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SCANNING ELECTRON MICROSCOPE EVIDENCE FOR YEARLY GROWTH ZONES IN GIANT BLUEFIN TUNA, *THUNNUS THYNNUS*, OTOLITHS FROM DAILY INCREMENTS

Atlantic bluefin tuna, *Thunnus thynnus*, are found throughout the Atlantic Ocean, the Mediterranean Sea, and the Gulf of Mexico (Gibbs and Collette 1967). Bluefin tuna are both commercially and recreationally important. Thus, it is important that the population dynamics of this species be understood in order that international policies can be developed.

Age determination and subsequent growth estimation are critical for tuna management. However, confusion and controversy surround age estimation in tunas. The earliest record of age and growth of tunas (probably bluefin) was by Greek fishermen nearly 2,000 yr ago as documented in Aristotle's "Historia Anumalium" (Bell 1964). In recent times, the aging of tunas has become much more important and has been critiqued by Hayashi (1958), Bell (1964), and Shomura (1966). These reviews point to the problems and difficulties in aging tuna. These problems and difficulties appear to be more evident in aging bluefin tuna.

Bluefin tuna are usually aged by counting growth increments on their hard parts. Vertebrae have provided acceptable ages (Rodríguez-Roda 1964; Butler 1971; Nichy and Berry 1976; Berry et al. 1976), but the aging of large or "giant" (>250 kg) bluefin tuna is suspect because the outer increments appear very close together. Otoliths have also been used to study age and growth of bluefin tuna (Butler et al. 1977). Berry et al. (1976) compared otolith age estimates with vertebra estimates and discovered a discrepancy. They found corresponding marks on both vertebrae and otoliths for the first 10 yr, but not thereafter, when otoliths had more incremental zones. They hypothesized that more than one incremental zone was deposited yearly in otoliths after the first 10 yr.

Daily increments in yellowfin tuna, *Thunnus albacares*, and skipjack tuna, *Katsuwonus pelamis*, otoliths were studied by Wild and Foreman (1980) and Uchiyama and Struhsaker (1981). Taubert and Tranquilli (1982) used daily increments to verify annuli in the otoliths of large mouth bass, *Micropterus salmoides salmoides*, and it is proposed that an analogous investigation would provide corroborative evidence for the annual nature of outer major increments in giant bluefin tuna otoliths.

Methods and Materials

Sagittal otoliths were collected in November 1978, from giant bluefin tuna which were reared in the sea ranching program of St. Margaret's Bay, Nova Scotia, Canada. Fish were weighed and measured (TL) and the otoliths were collected as described by Caddy et al. (1976). All otoliths were washed in water and stored dry.

Whole otoliths from four fish were placed in epoxy resin and sectioned on a Buehler Isomet[1] saw. Sections 200 μm thick were acquired from the region judged to contain the core. A diagrammatic view of a cross section of a bluefin tuna otolith is

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[1] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

shown in Figure 1. Ten to 15 sections were sawed from each otolith. The number of sections viewed was dependent upon the clarity of the increments.

Each otolith section was fastened to an aluminum scanning electron microscope (SEM) stub with 5-min epoxy. The otolith section was highly polished with 0.3 μm alumina paste and etched with 6% EDTA (ethylenediaminetetraacetic acid, adjusted to pH 8 with NaOH) for 1 to 20 min. The otolith sections were washed in water, dried, coated with gold, and viewed on a SEM at various magnifications. Observations and counts were made while the otolith section was in the SEM.

It was discovered that different areas of the rostral lobe of the otoliths were made clear by different etching times. Sequential etching made it possible to view microincrements in the outer 10 major increments. Individual sections were etched for different periods of time, with 15- to 20-min etching times showing the inner increments more clearly. The 10 outermost major increments were clearly visible in all sections and could be followed from section to section regardless of etching time. Each major increment was chosen to be from the center of one ridge to the center of the successive ridge; sequential etching revealed the microincrements between the ridges. It was not possible

to count the microincrements from the edge of the otolith inward past the 10th major increment on any individual section. Consequently, sequential cross sections from each otolith were etched for different periods of time, in steps of 1 min, in order to follow the progression of the microincrements.

