

Rhythmic Structural and Chemical Patterns in Otoliths of the Antarctic Fish *Notothenia larseni*: Their Application to Age Determination

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Summary. Sagittal otoliths from *Notothenia larseni* contain microincremental growth rings which are distinctly visible in otolith sections using Scanning Electron Microscopy. These microincrements are similar to those deposited daily in the otoliths of fishes from temperate and tropical waters. Microincrements were easily enumerated and fish length was related to increment number by a logarithmic curve. Otolith microstructure analysis appears to provide a technique to accurately determine age and growth rates in these fish. Sr/Ca ratios in otolith aragonite were analyzed along a radius from the outside edge to the core of an otolith section using an electron microprobe. The strontium/calcium (Sr/Ca) ratios varied with a cyclic periodicity apparently related to seasonal water temperature cycles and the number of cycles agreed closely with age estimated from "daily" microincrement counts. Sr/ca cycles can potentially be used to determine age, validate growth rates determined by other methods, and establish thermal conditions experienced during the life of a fish. Microstructural and chemical analyses of otoliths demonstrate great potential in helping to answer many questions about the growth processes and ecology of Antarctic fishes.

Introduction

The Antarctic coastal fish fauna is composed primarily of species not found elsewhere in the world (DeWitt 1971; Targett 1981). During the 40–65 million years since the isolation of the Antarctic Region, the original notothenioid fish fauna has undergone an evolutionary radiation with essentially no exchange with fishes outside the Antarctic (DeWitt 1971). Approximately 110 species in 14 families occur in coastal Antarctic waters. More

than 70% of the species and 90% of the individuals belong to four notothenioid families: Nototheniidae (Antarctic cods), Bathydraconidae (dragon fishes), Channichthyidae (ice fishes), and Harpagiferidae (plunder fishes). Approximately 95% of these species are endemic to the Antarctic.

Little information is presently available on the growth rates of Antarctic fishes, compared to the information on tropical, temperate and Arctic species (see Pauly 1978). Although existing data appear to demonstrate that Antarctic fishes grow slowly, comparably slow growth rates can be found in data on >200 non-Antarctic species (Pauly 1978). Information on growth rates is necessary to understand the growth patterns of Antarctic fishes occupying different trophic positions, to formulate population dynamics and production models, and to allow comparisons with the growth patterns of fishes in temperate, tropical and Arctic waters.

Scale annuli from *Notothenia rossii* (Olsen 1954) and otolith annuli from the ice fishes *Chaenocephalus aceratus* and *Champscephalus gunnari* (Olsen 1955) were used to obtain the first estimates of age for Antarctic fishes. The otoliths in these species are small and were found to be very difficult to analyze with methods and equipment available at that time. Scales were also used to estimate age in *Trematomus bernacchii* (Wohlschlag 1961, 1962), although the number of scale annuli appeared to underestimate age in these fish. It was necessary to add two to the number of annuli counted, because scales in this fish do not form until substantial growth has occurred and the last annulus could not ordinarily be identified. Hureau (1964) arrived at comparable age estimates for this species using otolith annuli. Everson (1970) utilized scales to age young *Notothenia neglecta* (one to four years old), and otolith annuli to age the older fish (>four years old) which have larger otoliths. Due to indistinct annuli, Everson could not accurately estimate age from the scales of either *N. neglecta* or *N. rossii* older than three or four years. Otoliths, scales, and

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bones were recently compared in determining age for *N. rossii* (Freytag 1980 a, b). Ring structure in the small otoliths was difficult to distinguish and interpret, and scales were most heavily relied upon for age estimates. Difficulties were noted, however, in discerning annual rings in scales from juveniles and fish older than eight years. Pectoral bones and scapulae were found to be useful, particularly in older fish, but few were analyzed. Other work on aging *N. rossii* used scale annuli (Crisp and Carrick 1975; Shcherbich 1975). In both of these studies, indistinct annuli and false checks were frequently encountered making it difficult to accurately determine age directly from annuli counts. Scale annuli counts were also used to estimate ages of *N. rossii*, *N. gibberifrons*, *N. kempfi*, *N. larseni*, *N. squamifrons*, and *T. hansonii* (Shust and Pinskaya 1978).

