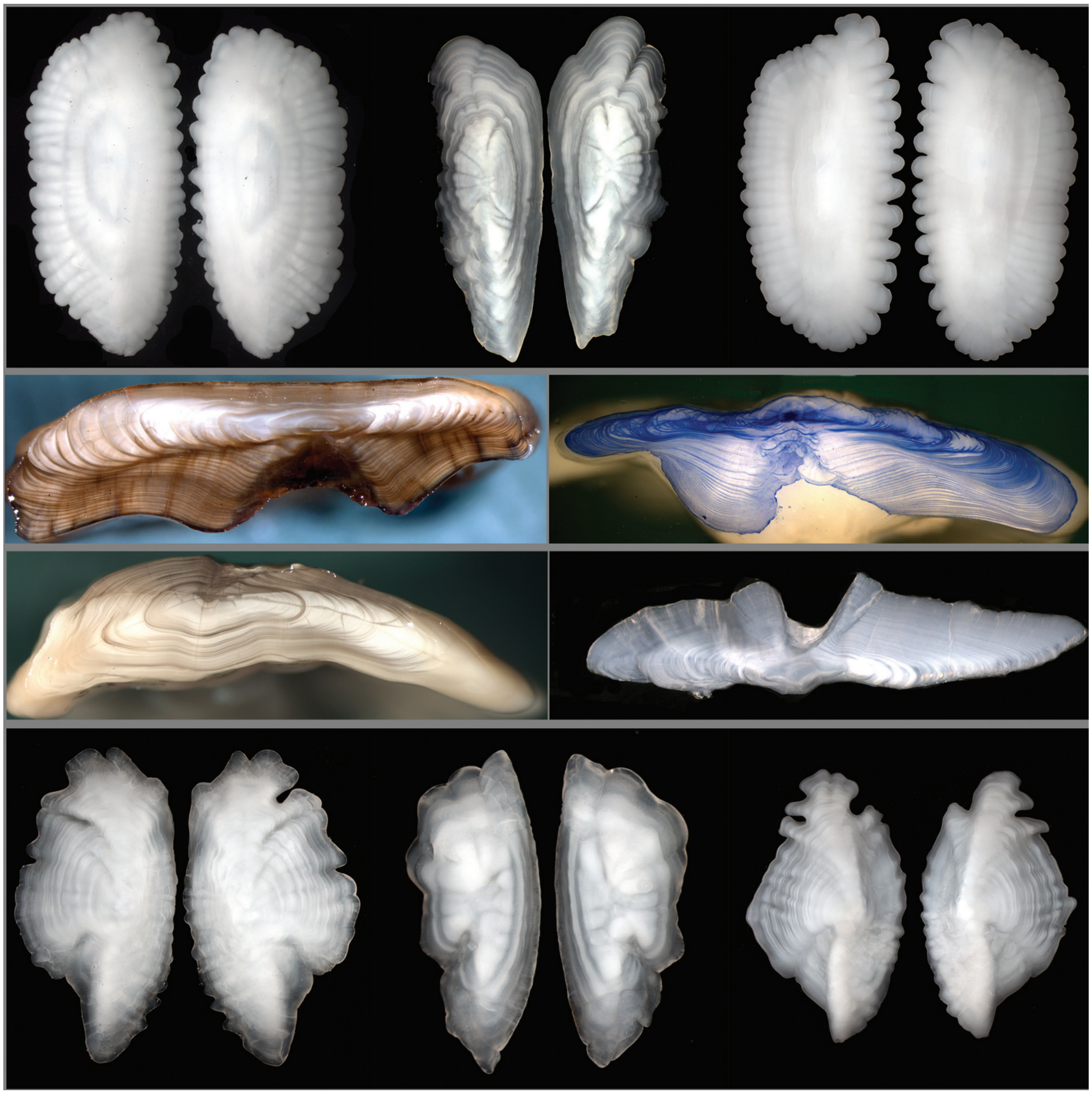
**NOAA Professional Paper NMFS 13**

# Age determination manual of the Alaska Fisheries Science Center Age and Growth Program

**Mary Elizabeth Matta and Daniel K. Kimura (editors)**



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# Age determination manual of the Alaska Fisheries Science Center Age and Growth Program

**Mary Elizabeth Matta and Daniel K. Kimura[1] (editors)**

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# CONTENTS

**Abstract**—The Age and Growth Program at the Alaska Fisheries Science Center is tasked with providing age data in order to improve the basic understanding of the ecology and fisheries dynamics of Alaskan fish species. The primary focus of the Age and Growth Program is to estimate ages from otoliths and other calcified structures for age-structured modeling of commercially exploited stocks; however, the program has recently expanded its interests to include numerous studies on topics ranging from age estimate validation to the growth and life-history of non-target species. Because so many applications rely upon age data and particularly upon assurances as to their accuracy and precision, the Age and Growth Program has developed this practical guide to document the age determination of key groundfish species from Alaskan waters. The main objective of this manual is to describe techniques specific to the age determination of commercially and ecologically important species studied by the Age and Growth Program. The manual also provides general background information on otolith morphology, dissection, and preparation, as well as descriptions of methods used to measure precision and accuracy of age estimates. This manual is intended not only as a reference for age readers at the AFSC and other laboratories, but also to give insight into the quality of age estimates to scientists who routinely use such data.

# Chapter 1: Introduction

**by Daniel K. Kimura and Mary Elizabeth Matta**

Age data can provide considerable insight into fish population dynamics. Age determination is particularly important for marine fishes because they are often difficult to census at every life cycle stage. At the National Marine Fisheries Service's Alaska Fisheries Science Center (AFSC), fish age data, including catch-at-age data collected from commercial fisheries and population age compositions estimated from scientific bottom trawl surveys, are used to develop age-structured stock assessment models. These models evaluate the overall health of fish populations and guide fishery managers in setting sustainable catch limits.

Age determination science relies on growth marks deposited on a daily or annual basis within the hard structures of marine organisms. Otoliths, sometimes referred to as ear bones, are of unique value for age determination of teleost fishes because across taxa they are the only hard structures that continue growing even after somatic growth has ceased. In cartilaginous fishes such as sharks and skates, otoliths are not well-calcified, so researchers must rely on other hard structures such as vertebrae or spines for age estimation.

Although annual marks in otoliths and vertebrae are similar to tree rings, their appearance is generally much fainter and more irregular. Another major problem in counting annual marks is that the timing of the deposition of new growth zones varies considerably by species, age, and exogenous factors such as geographic location and climate. These issues may increase the difficulty of interpreting annual marks in aging structures, thus requiring the specialized expertise of biologists trained in age determination.

The primary role of the AFSC Age and Growth Program is to provide age estimates to support stock assessments of commercially exploited groundfish species in Alaskan waters (Fig. 1). The program has aged thousands of fish since its inception in the late 1970s. However, we also conduct novel scientific research. Our program has performed numerous validation studies to evaluate the accuracy of our age estimates. We have also investigated the life histories of non-target species and developed new aging criteria for species that are difficult to age. Recent research has examined relationships between environmental variables and growth. A summary of the fish species aged and studied by the Age and Growth Program is listed in Table 1.

The purpose of this manual is to report age determination methodologies used by the Age and Growth Program. Without adequate documentation, unwanted drifts in age estimation practices could occur. This manual provides a means to ensure consistency over time and among different age readers by acting as a record of the criteria used to determine age for many of the key species studied at the AFSC. A subsequent goal of this manual is to provide insight into our data quality by describing the level of accuracy and precision associated with our age estimates. This manual represents the culmination of decades of work, benefitting from the expertise of a diverse group of scientists who have worked in or who are currently working in the Age and Growth Program.

In this extensive document, we begin by discussing fish otolith morphology and edge interpretation (Chapter 2) and the dissection, preservation, and preparation of otoliths for age determination (Chapter 3). In

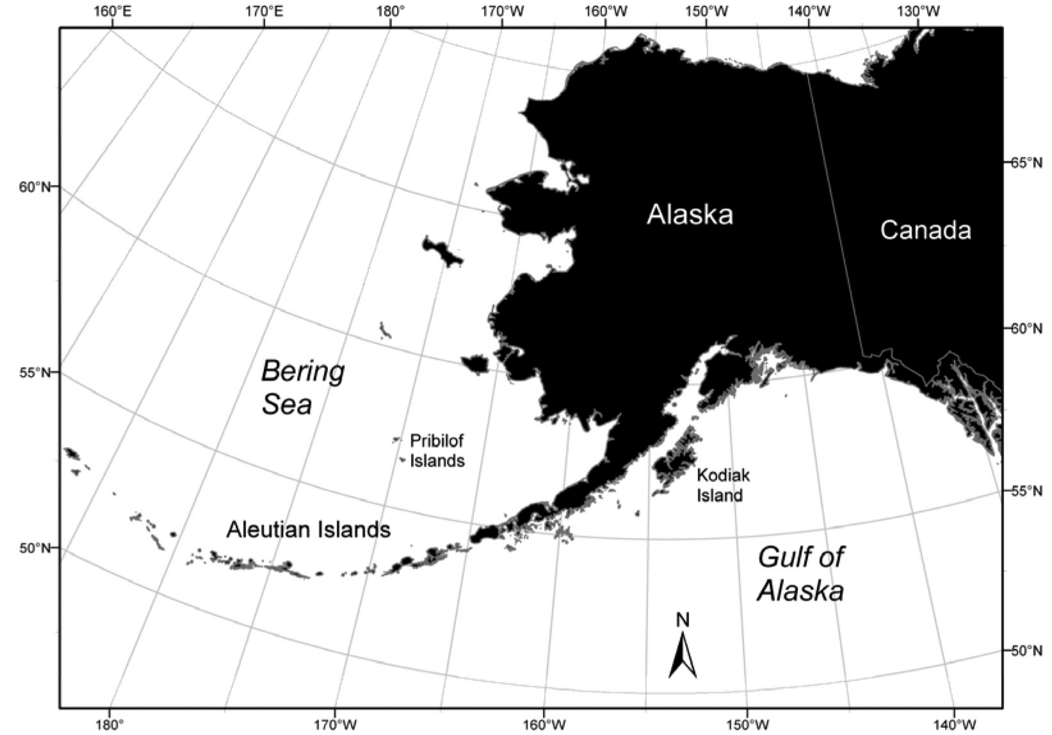

**Figure 1**

Major bodies of water where the groundfish species described in this manual are found and harvested.

Chapter 4, the most important chapter of this manual, we give descriptions of the age determination of all major species studied by the Age and Growth Program. Each species section within Chapter 4 includes relevant biological information, history of age determination methods, otolith preparation, growth pattern interpretation, age estimation, and availability of information supporting age estimate accuracy. The remaining chapters explain procedures used to maintain the precision of our age estimates (Chapter 5) and validation research (Chapter 6).

Age determination is a science that relies heavily on technical terminology. Definitions for these terms can be found in the glossary at the end of this manual. Images of otoliths have also been included throughout the manual to provide examples demonstrating how aging criteria are applied for a variety of species. Unless otherwise noted, all otolith images were taken with a digital camera mounted on a dissecting microscope, using reflected light emitted from a fiber-optic light source. Dots or numerals have been used to indicate the position of presumed annual marks in most otolith images.

Supplementary material for this manual, containing additional species chapters and more detailed information on age determination, is available from the AFSC Age and Growth Program website (Alaska Fisheries Science Center, http://www.afsc.noaa.gov/REFM/Age/Default.htm). The supplementary material is intended to serve as a working document for age readers and age data users by describing historic and current age estimation practices. Due to the evolving nature of fish age determination science, we expect to add new chapters to the online document with the advent of new technologies.

**Table 1**

Groundfish species currently aged by the Alaska Fisheries Science Center Age and Growth Program. Age type is classified as "production" (i.e., routinely aged on an annual or biennial basis to support stock assessment) or "limited" (i.e., aged infrequently for stock assessment or research purposes).

| Common name | Scientific name | Age type |
|---|---|---|
| Alaska plaice | *Pleuronectes quadrituberculatus* | Production |
| Alaska skate | *Bathyraja parmifera* | Limited |
| Arctic cod | *Boreogadus saida* | Limited |
| Arrowtooth flounder | *Atheresthes stomias* | Production |
| Atka mackerel | *Pleurogrammus monopterygius* | Production |
| Bering flounder | *Hippoglossoides robustus* | Limited |
| Bigmouth sculpin | *Hemitripterus bolini* | Limited |
| Big skate | *Raja binoculata* | Limited |
| Blackspotted rockfish | *Sebastes melanostictus* | Production |
| Capelin | *Mallotus villosus* | Limited |
| Dark rockfish | *Sebastes ciliatus* | Production |
| Dover sole | *Microstomus pacificus* | Production |
| Dusky rockfish | *Sebastes variabilis* | Production |
| Eulachon | *Thaleichthys pacificus* | Limited |
| Flathead sole | *Hippoglossoides elassodon* | Production |
| Giant grenadier | *Albatrossia pectoralis* | Limited |
| Great sculpin | *Myoxocephalus polyacanthocephalus* | Limited |
| Greenland halibut | *Reinhardtius hippoglossoides* | Production |
| Harlequin rockfish | *Sebastes variegatus* | Limited |
| Kamchatka flounder | *Atheresthes evermanni* | Limited |
| Longhead dab | *Limanda proboscidea* | Limited |
| Longnose skate | *Raja rhina* | Limited |
| Northern rock sole | *Lepidopsetta polyxystra* | Production |
| Northern rockfish | *Sebastes polyspinis* | Production |
| Pacific cod | *Gadus macrocephalus* | Production |
| Pacific ocean perch | *Sebastes alutus* | Production |
| Plain sculpin | *Myoxocephalus jaok* | Limited |
| Prowfish | *Zaprora silenus* | Limited |
| Redstripe rockfish | *Sebastes proriger* | Limited |
| Rex sole | *Glyptocephalus zachirus* | Production |
| Rougheye rockfish | *Sebastes aleutianus* | Production |
| Sablefish (black cod) | *Anoplopoma fimbria* | Production |
| Sharpchin rockfish | *Sebastes zacentrus* | Limited |
| Shortraker rockfish | *Sebastes borealis* | Production |
| Shortspine thornyhead | *Sebastolobus alascanus* | Limited |
| Southern rock sole | *Lepidopsetta bilineata* | Limited |
| Walleye pollock | *Theragra chalcogramma* | Production |
| Warty sculpin | *Myoxocephalus verrucosus* | Limited |
| Yellow Irish lord | *Hemilepidotus jordani* | Limited |
| Yellowfin sole | *Limanda aspera* | Production |

# Chapter 2: Otolith growth pattern interpretation

**by Mary Elizabeth Matta and B. J. Goetz**

## Otolith morphology

Fish otoliths, sometimes referred to as ear bones, are calcified structures that play a role in hearing, balance, and spatial orientation (Popper et al., 2005). Otoliths are composed of calcium carbonate precipitated upon a protein matrix (Degens et al., 1969; Campana, 1999). All teleost fish have three pairs of otoliths: the asterisci, lapilli, and the sagittae. The sagittae are typically the largest in size and are the otolith pair most often used in age determination applications (Fig. 2).