In the present study, a microincrement was defined as an unbroken incremental zone with discontinuous zones as boundaries (Radtke and Dean 1982) and was considered to be a daily increment.

Results and Discussion

SEM techniques made it possible to view microincrements in bluefin tuna otoliths from four individual fish. The most visually distinct increments were found on the rostral lobe of the otolith cross section (Fig. 1). Thus this area was used predominantly for SEM observations. The major increments of the otolith can readily be seen in Figure 2. Higher magnification (10,000 ×) revealed that the major increments were constructed of smaller increments which in turn were composed of microincrements (Figs. 3, 4).

Differential etching caused the problem that not all the increments could be viewed at the same time. This was overcome through the use of suc-
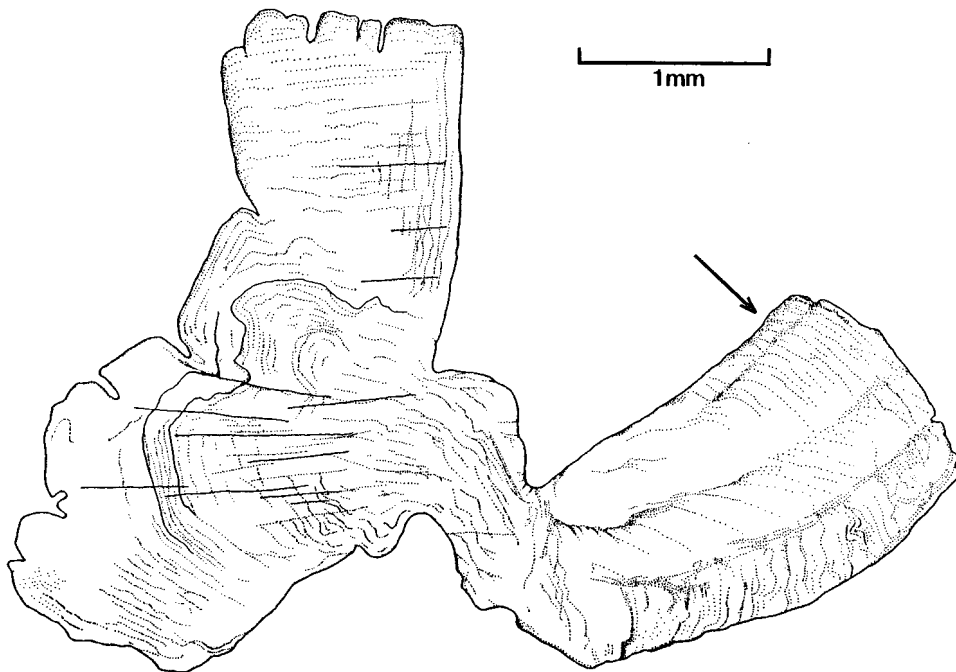

