed.

Six sagittae and two pairs of asterisci were chosen at random for X-ray diffraction analysis to determine their mineralogies.

To determine the relative age of particular specimens, thin sections from one sagitta of each fish were prepared.

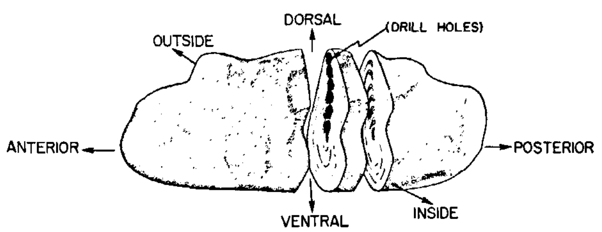


Figure 1

Illustration of the section of C. acrolepis *otolith used in thin section, and of the areas sampled for cross sectional isotopic measurements on the sagittae.*

The sagittae were embedded in plastic and a lateral section was cut approximately one-third of the way from the posterior end of each otolith. A second cut was made just to the anterior end of the middle of the otolith (Fig. 1). The latter cut was ground flat and mounted with resin to a petrographic slide. The section was then ground down until the growth rings and nucleus of growth were clearly visible. The finished thin sections were examined on a petrographic microscope in an attempt to determine the age of each fish.

Approximate age determinations were made by counting the pairs of translucent and opaque layers of the thin section which presumably represent annual growth rings. The method is described in Williams and Bedford (1974). In the case of *C. acrolepis*, the growth layers were very diffuse and it was extremely difficult to make reliable judgments as to each specimen's age. Possibly, burning (Williams, Bedford, 1974) or acetate replicas (Pannella, 1974), would improve these estimates.

The remaining sagitta of each fish was powdered with a mortar and pestle. Grinding was done gently so as not to create any intense frictional heat which would affect the isotopic measurements. Devereux (1967) suggests soaking the powdered samples in a 5% aqueous solution of sodium hypochlorite for 3 days to remove any organic matter which might be present. We experimented with various treatments of sodium hypochlorite on powdered samples but found no significant difference in oxygen and carbon isotopic values between samples so treated and those merely washed with deionized water. Therefore, all powdered otolith samples were washed through a Millipore filtering system with 200 ml of deionized water. The samples were then dried and stored in vials.

Isotopic variations within each otolith were determined. Four sagittae were chosen at random for this experiment. Using a 0.5 mm dental drill, six holes were drilled, consecutively, from the center of each otolith to the edge. This was done across a lateral section of the sagitta (Fig. 1). The grindings from each hole were collected and analyzed for isotopic composition. Samples removed by this procedure had an individual weight of approximately 0.5 mg.

In addition to these analyses, fifteen randomly selected entire sagittae were powdered, cleaned and analyzed in duplicate for their isotopic composition. All samples were reacted with phosphoric acid at 50°C in an on-line glass reaction system. The liberated carbon dioxide was analyzed for oxygen and carbon isotopic ratios on a VG Isotope Micromass 602 *c*. The data are reported in terms of per mil deviation relative to PDB. Analytical error by this procedure, on homogeneous material, is typically less than 0.1‰.

MORPHOLOGY AND X-RAY RESULTS

Otolith morphologies differ between species (Higgins, 1868; Scott, 1905), and the otoliths of *Coryphaenoides acrolepis* exhibit several characteristics which may be diagnostic. The sagitta tends to be ovate and elongate

(Plate 1 *a*). The ventral edge of the sagitta is smooth and slightly curved, contrasting with the dorsal edge which is irregular and tends to be higher towards the posterior. On the inside face, there is a marked depression just above the middle of the sagitta running the length of the body. Two deeper rounded depressions tend to develop within this depression; one located at the posterior and the other at the anterior end. The outside face of the sagitta is relatively smooth and slightly convex. The asteriscus of this species is globular and somewhat pyramidal in shape (Plate 1 *b*). Fingers of calcium carbonate tend to radiate out from the top of the "pyramid" and give the "base" the appearance of being composed of spheres.

The lapillus of this species seems to be the most diversely shaped of the three pairs. In general, the lapillus is a curved structure with the outer face being convex (Plate 1 *f*). The dorsal half of the lapillus is plate-like with no fingers of calcium carbonate visible. The edge of the dorsal half is smooth with no irregularities. The ventral half is characterized by protruding fingers of calcium carbonate which radiate out from the center of the structure. These fingers appear to protrude toward the anterior (Plate 1 *d*, 3). The outside surface is smooth under low magnification (Plate 1 *e*) and sponge-like under high magnification. The inside surface is very rough and irregular (Plate 1 *d*).