Otoliths vary widely in shape and size across taxa (Fig. 3). In general, otoliths from round-bodied teleosts are mirror images of each other, and either the left or the right otolith may be selected for age determination (Fig. 4). In contrast, flatfish have asymmetrical sagittal otoliths (Fig. 5). Generally, the otolith from the blind side of a flatfish's body has a centric core, whereas the otolith from the eyed side of the body has a core located posteriorly. Both otoliths can provide valuable information in age determination. The eyed-side otolith is generally more useful in estimating age from the surface, while cross-sectioning techniques are better applied to the blind-side otolith. (See Chapter 3 for a complete description of otolith preparation methods.)

Otoliths accrete material in concentric layers around the core, a process which continues even after a fish stops growing in length (Campana and Thorrold, 2001). The accreted material is composed of alternating layers that differ in density and optical properties. These layers are referred to as being either opaque or translucent, and their appearance depends on the microscope and light source used to view them. When viewed against a dark background using reflected light, translucent growth zones appear dark and opaque growth zones appear light in color (Wright et al., 2002). The inverse is true when these growth zones are viewed using transmitted light.

In general, a year of otolith growth consists of one opaque growth zone and one translucent growth zone (Figs. 2 and 6). In otoliths from North Pacific fish, the opaque growth zone is typically wider and corresponds to periods of fast growth, and the translucent growth zone is typically narrower and corresponds to periods of slow growth. Thus, the opaque growth zone is sometimes referred to as the "summer zone" and the translucent growth zone is sometimes referred to as the "winter zone." However, these nicknames can be somewhat misleading, since deposition of the translucent growth zone actually occurs in the spring or early summer for most of the groundfish studied

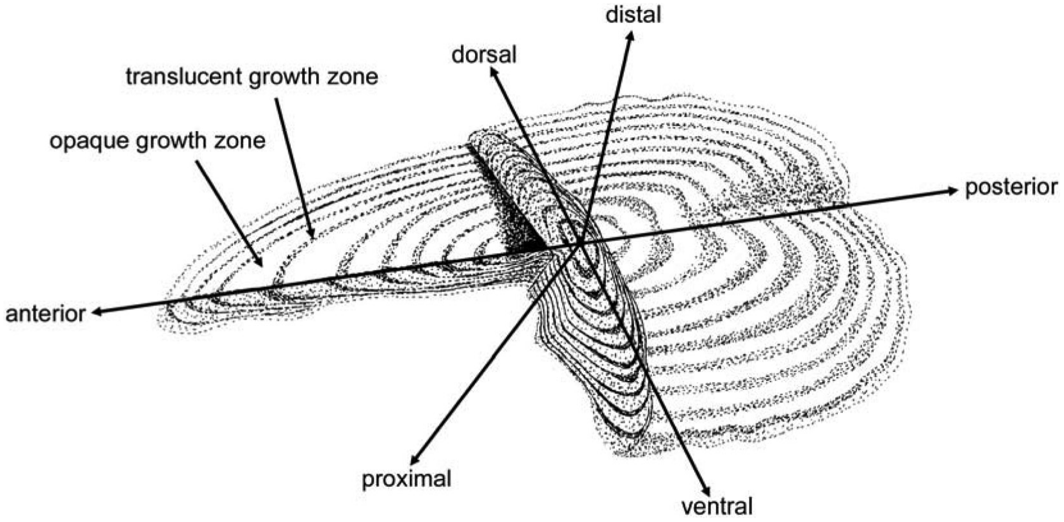

**Figure 2**

Diagram of a typical flatfish blind-side sagittal otolith, cross-sectioned to show concentric annual growth zones and general otolith morphology. Illustration courtesy of J. Forsberg (International Pacific Halibut Commission, Seattle, WA).

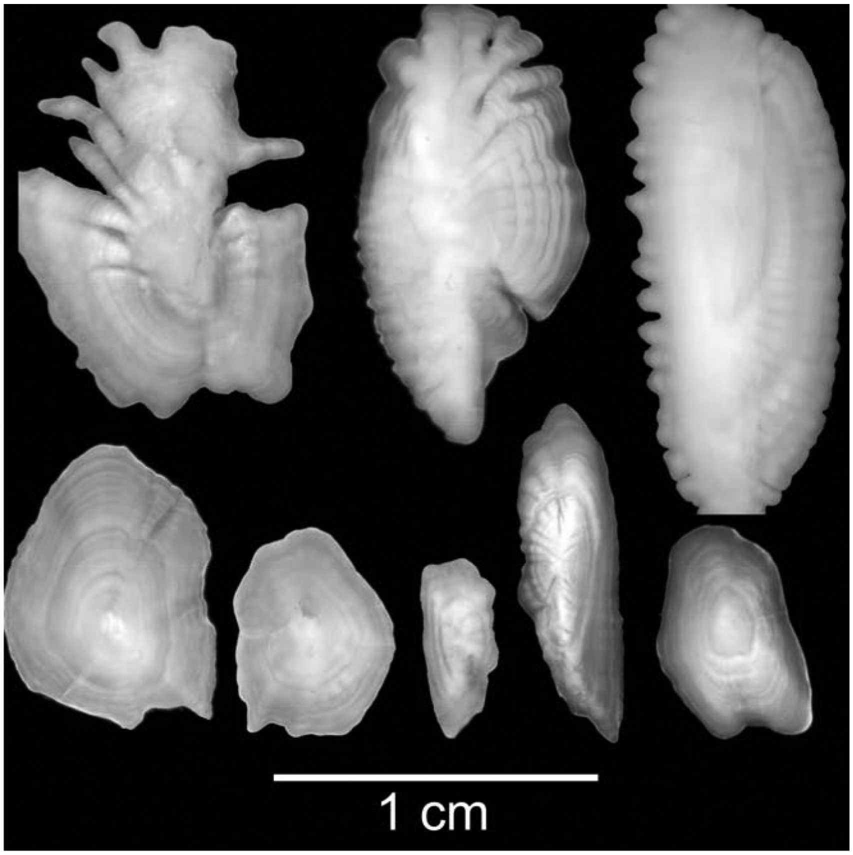

**Figure 3**

Composite image of otoliths from some of the groundfish species aged by the Alaska Fisheries Science Center Age and Growth Program. From left to right, top row: Greenland halibut (*Reinhardtius hippoglossoides*), Pacific ocean perch (*Sebastes alutus*), walleye pollock (*Theragra chalcogramma*); bottom row: flathead sole (*Hippoglossoides elassodon*), yellowfin sole (*Limanda aspera*), Atka mackerel (*Pleurogrammus monopterygius*), sablefish (*Anoplopoma fimbria*), Dover sole (*Microstomus pacificus*). Viewed with reflected light.

at the AFSC (Kimura et al., 2007). Exact timing of growth zone deposition depends on a suite of factors such as fish age, temperature, geographic region, and species.

Age readers often use the term "annulus" to describe an annual growth zone (typically the translucent growth zone). However, the phrase "annual mark" may be more apt because, as noted by Panfili et al. (2002) and Cailliet et al. (2006), the word "annulus" is derived from the Latin word "anus," meaning ring or circle, not from "annus," meaning year (Gove, 1961). The assumption of yearly growth zone deposition is critical to age determination, as it provides a timestamp matching otolith growth and fish age. Thus, throughout this manual we strive to use the term "annual mark" when referring to any annually deposited growth zone; the term "annulus" may also be used, although less commonly, to refer to concentric growth zones that may or may not be deposited on a yearly basis.

"Checks," irregular translucent growth zones, are sometimes present in otoliths (Fig. 6). Checks are not annual marks, and often occur as the result of physiological or environmental stresses experienced by the fish during life. Checks may correspond with life history events such as settlement, migration, maturation, or spawning (Penttila and Dery, 1988). Checks can be distinguished from true annual marks by their irregular spacing, relatively faint appearance, and lack of continuity throughout the otolith (Fig. 6). "Splitting" is a special case of checking, where two or more closely spaced translucent growth zones are deposited in a single year. The number of checks observed in groundfish otoliths

**Figure 4**

Sablefish (*Anoplopoma fimbria*) sagittal otoliths. Otolith pair is symmetrical, as is typical in round-bodied fishes. Viewed with reflected light.

often varies by species and geographic region and can increase the difficulty of growth pattern interpretation. Becoming familiar with the general growth pattern of a given species through experience helps an age reader differentiate between checks and annual marks.

At the AFSC, age readers typically count annual translucent growth zones from the core to the edge to obtain an age estimate. It is necessary to identify checks to avoid including them in the final age estimate. Age readers must also interpret any opaque growth observed at the otolith margin. Most age readers begin the age estimation process by viewing each whole otolith using a dissecting microscope with reflected light. Young fish with clear otolith growth patterns can often be aged solely from the otolith surface. In the case of older fish, examination of the otolith surface can act as a guide in interpretation of early annual marks in otolith cross sections.

Identification of the first annual mark is a critical step in age determination. Collections of juvenile fish are extremely helpful in this respect, as the act of matching length- and age-frequency modes of fast-growing young fish may be used to corroborate the first several annual marks (CARE, 2006). Checks are frequently observed within the first few years of life, often co-occurring with ontogenetic changes in diet or habitat; thus, life history information can also be useful in guiding age determinations (CARE, 2006). Knowledge of the spawning season can also give age readers an idea of the expected size of the first annual mark. For example, species that spawn earlier in the year may have more time to accrete opaque material around the core, resulting in a wider first annual mark, than those that spawn later in the year.

Morphology helps age readers identify reading axes in otolith cross sections (Fig. 6). A reading axis is the path from the core to the margin along which an age reader counts annual marks. Depending on the species and the clarity of the otolith, certain reading axes may be clearer and more accurate than others. Otoliths are not spherical, and thus new material does not accumulate at the same rate among reading axes; some grow much faster than others, a fact which may influence the type of preparation method used for age

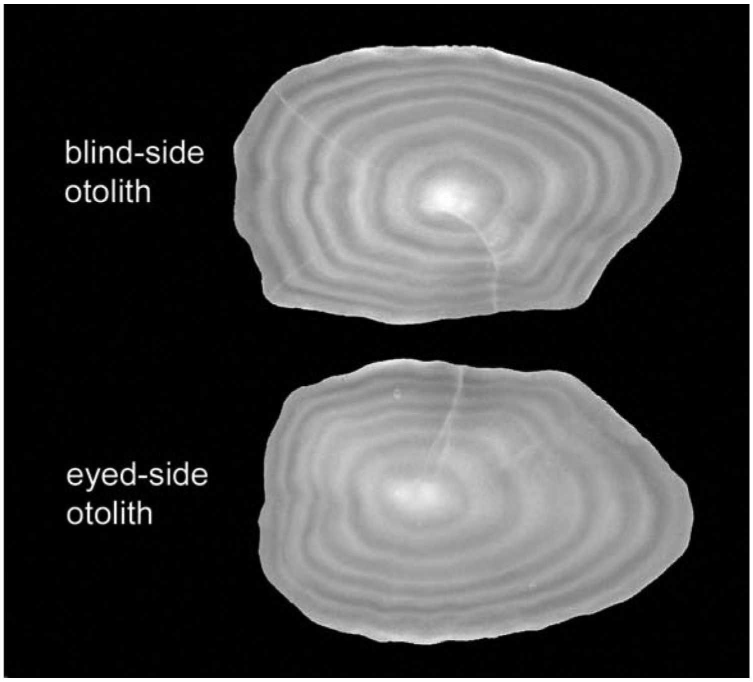

**Figure 5**

Northern rock sole (*Lepidopsetta polyxystra*) sagittal otoliths. Otolith pair is asymmetrical, as is typical in flatfishes. Viewed with reflected light.

determination (see Chapter 3). It is best to compare age estimates from different axes in the same otolith whenever possible to obtain consistent annual mark counts. The areas adjacent to the sulcus, a groove on the proximal surface, are critical reading axes in many species, as the translucent growth zones near the sulcus tend to be relatively clear.