FIGURE 1.—Cross section of a bluefin tuna otolith showing the area (arrow) studied for microincrements. This area is on the rostral lobe of the otolith.
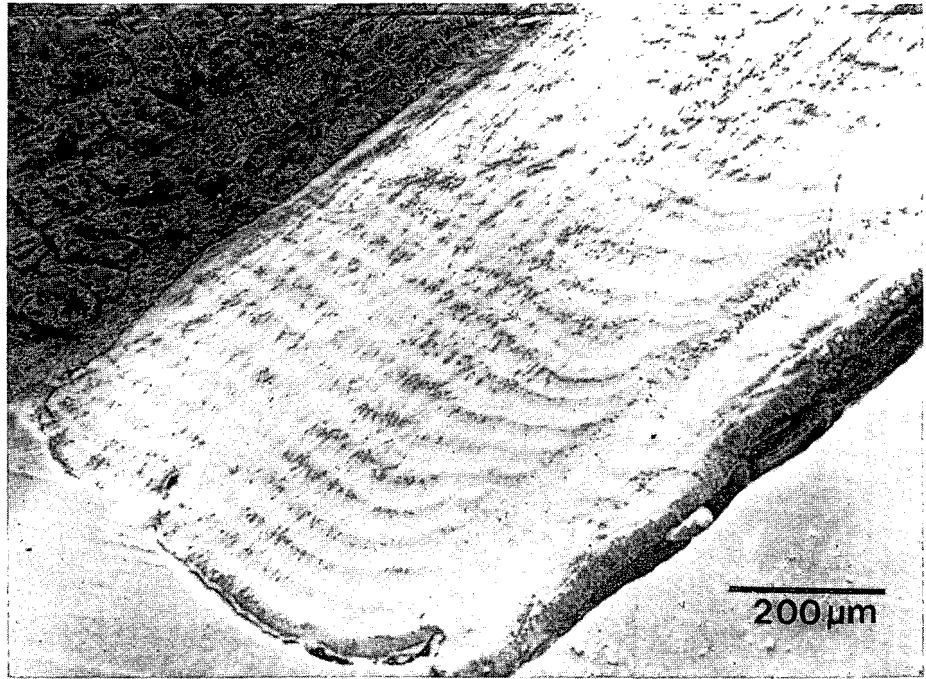
FIGURE 2.—Bluefin tuna otolith etched with EDTA which shows distinctive major increments. A short etching time gave good resolution to the outermost increments.
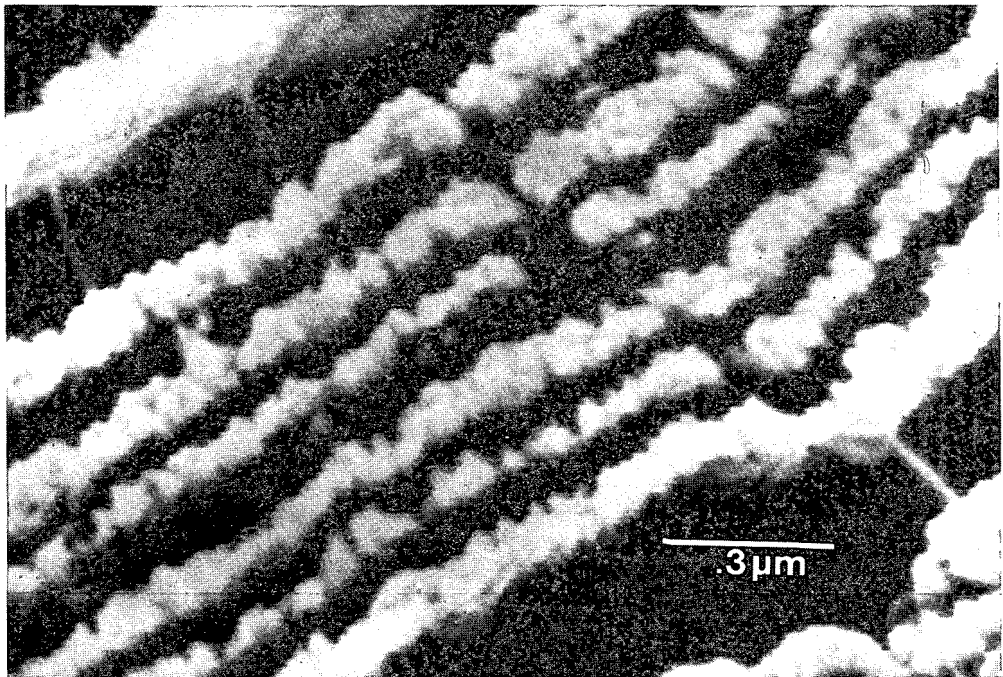

