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AGE DETERMINATION IN ATLANTIC BLUEFIN TUNA

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The National Marine Fisheries Service is developing an aging technique for Atlantic bluefin tuna (ABT), Thunnus thynnus thynnus, that appears to be a significant improvement over other aging methods used for this family of fishes.

Thin cross sections of otoliths show zones that we interpret to be annuli. In giant tuna (136.2 to 454 kg) the more recent annuli are much better defined than are those on scales or vertebrae.

This paper reports on the following aspects of ABT aging techniques:

1. previous ABT aging reports
2. scales
3. vertebrae
4. otoliths
    a. description
    b. removing and storage
    c. cutting sections
    d. description of zones.

PREVIOUS ABT AGING REPORTS

A history of tuna age determinations was given by Bell (1964). Tiews (1963) summarized prior reports on ABT age determinations, relative and absolute growth patterns and rates, age composition, and longevity. Butler (1971) reviewed ABT age and growth and discussed rings on hard parts and methods of aging vertebrae.

Three aging techniques have been used for ABT by several workers:

1. seasonal progression of dominant size groups
2. ring formations on scales
3. ring formations on vertebrae.

None of these have been completely satisfactory, especially for older age groups with the first two techniques.

Collectively, the ABT aging studies can be summarized as follows: length frequencies can separate the younger age classes, from 0 to 4, 5, or 6 years, especially when compared by monthly or shorter periods, and the number of rings on scales and vertebrae confirm these younger ages. The number of rings on the scales can be reliably used to an age of about 7 years, and are usually very imprecise beyond that age. Counting the number of rings on vertebral centra has been useful in assigning ages up to 14 years, but for larger fish the rings are illegible in some samples (ca. 30%) and counts of rings in most are imprecise (ca. plus or minus 1 year).

The most comprehensive report to date on aging western ABT is by Mather and Schuck (1960). Using scales and/or vertebrae, they determined the age of 196 ABT from the Cape Cod vicinity, ranging 34.2 to 249.0 cm FL, to be 0 through 14 years of age. These determinations were based on ABT samples collected between 1950 and 1960. Figure 17 of their report, plotting years of age against weight and fork length, has been most useful in subsequent estimation of ABT ages because no significant aging of western ABT has been done since to our knowledge. We note, however, that such indirect age estimations (based on fish length or weight) should be general and not specific, especially at larger sizes. For example, the size range encompassed by age 12 (for which Mather and Schuck had only 6 specimens) overlaps parts of the length and weight ranges that can be graphically projected from the table for ages 9, 10, 11, 13, 14, 15, and 16.

For maximum determined age, Bell (1964) cited 18 years (259 cm FL), but this was a subjective reading based on scale rings. Tiews (1963) postulated that extremely large bluefin may reach a maximum age of 17 to 18 years. Bigelow and Schroeder (1953) cited rumors and records of maximum size and mentioned a length of 14 feet or more and a weight of 1,600 pounds (427 cm, 726 kg). Butler (1971) speculated a maximum age of 20 plus years and calculated hypothetical maxima of 356 cm FL and 1550 pounds (702 kg).

SCALES

We have examined scales from a series of ABT from 5 to 700 pounds (2.3-318 kg). Our preferred body area for collecting scales is just in advance of the caudal keel and midway between the horizontal midline and the dorsal finlets, because these seem to be more uniform in shape, size, and structure, with less vacuolation and erosion.

This analysis is in process and still experimental. To prepare the scales for reading, we have used dry mounts, plastic impressions, and a modification of the eosin staining process used by Bell (1963). We note, as with previous reports, that most samples are discernible to an age of about 6 years and generally become increasingly imprecise in older fish.

Especially with older fish we note appreciable individual differences in sculpture of annuli between scales from the same body area of the same fish. For example, annuli in some scale fields are not continuous around the scale margin, and annuli may be legible near the focus and diffuse toward the periphery of one scale with the reverse condition on an adjoining scale. This suggests that collective scan and interpretation of a number of scales (25 or more) from a single fish may furnish a reasonably close age estimate of larger fish. This will be useful when no other parts are available for aging.

From our 1975 U.S. collection, we have scale samples for analysis from about 1,000 ABT, ranging 0.5 to 448 kg.

VERTEBRAE

We have examined vertebrae from a series of ABT 5-700 pounds (2.3-318 kg). Most of our samples are from the caudal peduncle. This part of the body is more readily available to our samplers, especially from the commercially valuable giants. Starting 13 August 1975, the U. S. law required all persons processing ABT greater than 300 pounds (136 kg) round weight in the U. S. to tag the caudal keel, remove 3 to 6 inches of the peduncle with the tag attached, and furnish it to the National Marine Fisheries Service.