## Marginal growth and the international birth date convention

Most fish age determination facilities have agreed by international convention to assign a birth date of 1 January to fish regardless of their actual spawning date (Chilton and Beamish, 1982; Penttila and Dery, 1988). The international birth date convention allows age readers to estimate birth year and assign each fish to the correct cohort, which is integral to effective stock assessment.

The birth date convention may affect an age estimate when opaque growth is seen on the margin (edge) of an aging structure. Age readers must assign the correct calendar year to the growth zone observed on the margin. In other words, age readers must decide whether opaque growth observed on the margin occurred during the year the fish was collected or during the previous year. The collection date is a vital piece of information that enables age readers to make this decision. For example, a fish caught in August will often have opaque marginal growth that was laid down during the collection year, and therefore the age estimate is equivalent to the number of observed translucent growth zones. However, a fish caught in January with the same amount of opaque marginal growth may not yet have deposited the translucent growth zone marking the end of the previous year. Therefore, opaque growth on the edge of a January-collected otolith is most likely attributable to the past growth cycle, and the estimated age is one year more than the observed number of translucent growth zones because 1 January has passed. The same fish would have been assigned an age one year younger if it had been collected in December, even if the edge characteristics were identical.

For many species, opaque edge growth is observed earlier in the calendar year in the otoliths of young fish than in those of older fish (Forsberg, 2001). For example, a 3-year-old walleye pollock (*Theragra chalcogramma*) caught in June typically has a greater proportion of opaque growth on the otolith margin than a 10-year-old walleye pollock caught at the same time. In other words, marginal growth is not identical across age groups.

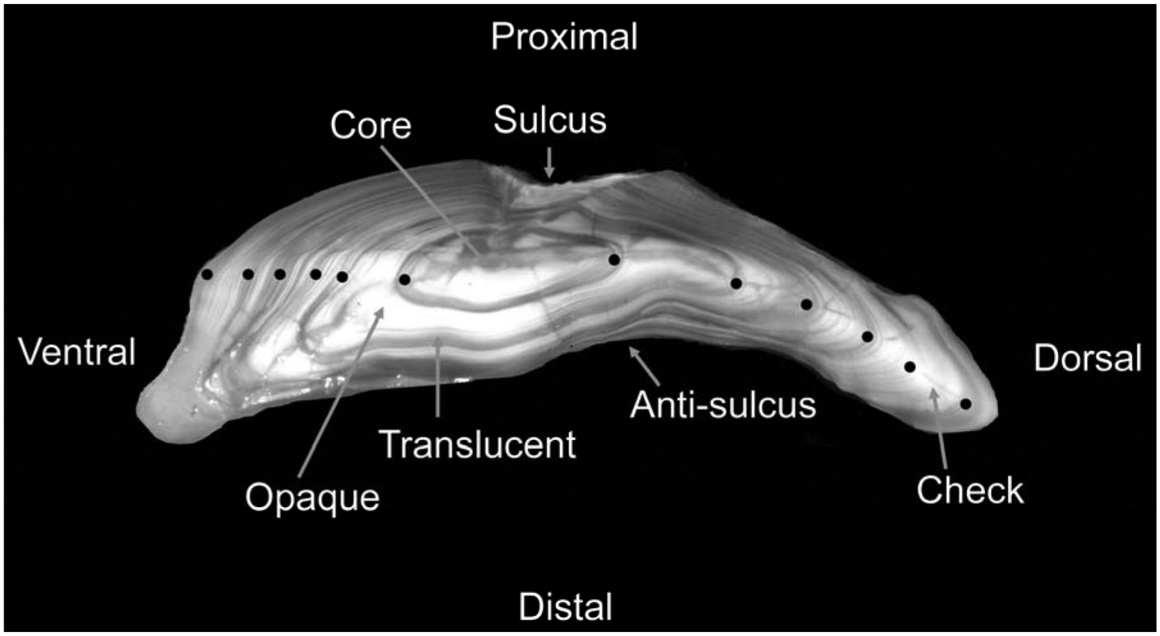

**Figure 6**

Otolith from a 6-year-old walleye pollock (*Theragra chalcogramma*), showing growth zones and terminology used to refer to otolith cross sections. Sectioned transversely through the core and baked in an oven for several minutes to improve the contrast between opaque and translucent growth zones. Viewed with reflected light.

Otoliths collected from spring to early summer often have the most problematic patterns to interpret because the translucent growth zone corresponding to the previous calendar year has often not yet been deposited (Forsberg, 2001). For example, in a March-collected otolith with three translucent growth zones, an age reader may observe an opaque growth zone at the margin which is almost equal in width to the previous opaque growth zone. The otolith would be estimated as 3 or 4 years depending on the interpretation of the edge. The age reader must decide which of two scenarios is more likely: 1) the opaque growth was laid down during the collection year, in which case it is determined to be 3 years; or 2) the opaque growth was deposited during the previous year and a translucent growth zone has not yet formed. In the second scenario, the opaque growth is attributed to the previous year and the age reader assumes that a translucent growth zone corresponding to the previous year will soon form; thus, the fish is determined to be 4 years. Even if a translucent growth zone was deposited late in the previous year, it is unlikely that a whole year's opaque growth would appear by March. Therefore, it is most likely that a translucent growth zone has not yet formed, and opaque edge growth can be attributed to the previous year, resulting in an age estimate of 4 years. If this otolith had been collected in December, it would be estimated as 3 years because of the conventional 1 January birth date, even if the growth pattern was identical.

For young fish caught early in the year, one factor that aids edge interpretation is whether opaque growth is visible on more than one reading axis, especially in cross section. It is somewhat common to see a large increment of opaque growth on one reading axis but not on others. This is probably due to an early growth spurt that appears only on the axis of greatest growth for that otolith; in such a case, the opaque growth is typically attributed to the collection year and not counted in the age estimate.

To illustrate the process of edge interpretation, two different Atka mackerel (*Pleurogrammus monopterygius*) otoliths collected in January and September 2005 are shown in Figure 7. Both otoliths have four fully-formed translucent growth zones. However, the specimen collected in September has a fifth translucent growth zone starting to form on the otolith margin. This fifth translucent growth zone (and the preceding opaque growth zone) most likely formed during 2005, and thus is not included in the age estimate. If this otolith had been collected in January 2005, the fifth translucent growth zone would be counted in the age estimate even though it isn't fully formed because it would have been attributable to the year 2004.

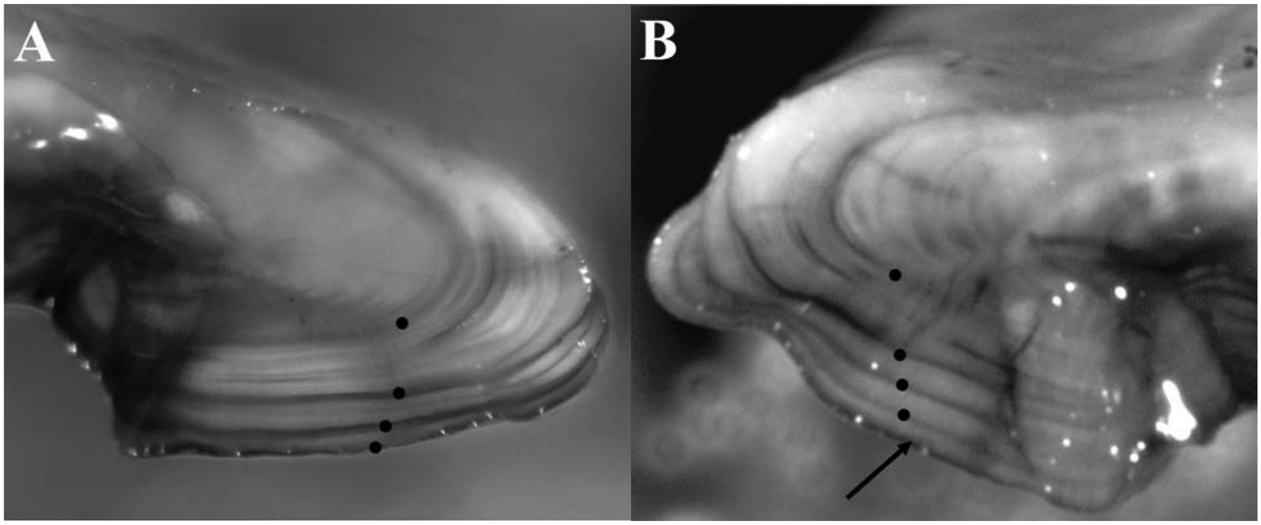

**Figure 7**

Otoliths from two different Atka mackerel (*Pleurogrammus monopterygius*), both with an age estimate of 4 years, demonstrating how collection date affects margin interpretation. (**A**) Break-and-burn preparation of an otolith collected in January 2005, with four fully-formed translucent growth zones. (**B**) Break-and-burn preparation of an otolith collected in September 2005, with four fully-formed translucent growth zones and a fifth translucent growth zone starting to form on the margin (indicated by arrow). This zone is not counted because it is not fully formed and most likely was deposited during 2005. If this otolith had been collected in January, the fifth translucent growth zone would be counted, resulting in an age estimate of 5 years. Viewed with reflected light.

Because age readers often note differences in the timing of marginal growth between areas, calendar years, and age groups, experience is often a key component in the interpretation of growth patterns. Unfortunately, it is rare to have samples collected throughout a single year, so age readers are often expected to make judgments about edge growth with very little information. In such cases, the reader must make a decision based on the most logical choice and make an effort to apply this criterion with precision, even if there is a lack of evidence as to its accuracy.

# Chapter 3: Collection and preparation of otoliths for age determination

**by B. J. Goetz, Charles E. Piston, Charles E. Hutchinson, Christopher G. Johnston, and Mary Elizabeth Matta**

## Otolith dissection

Sagittal otoliths are collected for the AFSC during scientific surveys or by domestic fishery observers working on commercial fishing vessels. A sharp knife is positioned with the blade just posterior to the preopercle to cut vertically (transversely) through the fish's head (Fig. 8A). As an alternative, the "haircut" method may be employed, whereby one holds the knife horizontally and slices into the head just above the eyes, cutting along the frontal plane toward the lateral line. Once the knife breaks through the bony skull, the snout of the fish is grasped in one hand and the body in the other, and the head is carefully cracked open to expose the brain. The sagittae, or sagittal otoliths, are located ventrally and to either side of the brain tissue (Fig. 8B). Each otolith is carefully removed using forceps.

It is important that otoliths are cleaned before storage to prevent degradation. This can be accomplished by placing otoliths on a wet sponge and carefully brushing off tissue and blood using a firm brush. Once the otoliths are cleaned, they are inserted into labeled vials containing preservative.

## Otolith preservation

Since the AFSC began collecting fish otoliths in the 1970s, we have used either glycerol-thymol solution or ethanol to preserve and store fish otoliths. Vertebrae and otoliths from round teleosts have been preserved using at least 50% ethanol. Flatfish otoliths have never been stored in ethanol at the AFSC because of concerns that it may cause their annual marks to over-clear. Denatured ethanol and formalin are acidic and can cause severe damage to otoliths (Butler, 1992; Morales-Nin, 1992), thus they should not be used as preservatives. Recently we switched to glycerol-thymol solution for preserving the otoliths of all species because it does not pose the safety hazards of ethanol. Ethanol is a flammable material, and special care is required for its transportation and storage.

Glycerol-thymol solution is made by crushing 5.5 g of thymol into a powder, which is then dissolved in 5 ml of 95% ethanol by stirring or agitating the mixture. (The thymol is added as a preservative to prevent mold and bacterial growth.) Once the thymol is dissolved, the thymol/ethanol solution is added to 0.5 gallons of glycerol and shaken well. Lastly, 0.5 gallons of water is added to the mixture and again shaken well. Care should be taken not to add the thymol solution to the water before adding the glycerol, as doing so will cause the thymol to precipitate.

## Methods of preparing otoliths for age determination

Examination of the surface of intact otoliths is generally the first step in age determination. However, there are several other techniques that can be used to visualize and further enhance otolith growth patterns. The most common method we use to determine fish age at the AFSC is the break-and-burn method, which entails halving the otolith transversely through the core and using an alcohol burner to burn the cut surface of one of the otolith halves (Beamish, 1979; Chilton and Beamish, 1982; MacLellan, 1997). The break-and-bake technique (Barto, 1999; Forsberg, 2001) is a recent variation of the break-and-burn method; it is best applied to delicate otoliths. Both methods enhance the contrast between translucent and opaque growth zones, resulting in clarification of the overall growth pattern. Thin-sectioning, a method typically used for species whose growth patterns are difficult to interpret, produces uniform reading surfaces. This method is often applied in special research studies.

When studying new species, different preparation methods should be tested to establish which is most appropriate. At the AFSC, the method chosen is the one that most efficiently produces growth patterns that are clear enough for age determination. Generally, surface examination is the least time-consuming method, followed by the break-and-bake method, the break-and-burn method, and finally, thin-sectioning. Validation of the age estimates generated by the chosen method should be the ultimate goal of any age determination laboratory (see Chapter 6). The four otolith preparation methods listed above are described in detail in the following sections, and their applications to individual species are further described in Chapter 4 of this manual.