FIGURE 3.—Protein ridges of microincrements from a bluefin tuna. Strands of protein can be seen to interconnect the ridges.
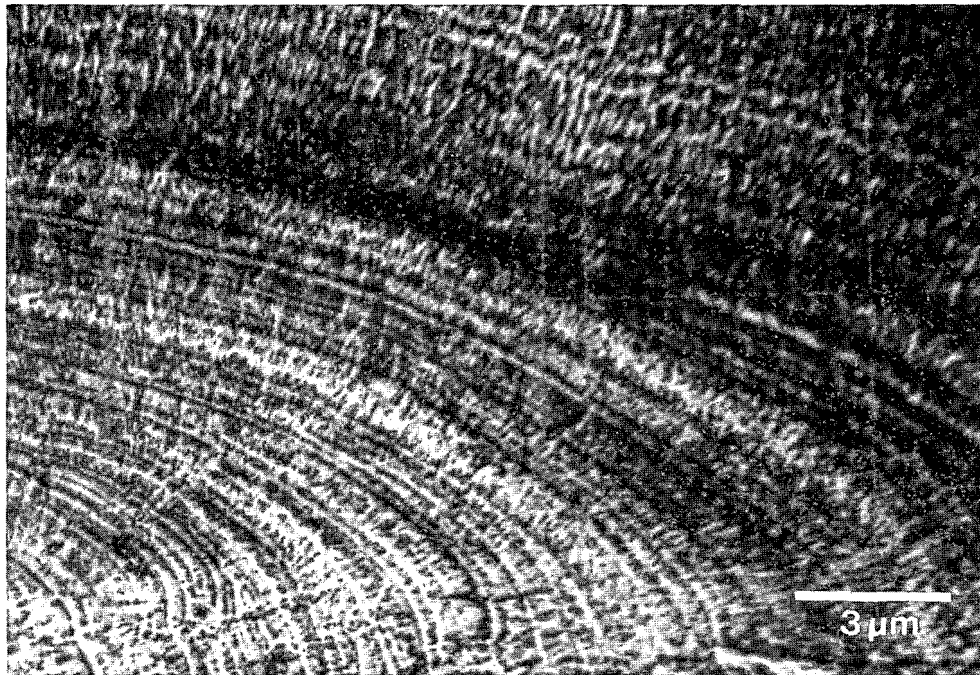
436

FIGURE 4.—Microincrements detected on the slope of a major protein ridge from a bluefin tuna. Differences in widths cause the yearly increments.

cessive cross sections which were etched for different time periods. This sequential etching made it possible to follow microincrements within the major increments. A difference in etching can be seen in Figures 2 and 5. Although major increments were clear in most etching times, the microincrements were not. Through the utilization of these techniques it was possible to obtain microincremental numbers for major increments (Table 1).

The microincrement counts in each major increment varied from 273 to 385 with the lowest count being found on the edge of the otolith. The summations of the microincrement counts for each fish were remarkably close and not significantly different ($P > 0.05$). Also, means of microincrements for each fish were not significantly different ($P > 0.05$) from the expected of 365 per year. These data increase the credibility of the microincrements being daily and present a plausible verification of the major increments as being annual.

Each microincrement is composed of a protein matrix with calcium carbonate crystals, in the aragonite crystal configuration, deposited within the matrix. Etching with EDTA dissolves the

TABLE 1.—Numbers of microincrements found in the major increments on the outer edge of the rostral lobe of the sagittae of four bluefin tuna, *Thunnus thynnus*.

Fish	1	2	3	4
Weight (kg)	496	381	405	470
Fork length (cm)	275	216	251	268
Sex	M	F	F	F
Estimated age[1]	25	19	19	24
Major increment	Counts	Counts	Counts	Counts
1	278	273	300	289
2	368	375	337	321
3	355	310	366	374
4	339	370	339	342
5	385	344	376	323
6	366	376	370	372
7	313	355	347	349
8	369	356	358	373
9	341	369	315	348
10	328	348	365	329
Total	3,442	3,476	3,473	3,420
Mean ± SD	344±32	348±33	347±25	342±27

