Manual of Best Practices for Age Determination of North Pacific Albacore Tuna

E. Chen and J.A. Holmes

Fisheries and Oceans Canada
Science Branch, Pacific Region
Pacific Biological Station
3190 Hammond Bay Road
Nanaimo, B.C. V9T 6N7

2015

Canadian Technical Report of Fisheries and Aquatic Sciences 3145

Fisheries and Oceans Canada
Pêches et Océans Canada

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts*.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. II n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques.*

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Canadian Technical Report of Fisheries and Aquatic Sciences 3145

2015

MANUAL OF BEST PRACTICES FOR AGE DETERMINATION OF NORTH PACIFIC ALBACORE TUNA

by

E. Chen and J.A. Holmes

Fisheries and Oceans Canada
Science Branch, Pacific Region
Pacific Biological Station
3190 Hammond Bay Road
Nanaimo, B.C., V9T 6N7

Cat. No. Fs 97-6/3145E-PDF ISBN 978-0-660-03507-9 ISSN 1488-5379

Correct citation for this publication:

Chen, E., and Holmes, J.A. 2015. Manual of best practices for age determination of north Pacific Albacore Tuna. Can. Tech. Rep. Fish. Aquat. Sci. 3145: v + 28 p.

TABLE OF CONTENTS

TABLE OF FIGURES

ABSTRACT

Chen, E., and Holmes, J.A. 2015. Manual of best practices for age determination of north Pacific Albacore Tuna. Can. Tech. Rep. Fish. Aquat. Sci. 3145: v + 28 p.

This manual describes the best practices currently available for the age determination of North Pacific Albacore Tuna. The described techniques and recommendations are the result of outcomes from the Pacific Bluefin Tuna and North Pacific Albacore Tuna Age Determination Workshop, held November 13-16, 2013, at the National Research Institute of Far Seas Fisheries in Shimizu, Shizuoka, Japan. We summarize some of the past tuna ageing studies done on various calcified structures and focus on methods involving tuna fin rays and otoliths, two of the more promising techniques when applied to North Pacific Albacore Tunas. We discuss the collection, preparation, and interpretation of the ageing structures, describe potential quality assurance measures for accuracy as well as precision, and propose some key tuna ageing protocol recommendations.

RÉSUMÉ

Chen, E. et Holmes, J.A. 2015. Manual of best practices for age determination of north Pacific Albacore Tuna Rapp. tech. can. sci. halieut. aquat. 3145: v + 28 p.

Le présent manuel décrit les pratiques exemplaires actuelles à adopter pour la détermination de l'âge du thon blanc du Pacifique Nord. Les techniques et recommandations qui y sont décrites résultent de l'atelier sur la détermination de l'âge du thon rouge du Pacifique et du thon blanc du Pacifique Nord, qui a eu lieu du 13 au 16 novembre 2013 à la National Research Institute of Far Seas Fisheries de Shimizu, Shizuoka au Japon. Nous résumons certaines des études antérieures de détermination de l'âge effectuées sur diverses structures calcifiées et mettons l'accent sur les méthodes impliquant les rayons de nageoire et les otolithes des thons blancs du Pacifique Nord. Nous discutons de la collecte, de la préparation et de l'interprétation des structures de détermination de l'âge, et décrivons les mesures possibles d'assurance de la qualité relativement à l'exactitude et la précision. Nous émettons des recommandations clés quant au protocole de détermination de l'âge du thon.

1.0 INTRODUCTION

Accurate and precise estimates of age and growth rates are essential parameters in understanding the dynamics of fish populations. Many contemporary stock assessment models require age and growth information to partition catch data by age. Interest in stock assessments of large oceanic pelagic fishes (tunas, billfishes, and sharks) has developed over the last three decades, during which exploitation has increased steadily in response to increases in worldwide demand for these resources. The rapidly growing international concern in managing large oceanic pelagic tunas in a sustainable manner, as well as the difficulties in ageing these species, prompted the production of this age determination manual.

The age of teleost fishes is traditionally estimated by counting growth bands on skeletal parts, size frequency analysis, tag and recapture studies, and raising known age fish in enclosures. However, problems related to determining the age of some highly migratory pelagic tunas are unique compared with other species. For example, representative sampling of fish for ageing structures, which is destructive, is difficult because of their size, value, and extensive movements and distribution throughout the oceans. In addition, movements of some oceanic pelagic fishes often transect temperate as well as tropical oceans, making interpretation of growth bands on skeletal parts more difficult than with more sedentary temperate species. Many oceanic pelagics are also long-lived, attaining ages in excess of 30 years, and their life cycles do not lend themselves to artificial propagation and culture. These factors contribute to the difficulty of determining ages and are generally characteristic of tunas, billfishes, and oceanic sharks.

Population size estimates, including recruitment and biomass levels, are highly sensitive to age and growth estimates used in assessment models. Although the growth models for North Pacific Albacore Tuna, *Thunnus alalunga* (NPALB), have improved, uncertainty related to accuracy and precision of age determination is an ongoing challenge for assessing these species. North Pacific albacore tuna ages have been estimated by many researchers using a variety of structures and techniques. Determining the age of a fish from growth structures (otoliths, fin rays, vertebrae, scales) is based on the assumption that growth increments occur at standard intervals and simultaneously within a cohort and that these increments continue to be formed, regardless of age. Compliance with these criteria: periodicity, synchronous and continuous development, is often uncertain in age determination studies of tunas and has contributed to uncertainty in the interpretation of the age results in stock assessments.

The Pacific Bluefin Tuna and North Pacific Albacore Tuna Age Determination Workshop (called the Workshop for here on) was developed in recognition that the Pacific Bluefin Tuna and North Pacific Albacore Tuna assessments shared common issues with respect to age and growth data and that common solutions might be available to address these uncertainties. The Workshp was sponsored by the International Scientific Committee for Tuna and Tuna-like Species in the North Pacfiic Ocean (ISC) and the Fisheries Research Agency of Japan and was held November 13-16, 2013, at the National Research Institute of Far Seas Fisheries in Shimizu, Shizuoka, Japan. The goals of the Workshop were to share information on age determination techniques among specialists and to develop standardized protocols for ageing methods in order to contribute to the development of more reliable growth models and improved stock assessments for both tuna species. Specific outcomes of the workshop include:

- Clarification of age determination issues for PBF and NPALB;

- Identification of practical techniques for ageing;
- Development of standardized protocols for ageing techniques where appropriate;
- Documentation of the issues, techniques and protocols in age determination manuals for PBF and NPALB; and
- Establishment of an age structure exchange process to facilitate inter-laboratory calibration of tuna age estimates.

This manual is a compendium of best practices for determining the age of north Pacific Albacore Tuna based on discussions and recommendations developed at the Workshop. We document a snap-shot of current techniques and best practices in an attempt to assist future researchers in producing reliable age and growth data for north Pacific Albacore Tuna. We expect that ongoing research on ageing methodologies will lead to the development of new approaches that will be documented in a future update of this manual. A companion manual of age determination techniques and best practices for Pacific Bluefin Tuna (Shimose and Ishihara 2015) represents a key output from the Workshop.

The objectives of this manual are to:

1. Provide guidance on standardized preparation and handling protocols for age structures to improve age determination in Albacore Tuna.
2. List the techniques, materials, and equipment necessary for large-scale studies on Albacore Tunas, and
3. Provide guidance on a standardized basis for interpretation of patterns on hard body parts for age determination.

2.0 BACKGROUND BIOLOGY

The North Pacific Albacore Tuna stock is valuable and has a long history of exploitation. The total reported catch peaked at 126,175 metric tonnes (t) in 1976 and then declined to the lowest observed catch of 37,274 t in 1991 (Fig. 1). Following this low point, total catch recovered to a second peak of 119,297 t by 1999 and then declined through the 2000s to a low of 63,654 t in 2005 and has increased slightly to between 65,000 and 92,000 t in recent years (2006-2012).

North Pacific Albacore Tuna exhibit complex movement patterns, particularly among juvenile fish (i.e., immature animals less than 5 years old and 85 cm FL), which generally inhabit surfacewaters (0-50 m) in temperate regions of the Pacific Ocean. Some juvenile albacore undertake trans-Pacific movements from west to the east and display seasonal movements between the eastern or western and central Pacific Ocean (Ichinokawa et al. 2008; Childers et al. 2011). Westward movements of juveniles tend to be more frequent than eastward movements (Ichinokawa et al. 2008), corresponding to the recruitment of juvenile fish into fisheries in the western and eastern Pacific Ocean and are followed by a gradual movement of maturing juveniles and mature (adult) fish to low latitude spawning grounds in the western and central Pacific Ocean. This pattern may be complicated by sex-related movements of large adult fish (> 125 cm fork length, FL), which are predominately male, to areas south of 20°N (ALBWG 2014), but the significance of these movements on the demography of this stock are uncertain.

Spawning occurs in tropical and sub-tropical waters between Hawaii (155°W) and the east coast of Taiwan and the Philippines (120°E) and from 10 to 25°N latitudes (Ueyanagi 1969; Chen et al. 2010). Spawning peaks between March-April in the western Pacific Ocean (Chen et al. 2010), but in the central Pacific Ocean much older and weaker evidence points to a probable peak spawning period between June and August (Ueyanagi 1957; Otsu and Uchida 1959a). Female albacore mature at lengths ranging from 83 cm fork length (FL) in the western Pacific Ocean (Chen et al. 2010) to 90 cm FL in the central Pacific Ocean (Ueyanagi 1957), and 93 cm FL north of Hawaii (Otsu and Uchida 1959a).

Growth is rapid in immature (juvenile) Albacore Tuna followed by a slowing of growth rates at maturity and through the adult period. Growth in the first year of life is uncertain since juvenile Albacore Tuna recruit into intensive surface fisheries in both the eastern and western Pacific Oceans at age 2. Albacore are ~ 60 cm FL at age 2 when they recruit into surface fisheries and grow at a rate of about 10 cm per year for ages 2-4. Growth slows after 5-6 years of age when albacore are mature (Clemens 1961; Otsu and Uchida 1959a; Chen et al. 2012; Wells et al. 2013). Chen et al. (2012) reported that growth was sexually dimorphic growth in adult north Pacific Albacore Tuna and reported that males attained a larger size and older age than females (114 cm FL and 14 years vs. 103.5 cm FL and 10 years, respectively). The maximum recorded size of a north Pacific albacore is 128 cm FL (Otsu and Uchida 1959a; Clemens 1961) and the oldest known age is 15 years (Wells et al. 2013).

3.0 CALCIFIED STRUCTURES FOR AGEING TUNAS

A variety of structures including otoliths, fin rays, vertebrae, various bones, and scales are used to determine the age of teleost fishes. Many of these structures have been used to estimate the age in Tunas. This section introduces each of these structures, describing their morphologies, and their locations on the fish.