This analysis is in process and still experimental. We are testing different methods of preserving the centra, including freezing, drying, boiling, isopropyl alcohol (50%), ethyl alcohol (70%), and formalin (10% buffered). In preparing the centra, we are experimenting with delysing (potassium hydroxide; ammonia and clorox) and decalicifying (hydrochloric acid; Decal). We are testing maceration of the flesh (fresh and saltwater). For staining we are using modifications of the alizarin technique described by Galtsoff (1952) and the silver nitrate technique described by Stevens (1975).

Our preliminary observations and opinions are: None of the several combinations of treatments of the centra used so far appreciably improve the approximately plus or minus one-ring variance in reading vertebrae from some giant ABT, and the experiments in technique warrant continuation. The ring sculpture on the centra is in the narrow external layer of connective tissue covering the cones, and this is occluded by some preservatives (alcohols and formalin). This will be useful aging process when techniques are improved and if this is the only hard part available.

From our 1975 U. S. collections, we have vertebrae samples from about 1300 ABT, ranging 0.5 to 478 kg.

OTOLITHS

Recent reviews of otolith studies are given by Blacker (1974, 1975) Struhsaker (MS) and Brothers (MS) presented reports on the structure and analysis of "daily growth rings" on the otoliths of smaller tuna (usually less than 2 years old). To our knowledge the use of otoliths for interpretation of annular rings in ABT has not been used.

Description

ABT otoliths (Figs. 1 and 2) are similar in shape and sculpture to that described and illustrated for albacore, Thunnus alalunga, by Fitch and Craig (1964). They range in length from approximately 7 mm in a 1-year-old ABT to 22 mm in an ABT identified as 27-years-old.

Removal and Storage

The most practical technique we have developed for removing otoliths from all sizes of ABT is illustrated on a following page (OTOLITH REMOVAL, ABT Tech Sheet No. 1). The front of the head is removed with a vertical cut halfway between the end of the upper jaw (maxilla) and the posterior margin of the preoperculum. This exposes the paired cranial canals that contain the sacculus chambers. The sagitta on each side is at most only a few millimeters deep in the chamber, and can be easily removed with forceps.

The otoliths are picked clean of any excess tissue. They may be gently rinsed in water and air dried. They are labeled and stored in cotton-plugged vials, in capsules, or in envelopes. Most of our samples have been stored dry. We have noted putrefaction of the accompanying tissue if they were not adequately cleaned and stored in air-tight vials. We are experimenting with various substances in trying to enhance the annular rings: storing one of a pair dry and the other in solution (as 50% glycerin and water: 70% ethyl alcohol; oil of wintergreen; anisole).

Cutting Sections

To cut sections from an otolith, a low-speed saw is used that is specifically designed for cutting small objects within micrometer accuracies The macrotome we use consists of a weighted arm that holds the object by gravity against the rotating blades. The rotation of the blades can be varied 0-300 rpm, and the arm can be moved laterally by a micrometer dial.

The blades are cleaned by passing the lower edge through a bath made of a solution of about 20% detergent and water. The instrument has an automatic stop that is activated when the blade has cut through the object.

Two blades separated by a thin spacer about the thickness of the sections desired are used so that a section is cut through with one pass of the blades. The otolith is held in place by imbedding it with melted wax that cements it to a small paper tab. Household wax is used, heated to about 115° C in a double boiler using glycerine for the heating bath. A non-hydroscopic powder (as Rottenstone or decolorizing carbon) is mixed into the wax to give it bulk and prevent running when cementing the otoliths to the tabs and to assist in polishing the surfaces of the sections as they are being cut.

Lines are marked on the tab to align the otolith so that the plane of the cut is automatically aligned with the blades when the tab is placed in the slotted holder of the macrotome. This makes the operation very routine, eliminating adjustments of the macrotome with each cut. The blades used are 3.0 inches in diameter, 0.006 inches thick, composed of metal with diamond particles bonded into the metal near the rim. Diamond size now in use is MD 410, which is large enough to ensure short cutting time, while maintaining a smooth finish to the surfaces of the sections so that visibility of the zones is not affected. A tuna otolith is routinely cut in 30 to 60 seconds.

