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The Propagation of Cod *Gadus morhua* L.

COD FISH OTOLITHS: INFORMATION STORAGE STRUCTURES

R.L. Radtke

Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, Columbia, South Carolina 29208 USA

Present address:
Hawaii Institute of Marine Biology, P.O. Box 1346, Coconut Island, Kaneohe, Hawaii 96744 USA

ABSTRACT

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The otoliths of cod fish may have incorporated within their structural and chemical components a large amount of life history information. This information may be revealed when appropriate analytical methods are utilized. External and internal examinations of all three otoliths (sagitta, asteriscus and lapillus) for otolith macrostructure and microstructure by light and Scanning Electron Microscopy indicate that yearly, daily and population rhythmic patterns exist. These data make it possible to estimate the age of adult, juvenile and larval cod fish. Chemical analyses of otolith carbonate by mass spectrometer for stable isotopic concentrations and by atomic absorption and electron microprobe for strontium and calcium concentrations provide valuable insights into the past history of an individual fish. Stable isotopic concentrations of ^{18}O and ^{16}O appear to be directly related to temperature, and stable isotopic concentrations of ^{13}C and ^{12}C appear to mirror the nutritional and metabolic history of a fish. Strontium-calcium concentration ratios seem to be inversely related to temperature and electron microbe analyses make it feasible to interpret almost daily changes in temperature. A combination of structural and chemical analyses would make it possible to link growth and mortality rates to nutritional and environmental occurrences.

INTRODUCTION

Cod (*Gadus morhua*) are one of the more economically important fish species and are of interest to countries worldwide. Cod abound in the eastern and western North Atlantic and are generally ecologically similar throughout the North Atlantic (Dannevig, 1933; Pinhorn, 1969). Consequently, ecological data on cod populations would be of benefit to the entire fishery.

Many ecological facets of cod may be interpreted from the structural and chemical patterns of otoliths. Otoliths are calcium carbonate concretions situated in the membraneous labyrinth of teleost fishes (Lowenstein, 1971; Popper and Coombs, 1980). There are three otoliths (the sagitta, asteriscus and the lapillus) on each side of the brain area, but only the largest otolith, usually the sagitta, has been utilized for most studies. The other two otoliths (the asteriscus and the lapillus) may also contain valuable ecological information, but need more investigation.

The use of the rhythmic structure of sagittal otoliths for yearly age determinations was suggested by Reibisch (1899) and since then otolith inspection for rhythmic depositional patterns has been the most widely practiced technique for age determinations (Bagenal, 1974). Sagittal otoliths have been demonstrated by Six and Horton (1977) to be the most consistent and accurate calcified structure for age determination. Consequently, they are probably the superior structure for age determination in most fish such as cod. In addition, the structural shape of sagittal otoliths is species specific (Hecht, 1978; Hecht and Hecht, 1978, 1979; Morrow, 1979). Otolith shape has been shown to be indicative of population or stock boundaries (Shinoda and Jayashingre, 1971; Messieh, 1972; Postuma, 1974; Yefanov and Khorevin, 1979). Otoliths can reveal trophic relationships in marine systems (Fitch and Brownwell, 1968), provide information on fish size (Templeman and Squires, 1956) and have been utilized in evolutionary studies of fish (Stinton, 1975). The macro-structural components of teleost otoliths contain a large amount of information that would be accessible if

the proper techniques were employed.

The micro-structural components of adult and especially larval and juvenile fish otoliths could provide answers to important ecological and life history questions. Micro-increments have been discovered in fish otoliths and have been postulated to be daily in formation. Pannella (1971) was one of the first researchers to detect daily increments in fish sagittae. Ensuing investigations have demonstrated that daily increments can be found in otoliths of larval, juvenile and adult fishes from a wide ecological range and can be utilized for individual and population biology (e.g. Brothers et al., 1976; Radtke and Dean, 1982). These studies on otolith micro-structure established the usability of otolith techniques and have revealed the merits of the rhythmic patterns in otoliths as indicators of ecological information. Further application of otolith techniques offers the means to determine growth, survivorship, feeding periodicities and other ecological parameters.

The chemical composition of otoliths may contain information about calcification mechanisms, evolution, environmental variations to which a fish has been subjected, trophic interactions, and other relevant information. Otoliths of most teleost fishes are mineralogically composed of aragonitic calcium carbonate (Carlstrom, 1963) situated within an organic matrix (Degens et al., 1969). The organic matrix for a limited number of teleost fish species was shown to consist mainly of highly oxygenated amino acids which were taxonomically separable by weight proportions (Degens et al., 1969). The organic matrix of cod otoliths has not yet been analyzed. Such analyses may provide insights into the calcification processes in cod otoliths and evolutionary or systematic relationships with other fish species.

The chemical composition of otoliths may be affected by environmental conditions and consequently otoliths could contain a record of the ecological variations to which a fish has been subjected. Specifically, otolith carbonate may contain a record of the temperature regime of a fish and thus the associated currents in which a fish has lived. The oxygen isotope ratio ($^{18}O/^{16}O$) of calcium carbonate has been demonstrated to be

dependent upon the temperature and isotopic composition of the sea water from which it was precipitated (Urey, 1947; Epstein et al., 1953). The stable isotopic composition of many forms of biogenic carbonate has provided information about prior environmental situations in which the organisms lived (Emiliani, 1955, 1966; Savin, 1977) as well as life histories of marine organisms (Emiliani et al., 1978; Killingley and Berger, 1979; Killingley, 1980). Likewise the oxygen isotope ratio ($^{18}O/^{16}O$) of otoligh calcium carbonate has been postulated to indicate habitat temperature (Devereux, 1967; Degens et al., 1969; Mulcahy et al., 1979) and has been shown to be related to the water temperatures in which a fish has lived (Radtke and Williams, 1980). Additional study of the stable isotopic composition of cod otoliths could provide valuable knowledge about calcification and life history parameters.