[1]From counts of major increments by light microscopy.

aragonite crystals leaving areas with a higher protein content to form discernible increments (Figs. 3, 4). Extended etching (times varied depending on the area of the otolith) can cause the protein ridges to collapse and prevent counting of the microincrements. Thus, etching times were critical to the acquisition of viewable increments.
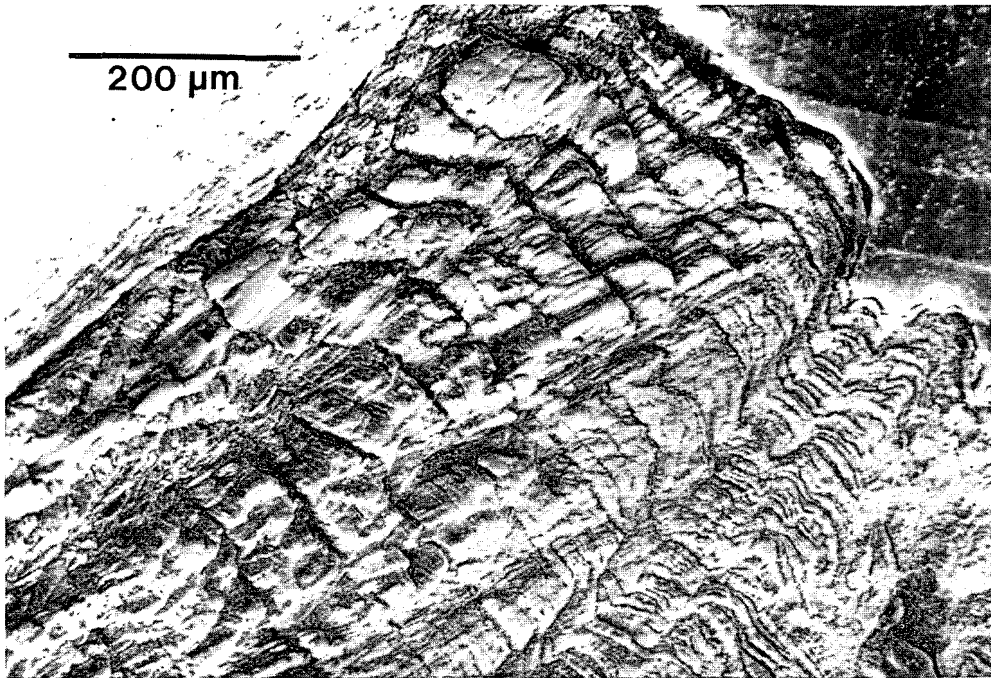
FIGURE 5.—Neighboring area of a bluefin tuna otolith shown in Figure 2 which demonstrates the uneven effects of etching.

The width of each microincrement varied in accordance with its position within a major increment. Microincrement width was probably a function of the time of the year when deposited. The widest microincrements were displayed between the ridges. Furthermore, the microincrements formed at the edge of the sagittae were wide and deposited during a time when the fish were fed large amounts of mackerel as part of the sea ranching operations. Observations on microincrement width suggest that wide microincrements were deposited during summer feeding and growth, while finer microincrements were deposited during the winter. It was these differences in width that accounted for the formation of yearly increments.

Most fish species investigated for daily age estimates have been found to possess daily increments in their otoliths (Pannella 1971; Brothers et al. 1976; Struhsaker and Uchiyama 1976; Taubert and Coble 1977; Methot and Kramer 1979; Steffensen 1980; Wild and Foreman 1980; Townsend and Graham 1981; Uchiyama and Struhsaker 1981; Radtke and Dean 1982). Thus, it is conceivable that the microincrements displayed in bluefin tuna otoliths are also daily. In tunas, Wild and