The width of the section removed from the otolith is regulated by the spacer placed between the two blades. For ABT we find sections 0.009-0.010 of an inch (0.229-0.254 mm) satisfactory. Sections thinner than this are frequently broken, especially at the narrow junction of the two leg-like segments. The sections removed from the otoliths are placed in depressions in black plastic trays, then flooded with a saturated solution of lead acetate in ethyl alcohol to prevent the section from clearing when it is mounted with Dammar-balsam or other mounting media. Various treatments are being used experimentally on the sections before mounting to enhance the contrast of the zones.

A cross section through the region of the sulcus is illustrated for two otoliths in Figures 3 and 4. In this region the connection between the two leg-like segments is very narrow. As cross sections are taken posteriorly through the broad portion of the otolith, the space increases between the two segments as the groove widens, and the length and configuration of the segments change. The formation of the otolith is considered to be quite complicated, for areas seem to be present inside the segments that are held together by connective tissue.

Description of Zones

We have been able to confidently interpret the zones as annuli on cross sections of otoliths of smaller ABT, from ages 1 through 8.

On cross sections from larger ABT, two areas of zones exist. One area represents early growth through approximately age 10 and is often difficult to interpret for aging. The other group represents later growth from about age 10 and older and is relatively much easier to interpret. This is a variable situation, but its occurrence is frequent enough to discuss. The zones of early growth, up to age 4 or 5 generally consist of broad, diffuse zones; only occasionally are they typically formed; i.e., a normal transition from opaque summer growth into winter hyaline, with an intensification of hyaline growth, so that the annulus is readily separated from adjacent rings. However, it is more likely for these zones to be vague and lack a definitive edging, so they require considerable interpretation. It is believed this is not characteristic of zone formation on otoliths, but due to the oceanic migrations of ABT. The problem is partly alleviated by cutting the sections away from the center of the otolith in a region where the zones are somewhat condensed. As sections are taken progressively further from the sulcus region, the zones become proportionately closer together because of the decrease in the width of the otolith, and a corresponding decrease in detail occurs within each zone. A series of sections are taken from the center through the broad portion of the otolith, so it is possible to examine the zones under varying conditions. Also, the two leg-like segments can be utilized in determining the number of rings present. The shorter leg ordinarily shows early growth more clearly, while older growth is better observed on the longer leg. Counts of the number of zones on each leg differ frequently by one or two rings, which is probably due to the vagueness of early growth on the longer segment of the otolith.

The broad diffuse pattern starts to change after age 5, becoming a series of closely spaced hyaline bands that coalesce near the edges of the segments forming what we interpreted as an annular zone. After approximately age 10, the hyaline bands of the annulus begin to combine into a singular type of growth pattern, so that the determinations become simpler.

Conclusion

Thus far 43 sections of ABT otoliths have been prepared that are believed to range in age from 1 to 27. Most of those from larger ABT required extensive examination for what were thought to be year marks. During the few months this work on evaluating tuna otoliths for age determination has been in progress, improvements have been made in preparing and storing the cross sections for aging. It is believed that further experimentation with the use of clearing agents will improve the visibility of the zones on the sections.

From our 1975 U. S. collections, we have access to 300 otolith samples of ABT of sizes 0.5 to 135 kg and 450 otolith samples of ABT of sizes 136 to 408 kg.

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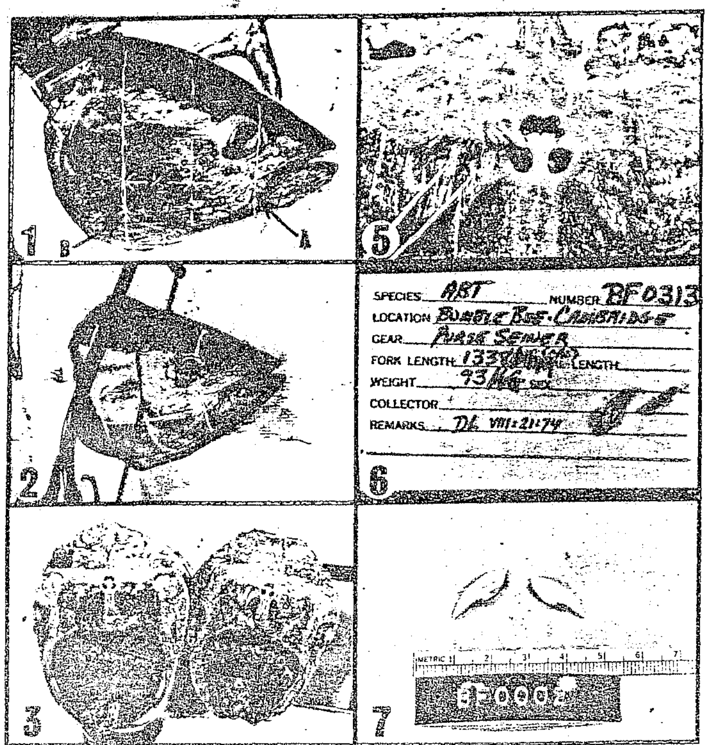
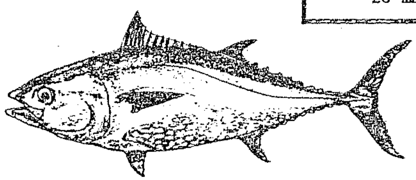