As mentioned previously, the growth rings of the otoliths of this macrourid are diffuse, which makes age determinations difficult. From the thin sections prepared, however, there are some trends visible. There are many pairs of translucent-opaque layers visible in the sections (Plate 1 *c*). There seems to be a pattern which shows a larger translucent-opaque layer with several less distinct pairs within. In the sections studied there are 15 to 25 distinct layers, the number increasing with increasing otolith size. If one assumes the distinct pairs to represent annual growth, then it would appear that these specimens are 15 to 25 years old. Savvatimskii (1972) studied North Atlantic rock grenadiers (*Macrurus rupestris* Gunn.). He estimated fish ages by counting the annual rings in their scales and reports female grenadiers to be as old as 27 years.

The X-ray diffraction data indicate that the otoliths in *C. acrolepis* are composed of aragonite and are monomineralic. This agrees with the observations of Degens *et al.* (1969). However, it should be noted that other minerals have been found to form otoliths. The trimorphs of calcium carbonate: calcite, aragonite, and vaterite seem to be the most common otolith forming minerals, according to Carlström (1963).

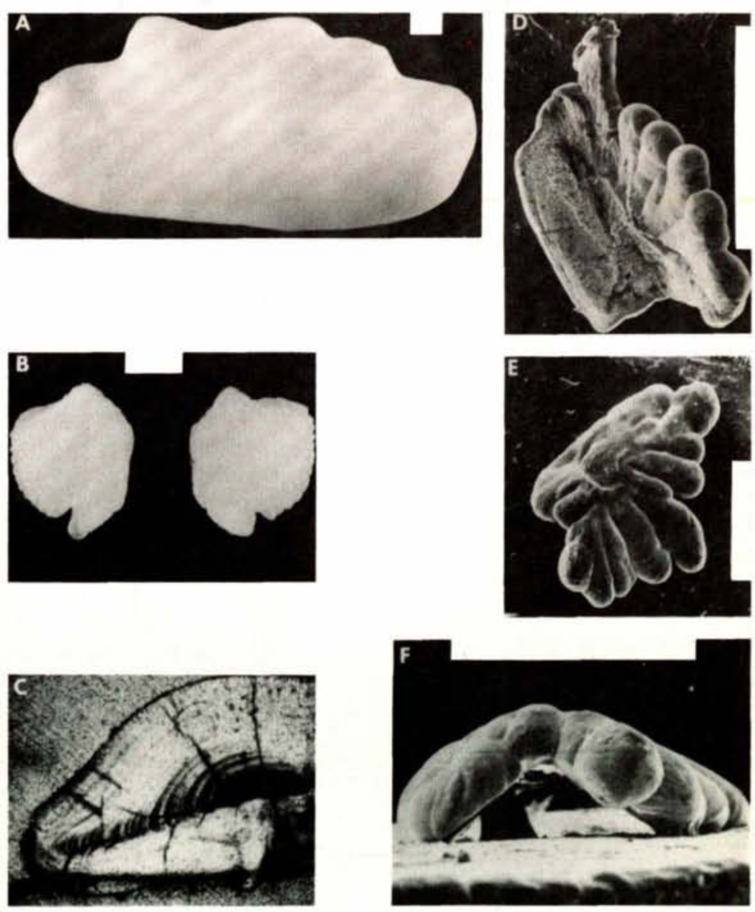
LIFE HISTORY AND STABLE ISOTOPES

Because of the difficulty in collecting eggs and larvae by Macrourid fishes, there is very little data available with which to reconstruct their life histories. Marshall (1965) has discussed possible life histories of benthopelagic Macrourid fishes. He suggests that the swimbladder drumming muscles in male fish may be used to attract females and that eggs, shed and fertilized near the bottom, slowly float upwards as they develop, buoyed by an oil droplet. Larvae hatch at 200 m or deeper and young rattails then progressively descend and inhabit near bottom depths as they mature into adults.