## Otolith surface examination

Surface examination, while rarely the sole method of age determination, provides supplementary information to the age reader that can be useful in making age deter-

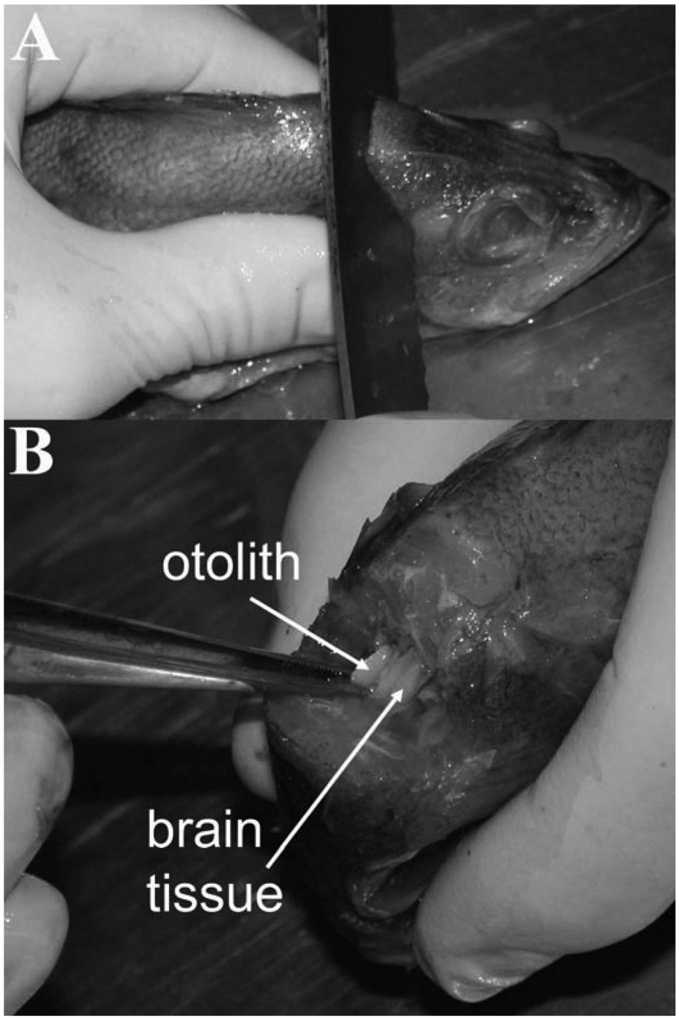

**Figure 8**

Otolith dissection from a Pacific ocean perch (*Sebastes alutus*). (**A**) Proper placement of knife immediately posterior to preopercle to cut transversely through head. (**B**) Right sagittal otolith and exposed brain tissue.

mination decisions such as identifying the first annual mark. Whole otoliths are submerged in water in a Petri dish lined with black felt and viewed with a dissecting microscope and reflected light. Surface examination is helpful in that it often makes it easier to distinguish between checks and annual marks, as translucent growth zones typically appear more coalesced than they do in cross section. Surface examination may be adequate for determining the age of young otoliths or otoliths with relatively clear growth patterns. However, in the case of older fish it is usually too difficult to interpret age from the otolith surface alone, and further treatment is required. This is because as fish grow older, their otoliths become thicker, effectively reducing the visibility of growth zones on the surface. Furthermore, annual marks become more closely spaced and more difficult to differentiate as otolith growth slows with age. Therefore, using only the otolith surface to estimate the ages of older fish may result in underestimation of true age.

## Otolith break-and-burn method

For most round-bodied teleosts, either otolith may be selected for cross-sectioning. However, flatfish otoliths are asymmetrical (Chapter 2), and it is usually preferable to section the blind side (centric-cored) otolith. Otoliths are cross-sectioned through the core using either a scalpel, a low-speed saw, or by snapping them in half. The method used depends on the species, the size of the otolith, and the degree to which the characteristics of the cut surface affect age determination. Very small otoliths such as those of Atka mackerel (*Pleurogrammus monopterygius*) or sablefish (*Anoplopoma fimbria*) may successfully be snapped in half using one's fingers, whereas most flatfish species are best cross-sectioned using a scalpel. Walleye pollock (*Theragra chalcogramma*) and Pacific cod (*Gadus macrocephalus*) otoliths should be sectioned using a low-speed saw, such as an Isomet™ (Buehler®, Lake Bluff, IL), to minimize topography on the cut surface which can interfere with age estimation.

When using a low-speed saw, a reference line should first be drawn transversely through the core to orient the otolith for sectioning. Some readers draw this line on the proximal surface whereas other readers prefer to draw it on the distal surface of the otolith. A microscope may be used to draw the line on smaller otoliths; on larger otoliths the line can be drawn with the naked eye. A clay support is inserted into a custom-made chuck on the saw and lowered onto the blade to press another reference line in the clay. The reference line on the otolith is aligned with the reference line in the clay. Using a light weight (25–75 g) to provide pressure, the otolith is lowered onto the turning saw blade and cut in half (Fig. 9). Heavier weights are not recommended because they may break the otolith or section it unevenly.

**Figure 9**

Walleye pollock (*Theragra chalcogramma*) otolith being sectioned along the transverse plane using a low-speed saw.

Some readers prefer polishing the cut surface of each otolith half before burning. Water is either added directly to fine grit sandpaper or the otolith half is dipped in water prior to polishing. Either a back-and-forth or a circular polishing pattern works well as long as one is gentle enough not to break the edges.

For all species, age readers usually examine the untreated cut surface ("the unburned face") of each otolith half prior to further processing, obtaining an age estimate from it when possible. The otolith half chosen for burning varies by species and general otolith clarity. For example, the posterior half is preferred over the anterior half for burning walleye pollock and Pacific cod otoliths. For these species, the anterior half yields better surface estimates and is typically preserved when possible, especially if the second whole otolith is broken, crystallized or otherwise damaged. For other species, the otolith half with the clearest growth pattern may be selected for burning.

Using forceps, the cut surface of the otolith half is passed back and forth through the alcohol flame until it is browned (Fig. 10A). With experience, age readers learn how long to burn otoliths. Under- or over-burning makes the growth pattern very hard to interpret. The amount of time required to obtain an optimal burn pattern varies by species and otolith size. Because walleye pollock otoliths are very brittle, they cannot be held in the flame for long periods of time without shattering. For this reason, otoliths should only be burned using safety goggles or behind a protective plastic shield to prevent eye injuries (Fig. 10B).

When sufficiently burned, the growth patterns are enhanced and more easily interpreted. The burned otolith is allowed to cool and is then inserted in a clay support and brushed with mineral oil to view the growth patterns. Break-and-burn growth patterns are interpreted using a dissecting microscope with reflected light. If the cut surface is not clean, the burning process can cause black deposits to appear. These deposits can interfere with the age determination process by occluding annual marks. Deposits can be removed by gently polishing the broken-and-burned surface on wet fine-grit sandpaper. The otolith is much more brittle after burning, so only gentle pressure should be used. A circular motion can cause breakage, so a simple, back-and-forth motion on the sandpaper is the safest way to remove deposits. Immersion in diluted hydrochloric acid also removes deposits but is more hazardous.

One disadvantage of the break-and-burn method is that otolith growth patterns may fade during long-term storage. This phenomenon does not affect all otoliths and is often unpredictable, but may be prevented by encasing otoliths in a clear casting resin (CARE, 2006).

## Otolith break-and-bake method

The break-and-bake technique is a variation of the break-and-burn method and was first used by the AFSC

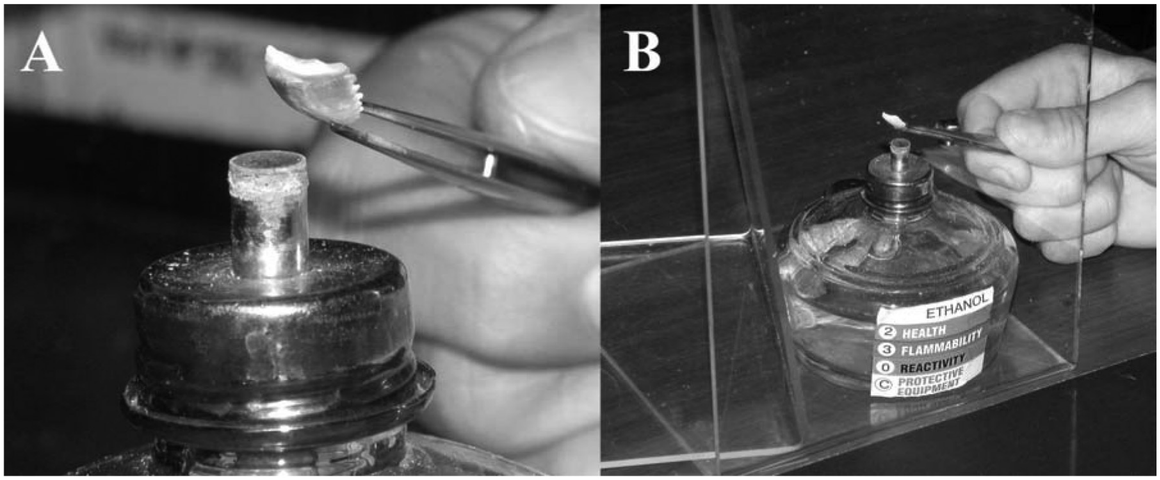

**Figure 10**

An alcohol flame is used to burn the cut surface of a walleye pollock (*Theragra chalcogramma*) otolith. (**A**) Close-up view. (**B**) Plastic shield used to protect the age reader from burning debris.

Age and Growth Program in 2003. Instead of being passed over a flame, the cut otolith is baked for several minutes in a conventional toaster oven. This technique works particularly well for otoliths that have a tendency to crumble when burned over an open flame.

Otoliths are transversely sectioned through the core using either a scalpel or low-speed saw, as described above. One half from each sectioned otolith is then baked in a toaster oven using a metal tray that has 50 separate cells. Optimal baking times and temperatures vary according to species, but otoliths are generally baked between 350°F and 500°F for several minutes.

The break-and-bake technique is often more convenient than the traditional break-and-burn method because it allows the age reader to prepare multiple specimens at the same time. Another advantage of oven-baking is that it produces relatively even contrast between opaque and translucent growth zones while reducing the possibility of charring or cracking otoliths. Barto (1999) found no significant difference between the break-and-burn and break-and-bake methods for Pacific halibut (*Hippoglossus stenolepis*) age estimates.

### Otolith thin-section method

At the AFSC, thin-sectioning is used to determine age for skates, some rockfishes, and other species for which the break-and-burn method is either not possible or does not produce reliable age estimates. Thin-sectioning results in an even reading surface and enhances both annual and sub-annual growth zones. Thin-sectioning can be a valuable method in studies on age validation, stock discrimination, and environmental effects on growth.

The AFSC thin-section method is a modified version of a similar thin-section technique developed in Australia (Smith et al., 1995). Multiple otoliths or vertebrae may be sectioned at once. Polyester resin is prepared and poured into blocks within custom-made rubber molds to form a thin uniform base layer about 2 to 3 mm thick. A tongue depressor is used to gently mix the resin to minimize the introduction of air bubbles, which can cause specimen loss during sectioning. Before mounting the otoliths or vertebrae, the resin is allowed to partially set to prevent them from sinking to the bottom of the mold.

Using a dissecting probe, the resin base layer is scored with parallel lines to create rows for aligning aging structures for cutting. Multiple otoliths are mounted along each row with the proximal surface facing down and the anterior-posterior axis perpendicular to the scored lines to result in transverse cuts directly through the cores (Fig. 11). For proper alignment of small otoliths, it may be necessary to draw a pencil line on the surface transversely through the core (Fig. 11). Vertebral centra are mounted so that their foci are aligned along the scored lines, with either the anterior or posterior side facing down to yield longitudinal sections. A label with specimen numbers is placed at the top of the block.

After mounting, another batch of resin is mixed and poured to cover the aging structures completely. Ideally, each aging structure should be completely encased in resin while keeping block thickness to a

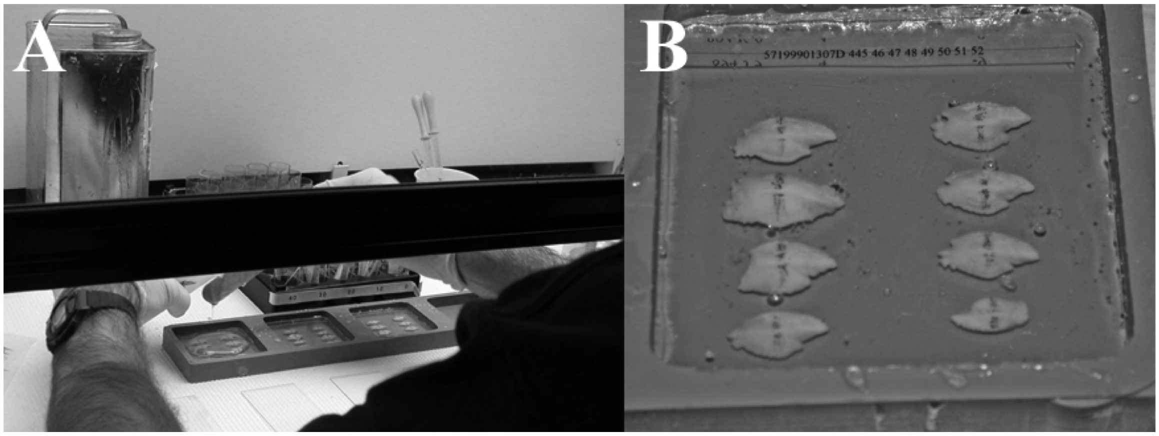

**Figure 11**

Preparation of rockfish (*Sebastes* sp.) otoliths for thin-sectioning. (**A**) Resin is poured into rubber molds to create blocks containing multiple otoliths. (**B**) Otoliths are mounted with their cores aligned to yield transverse thin sections.

minimum. Thick resin blocks take longer to cut and are more likely to cause overheating and binding of the saw blade.