The Fish Biology Working Party, of the Group of Specialists on Living Resources of the Southern Ocean, arranged a meeting of investigators in 1979 to consider the state of knowledge regarding growth in Antarctic fishes (see Everson 1980). Papers were presented on the use of scale annuli to estimate age in *Dissostichus eleginoides* (Hureau and Ozouf-Costaz 1980), on the use of length frequency distributions to provide age estimates for *C. gunnari* (Koch 1980), and on techniques for preparation and examination of otoliths and scales (Freytag 1980b; North et al. 1980; Schneppenheim and Freytag 1980; Townsend 1980).

Much of the previous work on aging Antarctic fishes has relied on analyses of scales. Results from these techniques have often been difficult to interpret. Also, a large percentage of scales may be regenerated (Freytag 1980a,b) and fish scales have in general been shown to be subject to resorption (see Mugiya and Watabe 1977). All of these problems become more acute in older fish. Although otoliths are not regenerated or resorbed and ring counts are often less difficult to interpret (Six and Horton 1977), they have been less frequently used than scales for determining age in Antarctic fishes. Otoliths from these fishes are frequently small and difficult to analyze using conventional methods.

The detection and description of daily growth increments in fish otoliths (Pannella 1971) established a technique for determining age which has shown great potential, in temperate and tropical fishes, to provide a new level of information about fish growth. Daily increments are present in the otoliths of fish from many environments and can be used to age a wide range of fishes (e.g., Pannella 1974, 1980; Brothers et al. 1976; Brothers 1979). The enumeration of daily microstructural growth increments has been used to age the larvae, juveniles, and adults of many fish species from temperate and tropical waters (Brothers et al. 1976; Radtke and Dean 1982; and many others). This research on otolith microstructure establishes techniques which could provide an accurate method for age determination in Antarctic fishes.

Townsend (1980), using Scanning Electron Microscope (SEM) techniques, showed that microstructural

growth increments are present in otoliths from the five Antarctic fish species he examined (*Aethotaxis mitopteryx*, *C. gunnari*, *N. gibberifrons*, *N. larseni*, and *N. rossii*) and suggested that these rings might represent daily growth patterns. Thus, otolith microstructure examination techniques have the potential of being used to increase the precision and resolution of aging Antarctic fishes, to eliminate ambiguity in distinguishing the otolith nucleus from the first annulus, and to distinguish annual growth marks from growth checks.

The presence of daily growth increments in otoliths of blue whiting (*Micromesistius poutassou*) from the Arctic (Townsend and Shaw 1982) further supports the potential use of otolith microstructure in the study of growth patterns in Antarctic fishes. Blue whiting, like Antarctic fishes, live in an environment where a pronounced light-dark cycle is interrupted for a portion of the year. Gjøsæter and Tilseth (1982) also demonstrated that Atlantic cod (*Gadus morhua*) larvae in Norwegian-Arctic waters display daily increment formation despite constant light in the natural environment. Laboratory work has indicated the importance of a biological clock, entrained by the 24h light-dark cycle, in forming daily growth increments in the otoliths of *T. mossambica* (Taubert and Coble 1977) and *F. heteroclitus* (Radtke and Dean 1982). However, it appears from the work on Arctic fishes and on several deep sea fishes (Brothers et al. 1976) that daily microstructural growth rings do occur in the otoliths of fishes which do not experience a pronounced solar light-dark cycle for at least parts of the year.

In the studies described here, sagittal otoliths of *N. larseni* were analyzed to examine the value of otolith microstructure enumeration techniques in determining precise age and growth rates for Antarctic fishes. SEM analysis was used to examine the rings in detail and permit their accurate enumeration in this slow growing fish. *Notothenia larseni* is an endemic Antarctic nototheniid abundant around South Georgia Island, the South Sandwich and South Orkney Islands (Targett 1981), the South Shetland Islands (Tarverdiyeva and Pinskaya 1980), and occurs as far south as the Antarctic Peninsula (DeWitt 1971).

Changes in the strontium/calcium (Sr/Ca) ratio across an otolith section were also examined to obtain data on whether differences were present which reflect seasonal temperature changes, and which might be used to age fish in seasons and verify age estimates determined from microincrement enumeration. Otoliths are composed of calcium carbonate in aragonite crystal form, precipitated into a protein matrix (Carlstrom 1963; Degens et al. 1969). A number of trace elements can be incorporated into aragonite (Amiel et al. 1973). Strontium is incorporated into coral aragonite, and the Sr/Ca ratio in coral skeletons is negatively correlated with the water temperature at the time of calcium deposition (Smith et al. 1979; Schneider and Smith 1982). It is thus possible to use Sr/Ca ratios as a recording thermometer in corals.