Most kinds of macrourids swim close to the sea floor but make feeding forays into the water column and may migrate considerable vertical distance off the bottom. Gut content analyses of *Coryphaenoides rupestris* from the Atlantic Ocean near Iceland have yielded more than 70% shrimps, probably consumed in midwater (Podrazhanskaya, 1967). Haedrich (1974) has reported the capture of *C. rupestris* between 270 and 1 440 m from the bottom in the Denmark Strait and *Coryphaenoides filifer* have been caught more than 500 m above the 2 700-2 800 m deep sea floor in the northeastern Pacific off central Oregon (Pearcy, 1976). More recently the cosmopolitan abyssobenthic macrourid, *Coryphaenoides armatus*, has been caught at depths ranging from 500 to 685 m above the bottom (3 800 to 5 700 m) in the central and eastern North Pacific (Smith *et al.*, 1979).

Plate 1

a) *Picture of whole left sagitta of* C. acrolepis *(inside view). Reference bar is 1 mm long.*
b) *Picture of whole asterisci showing pyramidal shape. Reference bar is 1 mm long.*
c) *Thin section, showing growth rings, of a sagitta from a specimen of* C. acrolepis *approximately 20 years of age.*
d) *Inside view of a left lapillus. Reference bar is 1 mm long.*
e) *Outside view of a right lapillus. Reference bar is 1 mm long.*
f) *SEM picture of the lapillus of* C. acrolepis *illustrating curved nature (looking at posterior end). Reference bar is 1 mm long.*



Studies on *C. acrolepis* show that the morphology and biochemistry of the swimbladder, an organ of hydrostatic buoyancy, differs considerably from that of shallow water fish. The swimbladder contains hyperbaric oxygen gas, in close association with a massive cholesterol-rich membrane system (Phleger, Benson, 1971; Phleger, Holtz, 1973). Oxygen gas, in proximity with cholesterol and spingoyamyelin in the membranes, may make vertical migrations simpler by providing a very sensitive buoyancy regulation device for migrations to and from the bottom and midwater.

Isotope measurements taken on whole powdered otoliths, as done previously (Devereux, 1967; Degens *et al.*, 1969) indicate the average isotopic composition of the otolith. Thus there is no information about any migratory cycles which the fish may follow or about changes in metabolic effects with age. The fifteen randomly selected sagittae of the individually crushed specimens analyzed for ^{18}O and ^{13}C in duplicate gave average values for $\delta^{18}O$ of 3.98 and 3.98 with standard deviations of 0.19 and 0.13°/₀₀ respectively. Average values of $\delta^{13}C$ were 0.07 and 0.06°/₀₀ with standard deviations of 0.28 and 0.25 respectively.

Isotope measurements taken across lateral sections (Fig. 1) of four sagittae revealed an interesting trend (Fig. 2). The $\delta^{13}C$ and $\delta^{18}O$ values increase as the fish ages. The increasing $\delta^{18}O$ suggests that the fish descends through the water column as it gets older. Haedrich and Polloni (1976) reported an increase in size and weight with depth for the North Atlantic macrourid *Coryphaenoides carapinus* which lives between 1 200 and 2 800 m off Southern New England. These fish were captured by trawl. R. H. Rosenblatt and T. Matsui (pers. comm., 1978) have not observed such a distinct correlation between size and depth for *C. acrolepis* which have been captured by free vehicle off the coast of California.

The carbon values show that a state of disequilibrium exists with respect to inorganic carbon in sea water. The increasing amount of ^{13}C may indicate an approach toward equilibrium values as the fish ages and its metabolic activity slows. A similar trend has been noted in the carbon isotopes of planktonic foraminifera (Berger *et al.*, 1978).