The cyclical growth patterns which form in the hard body parts used for age determination of fish depend on regularly occurring external factors. When patterns of growth are regular and attributable to an environmental stimulus, then increment formation periodicity can be determined. Temperature is the most common environmental factor governing the formation of growth increments in species inhabiting temperate latitudes. A growth increment consists of a dense narrow zone formed during exposure to cool temperatures and a wider, less dense zone formed at higher temperatures. These zones produce alternating concentric narrow and wide zones on the structure and the combination of a narrow zone and wide zone is called an annulus if they are formed once during a year. Verification that formation of these zones occurs once per year, i.e., annually, is an important, but often neglected, component of age determination studies.

3.1 OTOLITHS

Otoliths are small, pale structures primarily composed of aggregated calcium carbonate, located in the heads of teleost fish. These tiny structures, often called “earstones”, function similarly as the inner ears do for humans, aiding in balance, orientation, as well as hearing. Otoliths are located at the posterior of the cranium, within the semi-circular canals of the inner ears (Fig. 2). The shapes and sizes of otoliths can vary greatly between species, from simple ellipsoids to highly complex patterns with species-specific protrusions and depressions (Fig. 3). Otoliths are formed by the deposition of calcium carbonate and protein structures, making them especially suitable for age estimation because of their continuous growth. Growth of the otolith begins at the primordium, which is deposited prior to hatching, and continues outward. Knowledge of the location of the primordium is important for the correct interpretation of the banding pattern on an otolith.

Teleost fish possess three pairs of otoliths: sagittae, lapilli and asterisci. Sagittae otoliths are usually used for age determination studies because of their large size compared to the other otolith pairs. Albacore Tuna, as well as Tunas in general, have small sagittal otoliths relative to their body size. The otoliths, which may grow to be around 1 cm in size, are delicate structures, and require gentle handling during extraction and preparation to reduce chipping and breakage.

Otolith morphology is similar in most teleosts, but complex. The rostrum is the anterior section of the sagittal otolith, while the postrostrum is the posterior area (Fig. 3). The distal or dorsal surface is smooth and rounded while the proximal or ventral surface is characterized by a a wide groove called the sulcus (Fig. 4). The primordium, the initial part of the otolith where growth first begins, is a blob of dense material located in the central core of the otolith. Growth of the otolith radiates outward from the primordium.

Several different age determination studies utilizing slightly different otolith preparation and interpretation techniques have been carried out, focusing on the North Pacific Albacore Tuna population. Laurs et al. (1985) confirmed that increments are formed on the sagittae of north Pacific Albacore Tuna at a rate of approximately 1 per day based on an examination of 116 albacore between about 50 and 100 cm fork length caught, injected with tetracycline, tagged, released, and subsequently recaptured in various fisheries. Wetherall et al. (1987) applied this technique to whole otoliths to estimate the age of small Albacore Tuna caught off California. Recent age and growth studies of Albacore Tuna have used sectioned otoliths for age determination. Chen et al. (2012) used oblique thin sections close to the primordium to age fish from the western Pacific Ocean and reported sexually dimorphic growth after maturity. Wells et al. (2013) used transverse otolith sections through the primordium to age albacore caught in the western, central, and eastern Pacific Oceans, but did not address the sexually dimorphic growth hypothesis. Another study conducted by Renck et al. (2014) compared transversely and obliquely sectioned otolith sections, and determined transverse sections to be more suitable for reading and counting annuli.

3.2 FIN RAYS

Albacore Tuna possess two separate dorsal fins, consisting of a larger anterior fin which can fold down into a slot along the dorsal midline and a smaller posterior fin (Fig. 5). The two anterior spines of the first dorsal fin are the longest, with the following spines forming a groove midway through the fin. The second dorsal fin is about as high as the first, but has a shorter base. The anal fin is located below the second dorsal fin, and is roughly the same size and shape as the second dorsal fin. The long pectoral fins curve slightly downward, extending beyond the anterior of the anal fin. Fin rays from the second dorsal fin along with the anal fin were considered suitable for age readings by Beamish (1981), while Wells et al. (2013) studied the first dorsal fin spine in north Pacific Albacore Tuna. Otsu and Uchida (1959b) reported that the fin spines of Albacore Tuna caught near Hawaii had no usable marks for ageing, although their examination was cursory at best. The first ray from the first dorsal fin of Mediterranean Albacore Tuna was studied by Megalofonou (2000), and the first and second dorsal spines of Atlantic Albacore Tuna were studied by Santiago and Arrizabalaga (2005) and it was reported in both studies that spines are harder to interpret with increasing age due to the occurrences of resorption and vascularization obscuring the spine core (Megalofonou 2000; Santiago and Arrizabalaga 2005).

Wells et al. (2013) found that dorsal fin spines were most useful for estimating age of fish up to about 3 years of age, after which precision was low. They concluded that fin spines should not be the primary structure used to estimate absolute age, but could supplement ageing with other structures, particularly in young juvenile fish.

3.3 SCALES

Albacore scales are small, cycloid in shape, and have circuli which form rings around the scale origin. When a scale is lost, a new one is quickly grown in its place. However, these regenerated scales lack circuli near the centre and should not be used for age studies. Scale loss and regeneration are common on parts of the body subject to constant friction with hard substrates or other fishes (e.g., below the lateral line) and thus the sampling of scales for ageing studies tends to be restricted to dorsal areas where scale loss is less common.

Scales were the structure of choice for some of the earliest attempts to estimate the absolute age of Albacore Tuna. Otsu and Uchida (1959b) sampled scales from just below the second dorsal fin of Albacore Tuna caught near Hawaii and found that only 57% of the scales examined (N = 100) were readable and that the precision of counts within and between readers was low. Huang et al. (1990) estimated the age of Indian Ocean Albacore Tuna (N = 227) from scales sampled between the lateral line and the 6th/7th anal finlet and reported that 88% were readable and that precision of the readings declined with age of the animal. Given the low precision reported for age estimation with scales, this approach has not been pursued further.

3.4 BONES

The vertebral column in Albacore Tuna consists of 38 amphicoelous vertebrae, meaning that both the anterior and posterior ends of the centra are concave, as with most bony fishes. Alternating translucent and opaque rings are present on the inner centrum surface, while on the outer surface, the rings can be seen as ridges or valleys. In a given fish, the same number of rings may be observed in all of the vertebrae. Albacore Tuna vertebrae have been used for age estimation (Fig. 6), with various studies using different vertebral sections for age readings. Partlo (1955) was among the first to report that the absolute age of Albacore Tuna could be estimated from concentric banding patterns on the centrum of a vertebra. He used the 9th abdominal vertebra and found that the relationship between fish length and number of rings on a vertebrae was linear, although he noted that the first ring was often unclear leading to uncertainty in the assigned ages. A linear relationship between fish length and ring radii on the the 20th vertebra was also reported for 391 Indian Ocean Albacore Tuna (Lee and Liu 1992). Labelle et al. (1993) extracted the 35th and 36th caudal vertebrae from 494 south Pacific Albacore Tuna and used ring counts to estimate the age of fish ranging from 2-13 years. However, these authors assumed that the vertebral rings are annual features. In contrast, Otsu and Uchida (1959b) found that they could not successfully age 265 north Pacific Albacore Tuna using vertebrae because there was low precision in ring counts between different readers and in successive ring counts by the same reader. These authors speculate that the rings on the vertebrae that they were counting may be growth marks laid down randomly over time, i.e., they were not annual features.

Other calcified skeletal structures also have been used to estimate absolute age in teleost species (Faust 2013). For example, the opercula are commonly used to age yellow perch *Perca flavescens* (Baker and McComish 1998), cleithra are used to age northern pike *Esox lucius* (Casselman 1983), and clavicles are used to age white sturgeon *Acipenser transmontanus*

(Brennan and Cailliet 1989). However, Tuna age estimation studies using skeletal components have primarily used vertebrae, probably because they can be extracted fairly easily and their removal does not affect the value of the fish.

3.5 SUMMARY

Participants at the Workshop, noted that otoliths and fin rays are the most commonly investigated hard parts in Tuna age and growth studies, including those on Albacore and Pacific Bluefin Tuna. Vertebrae and scales proved to be unsuitable choices for estimating age of Albacore Tuna owing to low precision of the ring counts and the absence of formal validation of the rings as annual features on either structure. Fin ray sections have been shown to be acceptable age indicators for young tunas, up to about 3 years of age. As the fish age, their spine cross sections become increasingly difficult to interpret consistently, due to the vascularization and resorption occuring at the spine core, resulting in bias and lower precision in the resulting age estimates. Consequently, otoliths are widely viewed as the most reliable structure for ageing Tunas across the range of ages expected when sampling a stock, which is attributed to their continuous growth, and to their reduced susceptibility to vascularization and resorption. Fin rays can be used to assist age estimation using otoliths because the early annuli can be quite distinct. Farley et al. (2013) conducted a validation experiment to show that bands on otoliths from south Pacific Albacore Tuna are annual features, i.e, only one is formed per year of growth and that estimated ages are accurate. There are no formal validation experiments on other structures used to age Albacore Tuna historically. Although there is confidence among practioners that the south Pacific Albacore Tuna validation conclusions (Farley et al. 2013) apply to other Albacore stocks and tuna species, caution is warranted when interpreting these age estimates until the accuracy of the procedure is verified.

4.0 FIN RAYS

4.1 COLLECTION AND STORAGE

The first spine of the first dorsal fin (Fig. 7) is removed by cutting the membrane between the first and second fin rays. The first spine is pushed forward and twisted, alternating between the left and right directions until the ligament breaks. It is important to ensure the entire base of the spine is extracted because this is the portion of the spine used for ageing. Failure to extract the base may lead to biased estimates of age as some annuli, particularly the first annulus, may not be detectable in cross-sections higher up on the spine.

Tissue that adheres to the extracted spine should be removed and the spines air-dried prior to storage. After drying, spines can be stored in labelled paper envelopes, in a cool dry place. If the spine is broken in half, it may still be used provided the section near the bottom of the base remains, since the base is used for age readings.

4.2 PREPARATION FOR AGE DETERMINATION

A standardised methodology for sectioning and preparing fin rays for age determination was described by Rodríguez-Marín et al. (2007) for Atlantic Bluefin Tuna and was utilised by Farley et al. (2013) on south Pacific Albacore Tuna. When using fin rays/spines for age determination, it is important to clearly define the position of section cut on the base of the spine for all samples. First, the diameter of the spine just above the spine base, directly above the hollows is measured and the cut axis is marked above the measuring line, at half the length of the measured diameter (Fig. 8). A low speed diamond saw blade should be used to obtain several serial cross sections (consecutive cross-sections) 0.5 mm thick. These serial cross-sections should be mounted on a labelled slide (one slide per fish) using a mounting resin such as Eukitt.