The AFSC Age and Growth Program uses a Buehler Isomet® 5000 Linear Precision saw equipped with diamond blades to create thin sections. Because it is difficult to align the cores of all of the otoliths within a row, it is usually necessary to make three or four thin sections from each row. Optimum section thickness varies by species. Thin sections are affixed to glass slides with an adhesive resin after cutting and may be ground and polished to attain the desired thickness. Thin sections may be viewed using a dissecting microscope fitted with either a reflected or transmitted light source. The light source selected typically depends on the thickness of the specimen and the age reader's preference.

# Chapter 4: Groundfish age determination

Over 30 species of groundfish from Alaskan waters are aged at the AFSC, 18 of which are aged on a production basis for stock assessments. The remaining species are aged on an occasional basis for research purposes. The following sections describe age determination methods and aging criteria for some of the species most commonly studied by the AFSC's Age and Growth Program.

## Walleye pollock (*Theragra chalcogramma*)

**by B. J. Goetz, Irina M. Benson, and Daniel K. Kimura**

### Biology

Walleye pollock (*Theragra chalcogramma*) is a semi-demersal species found in the waters of the continental shelf and slope of the North Pacific Ocean; its range along the North American coast extends from southern Oregon to the Gulf of Alaska through the Bering Sea to the southern Chukchi Sea (Bakkala et al., 1986). Walleye pollock, a key species in the eastern Bering Sea ecosystem, is both a dominant predator and an important component of the diet of many upper trophic level consumers (Aydin and Mueter, 2007). Alaskan populations of walleye pollock, especially the Bering Sea stock, support one of the largest single-species fisheries in the world, representing over 40% of global whitefish production (Ianelli et al., 2007).

Walleye pollock are relatively fast-growing and short-lived (Ianelli et al., 2007), with a protracted spawning season lasting from January through August depending on geographic location. Recent studies indicate that shelf and deepwater basin walleye pollock in the Bering Sea form independent spawning groups that differ in timing and location of spawning, age at maturity, and possibly growth rate (Stepanenko and Nikolaev, 2004). In the Gulf of Alaska, spawning occurs from mid-February through April (Hughes and Hirschhorn, 1979; Dorn et al., 2010).

Larvae grow relatively slowly and metamorphose to the juvenile life stage at about 18 mm in length. Young-of-year juveniles reach 80 to 100 mm by 6 months and 120 to 140 mm by the end of their first year. Walleye pollock mature sexually at about age-4 and at a length of about 400 to 450 mm (Bailey et al., 1999). Maximum reported age differs by region, with walleye pollock in the Bering Sea generally growing older (maximum age=31 years) than those in the Gulf of Alaska. The relative differences in growth between the two regions are also reflected by fitting length-at-age data from each area with the von Bertalanffy growth function (VBGF), $L_t = L_\infty(1 - e^{-k(t-t_0)})$, where $L_t$=predicted length at age t, $L_\infty$=asymptotic length, k=growth coefficient, and $t_0$=theoretical age at zero length. Von Bertalanffy growth parameters were $L_\infty$=673.3 mm, k=0.1937/yr, and $t_0$=–0.205 yr (*n*=3147) and $L_\infty$=700.0 mm, k=0.2465/yr, and $t_0$=–0.364 yr (*n*=1177) for walleye pollock collected during trawl surveys in the Bering Sea and Gulf of Alaska, respectively, in 2008 (Fig. 12).

### Age determination history

Walleye pollock were first aged at the AFSC in 1971 using the surfaces of whole otoliths. The break-and-burn method was introduced in the 1980s, followed by the break-and-bake method in 2003 (Chapter 3). Today, surface examination is still used to age otoliths from small fish or otoliths with exceptionally clear growth patterns. The break-and-burn or break-and-bake methods are applied to otoliths that have patterns that are more difficult to interpret.

Around 1990, one of the aging criteria used to interpret growth patterns in walleye pollock otoliths was slightly modified. Prior to this time, otolith cross sections from older fish were typically aged from the core to a notch between the ventral and proximal surfaces. Additional growth zones on the proximal surface between the sulcus and notch were not counted (Fig. 13). However, after the criterion modification, all marks within this area were counted. The criterion change was relayed to data end users and only affected fish older than about 12 years, a very small percentage of the population.

The AFSC has aged large numbers of walleye pollock from the Bering Sea, Gulf of Alaska, and Aleutian Islands regions. To date, no obvious differences have been noted between walleye pollock otolith growth patterns from the Bering Sea and Aleutian Islands regions. However, walleye pollock otoliths from the Gulf of Alaska often have larger first annual marks than those in the Bering Sea and Aleutian Islands.

To date, the AFSC Age and Growth Program has aged over 300,000 walleye pollock otoliths. Inter-reader precision is relatively high (coefficient of variation [CV]=4.4%; *n*=55,282). Walleye pollock age estimates have been corroborated using radiometric methods (Kastelle and Kimura, 2006), length mode analysis (Kimura et al., 2006), and edge type studies (Kimura

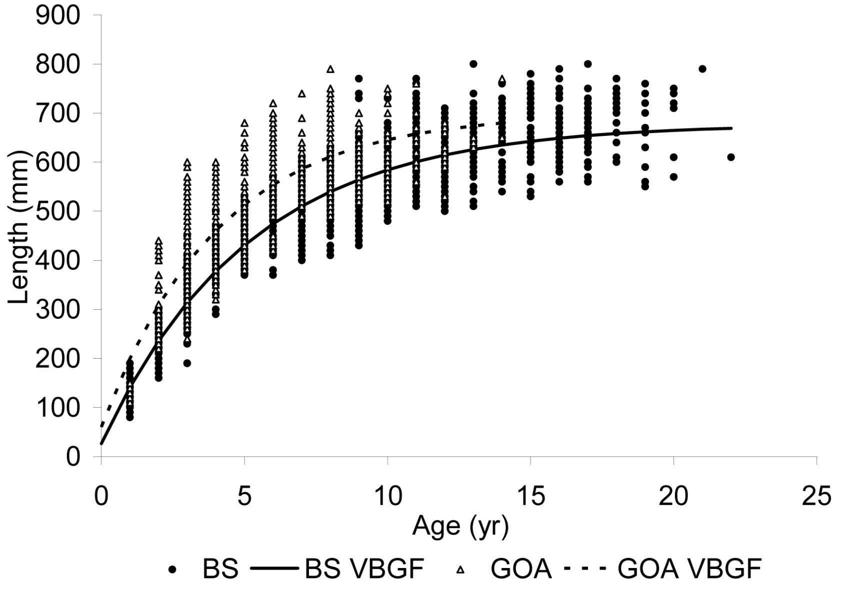

**Figure 12**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for walleye pollock (*Theragra chalcogramma*) collected during trawl surveys in the Bering Sea (BS; *n*=3147) and Gulf of Alaska (GOA; *n*=1177) in 2008.

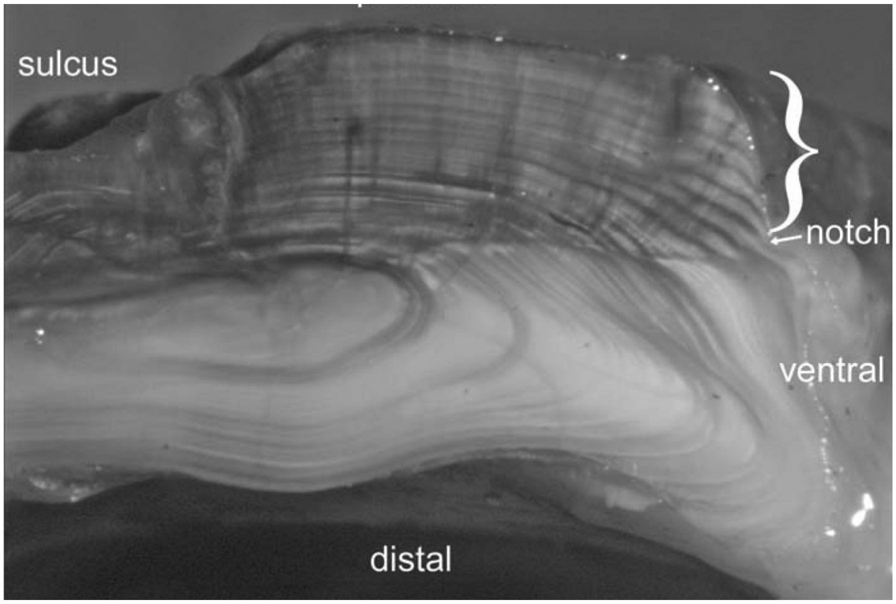

**Figure 13**

Break-and-burn preparation of a walleye pollock (*Theragra chalcogramma*) otolith collected from a 640 mm female on 6 April 2007 in the Aleutian Islands. Age estimate is either 16 or 17 years, with a questionable fifteenth annual mark. Before an aging criterion change in 1990, age readers did not count any translucent growth zones visible from the ventral-proximal notch to the margin along the proximal reading axis. (Area not aged prior to criterion change indicated by bracket.) Viewed with reflected light.

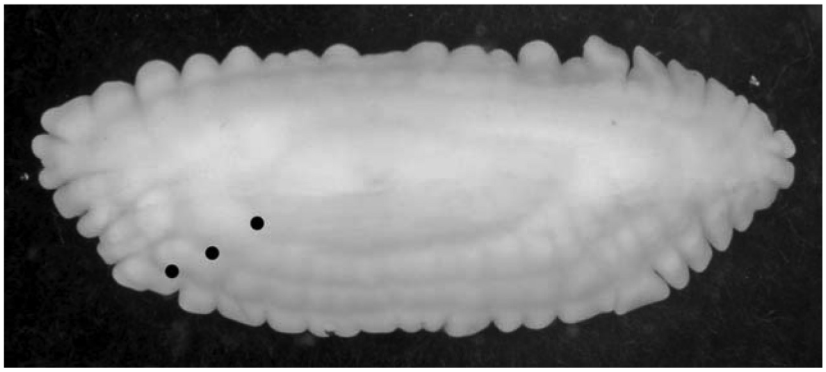

**Figure 14**

Clear walleye pollock (*Theragra chalcogramma*) otolith collected from a 380 mm male in the Bering Sea on 16 October 2001. Age estimate is 3 years. Viewed with reflected light.

et al., 2007) at the AFSC; these methods are described in further detail in Chapter 6 of this manual.

## Current age determination methods

Age determination of walleye pollock is moderately difficult, and the readability of otoliths from this species is variable. At the AFSC, walleye pollock otoliths are examined using a dissecting microscope and reflected light.

**Surface examination.** Annual marks are typically visible on the surfaces of whole otoliths (Fig. 14), and young, clear specimens can usually be aged from the surface alone. Surface age estimation is often more difficult on older, thicker otoliths. Large walleye pollock otoliths are generally aged from the proximal surface, and it is sometimes possible to improve visibility of annual marks by tilting the otolith to the side with forceps under low magnification. However, for thinner, younger otoliths, checks can often be distinguished from annual marks by turning the otolith over and examining the distal side.

Since the sagittae of walleye pollock are much thicker than those of many groundfish species, relying on surface examination alone can be problematic. For this reason, most walleye pollock are aged using the break-and-burn method. However, the occasional sample is comprised of younger or thinner otoliths which can easily be aged from the surface. New age readers should compare paired surface and break-and-burn age estimates from each otolith, but with experience age readers learn how to recognize young, clear surface patterns that do not require a break-and-burn preparation.

**Break-and-burn examination.** If a walleye pollock otolith cannot be aged from the surface, further processing is required. Age readers typically use either the break-and-bake or the break-and-burn method (Chapter 3) to enhance the growth pattern. Both methods have advantages and disadvantages: the break-and-burn method is better at darkening translucent growth zones but may result in crumbling, while the break-and-bake method leaves the otolith intact but generally yields slightly less contrast between opaque and translucent growth zones. However, the annual growth pattern is nearly identical in appearance using either method, and the choice of method is left up to the age reader.

It is difficult to break walleye pollock otoliths at the desired point manually, so a low-speed saw is used to section them in half. The best burn patterns occur when the otolith is slowly burned over an alcohol flame. Over-burning and under-burning create age determination problems, so it is best to experiment with an alcohol burner to find the best technique to achieve an optimal burn. Over-burning can result in erosion of the dorsal and ventral tips and a chalky white appearance, whereas under-burning will fail to produce enough contrast between opaque and translucent growth zones, making them more difficult to count. Walleye pollock otoliths are brittle and can shatter when exposed to heat, so they should be burned carefully. A protective shield or goggles should be used to prevent eye injuries.