The elemental composition of otoliths could also provide information on temperature. Strontium can interchange with calcium in aragonitic calcium carbonate in a temperature-related fashion. Strontium-calcium concentration ratios in coral skeletons have been related to temperature (Smith et al., 1979; Schneider and Smith, 1982) and it is conceivable that strontium is also substituted interstitially in the aragonite of fish otoliths in a temperature-dependent manner. The use of strontium-calcium concentrations has not been applied to fish otoliths. Furthermore, it is feasible with electron microprobe techniques to measure elemental differences theoretically in areas of one square micron. Such analyses of cod otoliths could provide a new level of information on migrations, growth and other ecological parameters.

An understanding of the structural characteristics, chemical composition and formation of cod otoliths would make it feasible to determine the ecological parameters of cod fish populations. If environmental changes are examined along with their effects on structural and chemical formation of otoliths, it is possible that cod otoliths and other fish otoliths could function as data storage units and thus could provide a chronicle of a fish's physiological and ecological past. In the present study, cod otoliths from reared and wild fish were analyzed for

chemical composition and external and internal structures.

MATERIALS AND METHODS

Otoliths were dissected from cod from both reared and wild populations on both the western and eastern north Atlantic. Larval, juvenile and adult cod were reared at several temperatures between 4°C and 10°C with a maximum deviation of ±0.65°C from the desired temperature. Cod were reared at the Biological Station, St. Andrews, New Brunswick, Canada and at the Fløde vigen Biological Station, Arendal, Norway. Wild individuals were collected from the Canadian and Norwegian coasts by 505µm mesh plankton nets and bottom trawls.

For internal structural analyses, otoliths from larvae and juveniles were mounted on viewing stubs in 5-min epoxy resins and otoliths from adults were sectioned with a low speed rock saw before mounting. The otoliths were ground with 600 grit sand paper and polished with 0.3 µm alumina polish. The polished surfaces were decalcified with 6% EDTA (disodium ethylene diamine tetraacetate) at pH 7.4 (pH adjusted with NaOH) for 5-15 min. The samples were coated with gold and observed in a SEM.

The chemical composition of cod otoliths was determined through the utilization of a multiplicity of analytical methods. The crystal structure was defined through the use of x-ray diffraction. Otoliths were pulverized in ethanol and the otolith mixture was allowed to dry on a glass slide. The sample (N=3) was analyzed in a Phillips manual x-ray diffractometer.

Examination of the organic components of cod otoliths was achieved by the isolation of protein matrix. Otoliths were pulverized, dried at 60°C for 24 h and weighed. The organic matrix was isolated for amino acid analyses by the techniques of Degens et al. (1969). In these methods otolith material was decalcified by 0.1 N HCl which was added in stoichiometric quantities along with trichloroacetic acid which was kept at 10% concentration. The precipitated protein was centrifuged, washed twice in 5% trichloroacetic acid and hydrolized with

6 N HCl for 24 h in vacuo. The hydrolized protein was analyzed by automatic ion-exchange chromatography.

Stable isotopic analyses were performed in accordance with established procedures (Williams et al., 1977) where the otolith materials were roasted for 1 h at 320°C in vacuo and reacted with 100% phosphoric acid at 50°C in vacuo. The resultant CO_2 was measured with a mass spectrometer. The ratios were calculated as:

$$\delta^{18}O\ (^{o}/_{oo}) = \left[\frac{^{18}O/^{16}O \text{ sample}}{^{18}O/^{16}O \text{ PDB}} - 1\right] 10^3;$$

$$\delta^{13}C\ (^{o}/_{oo}) = \left[\frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ PDB}} - 1\right] 10^3.$$

The oxygen isotopic composition of seawater ($\delta^{18}O_w$) was estimated from the salinity $\delta^{18}O_w$ relationship for the North Atlantic (Epstein and Mayeda, 1953). From the $\delta^{18}O$ data, isotopic temperatures were calculated from the temperature equation for aragonite, $T^{o}C = 13.85 - 4.54\ (\delta^{18}O_s - \delta^{18}O_w) + 0.04\ (\delta^{18}O_s - \delta^{18}O_w)^2$, where $\delta^{18}O_s = \delta^{18}O$ of the sample relative to the isotopic standard PDB and $\delta^{18}O_w = \delta^{18}O$ of seawater relative to the isotopic standard PDB (Horibe and Oba, 1972).

Elemental analyses of cod otoliths were accomplished by Atomic Absorption (A.A.) spectrophotometry and by electron microprobe analyses. For A.A. analyses otolith material was dissolved in 5% HCl and analyzed for strontium (Sr) and calcium (Ca) using an A.A. spectrophotometer with an auto sampler. Air-acetylene reducing flames were used for all analyses and elements were analyzed from the same sample to avoid dilution error. Commercial A.A. standards were mixed in proportions approximating otolith aragonite.