4.3 INTERPRETATING FIN RAY PATTERNS

Banding patterns on fin rays/spines are best viewed with a compound microscope and transmitted light at a magnification appropriate for the reader. A growth increment (annulus) on any hard structure including fin rays/spines consists of a fast growth zone (wide) and a slow growth zone (narrow), which appear as translucent and opaque areas when transmitted light is used for viewing. Since the combination of one translucent and one opaque zone forms one annulus (and this is confirmed by appropriate validation studies), then it is recommended that counts of bands for age estimation focus on the opaque zone and use the distal edge (relative to the structure center) as the counting edge. As the fish age, growth zones at the centre of spine sections may be obscured by resorption, so the diameters of opaque zones in cross sections obtained from younger fish may be used to assign a more suitable age for the first observed opaque band in spines of larger fish (Fig. 9).

Single, double, or triple translucent zones separated by narrow opaque zones may be observed and are called check marks. Check marks are visible discontinuities formed in a growth structure as a result life history transitions (e.g., hatching), physiological events, extreme thermal events, or habitat changes affecting growth of the structure. When counting, multiple translucent zones should be counted as part of the same zone, if the distance between them is less than the distance to the previous and following translucent zones, and the multiple translucent zones converge at the spine vertex (Farley et al. 2013). Count the growth zones starting from the core to the outer edge of the cross section, following a perpendicular path to the growth increments. Using the same counting axis on all structures examined is an important quality control procedure for ensuring high precision within and between readers. If the counts are made off of images, then it is useful to mark the counting axis on the images.

5.0 OTOLITHS

5.1 COLLECTION AND STORAGE

The otoliths are extracted by cutting the head open either above the eyes, in the frontal plane, or directly behind the eyes, along the transverse plane (Fig 2). The semi-circular canals containing the otoliths are located in the ventral section of the cranial cavity and may require removal of the brain to see them fully. The otoliths are carefully removed from the semi-circular canals with fine forceps. The sagittal otolith is the largest of the three otoliths and is commonly used for age determination.

A drilling procedure was developed to extract otoliths from Southern Bluefin Tuna (*Thunnus maccoyii*) when standard extraction procedures cannot be used because they reduce the value of the fish (Anonymous 2002). This procedure has not been tested on Albacore Tuna, but could be attempted if the normal sampling protocols is not possible.

Each pair of sagittal otoliths should be cleaned using distilled water to remove membrane tissue adhering to them, air-dried and stored separately in labelled paper envelopes or plastic capsules. It is good practice to consistently use either the left or right sagittal otolith for age determination, retaining the other otolith for other types of studies such as microchemistry assessment of natal areas.

5.2 PREPARATION FOR AGE DETERMINATION

5.2.1 Surface Reading

Whole otoliths may be read intact, without any sectioning (Fig. 10). Because of their size, whole otoliths are usually examined with a dissecting microscope and reflected light. Opaque zones will appear as bright white bands around the surface of the otolith separated by darker translucent zones under reflected light. Surface reading is useful in establishing preliminary estimates of age, but it is not always possible to apply this technique. Select one from each sagittal pair to be read whole, while the other otolith may be read with the thin sectioning technique.

5.2.2 Thin Sectioning

Thin-sectioning of otoliths embedded in epoxy resin is the most common preparation technique applied to teleost otoliths. Each clean and dry otolith is embedded in epoxy resin in a silicone mold, and left to harden for 24 hours prior to sectioning. The core of each otolith (primordium), is lightly marked with a pencil on the resin block prior to sectioning and the first section should always be through the primordium (Fig. 11), to ensure the first inflection point is captured. Thin sections are cut with a high-speed saw at a 90° angle to the longest axis of the embedded otoliths. Serial transverse thin sections 0.3-0.5 mm thick on either side of the primordium are recommended to obtain the best readings, especially at a thickness of 0.4 mm. All serial sections should be mounted on the same slide and the first section with the primordium should be noted on the slide.

Once the thin sections are mounted on glass slides with a mounting medium, the surface is polished with aluminum powder or coated with epoxy embedding resin to obtain smooth surfaces for reading. The polishing procedure may also enhance the contrast between opaque and translucent zones.

5.2.3 Baking and Burning

Burning and baking procedures have been developed to enhance the banding patterns on otoliths used for age estimation in teleosts. These techniques have been shown to improve precision in ageing studies of other valuable marine species such as Pacific Halibut, *Hippoglossus stenolepis* (Chilton and Beamish 1982), but their value to Tuna age estimation is unclear at present.

In order to apply these procedures, the otolith is bisected transversely, held in place with a plasticene plug so that the cross sections are exposed. The sections can then be burned or baked in order to accentuate growth patterns.

Burning involves holding the otolith cross section over an alcohol flame until it is evenly dark brown. Once burning is complete, the otolith section is mounted in plasticene and the exposed cross section is painted with mineral oil to enhance the otolith growth patterns. Alternatively, the bisected otoliths can be baked at 400°C in a muffle furnace for five minutes to enhance banding patterns.

5.3 INTERPRETATING OTOLITH PATTERNS

A complete yearly growth increment (annulus) consists of both a slow and fast growth zone that are opaque and translucent, respectively, in transmitted light. Counts for ageing can be made to the distal edge (relative to the primordium of the otolith) of either zone, but whatever choice is made, it must be used consistently for all ageing. It is recommended that counts be made at the distal edge of the opaque zone.

Both dissecting and compound microscopes may be used to count annual otolith growth patterns, or annuli, using transmitted light (Fig.12). Burnt or baked otolith sections can be read with reflected light. An annulus is composed of a translucent zone formed during fast growth, along with an opaque zone of slow growth, with the translucent zone generally covering a wider area. Using transmitted light, translucent zones appear light, and opaque zones appear dark. Under reflected light against a black background, translucent zones appear dark, and opaque zones appear light. The zone edges furthest from the primordium of either the opaque or the translucent zones may be used to count annuli, as long as what is being counted is consistent throughout.

The location of first annulus is not always apparent on Tuna otoliths. Failure to establish the location and count the first annulus will bias the resulting age estimates. Workshop participants recommended that age readers estimate the average length from the primordium or inflection point on the ventral arm of the otolith to the distal edge of the first annulus on otoliths with a clear first annulus and use these measurements to establish the first annulus location when it is not readily detectable on other otoliths (Fig. 13). It was also noted that these measurements may need to be used for the first two annuli in some cases. Counting daily growth rings was also suggested as a method for establishing the location of the distal edge of the first annulus (365 growth rings per year), but it was recognized that it may be possible only in rare cases.

Otoliths should be read at least two times by the same reader without reference to the previous reading, size of fish or capture date in order to assess confidence in the reading. If the two readings are in agreement, then this estimate can be used as the final count for the otolith. If the readings differ, then a third reading should be made to decide on a final count and confidence score. If no obvious pattern could be seen in the otolith section, a confidence score of 0 should be assigned to the sample and a count not recorded.

Fin rays may be a useful cross-check when estimating age using otoliths because the first 2-3 annuli are often readily detectable in properly prepared fin-ray cross-sections.

Check marks may be distinguished from actual annuli by studying the relative spacing between the annuli. Additionally, the edges on either sides of the counting plane may be examined for crenulations, which may indicate the presence of an annulus in some cases.

Marginal increment interpretation is important for the correct assignment of age. The slow growth opaque zone usually forms on an otolith in the winter months. The terminal or marginal edge of each otolith should be inspected and whether the last opaque zone is “wide”, “narrow”, or possesses a translucent edge beyond it should be recorded. The first observation (a wide opaque zone) means that a full year of growth has occurred while the latter observation (translucent edge) indicates that the new year of growth has begun.

A counting axis should be established for estimating age. Counts on tuna otoliths are made along the long ventral arm of the otolith (Fig. 13), and switch between the sulcal and distal edges where the banding pattern is clearest as necessary.

6.0 QUALITY ASSURANCE

6.1 ACCURACY

Validation of an ageing technique on North Pacific Albacore Tuna is a necessary but often neglected step, to establish the accuracy of the technique. The technique should be validated for all ages in the population being studied, considering that any extrapolation beyond the maximum validated age is unreliable (Beamish and McFarlane 1983). Successful validation demonstrates that only one growth increment or annulus (consisting of fast and slow growth zones) is formed per year.

Direct methods are the best approach to validate age estimation techniques using hard parts of teleost fish. The most common method of directly validating an ageing technique is the application of oxytetracycline (OTC) marker (Laurs et al. 1985; Farley et al. 2013). OTC is preferentially deposited in bones at the growing surface and fluoresces when exposed to ultra violet light at a wavelength of about 360-400 nm. A 200 mg/mL OTC solution is commonly injected into the muscle tissue of captured fish using volumes in the range of 25-50 mg/kg of body weight (Farley et al. 2013). OTC-injected fish should be tagged using conventional plastic dart tags prior to their release.

Once the tagged fish are recaptured, the otoliths or other hard body part are extracted and examined for OTC marks, using ultraviolet light. The location of the OTC mark and the number of presumptive annuli after the mark are compared to the amount of time that the fish was at liberty between injection and recapture to establish the validity of the ageing technique. It is important that the initial detection of the OTC mark and the number of bands after the mark be made without knowledge of the time the fish was at liberty.

Indirect methods may aid in validating age determination methods and are sometimes used when direct validation studies are not possible. These methods include counting daily rings (or microincrements), and cohort analyses following size frequency modes in successive years.

Daily rings may be viewed by polishing thin sections to the extent where microincrements may be seen along the counting path. Counting daily rings may help to validate annulus counts, by counting approximately 365 microincrements between consecutive annuli. Laurs et al. (1985) reported that daily increments formed on the sagittal otoliths of North Pacific Albacore Tuna between 51 and 97 cm FL at an average rate of 0.954 increments per day, thus confirming the occurrence of daily increments. The observed deviation from one increment per day could be caused by intermittent interruptions in growth as a result of events such as starvation, environmental conditions, and other factors.

Size frequency analysis involves measuring the fork lengths of fish caught over a period of several years to the nearest centimeter, and assuming that the successive size-frequency modes are consistent with successive year classes in the population sampled. Compiling size-frequency histograms through the years will show the progression of the modes, and approximate sizes for each year class may be determined.

6.2 PRECISION

Precision is a measure of the reproducibility of the results obtained from the applied ageing technique. Both accuracy and precision are important characteristics of an ageing technique, and should be demonstrated in any study. Accuracy is established using a validation technique while precision is estimated statistically. Two types of precision can be estimated: (1) precision among age readers within a lab, and (2) precision in age readings on the same structure between labs. In this section, we discuss the former (precision among age readers within a lab. Precision between labs is addressed using reference collections (see Section 5.3 below).