Foreman (1980) studied daily increments in yellowfin and skipjack tuna, and Uchiyama and Struhsaker (1981) also investigated daily increments in yellowfin and skipjack tuna. Yellowfin tuna are found to deposit daily increments in both studies, whereas Wild and Foreman (1980) suggested that skipjack tunas have 25% fewer increments than would be expected if the increments occurred daily, while Uchiyama and Struhsaker (1981) advocated that daily increments did occur in skipjack tuna. In light of the present data, Wild and Foreman (1980) may have not detected increments formed during winter or colder periods. For giant bluefin tuna it is suggested that the microincrements are formed daily. If bluefin tuna did not deposit microincrements on a daily schedule, it would be expected that fewer daily increments would be detected in each major increment. Since this is not the case, it corroborates the idea that daily increments are formed in bluefin tuna otoliths and groups of daily increments form annual increments.

Otoliths may be the most useful hard structure for aging fish. Vertebrae and other hard structures are much more susceptible to resorption during times of physiological stress, while otoliths are

capable of permanently storing important ecological information since they are not susceptible to resorption (Mugiya and Watabe 1977). Otoliths have been shown to be the more accurate method of age determination in several fish species (Six and Horton 1977; Kimura et al. 1979). Otoliths are probably the most accurate means of age resolution in bluefin tuna.

In conclusion, the observation that microincrements in the sagittae of giant bluefin tuna about 365 in number for each outer major increment verifies the annual nature of these structures and strongly suggests that the microincrements are daily. Although it is not feasible to view large numbers of tuna otoliths by SEM techniques, the application of such techniques can provide answers to important biological questions.

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YEARLY CHANGES IN ABUNDANCE OF HARBOR SEALS, *PHOCA VITULINA*, AT A WINTER HAUL-OUT SITE IN MASSACHUSETTS

Information on the abundance of the harbor seal, *Phoca vitulina concolor*, population in New England consists of outdated estimates in the literature (King 1964; Maxwell 1967; Hewer 1974; Bonner 1976). A more recent series of unpublished reports (Richardson[1]; Knapp and Winn[2]; Kraus[3]; Gilbert and Stein[4]) suggests a harbor seal population which is increasing in numbers from its present breeding range north of Massachusetts southward into southern New England. A primary research need identified by Prescott et al.[5] was confirmation of this suspected increase in the harbor seal population throughout New England.

This study summarizes available data on annual fluctuations in seal numbers since 1972 at one site in southeastern Massachusetts.

The study was conducted at Stage Point, Manomet, Mass. (lat. 41°55′N, long. 70°32′W). Harbor seals occur seasonally at Stage Point from late October through May (Schneider and Payne 1983). A rapid decrease in numbers occurs at this site in May (Schneider and Payne 1983), prior to the pupping season which occurs mid-May to mid-June in Maine (Richardson footnote 1; Wilson[6]). A few seals are reported throughout the summer but most move northward out of the study area by June.

The study site consists of a shoreline with a sandy cliff to 25 m. Sand, rock, and cobble extend from the base of the cliff into the water. Seals haul out exclusively on the larger rocks in the immediate subtidal zone from about 1-2 h before to 1-2 h after low tide (Schneider and Payne 1983). A similar haul-out pattern has been described at other rock-ledge sites in New England (Richardson footnote 1; Wilson footnote 6). Because of the synchronized haul out observed at Stage Point, the number of seals seen on the rocks is considered representative of the number of seals in the immediate vicinity (Schneider and Payne 1983) and, therefore, a useful index for monitoring changes in the abundance of harbor seals at this location.

Methods

Counts at Stage Point were made by direct observation within 2 h of low tide from the cliffs above the haul-out site. Schneider and Payne (1983) found that during 1979-80 the average number of seals observed at Stage Point peaked in January; therefore, the average number of seals ($\pm$SE) seen per daily count in January of each year was used in analyses among years. We transformed the January averages into logarithmic values, and the coefficient of correlation (r) from the linear regression was used to describe the relationship between the average number of seals seen per daily count in January 1972 and 1983.

In addition, air temperature, wave intensity, and human disturbance influence the total number of seals seen per daily count at Stage Point

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