To accomplish measurements of Sr and Ca on the electron microprobe, sagittal otoliths were first ground on the proximal side until the core regions were revealed. The ground sagittae

were imbedded in epoxy resin to form one-inch diameter discs and then highly polished. The surface of the specimens to be examined must be extremely smooth or large diffractions of x-rays and thus analytical error result. The specimen discs and standards were coated with carbon in order to further dampen diffraction of the resultant x-rays and increase electron conductance. The standards and samples were analyzed on a CAMBAX x-ray electron microprobe with the electron beam focused on a 5 μm^2 area. Analyses of Sr and Ca were executed at 5 µm intervals across the longest axis of the sagittae and Sr/Ca ratios calculated for each area analyzed. Sr/Ca ratios were multiplied by 10^3 for presentation.

RESULTS

The sagittae were from 200 to 300 times larger by weight than the asteriscus and the lapillus (Fig. 1). Cod sagittae

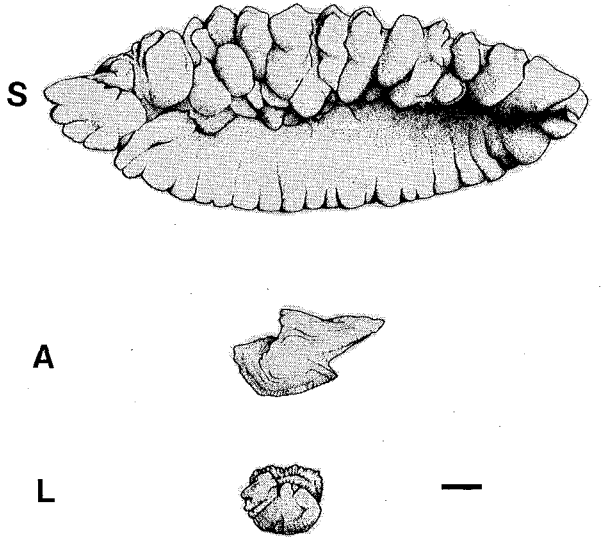

Fig. 1. The otoliths from the right side of a 3 kg cod. S - sagitta; A - asteriscus; L - lapillus. Bar equals 100 µm.

from all collections displayed similar morphological features. They were large in relation to the size of the fish (Morrow, 1979) and generally oval in shape. The proximal surface of each sagitta was oriented towards the central axis of the fish. The convex surface was demarcated by a shallow sulcus with the depth of the sulcus increasing with fish size. The sagittae have a rounded rostrum with an undifferentiated antirostrum (nomenclature from Hecht, 1978). The margins of the sagittae were denticulated with no discernible increase in dentation in any areas. No distinct notches were present. The sagittae were robust with a shape which was distinguishable from other gadoid species.

The sagittae of adult cod displayed zones which have been utilized to provide annual records of age. These yearly zones are present in most cod sagittae (Fig. 2). The annual nature of these zones is widely accepted by most fish biologists.

Each asteriscus had a distinct sulcus, but it was not known if nerve fibres extend into this grove as they do for sagittae.

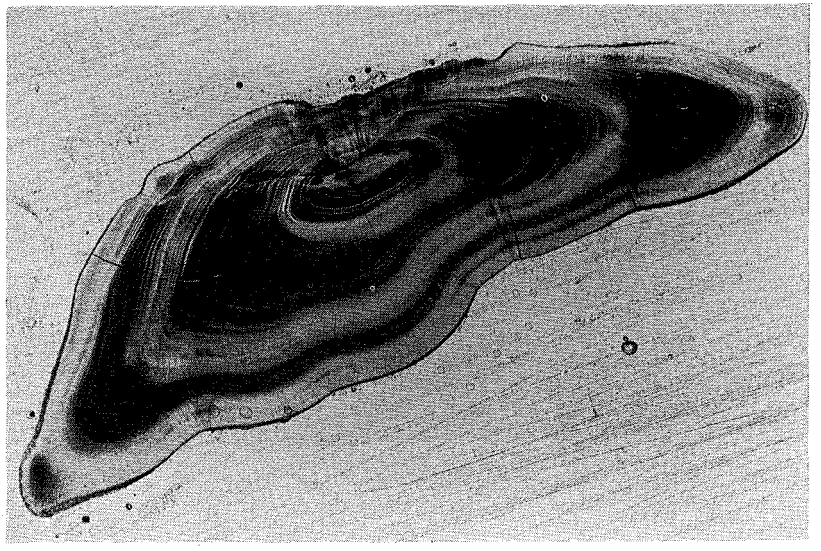

Fig. 2. Sagitta from a 42 cm (TL) adult cod captured in February estimated to be 3 years old.

The asteriscus lacked a collum and anterior or posterior cristae. The excisural notch was very distinct and the rostrum was exaggerated with the antirostrum smaller. Visual differences in antirostrum shape were discovered between southern coastal Norwegian cod reared in the laboratory and northern oceanic Norwegian cod (Fig. 3). It is suggested that these visual variations may be indicative of stock or population entities. A more strict documentation of asterisci shapes may provide information on the geographical boundaries of cod.

The lapilli of adult cod were moderately spherical in shape and heavier in structure than the asterisci. No distinct morphological deviations were visually evident. Further investigations on the lapilli and the asterisci could furnish new knowledge for fishery biologists.