All structures should be read by a single reader at least twice, with the second reading occurring at least a week after the first, and without knowledge of the results from the previous reading, the size of the fish, or i.e., blindly. A second reader should conduct an audit on a randomly selected sample of completed otolith readings to estimate a final opaque zone count and edge classification, without knowledge of any of the results obtained by the first reader. If the counts of the second reader differ from the first reader, then the differences should be discussed by both readers and a resolved count recorded for that fish. The magnitude of difference that triggers discussions leading to a resolved age depends on the life history of the fish: a difference of 1-2 years may be significant in fish whose longevity is estimated to be 10-15 years whereas a difference of less than 5-10 years may not be significant for fish with longevities of 50 years or more.

The precision of age readings is commonly estimated with two types of statistics: coefficient of variation (CV) and average percent error (APE). The APE (Beamish and Fournier 1981) measures the average precision over all N events in a data set and is used in fish-ageing literature to compare the precision of different ageing methods, observers, or ageing of different species. The APE is calculated as

$$APE = \frac{1}{N_A} \sum_{j=1}^{N_A} \left(\frac{1}{R} \sum_{i=1}^{R} \left| \frac{X_{ij} - \bar{X}_j}{\bar{X}_j} \right| \right) x\ 100,$$

where N_A is the number of fish aged, R is the number of readings of each fish, X_{ij} is the *i*th reading of the *j*th fish, and $\bar{X}_j$ is the average count of the *j*th fish. The CV is an alternative metric used to estimate the precision of multiple counts from one observer or counts from multiple observers for the *jth* fish and is calculated as

$$CV = \sqrt{\frac{\sum_{i=1}^{R} (X_{ij} - \bar{X}_j)^2}{\bar{X}_j^{\,2}}} \quad X \quad 100$$

where the terms are as described for the APE. The estimated CV requires at least three observations of the *jth* fish to be a meaningful measure.

7.0 TUNA AGE PROTOCOL RECOMMENDATIONS

7.1 BIRTHDATE

Absolute estimates of age are based on an assumed birthdate. A birthdate of January 1st is often used for fishes in northern temperate waters as it often corresponds to the formation of the opaque, slow growth zone on otoliths. Using a birthdate corresponding to the middle or peak of the spawning season is common in tunas. For example, Wells et al. (2013) assigned ages to North Pacific Albacore based on a birthdate of May, which corresponds to peak spawning (Chen et al. 2010). In contrast, Farley et al. (2013) assumed a birth date of 1 December for south Pacific Albacore Tuna because this date is in the middle of the spawning season. The choice of birthdate should be clearly identified and consistently applied when absolute ages are estimated.

7.2 FIRST ANNULUS LOCATION

Workshop participants strongly recommended that researchers compile measurements identifying the location of at least the first annulus (distal edge of the opaque zone) on tuna otoliths. This recommendation is based on experience showing that first annulus is not clear on the majority of otoliths examined. These measurements can be made from otoliths in which the first annulus is clear and in some cases can be estimated through microincrement (daily) counts. It was also noted that properly extracted and prepared fin rays can be used to assist researchers in establishing the location of the first annulus, at least in relatively young fish.

7.3 REFERENCE COLLECTIONS

A collection of known-age reference structures should be established where possible for quality control purposes. A reference collection of known age structures has two primary purposes: (1) as a quality assurance measure to assess age readers for changes in banding pattern interpretation over time (i.e., drift), and (2) as a tool for inter-laboratory calibration to identify laboratory specific biases that can be used to adjust age data if necessary. Reference collections also have value as a training tool for new age readers, ensuring consistency in annulus pattern interpretation within and between labs (i.e., precision).

7.4 INTER-LAB CALIBRATION

Workshop participants noted that inter-laboratory calibration of age determination is important when more than one laboratory is providing age estimates for a particular species or stock. An inter-laboratory calibration is conducted by having each laboratory estimate ages based on the same set of structures (e.g., otoliths) without reference to the results from other laboratories or the size of fish from which the structures were sampled. Inter-laboratory calibration is used to assess the nature and magnitude of bias among laboratories and develop corrections for those biases and to ensure high precision of age data, regardless of the laboratory providing the data. Two types of bias may be present: constant and systematic. Corrections for constant biases may be as simple as all laboratories adopting the same birthdate for assigning ages. Correcting for systematic bias is more complicated and may be related to the absence of technique validation, reader drift, or differing pattern interpretations with age.

8.0 LITERATURE CITED

Anonymous. 2002. A manual for age determination of southern bluefin tuna *Thunnus maccoyii*. Otolith sampling, preparation and interpretation. The Direct Age Estimation Workshop of the CCSBT 11–14 June, 2002, Queenscliff, Australia. 1–36 pp.

Baker, E.A., and McComish, T.S. 1998. Precision of ages determined from scales and opercles for yellow perch *Perca flavescens*. J. Great Lakes Res. *24*: 658-665.

Beamish, R.J. 1981. Use of fin-ray sections to age walleye pollock, Pacific cod, and albacore, and the importance of this method. Trans. Am. Fish. Soc. 110: 287-299.

Clemens, H.B. 1961. The migration, age, and growth of Pacific albacore (*Thunnus germo*), 1951-1958. Calif. Dept. Fish Game Fish. Bull. 115: 1-128.

Beamish, R.J., and Fournier, D.A. 1981. A method for comparing the precision of a set of age determinations. Can. J. Fish. Aquat. Sci. *38*: 982-983.

Beamish, R.J., and McFarlane, G.A. 1983. The forgotten requirement for age validation in fisheries biology. Trans. Am. Fish. Soc. 112: 735-743.

Brennan, J.S., and Cailliet, G.M. 1989. Comparative age-determination techniques for white sturgeon in California. Trans. Am. Fish. Soc. 118: 296-310.

Casselman, J.M. 1983. Age and growth assessment of fish from their calcified structures- techniques and tools. NOAA Tech. Rep. NMFS 8: 1-17.

Chen, K.-S., Crone, P.R., and Hsu, C.-C. 2010. Reproductive biology of albacore *Thunnus alalunga*. J. Fish Biol. 77: 119–136. doi:10.1111/j.1095-8649.2010.02662.x

Chen, K.-S., Shimose, T., Tanabe, T., Chen, C.-Y., and Hsu, C.-C. 2012. Age and growth of albacore *Thunnus alalunga* in the North Pacific Ocean. J. Fish Biol. 80: 2328–2344. DOI: 10.1111/j.1095-8649.2012.03292.x

Childers, J., Snyder, S., and Kohin, S. 2011. Migration and behavior of juvenile North Pacific albacore (*Thunnus alalunga*). Fish. Oceanogr. 20: 157–173. doi:10.1111/j.1365-2419.2011.00575.x

Chilton, D.E., and Beamish, R.J. 1982. Age determination methods for fishes studied by the groundfish program at the Pacific Biological Station. Can. Spec. Publ. Fish. Aquat. Sci. 60: 102 p.

Compeán-Jiménez, G. 1980. Comparaison de techniques de détermination de l´age chez les principals espéces de thonidés atlantiques (Ph.D. thesis). Univ. Aix Marseille II, 153 p. (as used in Ruiz et al. 2005).

Farley, J.H., Williams, A.J., Clear, N.P., Davies, C.R., and Nicol, S.J. 2013. Age estimation and validation for South Pacific albacore *Thunnus alalunga*. J. Fish. Biol. 82: 1523–1544.

Faust, M.D., Breeggemann, J.J., Bahr, S., and Graeb, B.D. 2013. Precision and bias of cleithra and sagittal otoliths used to estimate ages of northern pike. J. Fish Wildl. Manage. 4: 332-341.

Hart, J.L., and Clemens, W.A. 1973. Pacific fishes of Canada. Bull. Fish. Res. Board Can. 180. 740p.

Huang, C.-S., Wu, C.-L., Kuo, C.-L., and Su, W.-C. 1990. Age and growth of the Indian Ocean albacore. FAO/UNDP Indo-Pacific Tuna Development and Management Programme, Colombo (Sri Lanka), (TWS/90/53).

Ichinokawa, M., Coan, Jr., A.L., and Takeuchi, Y. 2008. Transoceanic migration rates of young North Pacific albacore, *Thunnus alalunga*, from conventional tagging data. Can. J. Fish. Aquat. Sci. 65: 1681–1691.

Labelle, M., Hampton, J., Bailey, K., Murray, T., Fournier, D.A., and Sibert, J.R. 1993. Determination of age and growth of south Pacific albacore (*Thunnus alalunga*) using three methodologies. Fish. Bull. 91: 649-663.

Laurs, R.M., Nishimoto, R., and Wetherall, J.A. 1985. Frequency of increment formation on sagittae of North Pacific albacore (*Thunnus alalunga*). Can. I. Fish. Aquat. Sci. 42: 1552-1555.

Lee, Y.-C., and Liu, H.-C. 1992. Age determination, by vertebrae reading, in Indian albacore, *Thunnus alalunga* (Bonneterre). J. Fish. Soc. Taiwan 19: 89-102.

Megalofonou, P. 2000. Age and growth of Mediterranean albacore. J. Fish Biol. 57: 700-715.

Otsu, T., and Uchida, R.N. 1959a. Sexual maturity and spawning of albacore in the Pacific Ocean. Fish. Bull. 50(148): 287-305.

Otsu, T., and Uchida, R.N. 1959b. Study of age determination by hard parts of albacore from central north Pacific and Hawaiian waters. Fish. Bull. 59(150): 353-363.

Partlo, J.M. 1955. Distribution, age and growth of eastern Pacific albacore (*Thunnus alalunga* Gmelin). Journal of the Fisheries Board of Canada, 12: 35-60.

Rees, A.J., Gunn, J.S. and Clear, N.P. 1996. Age determination of juvenile southern bluefin tuna, *Thunnus maccoyii*, based on scanning electron microscopy of otolith microincrements. CCSBT/SC/96/8 (Appendix). Second Meeting of the Commission for the Conservation of Southern Bluefin Tuna (CCSBT), 26 Aug-5 Sep 1996, CSIRO Marine Laboratories, Hobart, Tasmania. 122 pp. (as used in Anonymous 2002).

Renck, C.L., Talley, D.M., Wells, R.D., and Dewar, H. 2014. Regional growth patterns of juvenile albacore (*Thunnus alalunga*) in the eastern North Pacific. Cal. Coop. Ocean. Fish. Invest. Rep. 55: 135-143.

Rodríguez-Marín, E., Clear, N., Cort, J.L., Megalofonou, P., Neilson, J.D., Neves dos Santos, M., Olafsdottir, D., Rodriguez-Cabello, C., Ruiz, M. and Valeiras, J. 2007. Report of the 2006 ICCAT workshop for bluefin tuna direct ageing. ICCAT Col. Vol.Sci. Pap. 60: 1349-1392.

Ruiz, M., Rodríguez-Marín, E., and Landa, J. 2005. Protocol for sampling of hard parts for bluefin tuna (*Thunnus thynnus*) growth studies. Report of the bluefin tuna direct ageing network (under the BYP framework). Collective Volume of Scientific Papers ICCAT, 58(4): 1403-1419.