The same mechanisms may function in fish otolith aragonite.

The present study investigates the use of otolith microstructural and chemical analyses to provide accurate age determinations and thus eliminate ambiguity in interpreting annual marks on scales and otoliths. Such techniques would be particularly valuable in aging species which lack scales (ice fishes) or have small otoliths which are difficult to analyze and interpret with other methods. The potential to accurately determine growth patterns and construct growth curves and models with precision is important to understanding the age structure of Antarctic fish stocks, the biology and ecology of fishes in the Antarctic ecosystem, and the functional relationship of fishes to other components of that system.

Materials and Methods

Notothenia larseni were captured on 19 May 1975 in 143 m of water at 54°05.6'S and 36°30.8'W, approximately 10 km from the northeast shore of South Georgia Island. Collections were made with a 3.0 m Blake trawl from the research ship *ARA Islas Orcadas* (formerly *USNS Eltanin*). Upon capture, fish were measured to the nearest mm (total length) and their sagittal otoliths were removed, air dried, and stored.

In the laboratory otoliths from 14 *N. larseni* of various sizes were cleaned of extraneous material, washed with water, dried at 60°C for 24 h, and weighed. Before sectioning, otoliths were examined with a light dissection microscope to determine the best plane in which to section for enumeration of microstructural growth increments. The otoliths were then embedded in epoxy resin and sectioned with a low speed stone saw. Sections 200 µm thick were taken from the medial cross sectional plane, the region which intersects the core area. After initial viewing with a compound light microscope, the sections were prepared for SEM examination. Otolith sections were attached to SEM viewing stubs with 5 min epoxy, and polished with 0.3 µm alumina paste. The polished sections were then etched for 1 to 15 min using 7% EDTA (Ethylene Diamine Tetra Acetate - pH adjusted to 8 with NaOH) to reveal incremental patterns in the surface relief. The etched otolith sections were washed in water, dried, coated with gold, and viewed with the SEM. Microstructural increment counts were then made for each otolith.

In regression analyses, total body length was used as the dependent variable related to number of increments and otolith weight. The use of total body length as the dependent variable is consistent with accepted methodology (Ricker 1973).

A Cambax electron microprobe was used to qualitatively and quantitatively measure changes in the Sr/Ca ratio across the surface of an otolith section from one specimen. The electron microprobe can analyze volumes as small as 3 µm³, making it possible to detect changes in otolith chemistry at very small spatial intervals. The wavelengths and energy dispersion of the X-rays resulting from electron bombardment are related to the nature and amount of the elements being analyzed. The otolith section was embedded in epoxy and highly polished with 0.1 m alumina paste to produce a flat surface which would not cause diffraction of the emitted X-rays. The surface was then coated with a thin layer of carbon to further dampen X-ray diffraction and to increase conductance of electrons. The section was analyzed at 25 points along a line from the edge to the core. Apatite and SrF² were used as standards.

Results

The sagittal otoliths of *N. larseni* are small and generally oval with a well defined rostrum and anti-rostrum (Fig. 1) (nomenclature from Hecht 1978; Morrow 1979).

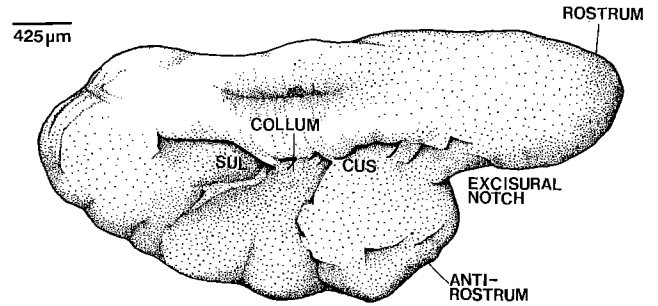


Fig. 1. The right sagitta of *Notothenia larseni* with the major external morphological features noted. The sagitta is from a specimen 16.4 cm total length