The otoliths of larval and juvenile contain a tremendous amount of information about the life history of cod. The sagittae and lapilli of developing cod were the first tissue to calcify as determined by polarized light examination of the egg. Calcification of these otoliths occurred just before eye pigmentation during the embryological stage. The asteriscus did not become evident until after yolk sac absorption. At hatching

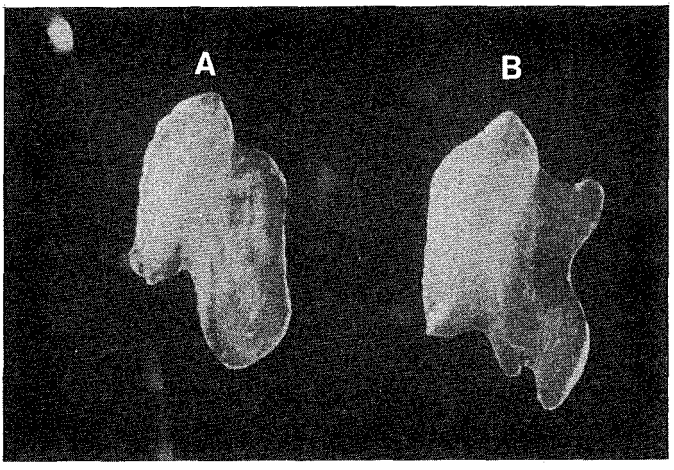

Fig. 3. Asterisci of Norwegian cod which displayed morphological variation. A - 1 kg northern oceanic cod; B - 1 kg southern coastal cod.

the lapilli were slightly larger than the sagittae, but this size difference was reversed within several days of hatching. Diameters averaged .0268 ± .0017 mm at hatching for lapilli with sagittae .0040 mm smaller than lapilli at hatching. The size of the otoliths increased with an increase in fish size. The larger fish had larger otoliths and fish size could be inferred from otolith size.

Sagittal otoliths were spherical in form and prefactory data indicated that daily increments were deposited concentrically to the core (Fig. 4). These daily increments were documented through the first 30 days in laboratory reared fish. It appeared that the increments were composed of incremental and discontinuous zones constructed of mineral crystals in a protein matrix with disruptions in the protein matrix as the source for increment formation. Daily increments in larval cod fish otoliths could provide many new insights about a period of cod life history which is difficult to investigate.

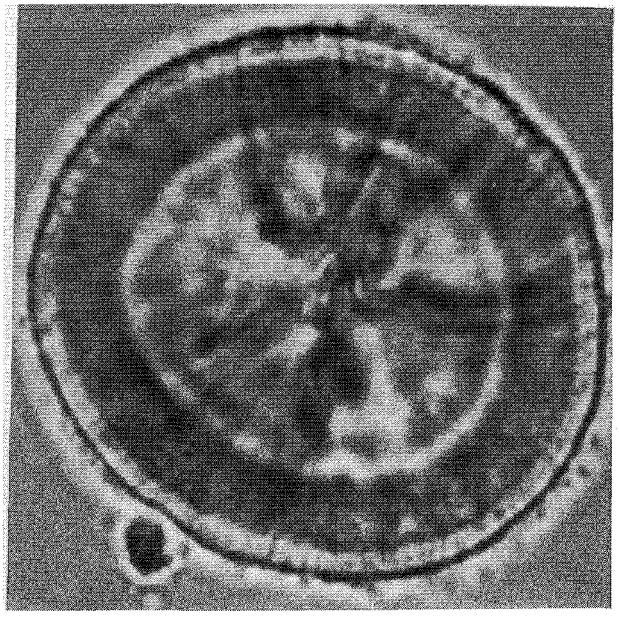

Fig. 4. Sagitta from a 3 day old cod larva which displayed two increments. 3625 X.

Information on feeding success and/or rate may also be accumulated in the structural components of larval and juvenile cod otoliths. Fish which had higher growth rates displayed wider increments in both the sagitta and lapillus. The initiation of feeding in larvae produced heavier more distinct increments (Fig. 5). Starved cod larvae exhibited faint increments which were often difficult to interpret. However, after feeding more distinct increments were produced. This phenomenon occurred even when larvae were starved for more than 10 days. The faint increments which formed before normal first feeding continued on a daily schedule when feeding was not initiated, but could easily be misinterpreted if not observed with the necessary techniques. Furthermore inviable larvae (larvae which would die no matter what the extent of the feeding rate) did not exhibit increment formation and could cause difficulty of interpretation if these larvae were examined with viable larvae. Extreme caution must be exercised when interpreting events from the otoliths of laboratory cultured larvae.

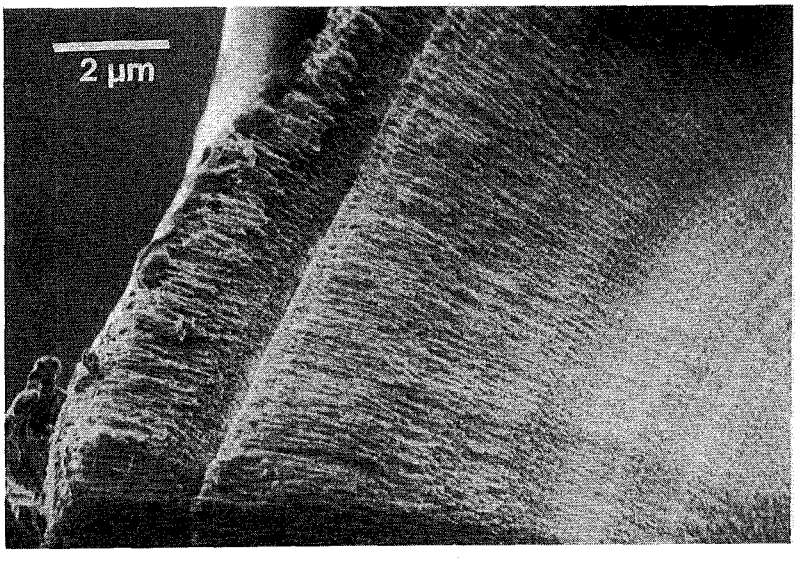

Fig. 5. A scanning electron micrograph of a sagitta from a 7 day old cod with a distinct incremental check that corresponds to the times of first feeding.