Santiago, J., and Arrizabalaga, H. (2005). An integrated growth study for North Atlantic albacore (*Thunnus alalunga* Bonn. 1788). ICES Journal of Marine Science: Journal du Conseil, 62: 740-749.

Secor, D. H., Laban, E. H., and Dean, J.M. (1991). Manual for otolith removal and preparation for microstructural examination. Technical Publication No. 1991-01. *Belle W. Baruch Institute for Marine Biology and Coastal Research, Columbia, SC*.

Shimose, T., and Ishihara, T. 2015. A manual for age determination of Pacific bluefin tuna Thunnus orientalis. Bull. Fish. Res. Agency, 40: 1-11.

Ueyanagi, S. 1969. Observations on the distribution of tuna larvae in the Indo-Pacific Ocean with emphasis on the delineation of the spawning areas of albacore, Thunnus alalunga. Bull. Far Seas Fish. Res. Lab. 2: 177–256.

Ueyanagi, S. 1957. Spawning of the albacore in the Western Pacific. Rep. Nankai Reg. Fish. Res. Lab. 6: 113-124.

Wells, R. D., Kohin, S., Teo, S. L., Snodgrass, O. E., and Uosaki, K. (2013). Age and growth of North Pacific albacore (*Thunnus alalunga*): Implications for stock assessment. *Fisheries Research*, *147*, 55-62.

Wetherall, J.A., Laurs, R.M., Nishimoto, R.N, and Yong, M.Y.Y. 1987. Growth variations and stock structure in north Pacific albacore. 10th North Pacific Albacore Workshop Working Paper, 11-13 August 1987, Nat. Res. Inst. Far Seas Fish., Shimizu, Japan, 21 p.

9.0 GLOSSARY OF TERMS

Accuracy—The degree to which a measured or computed result is close to its true value.

Annulus— Concentric marking present in series on different hard parts such as fish otoliths and vertebrae, which may be counted to interpret the age of an organism (**plural: annuli**).

Check marks—Markings which appear similar to annuli, but which are actually formed as a result of stress-related factors, unrelated to yearly occurrences.

Inflection point— The point where the curvature direction of growth increments changes.

Microincrements— Very narrow increments believed to be deposited daily.

Precision—The degree to which a measured or computed result can be replicated consistently.

Primordium—The central area of the otolith that was first formed.

Validation—The process of ensuring the accuracy of an applied technique. In the context of age estimation studies, it involves showing that the counted annuli are truly formed once a year.

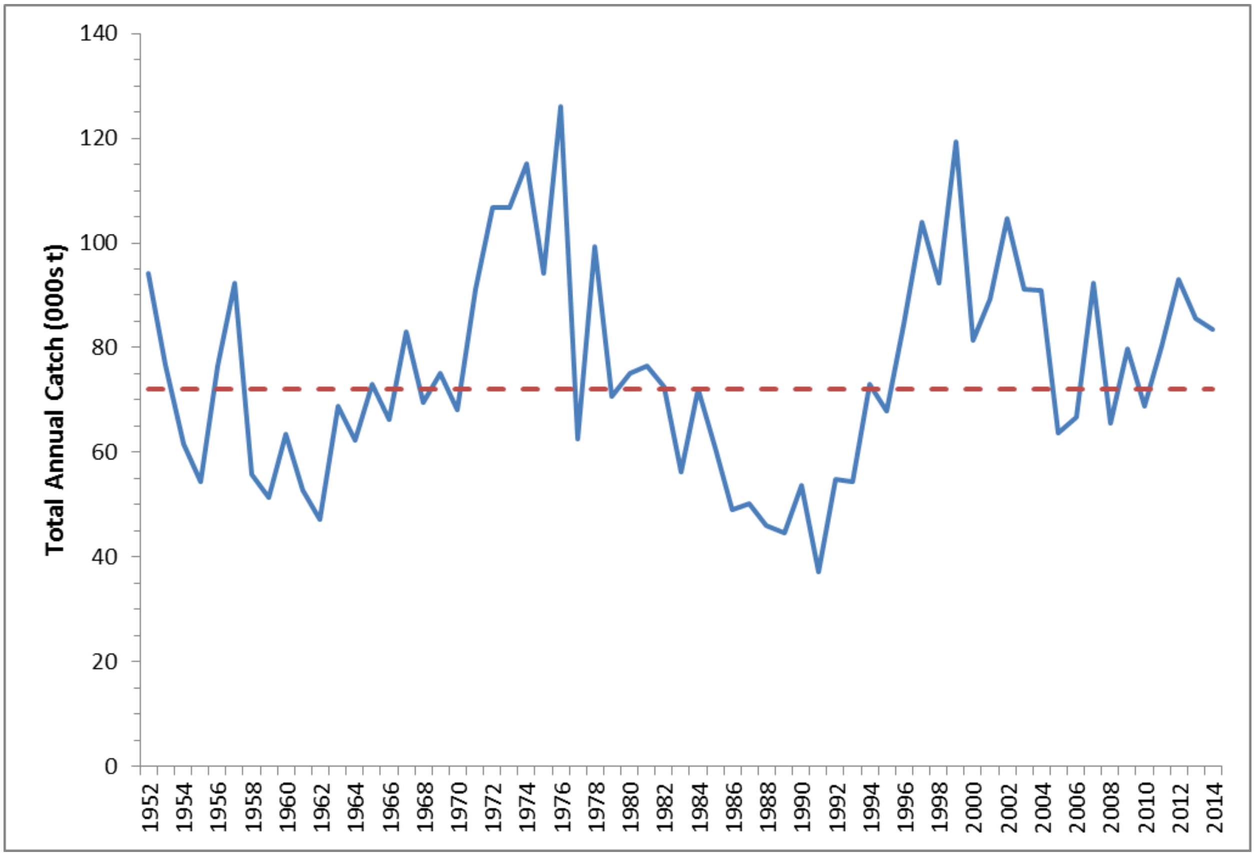

Figure 1. Total catch of north Pacific albacore from all sources, 1952-2014. The red dashed line is the 30-year average (1981-2010) of 72,128 t.

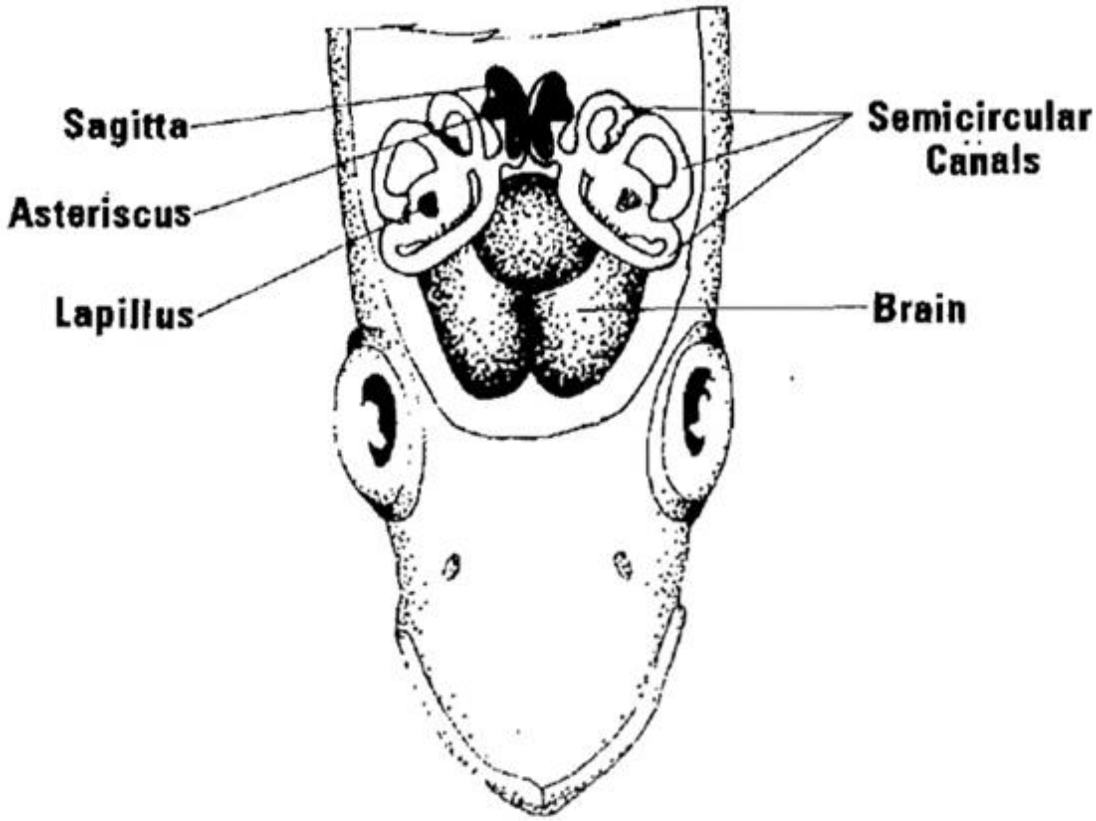

Figure 2. The head of a typical teleost fish, with the top cut open to reveal the semicircular canals and the otoliths in relation to the brain (from Secor et al. 1991).

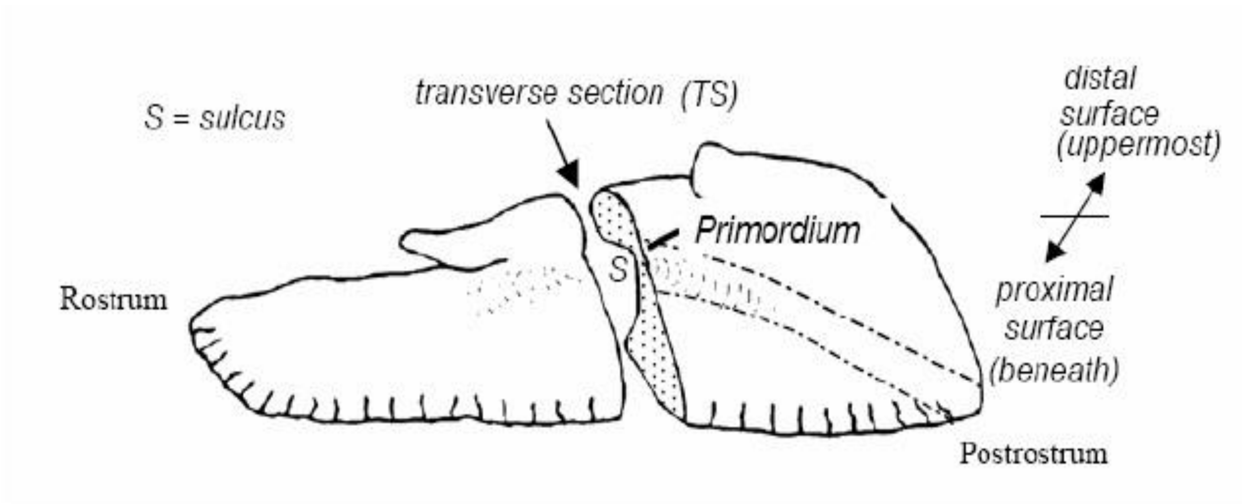

Figure 3. The left sagittal otolith of a southern bluefin tuna, with a transverse cross-section shown (adapted from Rees et al. 1996 and taken from Anonymous 2002).