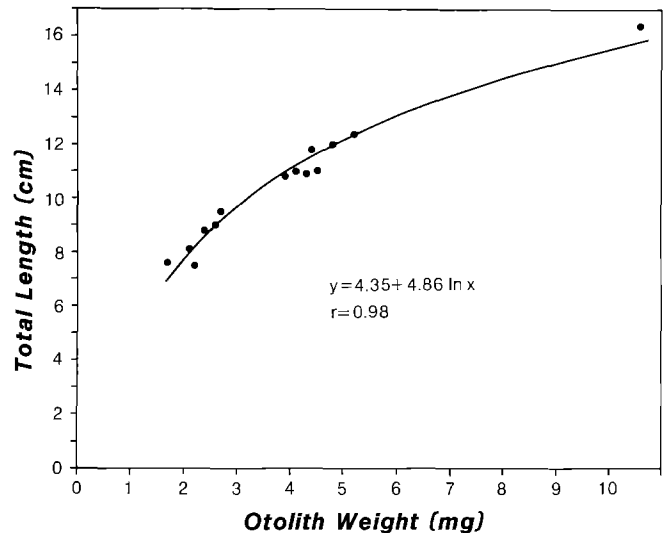


Fig. 2. Regression of total fish length on otolith weight for *Notothenia larseni*

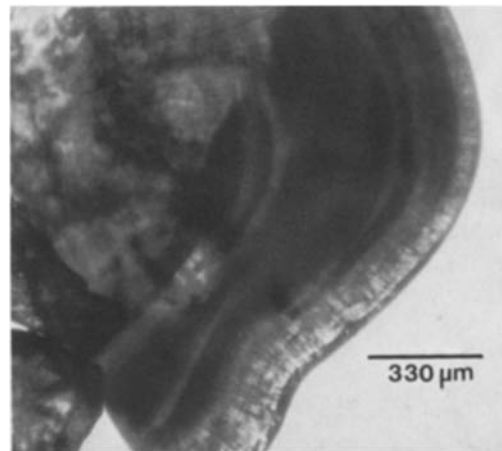


Fig. 3. Light micrograph of a sagitta from an 8.8 cm total length *Notothenia larseni*, showing increments which proved difficult to interpret

The rostrum is proportionally larger in larger fish. A well defined sulcus is transected by a collum and terminates to from an excisural notch accentuated by the rostrum and anti-rostrum. The core region of the otolith is in the col-

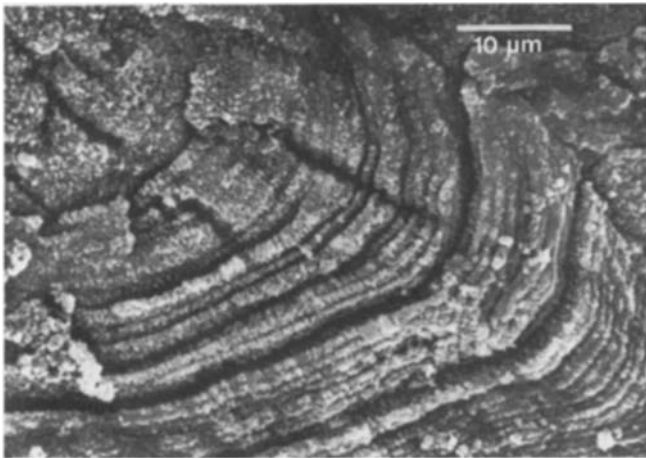


Fig. 4. Scanning Electron Micrograph of microincrements in a sagittal otolith from an 11.0 cm total length *Notothenia larseni*

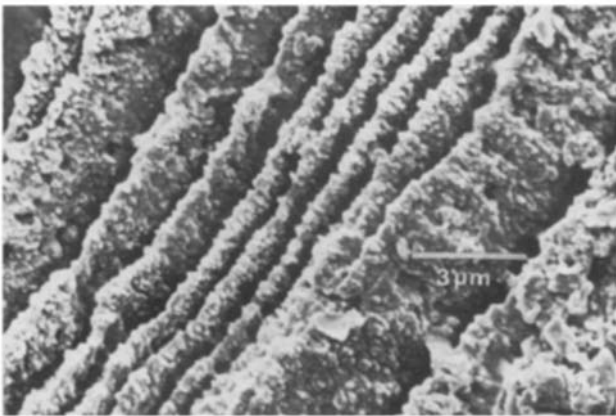


Fig. 5. Scanning Electron Micrograph of microincrements in a sagittal otolith from an 11.8 cm total length *Notothenia larseni*