SEM examination of larval, juvenile and adult sagittal otoliths revealed mineral crystals in a protein matrix which formed increments. An example of such increments is demonstrated in Fig. 6. X-ray diffraction analyses (Fig. 7) of cod otoliths showed that the mineral crystals were pure $CaCO_3$ in the aragonite crystal structure. In SEM inspections the aragonite crystals were shown to radiate from the core region with multiple primordia. Starting from the core region, rhythmic microstructural patterns of protein deposition produced distinct increments which could be enumerated from the rugose surface of prepared otoliths. Through the utilization of progressive grinding and etching techniques it was feasible to count increments in the sagittae of cod reared for more than two years. Initial data demonstrated that daily increments were formed in the sagittae of cod reared from larvae to adults for a period of more than two years. Further SEM investigations will provide more data on otolith formation.

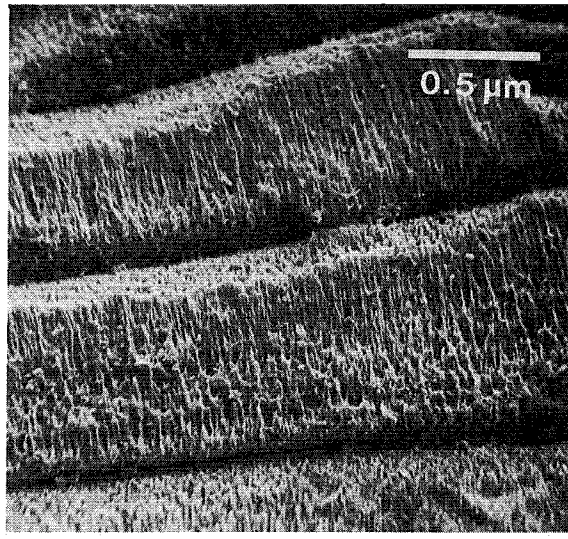

Fig. 6. SEM photograph of the daily increments studied in the sagitta of an adult reared cod. Upon decalcification the protein matrix of each increment formed ridges.

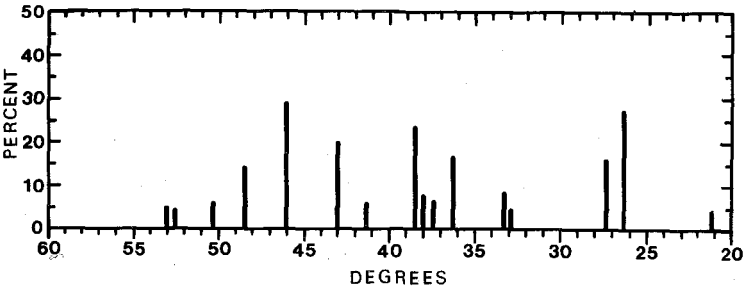

Fig. 7. X-ray diffraction pattern for cod otoliths that demonstrated their monomineralic aragonite composition.

The organic material of cod otoliths was proteinaceous and comprised approximately 5% of the otolith by weight. Free amino acid quantitative analyses of otolith protein matrix disclosed that the organic material was mainly comprised of acidic amino acids with a large abundance of aspartic acid, glutamic acid, glycine, leucine, proline and serine (Table 1). These amino acids have been implicated in the calcification processes (Degens et al., 1969) and the differences in quantities of specific amino acids may be a taxonomic feature.

TABLE 1

Free amino acid quantitative analysis of cod otolith protein. (in residues per 1000)

Alanine	52.4	Leucine	93.2
Arginine	54.4	Lysine	40.00
Aspartic Acid	117.05	Methionine	8.25
Cystathionine	Trace	Ornithine	Trace
Cysteic Acid	3.6	Phenylalanine	36.7
Galactosamine	Present	Proline	98.5
Glucosamine	Present	Serine	94.9
Glutamic Acid	99.35	Taurine	Trace
Glycine	100.2	Threonine	80.8
Half cystine	14.8	Tyrosine	23.75
Histidine	16.00	Valine	49.5
Isoleucine	15.75		

The oxygen and carbon stable isotopes of cod otoliths demonstrated significant relationships. Isotopic temperature equations assume isotope deposition to be in equilibrium with the water. For cod, neither the calcite (Epstein et al., 1953) nor the aragonite (Horibe and Oba, 1972) equation could predict the temperature in the cod's habitat. However, when the $\delta^{18}O$ values for cod otoliths from reared specimens were plotted against temperature (Fig. 8) the line produced was parallel and the slope was not significantly different ($p>.05$) from those lines created by the $\delta^{18}O$ values of the isotopic temperature equations. Hence, even though the oxygen isotopes were not deposited in isotopic equilibrium with the surrounding environment, the $\delta^{18}O$ values were temperature dependent. An intercept which differs from equilibrium may be caused by biological processing of the isotopes in the cod. Thus a new equation