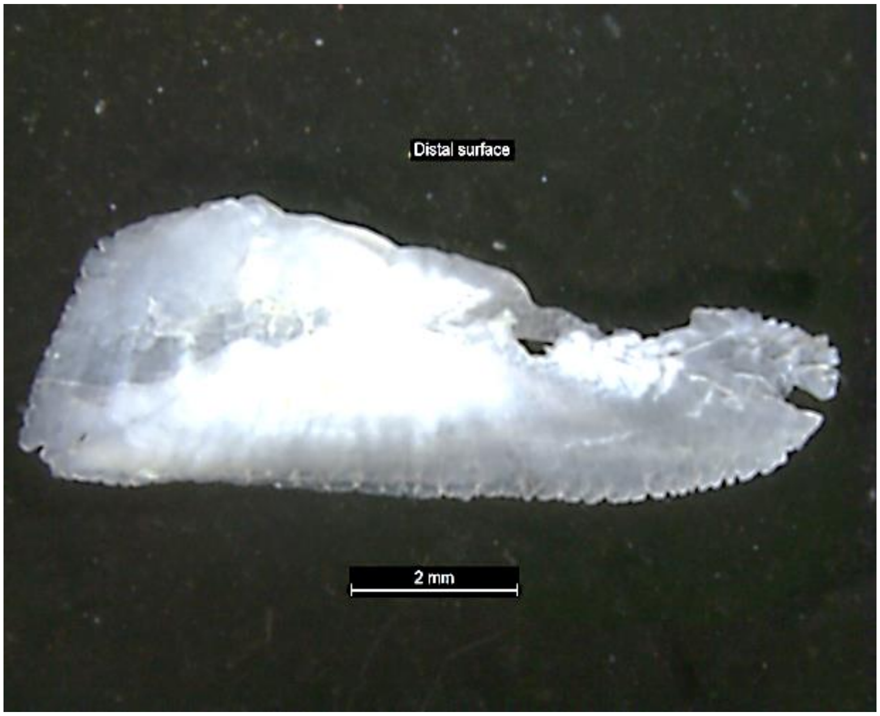

Figure 4. Distal (top) and proximal (bottom) views of an Albacore Tuna otolith. The sulcal groove is visible from the left to center of the bottom view and the primordium is visible as the dense white material in both views. Images from Darlene Gillespie, Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, Canada.

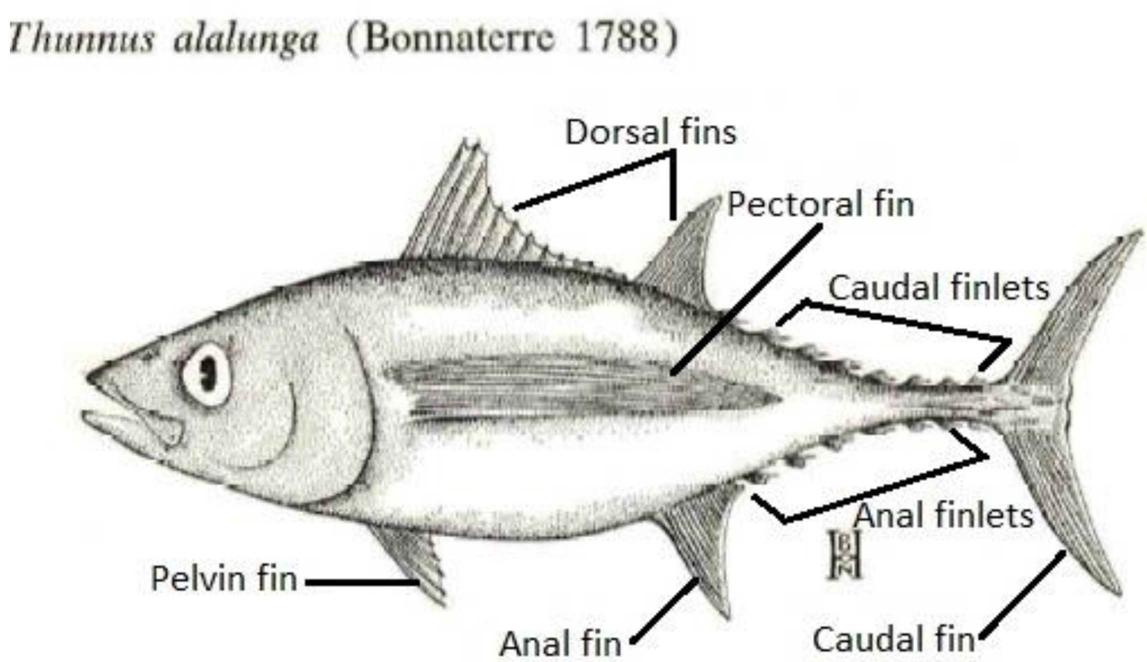

Figure 5. Drawing of an Albacore Tuna (*Thunnus alalunga)*, showing relative fin ray sizes and locations (adapted from Hart 1973).

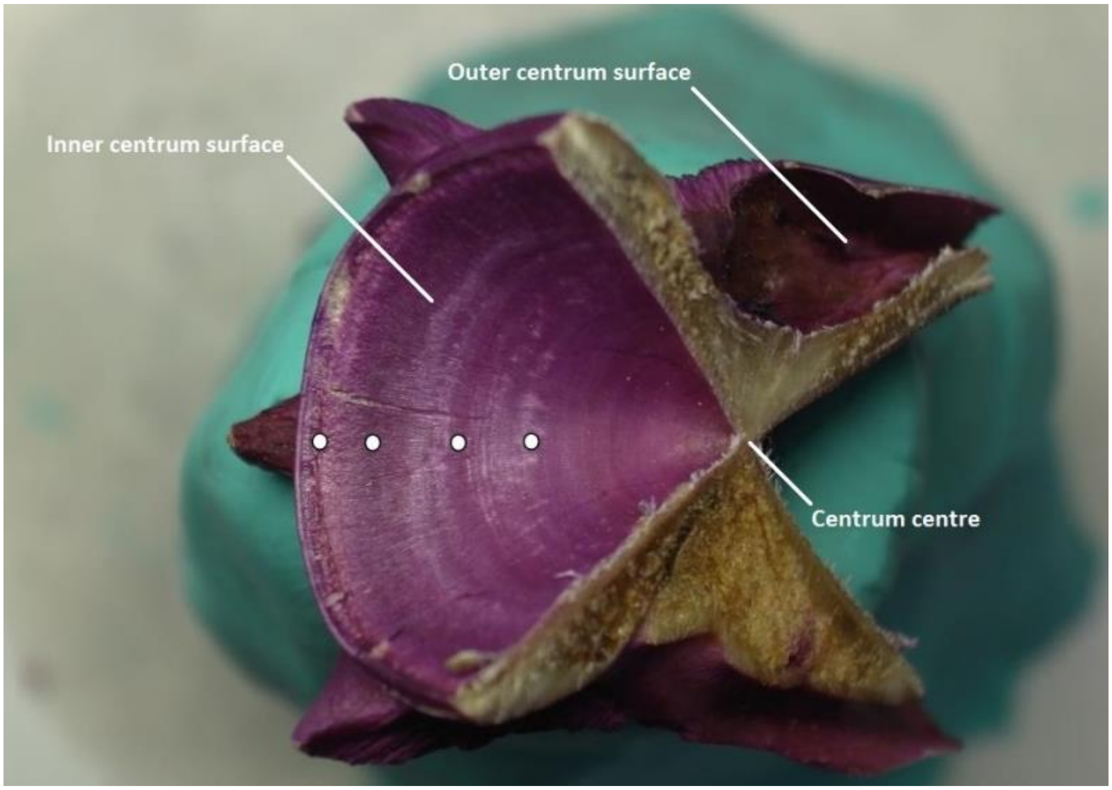

Figure 6. Stained vertebral section of a Pacific Bluefin Tuna. Age is estimated to be four years old, with the annuli marked by white dots. Image adapted from Shoto Shimizu, Tokyo University of Marine Science and Technology, presentation made at The Pacific Bluefin Tuna and North Pacific Albacore Tuna Age Determination Workshop, held November 13-16, 2013, at the National Research Institute of Far Seas Fisheries in Shimizu, Shizuoka, Japan.

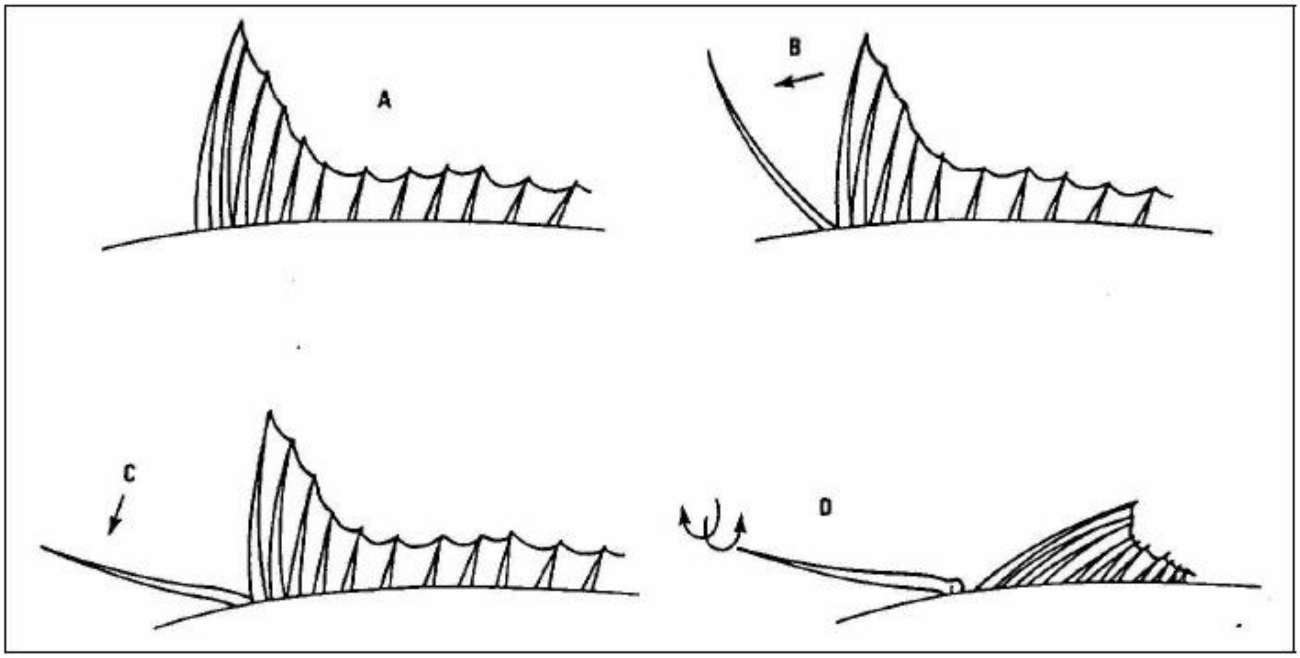

Figure 7. Diagram showing the first dorsal fin (A), the first spine being pushed forward (B) until a nearly horizontal position (C), and twisting the spine to the left and right directions until the ligament breaks. (Figures from Compeán-Jiménez 1980, taken from Ruiz et al. 2005).