OTOLITH REMOVAL

ATLANTIC BLUEFIN TUNA

A B

1. Locate end of upper jaw/and rear edge of preoperculum. Mark a vertical line half distance between these.
2. Along this line, saw through head in a vertical cut.
3. Front end of the cut head contains the otoliths.
4. Otoliths are in the 2 bilateral cavities on each side of the midline in the upper third of the head.
5. With forceps, gently locate and extract each otolith.
6. Remove fibrous capsule from each otolith (if it is still intact). If an otolith breaks, save both pieces. Rinse briefly in water. Dry in air.
   Store in vial or envelope (no pressure). Include data on Fork Length (caliper or contour specified). Weight (round, or other specified), Location and Date (caught and/or collected), and collection number (if assigned).
7. Otoliths (pair) from a 560-lb Atlantic Bluefin Tuna. Otoliths range in length from 7 mm (1-year-old) to 20 mm (giants).



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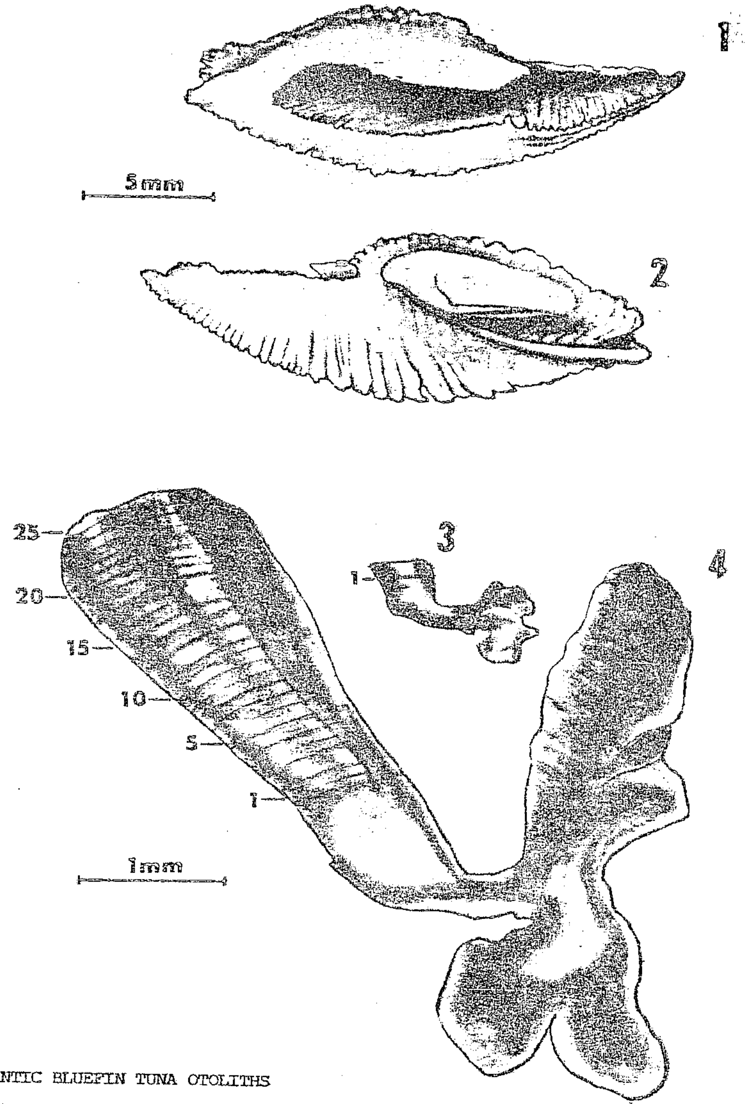
ABT TECH SHEET NO. 1



ATLANTIC BLUEFIN TUNA OTOLITHS

1. Inner face of left sagitta from giant ABT.
2. Outer face of same otolith.
3. Cross-section (0.15 mm thick), one-year old, 51.2 cm FL, 2.9 kg.
4. Cross-section, 25-year old, 277 cm, 415 kg.