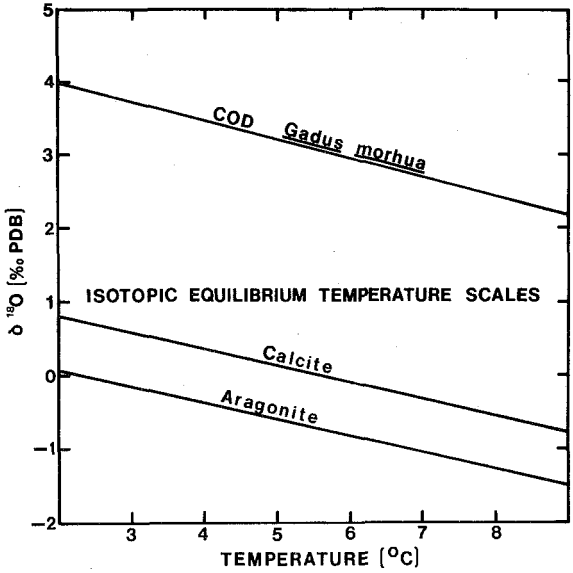

Fig. 8. Relationship of $\delta^{18}O$ in otolith aragonite to temperature in cod. The temperature relationship was not equal to the equilibrium temperature scales, but the slopes were not significantly different.

based on fish reared at known temperatures could be used to predict environmental temperatures of wild cod or reveal a record of the fish's physiological past.

The carbon isotopes could also be related to biological processes. Cod otoliths were not deposited in carbon isotopic equilibrium with the ambient total dissolved CO_2. Cod otoliths were depleted of $\delta^{13}C$ which indicated a temperature and/or metabolic relationship. The more negative $\delta^{13}C$ values of fish reared at higher temperatures could represent a period of higher metabolism. The stable isotopes of cod otolith carbonate, therefore, appeared to provide a record of the temperature and metabolic history of cod.

The ratio of Sr to Ca in cod fish aragonite could also be utilized as a recording thermometer at a finer analytical level. Sr/Ca ratios of cod otoliths mearsured by A.A. established that strontium was deposited in an inverse relationship to temperature. The ratio of Sr to Ca was temperature related, but was not in equilibrium with inorganically precipitated aragonite (Fig. 9). A comparison of slopes between the equations for inorganic aragonite and cod otolith aragonite showed no significant difference ($P>.05$). The Sr/Ca ratios in cod otoliths were temperature predictive, but shifted, possibly by biological processes.

The application of the electron microprobe to the measurement of Sr/Ca concentration ratios made it plausible to detect changes on the micro level. The Sr-Ca profile for a juvenile cod captured from the wild (Fig. 10) indicated that the fish may have been in contact with changing water masses. This profile suggested that after hatching, the larva was situated in a water mass of approximately $6^{o}C$ and then moved into warmer conditions. Later it appeared the fish was in contact with colder water for an extended period of time with a resultant decrease in growth rate. These data may also suggest a lunar periodicity in migrations as the fish studied was estimated to be 46 days old and would have been through one complete lunar cycle. It was difficult to predict the exact cause of the suggested migrations, but biological changes were discernible from the Sr/Ca concentration ratios. The comparison of increment

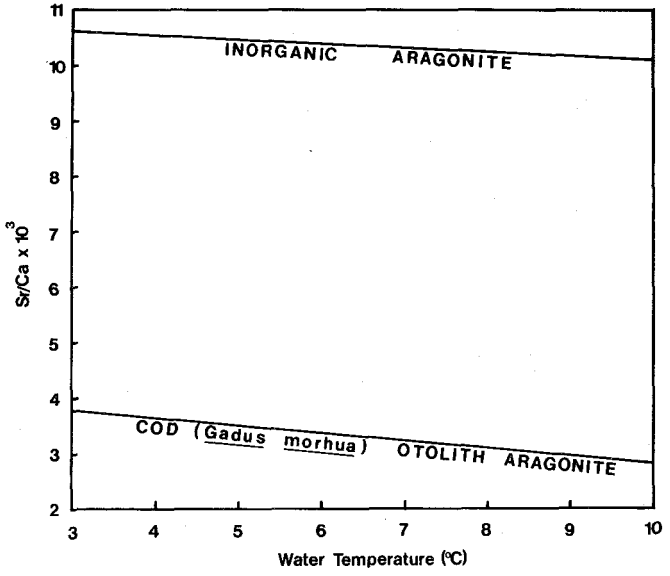

Fig. 9. The relationship between Sr/Ca ratios in otolith aragonite in cod and temperature. Comparison with equilibrium precipitated aragonite demonstrated equal slopes and a possible biological fractionation.