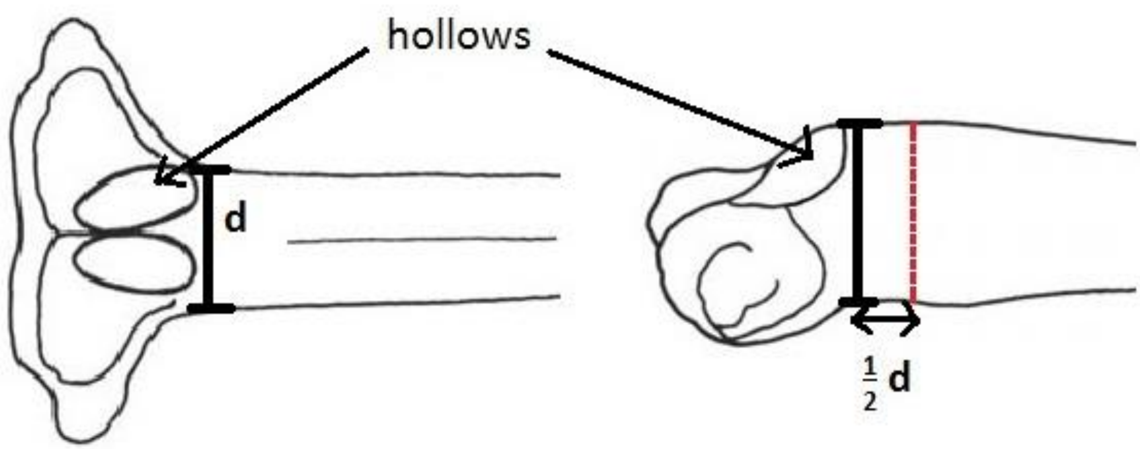

Figure 8. Anterior view of a ray base (left), showing the location of where the diameter is measured (d), directly above the "hollows". Horizontal view of the same ray base (right), with the location of the cut axis (dotted red line) located at half the length of the diameter ($\frac{1}{2}\,d$) above the measured line (adapted from Rodríguez-Marín et al. 2007).

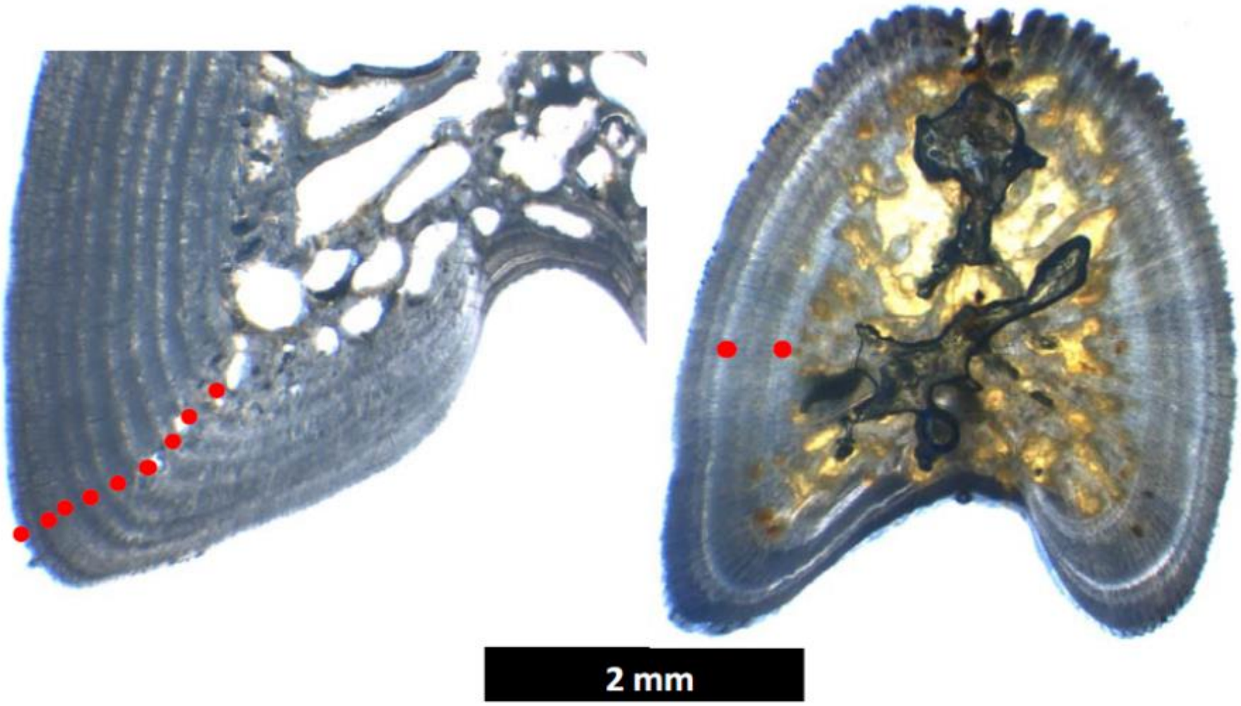

Figure 9. Fin spine cross sections from two different albacore. On the left, 3 zones were estimated to be obscured by resorption and added onto the number of visible zones counted, for a final age of 12 years. On the right, age was estimated to be two years (from Farley et al. 2013).

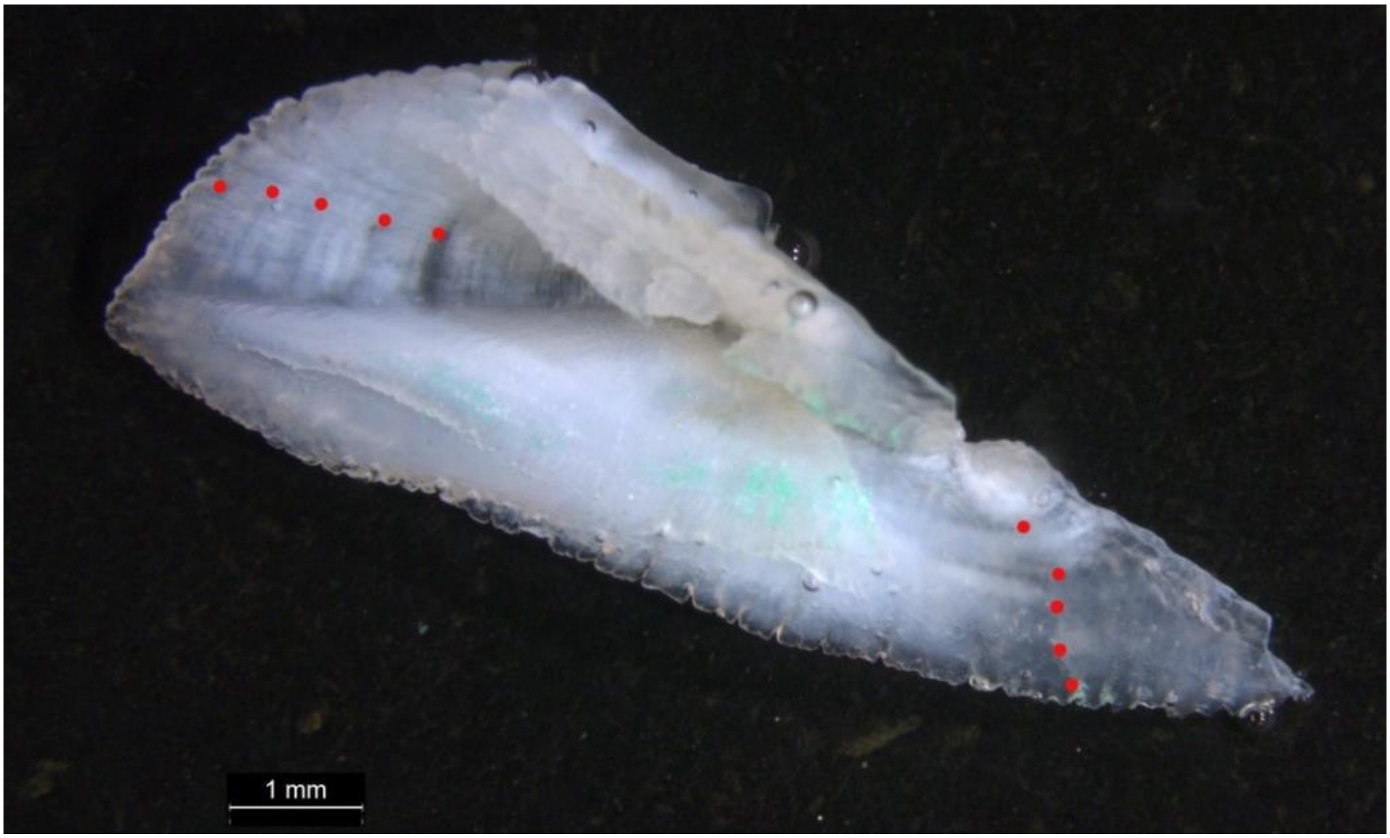

Figure 10. Sulcal side of a whole otolith, with annuli marked by red dots. Image from Darlene Gillespie, Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, Canada.