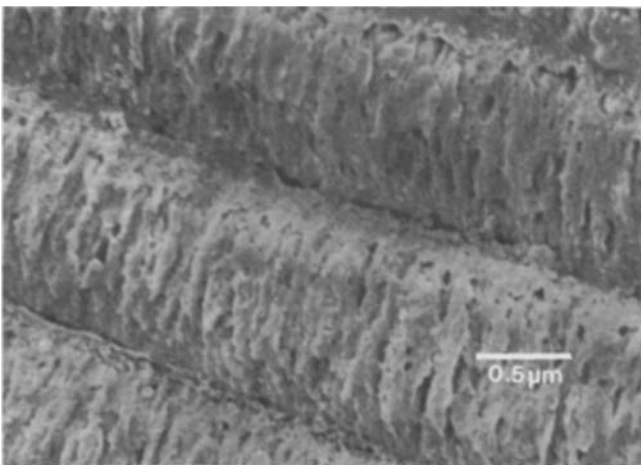


Fig. 6. Scanning Electron Micrograph of microincrements in a sagittal otolith from a 7.5 cm total length *Notothenia larseni*. Increments are composed of protein left after chelation of the calcium carbonate. Disruptions in the protein matrix account for the incremental patterns

lum area. The external surface is smooth and gently rounded. Fish lengths were related logarithmically to otolith weight (Fig. 2).

Otolith sections viewed by light microscopy (Fig. 3) showed small and large scale incremental patterns which were difficult to interpret and annuli were not interpretable. With the SEM, distinct microincrements were visible (Fig. 4). The increments displayed a range in widths (Fig. 5) which were likely related to differences in daily growth rates. Closer examination (Fig. 6) revealed that the increments were alternating protein ridges, made visible by use of EDTA. EDTA chelates CaCO_3 while leaving the protein unchanged. Once the correct plane and etching time were resolved, the microincrements (Fig. 7) could be counted regardless of changes in width.

Fish lengths were related to increment number by a logarithmic curve (Fig. 8). This relationship demonstrates the potential that each microincrement reflects daily growth or at least a growth interval related to fish size in a manner consistent with growth processes.

Analyses of Sr/Ca ratios across the surface of an otolith section provided additional support that the frequency of microincremental growth rings reflects daily growth. Electron microprobe examination showed that strontium was present and that Sr/Ca ratios varied regularly across the section, with 3+ distinct peaks (Fig. 9). If Sr/Ca ratios in otoliths are temperature dependent, as in corals, the present data demonstrate 3+ seasonal temperature cycles, with higher Sr/Ca ratios reflecting colder water temperatures. Assuming microincrements are added daily, this fish was 3.52 years old, an age agreeing closely with that determined from Sr/Ca ratios.

All *N. larseni* otoliths examined contained anomalous protein ridges (Fig. 10) which could not be attributed to preparation procedures. To our knowledge similar ridges have not been reported in otoliths from other fishes. These ridges may indicate growth disruption zones or periods of life history change, as they were found at approximately the same distance (420 m) from the core in all otoliths examined. Although the detailed nature of these ridges is not known, their structure and the physiological/ecological cause for their formation may provide additional information on growth processes and life history in this fish. Some evidence for lunar periodicity was observed in incremental patterns (Fig. 7), although such periodicity could not be definitively established.

Discussion

The otoliths of *N. larseni* contain microincremental growth rings which are easily counted using SEM techniques. Several lines of evidence support the assumption that the observed growth increments reflect daily growth. 1) Similar microincrements have been verified as daily in many of the temperate and tropical fishes previously investigated. 2) The number of increments in patterns con-