**Reading axes.** Although there is no single preferred reading axis on walleye pollock otolith cross sections, the areas on either side of the sulcus are often clearest. It is useful to compare patterns on multiple reading axes to see if translucent marks are continuous around the otolith (Fig. 15). The anti-sulcus is usually the least

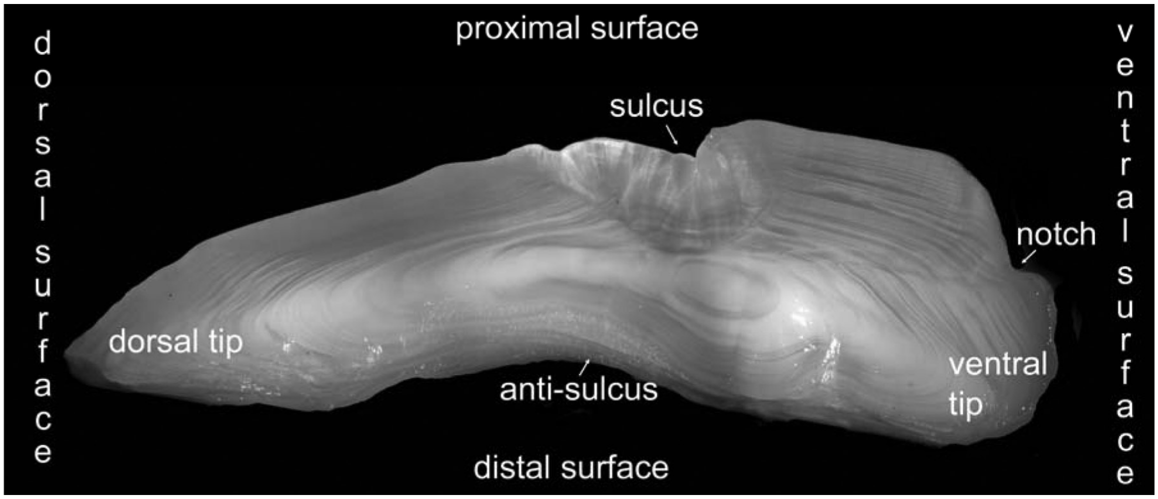

**Figure 15**

Sectioned walleye pollock (*Theragra chalcogramma*) otolith showing general age reading axes. Collected from a 550 mm female in the Gulf of Alaska on 11 July 2006. Age estimate is 16 years. Viewed with reflected light.

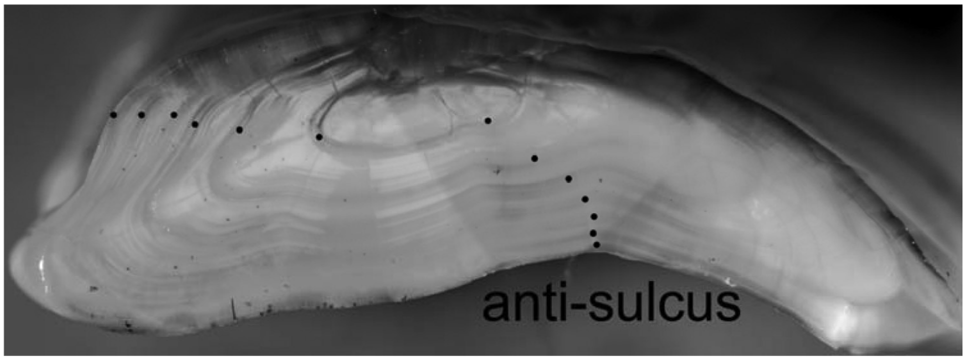

**Figure 16**

Otolith break-and-burn pattern from a 510 mm female walleye pollock (*Theragra chalcogramma*) collected on 19 February 2008 from the Bering Sea. Using other reading axes, one might assign an age estimate of 6 years, but this specimen is clearly 7 years on the anti-sulcus. Viewed with reflected light.

useful reading axis, although in rare cases it is the clearest (Fig. 16).

Marginal growth deposition is not uniform among all cross-section reading axes. For example, some walleye pollock otoliths tend to grow most quickly on the dorsal tip, whereas others, especially those from older fish, accrete new material most quickly near the ventral tip.

**Identification of the first annual mark.** The first translucent growth zone, or annual mark, is typically visible on the surface of whole walleye pollock otoliths. Bering Sea walleye pollock stocks spawn over a broad time period, and thus the size of the first annual mark may depend on the hatch date of the fish. We expect the first annual mark of a late-spawned (summer or early fall) fish to be smaller in size than that of a fish spawned early in the spring. Age readers should be aware of variation in size of the first annual mark (Fig. 17), since the time and location of walleye pollock spawning in the Bering Sea varies widely.

Information on the first annual mark's microstructure is a valuable aid in its identification. The central area of the first annual zone (Fig. 18) appears darker

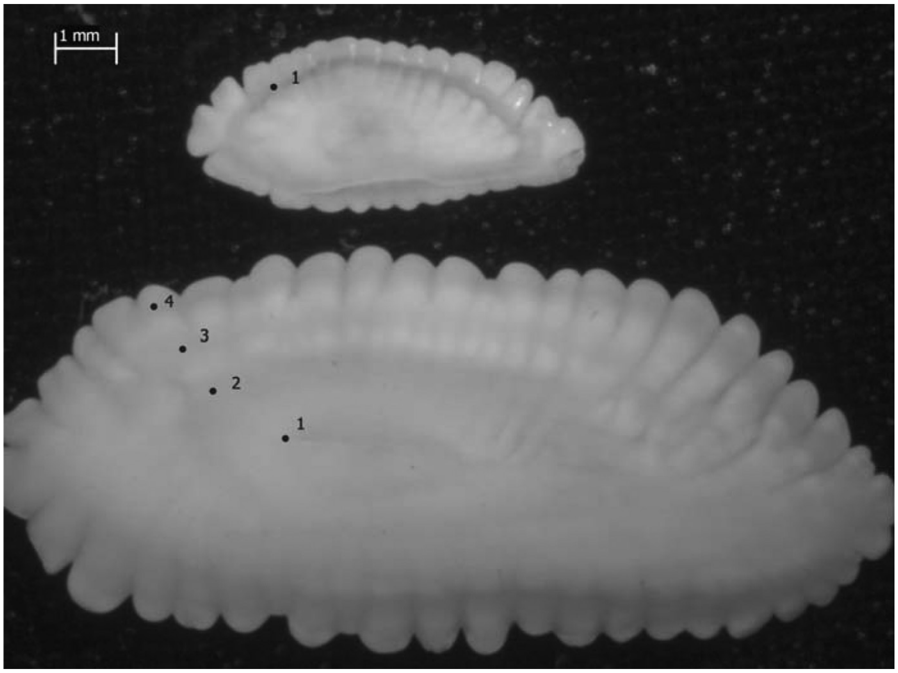

**Figure 17**
Variation in first annual mark size in Bering Sea walleye pollock (*Theragra chalcogramma*) otoliths. First annual mark of the 1-year-old (upper) otolith is larger than the first annual mark of the 4-year-old (lower) otolith. Upper otolith collected from a 150 mm male on 21 June 2003. Lower otolith collected from a 410 mm female on 3 July 2003. Viewed with reflected light.

because daily increments are arranged densely around the otolith core (Nishimura and Yamada, 1988). While the core of some otoliths is very dark, in others it is barely visible. During the first year of growth, daily increments gradually widen in the first annual opaque growth zone, which usually forms during spring and summer (Nishimura and Yamada, 1988). As growth slows down in winter, daily increment widths diminish until increments become obscure within the first translucent growth zone (Nishimura and Yamada, 1988).

One of the problems encountered when interpreting the first annual mark on otoliths of walleye pollock is the occasional formation of checks in the first year (Fig. 19). One or two translucent growth zones sometimes precede the first annual mark on otoliths of young walleye pollock (LaLanne, 1979). These zones are seldom apparent after the third and fourth year of growth because otoliths thicken with age (LaLanne, 1979). An otolith growth check is formed during the postlarval period at body lengths of 8–11 mm, independently of age and growth patterns and corresponding to initiation of calcification in the endoskeletal system (Nishimura, 1993). Nishimura[1] recognized three different growth patterns in age-1 otoliths: 1) otoliths with a single translucent growth zone, 2) otoliths with two translucent growth zones, and 3) otoliths with three translucent growth zones. In each of these three growth patterns, only the outermost translucent growth zone, ranging between 4.5 and 5.1 mm in diameter, was identified as the first annual mark because, unlike the other translucent growth zones, its daily growth increments were compressed (Nishimura[2]).

When the first annual mark is not clear on the otolith surface, corresponding break-and-burn or break-and-bake preparations may increase the prominence of the first annual mark and make checks less visible (Fig. 20). However, age readers should be aware that variation in

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[1] Nishimura, A. 1998. Personal commun. at workshop, as noted in: Report from the second workshop on ageing methodology of walleye pollock (*Theragra chalcogramma*), Alaska Fisheries Science Center, Seattle, WA, 17–20 March 1998, [Available from http://www.afsc.noaa.gov/REFM/Age/age_pollock_workshops.htm, accessed March 2011.]

[2] Nishimura, A. Unpubl. manuscr. False ring observed in the otolith of age 1 walleye pollock collected in the Bering Sea. Kumaishi Branch, Hokkaido Fish Hatchery, 043-04 Kumaishi, Hokkaido, Japan.

size and spacing of annual marks may make it difficult to interpret the growth pattern and determine the first annual mark.

**Age determination problems.** A common growth pattern in Bering Sea walleye pollock is a nebulous second annual mark. A strong, clear first annual mark is commonly followed by one or two diffuse, darkened areas that could be interpreted as either two discrete annual marks or a single broad translucent growth zone. Sectioning the otolith and viewing the unburned face or burning the otolith may improve clarity. If the mark is well-defined, discrete, and continuous, it is considered to be an annual mark.

Crystallization is a common problem in walleye pollock otoliths (Fig. 21). In most cases, only one otolith is crystallized. Crystallized otoliths may exhibit dense nodules on the proximal surface or they may be diffusely crystallized over a large area, sometimes over the entire otolith. Annual marks are typically obscured in crystallized areas, so otoliths with extensive crystallization usually cannot be aged.

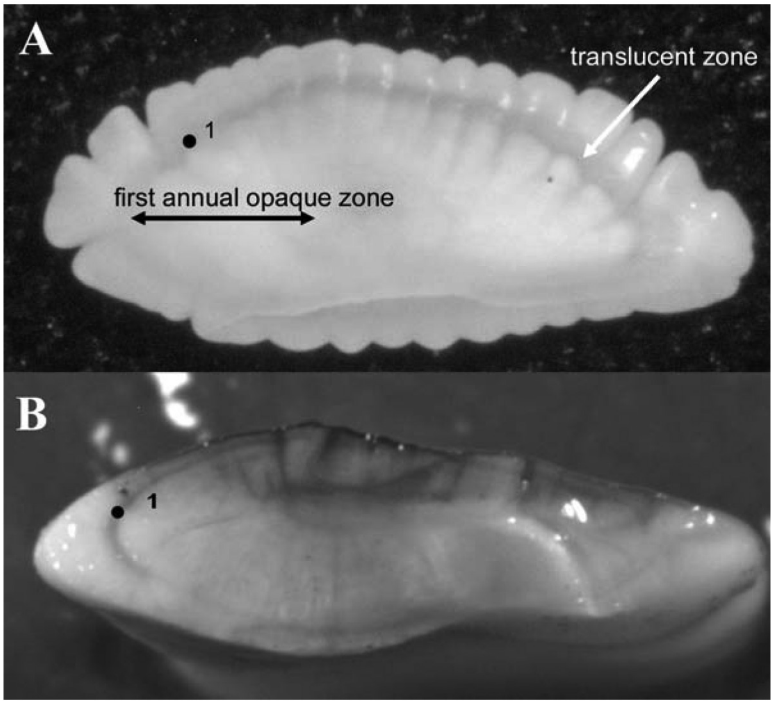

**Figure 18**

(**A**) Otolith surface and (**B**) break-and-burn pattern from a 1-year-old, 150 mm male walleye pollock (*Theragra chalcogramma*) collected from the Bering Sea on 21 June 2003. First annual mark consists of an opaque (light-colored) growth zone around the core and a translucent (dark-colored) growth zone. Opaque growth around the otolith edge is not included in the age estimate because it likely occurred during the collection year. Viewed with reflected light.

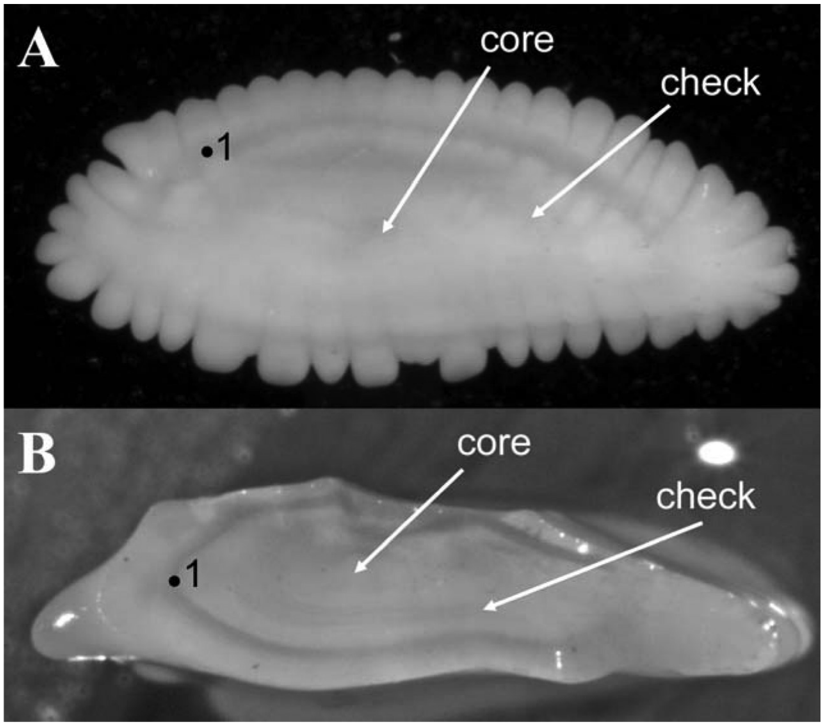

**Figure 19**

One-year-old walleye pollock (*Theragra chalcogramma*) (**A**) otolith surface and (**B**) break-and burn patterns. Checks are evident within the first year. Collected from a 190 mm male in the Bering Sea on 3 July 2003. Opaque growth around the otolith edge is not included in the age estimate because it likely occurred during the collection year. Viewed with reflected light.