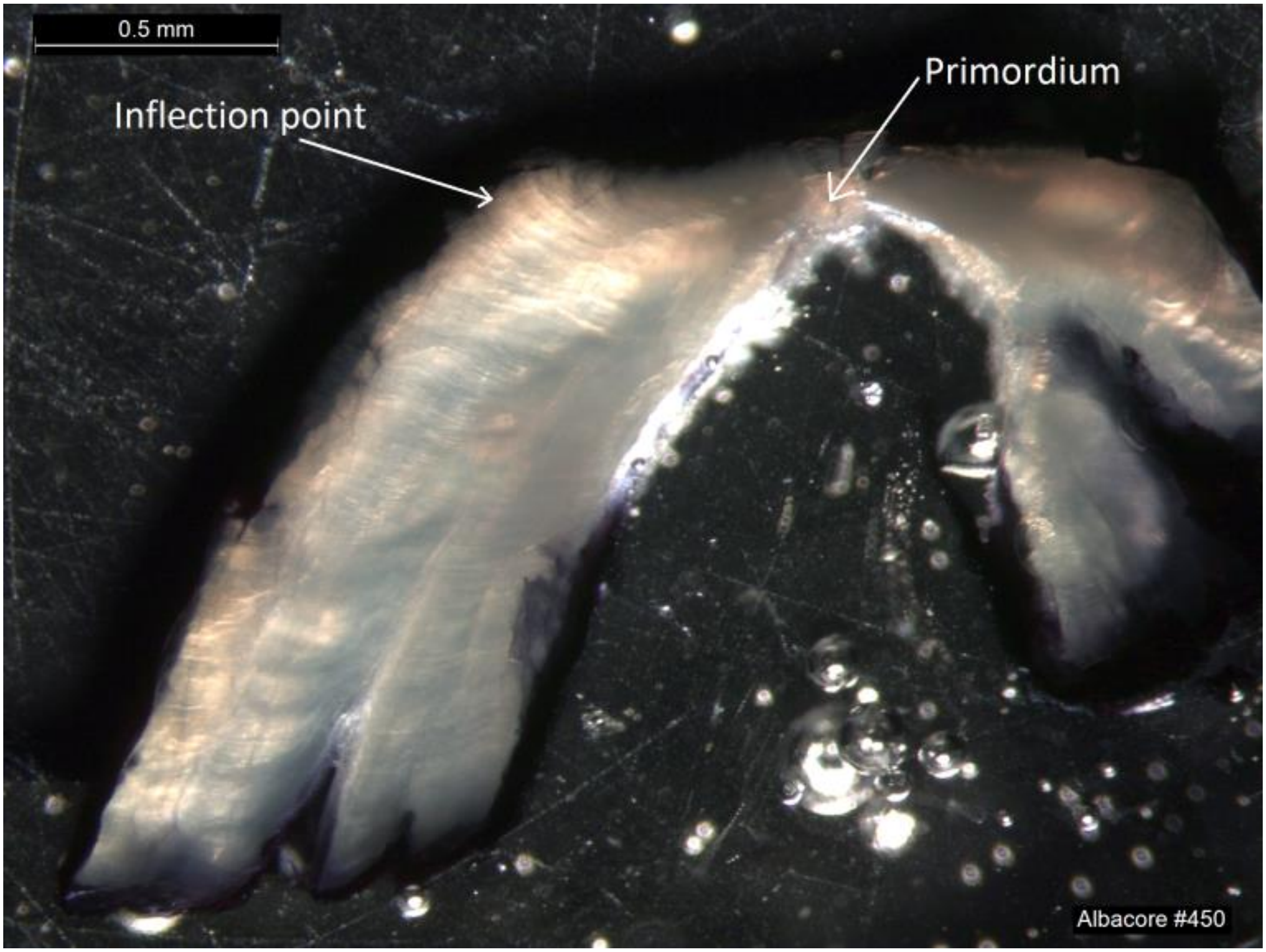

Figure 11. Image of an albacore otolith thin section, showing the primordium and inflection point. Image adapted from Darlene Gillespie, Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, Canada.

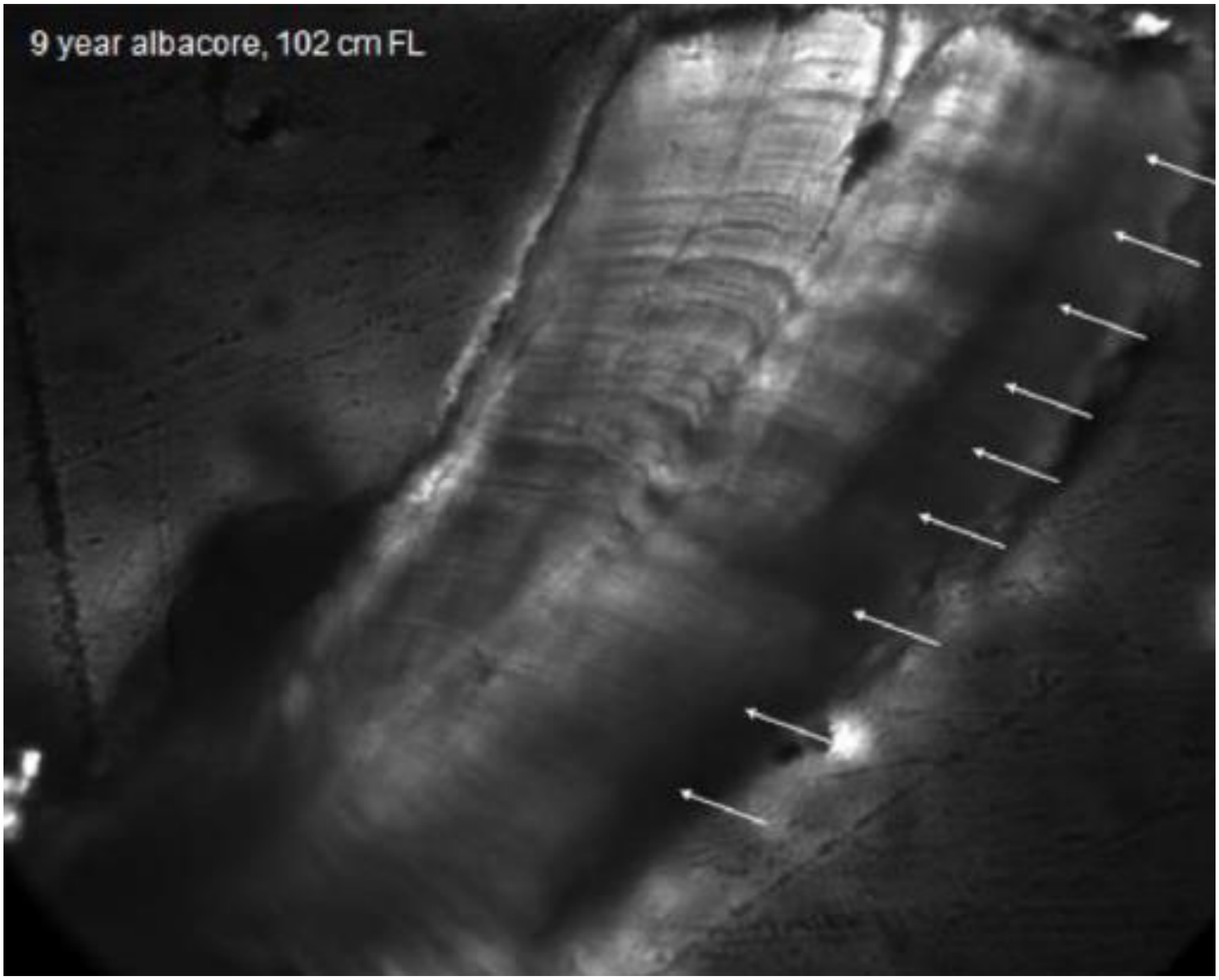

Figure 12. Transverse section of a North Pacific albacore sagittal otolith, with observed annuli indicated by white arrows (from Wells et al. 2013).

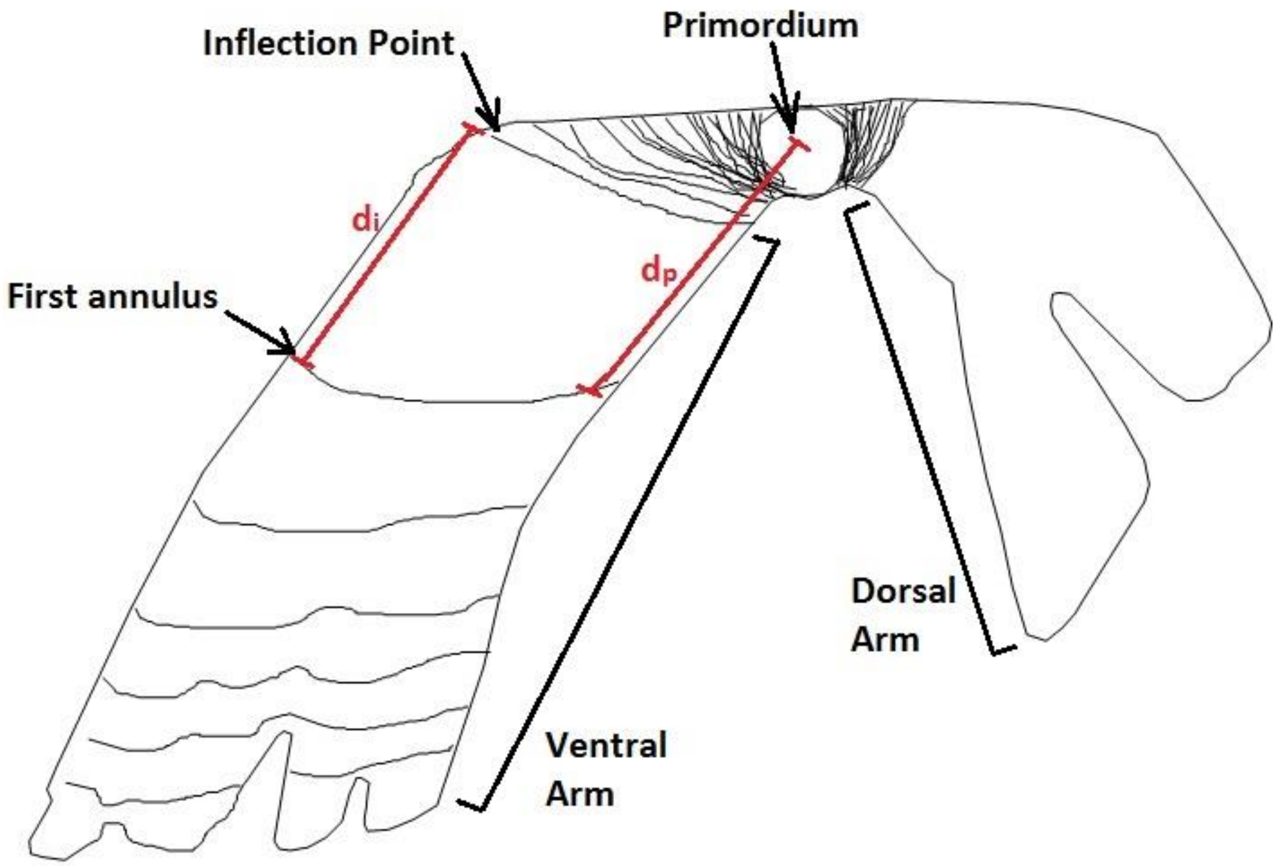

Figure 13. Diagram of an Albacore Tuna otolith thin section, showing the primordium, the inflection point, and the first annulus. The distance $\mathbf{d_i}$ represents the distance between the inflection point and the first annulus. The distance $\mathbf{d_p}$ represents the distance between the primordium and the first annulus. Either $\mathbf{d_i}$ or $\mathbf{d_p}$ on multiple otoliths may be measured to establish an average distance, as an aid in locating the first annulus of otoliths with unclear increments. Counting is usually carried out on the ventral arm, instead of the dorsal.