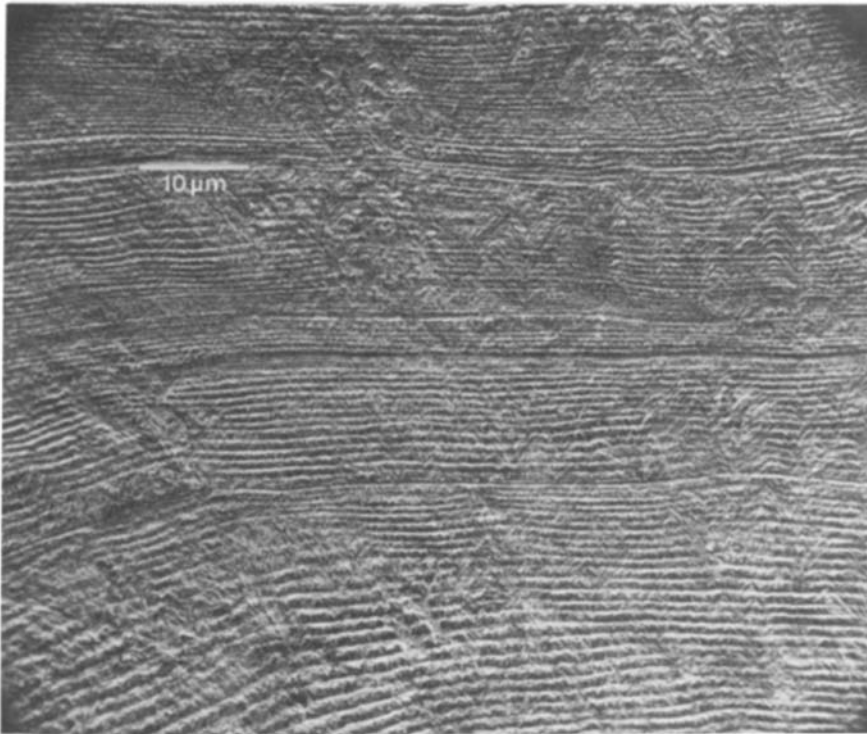


Fig. 7. Scanning Electron Micrograph of increments from the cross sectional plane of a sagittal otolith from a 12.0 cm total length *Notothenia larseni*, as utilized for daily increment enumeration. Possible lunar periodicity in increment patterns is evident

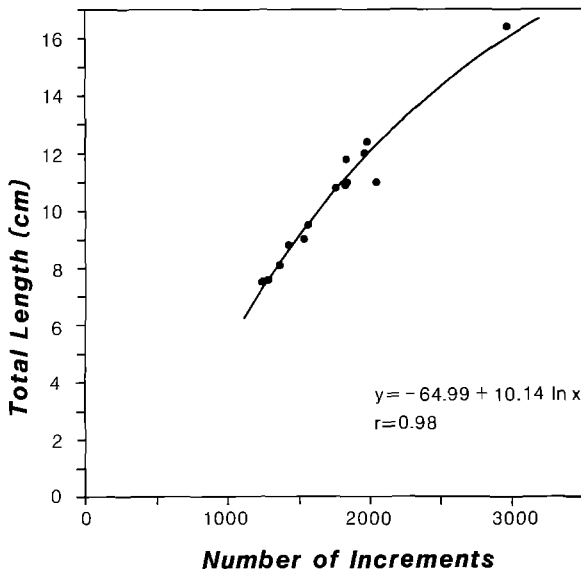


Fig. 8. Regression of total fish length on number of sagittal otolith microincrements for *Notothenia larseni*

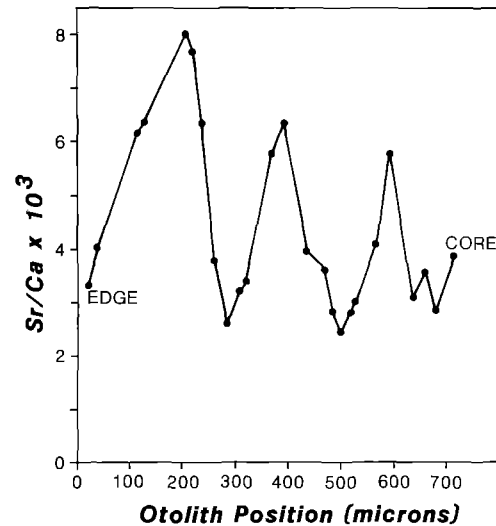


Fig. 9. Sr/Ca ratios across the surface of a sagittal otolith section from a 7.6 cm total length *Notothenia larseni*, as determined by electron microprobe analysis. The estimated age of this fish from microincrement counts (3.52 years) corresponds closely to the 3+ temperature cycles indicated in Sr/Ca ratios

sidered to be of possible lunar periodicity also suggests that one increment is formed per day. Incremental patterns presumably reflecting lunar periodicity have been previously reported for *N. larseni* (Townsend 1980). 3) Cyclic periodicity in Sr/Ca ratios supports estimates of age from microincrement counts. Thus, otolith microstructure analysis shows promise as a technique to accurately determine age and growth rates in Antarctic fishes.