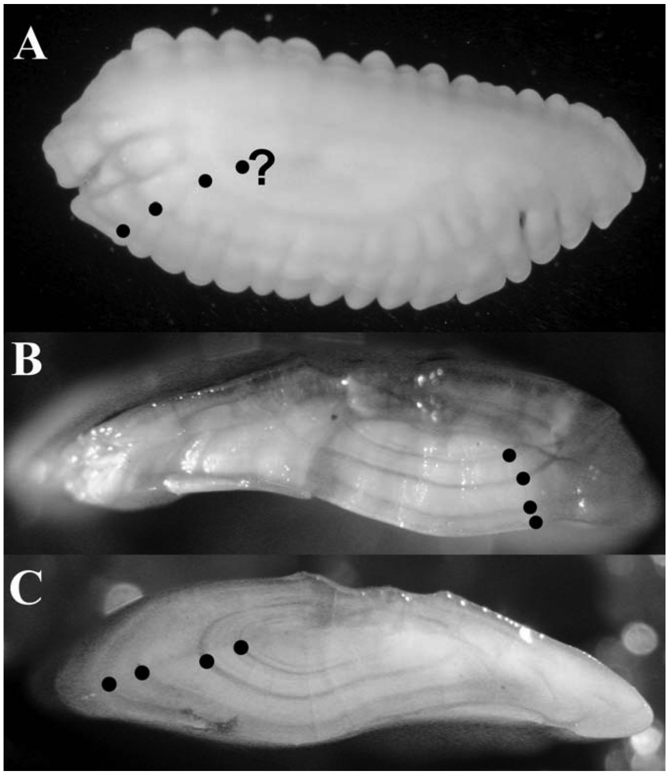

**Figure 20**

(**A**) Surface, (**B**) break-and-burn, and (**C**) break-and-bake patterns from a Bering Sea walleye pollock (*Theragra chalcogramma*) otolith. Surface pattern is clear with the exception of the first annual mark, which appears to be preceded by a faint check (indicated by question mark in **A**). In (**B**) and (**C**), the first translucent growth zone is more prominent, likely making this a 4-year-old with a small first annual mark. Collected from a 330 mm male on 21 July 2004. Viewed with reflected light.

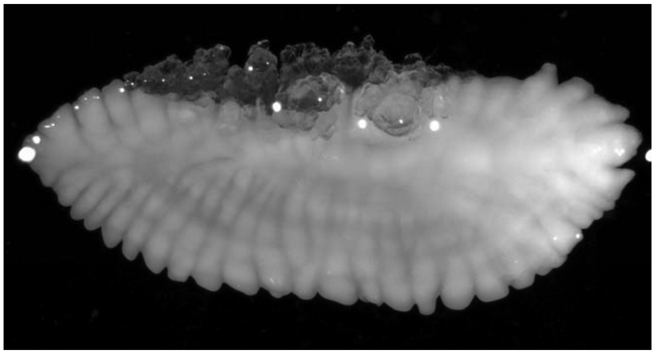

**Figure 21**

Distal surface of a walleye pollock (*Theragra chalcogramma*) otolith with moderate crystallization along the ventral edge. Viewed with reflected light.

## Pacific cod (*Gadus macrocephalus*)

**by Christopher G. Johnston and Delsa M. Anderl**

### Biology

Pacific cod (*Gadus macrocephalus*) are found in continental shelf and upper continental slope waters of the North Pacific Ocean, from China's northern Yellow Sea along the North Pacific Rim, in the Bering Sea as far north as the Chukchi Sea, and south along the Gulf of Alaska and the coast of North America to Santa Monica Bay, California (Love, 1991; Westrheim, 1996). This species is the target of a major multiple-gear fishery in Alaskan waters.

Pacific cod are moderately fast-growing and short-lived compared to many other Alaskan groundfish species. Spawning generally occurs between January and April at depths of 40 to 120 m (Klovach et al., 1995). Hatching occurs in 8–28 days depending on water temperature and salinity (Alderdice and Forrester, 1971; Hart, 1973). Larvae are approximately 3–4 mm in length at the time of hatching (Palsson, 1990) and 40 mm at the time of settlement (Laurel et al., 2009). Adults inhabit depths ranging from 10 to 875 m, although they are most frequently found between 50 and 300 m (Allen and Smith, 1988; Love, 1991). Tagging studies suggest Pacific cod make seasonal migrations to spawn and feed, moving considerable distances within the Bering Sea, eastern Aleutian Islands, and Gulf of Alaska (Shimada and Kimura, 1994). According to AFSC Age and Growth Program data, Pacific cod in the Bering Sea tend to grow more slowly and to larger sizes than those in the Gulf of Alaska. Von Bertalanffy growth parameters were $L_\infty$=1220.9 mm, k=0.1353/yr, and $t_0$=–0.144 yr (*n*=1440) and $L_\infty$=876.3 mm, k=0.2376/yr, and $t_0$=–0.118 yr (*n*=471) for Pacific cod collected during trawl surveys in the Bering Sea and Gulf of Alaska, respectively, in 2007 (Fig. 22). The maximum age reported to date by the Age and Growth Program for Pacific cod is 17 years.

### Age determination history

Pacific cod is one of the most difficult Alaskan groundfish species to age. Various hard parts have been tested as aging structures, but no structure consistently shows clear early annual marks (Kennedy, 1970; Ketchen, 1970). From 1976 to the early 1980s, the AFSC Age and Growth Program used scales to determine ages of Pacific cod. Thereafter, otoliths became the exclusive aging structure for Pacific cod, primarily using the break-and-burn or break-and-bake methods (Chapter 3).

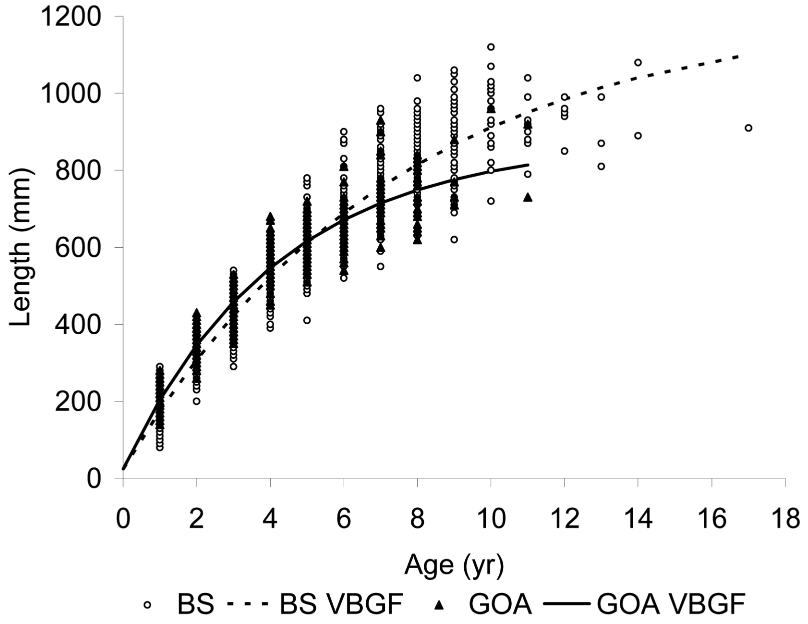

**Figure 22**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for Pacific cod (*Gadus macrocephalus*) collected during trawl surveys in the Bering Sea (BS; *n*=1440) and Gulf of Alaska (GOA; *n*=471) in 2007.

An unexplained decline in length-at-age observed in the early 1990s, combined with the inherent difficulty associated with Pacific cod aging, resulted in a decade-long suspension of production age determination of Pacific cod at the AFSC. A study to reevaluate Pacific cod aging criteria and investigate the length-at-age shift was initiated in 1998 (Roberson, 2001; Roberson et al., 2005). Otoliths collected from tagged and recaptured Pacific cod were embedded in black resin and thin-sectioned to approximately 0.2 mm thick. Back-calculation of fish lengths from these otoliths showed that criteria-based identification of checks and annual marks was correct. Otoliths from this study seemed to have better pattern clarity than those prepared by the break-and-burn method. However, thin-sectioning is a very time-consuming method of preparation, and Roberson's age estimate precision was no higher using thin-sections than break-and-burn preparations. With regard to the decline in length-at-age, it was not possible to identify a single contributing factor; Roberson's results indicated that both a true shift in population growth parameters and changes in otolith aging criteria may have occurred (Roberson et al., 2005).

Since 1990, the AFSC Age and Growth Program has aged over 23,000 Pacific cod otoliths from the Gulf of Alaska, Bering Sea, and Aleutian Islands. Estimates of inter-reader precision reflect the relative difficulty of age determination for this species (CV=7.74%; *n*=7531). Pacific cod age estimates have been corroborated by Roberson's work and by tracking the length mode of the strong 1977 year class in the eastern Bering Sea (Lai et al., 1987; Kimura and Lyons, 1990). Stable isotope and bomb radiocarbon studies (Chapter 6) are currently underway at the AFSC to provide a stronger form of age validation for Pacific cod.

## Current age determination methods

**Surface examination.** Surfaces are viewed using a dissecting microscope and reflected light. Pacific cod growth patterns range from being barely visible to being invisible on the otolith surface. However, surface examination gives age readers a relative idea of the expected size and age of the fish. With the exception of very small otoliths, determining age from the surface alone is not usually an appropriate method for this species.

**Break-and-burn/break-and-bake examination.** Otoliths are cut transversely through the core using a low-speed saw (Chapter 3). Care is taken to ensure that the saw blade cuts through the otolith cleanly in an even plane. Bumps and fractures on the otolith reading surface can produce pattern disruptions, further complicating the identification of annual marks. The saw cutting speed should be set slower for small otoliths than for larger ones. The otolith must be embedded firmly in the clay and held in place with either the reader's thumbs or a pair of forceps. This is particularly important at the end of the cutting process when loosely-anchored otoliths tend to snap, resulting in an uneven reading surface. If necessary, ledges and other surface roughness may be sanded using 600-grit sandpaper. Sanding should only be done sparingly because it can sometimes blur the exposed pattern.

Before further treatment, the otolith halves are placed under water in a Petri dish to examine the cut surfaces. Occasionally the pattern appears sharper on one of the halves than the other, in which case the clearer of the two should be selected for further treatment. If possible, the reader should attempt to determine age from the untreated surface, sometimes referred to as the unburned face.

Contrast between opaque and translucent growth zones is increased by either the break-and-burn method or break-and-bake method (Chapter 3). Method choice is left to the age reader's discretion and may be decided on a per-otolith basis. Using the break-and-burn method for Pacific cod otoliths requires care and attention, but a well-controlled burn can result in clear patterns. Burning these otoliths in a very hot flame quickly produces unevenly burned or over-burned patterns. A very hot flame also can cause fractures in the otolith surface, leading to loss of vital material, especially at the dorsal and ventral tips. Thus, the break-and-bake method is generally the preferred method for age determination of Pacific cod at the AFSC. Pacific cod otoliths are usually toasted for a minimum of 4.5 minutes at 390°–460°F, but baking time and temperature can be varied to optimize contrast between growth zones.

Oven-baked Pacific cod otoliths typically show more uniform contrast and finer growth pattern details compared to burned otoliths. One drawback of baking is that it is more likely to expose checks, whereas flame burning tends to burn checks away. Unfortunately, it is hard to control the flame to avoid burning away the annual marks, too. Baked otoliths usually have a fainter but more regular pattern of opaque and translucent growth zones compared to burned otoliths. Some age readers bake the first otolith half, and if they cannot decide upon an age using the baked section, they will burn the second half. All Pacific cod otolith cross sections are viewed with a dissecting microscope and reflected light.

**Applying aging criteria.** A few Pacific cod otoliths have distinct, strong annual patterns (Fig. 23), though most patterns are faint and thread-like (Fig. 24). Some specimens are easy to prepare for age determination while others shatter with minimal heat. Checks are frequently

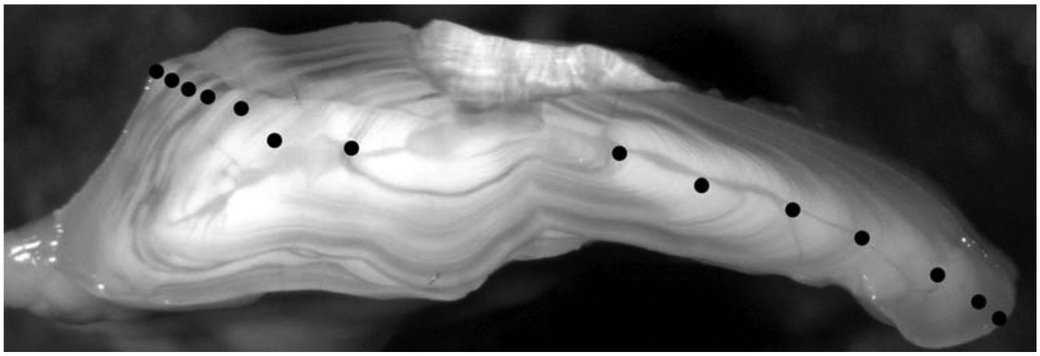

**Figure 23**

Unusually clear Pacific cod (*Gadus macrocephalus*) otolith collected from an 830 mm female captured in March. Age estimate is 7 years. Viewed with reflected light.