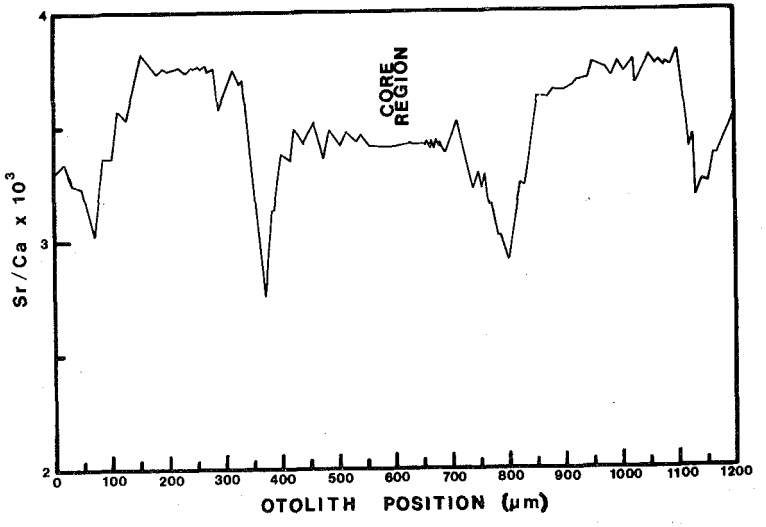

Fig. 10. Sr/Ca concentration ratio profile of the sagitta of wild cod juvenile with a total length of 36.8 mm and an estimated age of 46 days.

counts, lunar periodicity, feeding, etc. with the Sr/Ca ratio data could provide an immense amount of information about the past ecological history of a fish.

DISCUSSION

Cod otoliths function as data storage units in that they contain a large amount of information about the individual's life history and past. This information is accessible if the proper techniques are employed.

The external morphology of cod otoliths was a species discriminating component. External features from the sagittae demonstrated the species specificity of these otoliths and their conceivable utilization in phylogenetic research. Bingel (1981) suggested that for cod, differential addition of carbonate material to sagittae produced a species-specific shape. Studies by Hecht (1978), Hecht and Hecht (1978, 1979) and Morrow (1979) have used the morphological traits of other fish sagittal otoliths as indicators of taxonomic classification. Furthermore, fish sagittae have been extensively employed to describe fossil fish assemblages and sagittae have contributed immensely to the elucidation of fish phylogeny and evolution (Stinton, 1975). Research on the external morphology of sagittae would provide fresh insight into the complex evolution of teleost fish species and the resultant taxonomic classification.

The sagittae of adult teleost fish have been the chosen hard structure for age determination (Williams and Bedford, 1974; Pannella, 1980) and have been suggested as being the more accurate aging structures (Six and Horton, 1977). Sagittal otoliths of cod have been investigated for a long period of time in age studies. In more recent times Trout (1954) tried to document cod sagitta growth as it related to the deposition of annual zones while Gulland (1958) utilized annual marks in both spines and sagittae. The final judgement by Gulland (1958) was that sagittal otoliths appeared to be better indicators of age in

adult cod. Blacker (1969) investigated the structure of opaque and hyaline zones in cod sagittae and later expanded upon this information (Blacker, 1974a) so resulting in an international exchange of cod sagittae for population dynamics studies (Blacker, 1974b). Sagittae from cod can be strongly advocated for annual age determinations as these data appeared to be retrievable from cod sagittae.

The other otoliths (asteriscus and lapillus) were small when compared to the sagitta which may explain why they have been overlooked in previous studies of cod or other fish species. However, with careful dissections and the utilization of the necessary techniques it was practicable to examine these otoliths for morphological differences and recurrent depositional patterns. The lapillus did not demonstrate visible differences among distinct populations while the asteriscus did. Both otoliths displayed depositional patterns that have not yet been related to external influences. In preliminary visual observations the asterisci showed distinct external morphological characteristics which may be indicative of population or stock structure. The asterisci were not formed at the same time as the other otoliths and consequently may be more susceptible to environmental conditions.

The data are presented here to stimulate further research, but strong indications of population discrimination have been witnessed. Sagitta of other fish species (McKern et al., 1974; Postuma, 1974; Yefanov and Khorevin, 1979) have been utilized to denote population structure. Studies by Rollefsen (1933) and Mina and Markevich (1968) used the internal and external sagitta parameters in cod as indexes of intraspecific differentiation. All the cod otoliths were species specific, but the difference in morphological characteristics of the asterisci supports the supposition that these otoliths were also population specific and may be able to define stock boundaries on a more precise level. More samples and study should demonstrate the value of the lapillus and asteriscus in fish research.

The physiological activities of cod are controlled to an extent by environmental changes synchronized to the diurnal astronomical cycles and these events may be recorded in the

chemical structural components of cod otoliths. X-ray diffraction analyses of cod otoliths established that they were composed of $CaCO_3$ crystallized as aragonite. This finding was in agreement with Carlstrom's (1963) investigations that otoliths from teleost fish are formed of the aragonite polymorph of calcium carbonate. Aragonite appears to be a fundamental component of gravity receptors in the animal kingdom, although the other crystal forms of calcium carbonate (calcite and vaderite) can be found in otolithic concretions (Carlstrom, 1963). The type of crystal polymorph can affect the interpretation of other chemical data and consequently is important to know.