Strontium was found in the aragonite crystals of *N. larseni* otoliths, as it is in coral aragonite. Corals deposit strontium and calcium at differential rates depending upon temperature related processes such as metabolism and growth. Although the magnitude of the Sr/Ca ratio may vary from species to species, the ratio always decreases with increasing temperature (Weber 1973; Livingston and Thompson 1971). This same process appears to

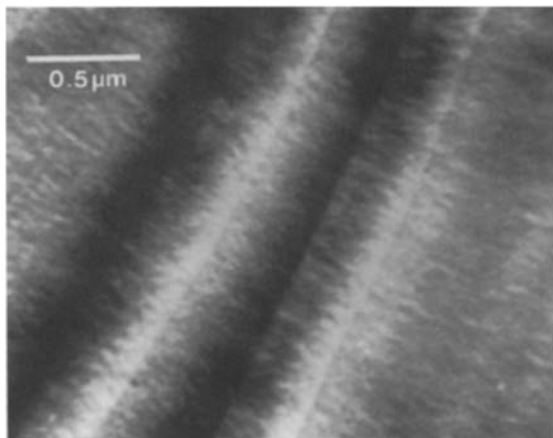


Fig. 10. Scanning Electron Micrograph of distinct protein ridges in a sagittal otolith from a 9.0 cm total length *Notothenia larseni*

be reflected in fish otolith aragonite. Otoliths from laboratory reared Atlantic cod displayed Sr/Ca ratios that were inversely related to temperature although the absolute levels of Sr and Ca were not in equilibrium with inorganically precipitated aragonite (Radtke, unpublished data). The Sr/Ca ratios in *N. larseni* otoliths also displayed changes apparently related to temperature, although these changes do not fit the regression model developed for Atlantic cod otoliths. These changes indicate that the application of Sr/Ca “thermometer” techniques to otolith aragonite are precise enough to provide a record of thermal history, and thus an age estimate, for individual Antarctic fish experiencing annual temperature changes generally of $<4^{\circ}\text{C}$. This technique could prove to be valuable in studies of Antarctic fishes as well as fishes elsewhere in the world.

Care should be taken in interpreting the rise in absolute level of the three Sr/Ca peaks from the *N. larseni* otolith section (Fig. 9). This trend is not necessarily due to the fish experiencing colder water temperatures with growth. Seven measurements per cycle are likely not sufficient to precisely determine relative peak heights.

The growth curves for *N. larseni*, determined in terms of total body length vs. otolith weight and total body length vs. microincrement count, were logarithmic. Otolith weight appears to be direct corollary of fish age since both are related to fish length in a similar fashion. The part of a growth curve representing most rapid growth, that portion before growth slows markedly toward an upper asymptote, is normally nearly linear. The growth pattern determined in the present study is based on intermediate size fish and further study using more otoliths, especially from small and large individuals, will be necessary to determine the precise nature of the entire growth curve. In particular, the slope of the lower portion of the curve relating body length to microincrement count must be accurately established since the present curve, when extended downward, does not pass through the origin. Because of these factors, no attempt was made to fit the-

oretical growth models such as Gompertz or von Bertalanffy curves (Ricker 1975) to the data.

The only previous work on *N. larseni* utilized scales to age 32 fish from near South Georgia Island (Shust and Pinskaya 1978). Ages were assigned to fish directly from scale annuli counts and mean length at each age was back calculated by direct proportional dependence. Comparison of the growth curve determined in the present study with that of Shust and Pinskaya (1978) shows that microincrement counts provide older age estimates than do those from scale methods (Fig. 11), suggesting slower growth.

It seems unlikely that microincrement enumeration greatly overestimated *N. larseni* age in the present study. Subdaily growth increments are infrequently encountered in fish otoliths. They have been found primarily in portions of otoliths deposited during the rapid early stages of larval and juvenile growth and in otoliths of tropical fishes within thick daily increments deposited during rapid growth (see Pannella 1980). If, on the other hand, microincremental growth rings are deposited less frequently than daily as growth rate slows in older fish, or for the portions of each year when the 24 h light-dark cycle is indistinct or lacking in the Antarctic, age estimates from microincrement counts would underestimate rather than overestimate age. Although areas in otoliths were detected in which increment width decreased, none of these appeared to indicate cessation of increment formation. More work on otolith microstructure enumeration in this fish is necessary before age and growth rate can be conclusively determined.