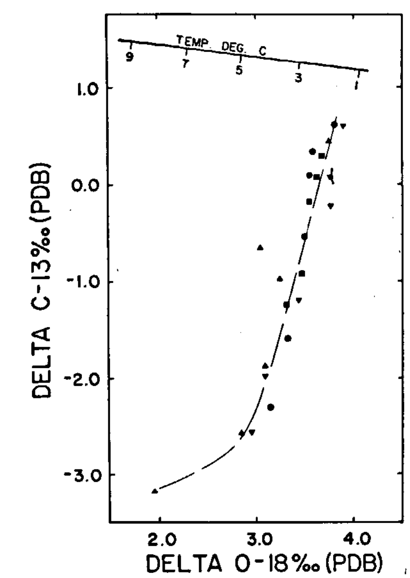


Figure 2

Graph of oxygen versus carbon isotope measurements within cross sections of sagittae. The lowest points represent the values at the center of the sagittae (young fish); the highest points represent the edge of $-0.0°/_{00}$ is assumed for the sagittae (old fish).'Specimen ≠ 1, ▲; specimen ≠ 2, ●; specimen ≠ 2, ■; specimen ≠ 4, ▼. The temperature calibration shows the expected equilibrium values using the Epstein and Mayeda (1953) equation for oxygen-18 and the Emrich, Ehhalt and Vogel (1970) relationship for carbon-13. A value of $-0.10°/_{00}$ is assumed for ocean water $\delta^{18}O$ and a value of $0.0°/_{00}$ for the $\delta^{13}C$ of dissolocean water $\delta^{18}O$ and a value of $0.0°/_{00}$ for the $\delta^{13}C$ of dissolved bicarbonate at the given water depths.

The temperature scale plotted in Figure 2 was calculated using the paleotemperature equation by Epstein and Mayeda (1953) where

$$t°C = 16.5-4.3\,(\delta_s-S_w)+0.14\,(S_s-\delta_w)^2,$$

where δ_s is the $\delta^{18}O$ of CO_2 from calcium carbonate by reaction with phosphoric acid at 25°C and δ_w is the $\delta^{18}O$ of CO_2 in equilibrium, at 25°C, with the water from which the carbonate was precipitated (estimated as $-0.1°/_{00}$ PDB for the present case). Horibe and Oba (1972) proposed a paleotemperature equation for aragonite rather than calcite. Use of their equation yields temperatures approximately 5°C colder than using the Epstein and Mayeda (1973) equation. These would be unreasonable temperatures in the present context. An alternative hypothesis would be that the otoliths were deposited out of oxygen isotopic equilibrium by more than $1°/_{00}$ (positive) and that the observed agreement with the Epstein-Mayeda equation is coincidental. [Recently Epstein (pers. comm., 1979) has proposed a revised paleoequation but temperatures calculated with the revised equation are not significantly different from those calculated in this work.] The expected temperature range corresponding to the *C. acrolepis*'s depth range is 2 to 6°C (Reid, 1965). Most of the cross sectional $\delta^{18}O$ values of the four sagittae in Figure 2 correspond to temperatures within this range which strongly suggests that the aragonite is deposited in isotopic equilibrium with the ambient sea-water, as previously suggested for whole-otolith analyses (Devereux, 1967; Degens *et al.*, 1969). If this is correct, the trend in $\delta^{18}O$ values seen during growth corresponds to a general downward migration of at least 1 400 m, by the fish, as it ages. The indication that most otolith centers have $\delta^{18}O$ values corresponding to temperatures lower than 6°C supports the findings of Merrett (1978) that the migration of macrourids to the depth range of adults is accomplished early in the life cycle.

The apparent increase in depth with age for *C. acrolepis* is, however, contrary to the observation that *C. rupestris* juveniles appear to develop in the deepest part of the depth distribution for that species (Geistdoerfer, 1977).

Although the isotopic evidence suggests a progressively increasing average depth of habitation for *C. acrolepis* it should be explained that each subsample of the otoliths taken for analysis probably represents several years of growth. Therefore we do not resolve isotopic changes associated with periodic excursions of the fish into the midwater regime, although such isotopic fine structure may be present in the otoliths.

CONCLUSIONS

1. Age determinations made from thin sections of otoliths showed that the fish studied were probably between 15 and 25 years old.

2. X-ray analyses show that the otoliths of *Coryphaenoides acrolepis* are composed of aragonite and are monomineralic.

3. Cross sectional isotope measurements revealed a trend in which the amount of ^{18}O and ^{13}C in the aragonite increases as the fish gets older.

4. Calculated water temperatures agree with the expected ones for these specimens using the paleotemperature equation of Epstein and Mayeda (1953). A downward migration of adult fish, during aging, is suggested.

5. From the data examined, it may be concluded that the otoliths of *Coryphaenoides acrolepis* are not deposited in isotopic equilibrium with carbon throughout the fish's lifetime.

6. Our results suggest that stable isotopes in otoliths of deep-sea fishes are useful in elucidating the life history of these organisms, and are potentially useful in paleoceanographic research, in areas where such otoliths are preserved.

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