The protein matrix of cod otoliths was a mucoprotein which was similar to keratin in as much as protein from otolith matrix displayed relatively abundant glycine, histidine, leucine, lysine, threonine and valine. The relative concentrations of amino acids were parallel to those detected in other fish otoliths (Degens et al., 1969). The amino acid components of organic matrices define the mineralization model for the involved tissue. The protein matrices supply locations for the consolidation of ions and regulate the chemistry, mineralogy, crystal size and alignment of the mineralized tissue. The protein matrix of cod otolith protein contained a high abundance of oxygen rich amino acids. The carboxyl groups of acidic amino acids accounts for the ease of mineralization of the protein template due to the initial fixation of ions (Hare, 1963; Hallsworth, 1964). Of the acidic amino acids present, aspartic acid appears to be prominent to mineralization systems. Aspartic acid rich protein has been discovered to be essential in the process of biological mineralization (Hare, 1963; Weiner and Hood, 1975; Mitterer, 1978; Weiner, 1979). The process of mineralization of cod otoliths would parallel the model of Degens et al., (1969). Oxygen situated on the carboxyl groups would arrange the calcium ions to form metal ion coordination polyhedra (Matheja and Degens, 1968). Hydrogen linkages would attach the carbonate groups to the protein template. Oxygen from the carbonate assemblages would exchange with oxygen from the metal ion coordination polyhedra to stabilize the structure. In this fashion the aspartic acid rich protein

would assist in the nucleation and growth of calcium carbonate crystals.

The stable isotopic composition of aragonite in cod otoliths appears to be influenced by the metabolism of the fish and is not precipitated in isotopic equilibrium with seawater. This may also be the case for other fish species. However, the $\delta^{18}O$ of cod otoliths was directly related to the temperature of the hydrographic environment. The value of stable isotope studies in fishery biology and ecology is strengthened by these findings. Through the combined use of rhythmic otolith increments and stable isotope studies, the record of a fish's past life history could be reconstructed. Hence, otolith studies could be extremely useful as metabolic, biological and ecological tools.

The question of biological isotopic fractionation or "vital effects" has been studied in other carbonate secreting organisms (Shackleton et al., 1973; Williams et al., 1977; Berger et al., 1978; Dudley and Goodney, 1979), although the exact mechanisms are not always clearly defined. The isotopic data for cod otoliths demonstrated that otolith formation occurred in isotopic disequilibrium with the ambient seawater and that the temperature fractionation equations for calcium carbonate must be applied with caution. Previous studies (Devereux, 1967; Degens et al., 1969; Mulcahy et al., 1979) applied the calcite-$\delta^{18}O$ fractionation equation for otoliths even though those otoliths were composed of aragonite (Irie, 1955; Carlstrom, 1963; Degens et al., 1969). The x-ray diffraction analysis of cod otoliths showed them to be pure aragonite and so it would be necessary to use the aragonite-$\delta^{18}O$ fractionation equation.

In view of the results, the observed agreement (Devereux, 1967; Degens et al., 1969; Mulcahy et al., 1979) to the calcite fractionation equation may be due to the fractionation of oxygen isotopes in a positive direction. Since most teleosts are poikilotherms and thus their metabolism is directly related to the temperature of their habitat, the isotopic ratios of most teleost otoliths can still be used to determine the water temperature to which a fish was exposed. It would be necessary to determine the particular isotopic fractionation equations

for species-specific otoliths as a function of temperature and salinity.

In the present study, A.A. and microprobe analyses of strontium concentrations in otolith aragonite suggested that the ratio of strontium concentrations to calcium concentrations was correlated negatively with temperature. However, the proposed temperature dependence of strontium was not the same as inorganically precipitated aragonite (Kinsman and Holland, 1969). The slopes of the two models were not significantly different ($p>0.5$), but the intercepts were different. The Sr/Ca levels in seawater are constant within the environmental area which most fish would inhabit (Kinsman, 1969) so it would appear that some factor, probably metabolism, was controlling the amount of strontium incorporated into otolith aragonite.

The relationship between temperature and strontium concentration in fish otoliths has not been demonstrated before, but it could be analogous to the correlation found in aragonitic corals. Weber (1973) found strontium to be temperature dependent in a host of coral species and advocated that strontium incorporation was governed by physiological processes. Recent studies by Schneider and Smith (1982) have supported these findings. It is conceivable that strontium was also incorporated into otolith aragonite related to physiological processes. Further studies, especially holding experiments should provide more information about this phenomenon.

Other knowledge about cod ecology which was not presented in the present study could be obtained from the otoliths. The weights of sagittal otoliths in cod (Radtke, unpublished data) appeared to indicate whether the fish was fast or slow-growing. The same relationship has been demonstrated for another gadoid, the haddock, *Melanogrammus aeglefinus*, by Templeman and Squires (1956). During the time of fast growth more protein appeared to be deposited in the otolith with a resultant lighter otolith. If a fish were to grow slower a higher proportion of carbonate would be deposited which would result in a lighter otolith. Sagittae weights could provide a quick estimate of relative fish growth.

Radiometric analyses of the otoliths of the splitnose rock-

fish, *Sebastes diploproa*, have been used to confirm logevity (Bennett et al., 1982) and provide other information on growth. Such analyses of cod otoliths could also provide new information on calcification and growth ecology. The analyses presented in the present study along with other analyses yet to be applied to cod otoliths will truly demonstrate that otoliths are data storage units when the proper techniques are applied.

In cod otoliths we are presented with countless patterns from their structural and chemical composition. The patterns displayed are sometimes different, but they have one thing in common: they all have a distinct function and origin which is special for each fish. Otoliths serve neurophysiologic functions, are composed of calcium carbonate in aragonite crystal form, have aspartic acid rich protein matrices, contain rhythmic incremental depositions, and can provide environmental life histories from the stable isotopes in their carbonate. The information recorded in cod otoliths would be invaluable to population, ecological and evolutionary studies of cod and would supply a new level of knowledge for fisheries biologists.

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SECTION III

Sea surveys. Distribution and ecology

CHAIRMEN

O. Bagge – T. Jakobsen