Other characteristics of otoliths, such as weight, could provide additional information on the ecology of *N. larseni*. Sagittal otolith weights were related logarithmically to fish size. There was evidence that fast growing (younger) fish have lighter otoliths at a given body weight than do slow growing (older) fish. Thus, it may be

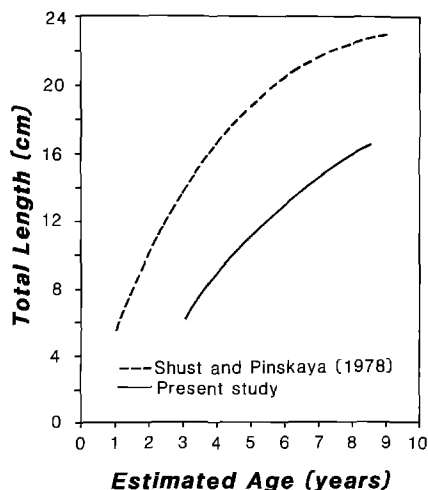


Fig. 11. A comparison of the growth curve determined for *Notothenia larseni* using otolith microincrement counts in the present study, with that from Shust and Pinskaya (1978) determined by back calculation from scale annuli readings

possible to compare otolith weights to fish size and arrive at quick estimates of relative growth. A similar relationship between otolith weight and fish growth has been demonstrated in haddock (*Melanogrammus aeglefinus*) (Templeman and Squires 1956). The processes by which differentiation in otolith weight occurs have been reported to involve greater protein deposition in the otolith during fast growth, producing a lighter otolith, and a higher proportion of calcium deposition during slow growth, producing a heavier otolith (see Bagenal 1974; Mugiyu et al. 1981; Tanaka et al. 1981). Similar processes may also operate in other Antarctic fish species. Such a technique could be useful in comparing growth rates of individuals in different geographical locations experiencing different prey resources or other ecological conditions.

The external features of *N. larseni* otoliths are distinctive compared to those from other Antarctic fishes examined (*Chaenocephalus aceratus*, *Champsocephalus gunnari*, *N. angustifrons*, *N. gibberifrons*, and *N. nudifrons*) (Radtke and Targett, personal observations). Species specific otolith features may be useful in identifying Antarctic fishes from cetacean, bird, or fish stomachs (North et al. 1984). Otoliths have been used in other areas of the world to identify fishes from predator stomachs (Fitch and Brownell 1968; Frost and Lowry 1981). Otolith morphological characteristics may also provide information on taxonomic relationships between Antarctic fishes, as they have in other fish groups (Hecht 1978; Hecht and Hecht 1978, 1979; Morrow 1979).

The otoliths of *N. larseni* appear to contain a large amount of biological and ecological information. This information can be assessed if proper equipment and techniques are used to analyze otolith microstructure and patterns of otolith chemistry. The present study demonstrates that easily counted microincremental growth rings and chemical rhythms show great potential in being used to estimate the age and growth pattern in *N. larseni*. The cyclic periodicity of Sr/Ca ratios can be applied to determine not only growth rates and validate those arrived at by other methods, but also to establish thermal regimes experienced by the fish. The existence of similarly appearing microincrements in other species of Antarctic fish (Townsend 1980; Radtke and Targett, unpublished data) indicates that these techniques can be used to help answer many questions about the growth processes and biology of Antarctic fishes.

Experimental work is necessary to validate the periodicity of microincrement formation in these fishes. The timing of formation must be firmly established. The effects of age, indistinct 24 h photoperiod, and feeding frequency need to be examined to determine their effect on the rate of microincrement formation and to allow proper interpretation of increment counts in these fishes. If environmental and nutritional changes affecting fish growth are reflected in otolith microincrement width, measurement of widths of increments and sequences of increments could be used as a chronicle of the fishes envi-

ronmental and ecological past. Additional work is also needed to firmly establish the reliability of otolith Sr/Ca ratios to detect seasonal temperature differences. The coupling of structural and chemical analyses could make it possible to link growth in Antarctic fishes to life history, feeding biology, and environmental events.

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