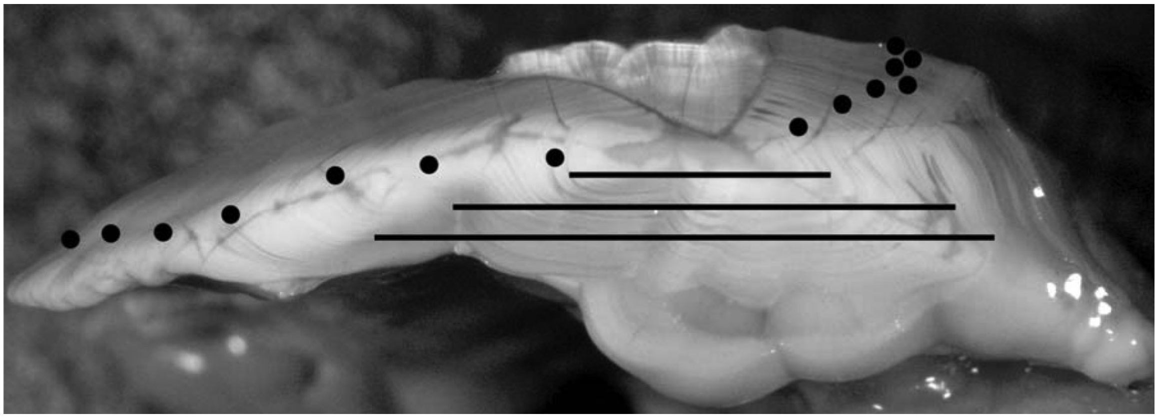

**Figure 24**

A typical Pacific cod (*Gadus macrocephalus*) otolith, collected in March from a 780 mm female. Identification of the first three annual marks (represented by bars) is critical to avoiding over-aging. Age estimate is 7 years. Viewed with reflected light.

observed during periods of rapid growth early in life. Pacific cod otoliths develop multiple growth checks that can be easily mistaken as annual marks during their first three years. The difficulty in determining age for this species is that the general properties defining checks and annual marks do not appear to be applicable to Pacific cod. Checks are normally easily identified as faint, discontinuous rings between darker, more distinct annual marks (Fig. 25). However, difficult early growth patterns are fairly common in Pacific cod otoliths (Fig. 26). Checks in the first two years are sometimes as strong as annual marks, and occasionally the first three annual marks are as faint and discontinuous as checks.

Specific criteria have been defined to aid age readers in identifying the first three annual marks in break-and-burn and break-and-bake preparations. From an initial set of clear otoliths, measurements of the distance from the dorsal to ventral side of each annual mark were used to define expected size ranges for the first three annual marks. These are 2.3–3.8 mm for the first annual mark, 4.6–5.6 mm for the second annual mark, and >6.15 mm for the third annual mark. These width ranges are used as supplemental information in identifying the first three annual marks when the pattern is difficult to discern (Figs. 24 and 27).

Identifying annual marks after the third year requires close examination of the five reading axes in Pacific cod break-and-burn otolith preparations. These reading axes are transects between the otolith core and the following points: the ventral side of the sulcus, the dorsal side of the sulcus, the ventral tip, the dorsal tip, and the anti-sulcus (Fig. 28). The best reading axes are the ventral sulcus and the dorsal sulcus, followed by the anti-sulcus and the dorsal tip. Generally, the least helpful axis is along the ventral tip. Rarely, the same number of annual marks can be observed in more than two of the five reading axes (Fig. 29). When possible, annual marks should be followed around the otolith cross section from one reading axis to another.

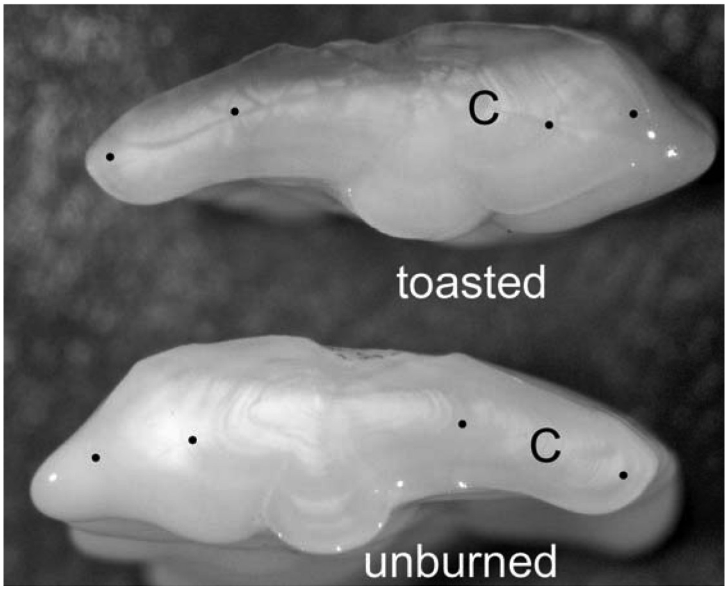

**Figure 25**

Faint Pacific cod (*Gadus macrocephalus*) otolith with checks (indicated with a C) before first and second annual marks. Collected in June from a 340 mm male. Age estimate is 2 years. Viewed with reflected light.

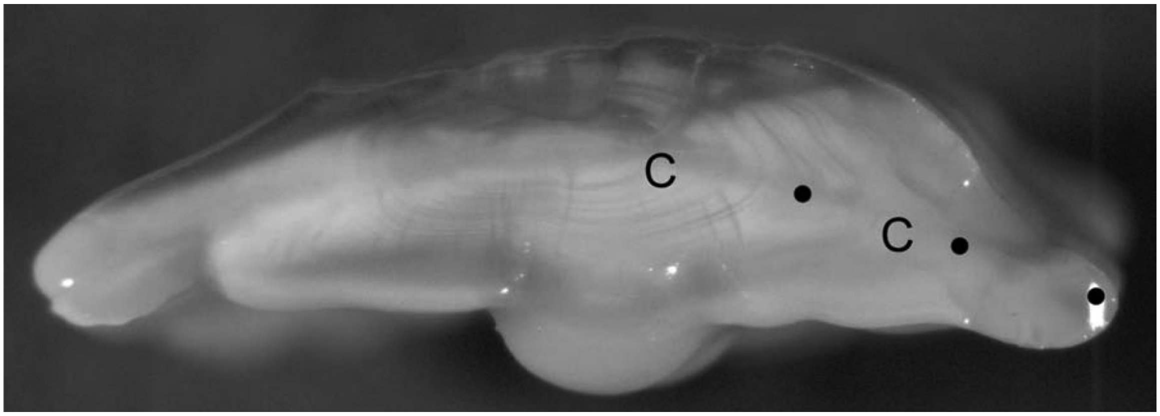

**Figure 26**

Faint Pacific cod (*Gadus macrocephalus*) otolith with multiple checks (indicated with a C). Collected in June from a 340 mm male. Age estimate is 3 (or possibly 2) years. Viewed with reflected light.

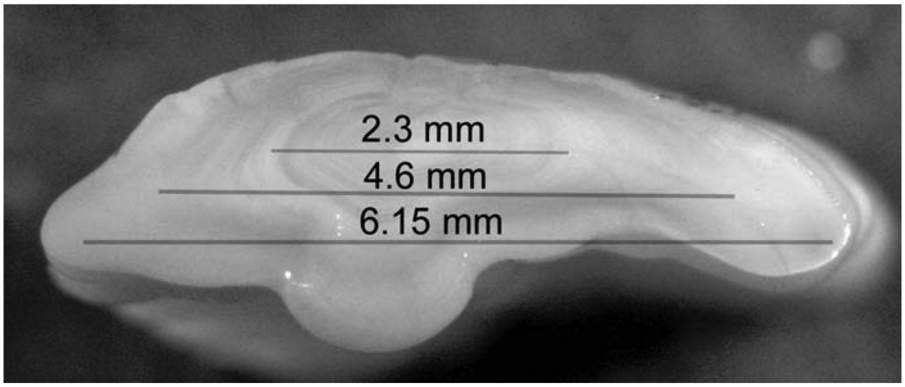

**Figure 27**

Dorsal-ventral measurements of first, second, and third annual marks in a Pacific cod (*Gadus macrocephalus*) otolith cross section. Collected in June from a 320 mm female. This fish is small in length for its age, but the diameter of the third (outermost) annual mark fits the aging criteria for a 3-year-old. Viewed with reflected light.

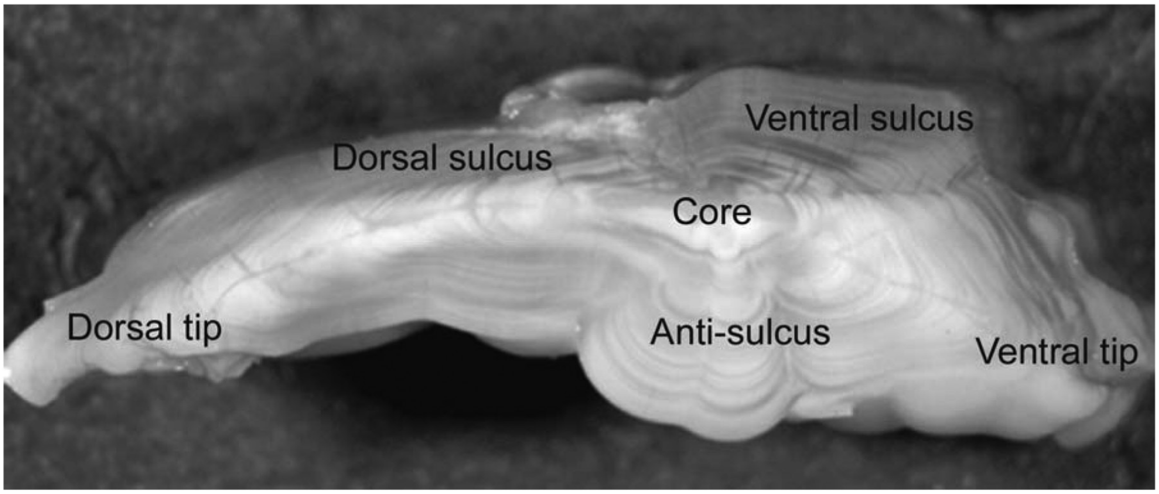

**Figure 28**

Reading axes for Pacific cod (*Gadus macrocephalus*) otoliths. Viewed with reflected light.

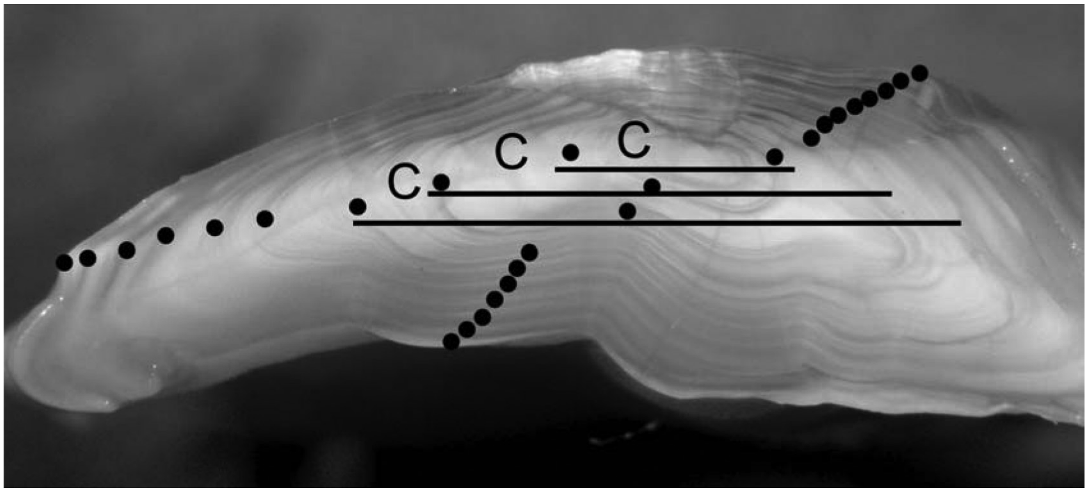

**Figure 29**

Pacific cod (*Gadus macrocephalus*) otolith with checks (indicated by C) before first, second, and third annual marks. Annual mark counts along the dorsal sulcus, dorsal tip, and anti-sulcus axes are identical, but the pattern near the fourth and fifth annual marks is blurry along the ventral sulcus axis. Collected in June from a 700 mm female. Bars represent expected diameters of the first, second, and third annual marks. Age estimate is 9 years. Viewed with reflected light.

## Atka mackerel (*Pleurogrammus monopterygius*)

**by Delsa M. Anderl**

### Biology

Atka mackerel (*Pleurogrammus monopterygius*) are common in the northern Pacific Ocean, ranging from the Kamchatka Peninsula to southeast Alaska. Atka mackerel abundance is highest around the Aleutian Islands, where adults are found in dense and highly localized aggregations at depths less than 200 m (Lowe et al., 2009). Atka mackerel are also the target of a large trawl fishery in the Aleutian Islands. They are an important food source for Steller sea lions (*Eumetopius jubatus*) and other predators (Sinclair and Zeppelin, 2002; Lowe et al., 2009).

The Atka mackerel spawning season lasts from July to October (McDermott and Lowe, 1997). Eggs hatch 44–100 days after spawning, depending on temperature (Lauth et al., 2007). Neuston and bongo net surveys by the AFSC's Fisheries-Oceanography Coordinated Investigations (FOCI) program have found Atka mackerel larvae in nearshore surface waters from September to June. The average standard length (SL) of larvae in September is 8.6 mm, increasing to 34.7 mm by the following June, and larvae become scarce in nearshore collections after April when average standard lengths are greater than 19.2 mm (Anderl et al., 1996). Juvenile Atka mackerel have been collected in surface tows in the central Bering Sea during June, July, and August, with average monthly lengths of 49, 54, and 101 mm SL, respectively (Anderl et al., 1996). During the spring months, young Atka mackerel of about 180 mm fork length (FL) start to appear in bottom trawl surveys (Anderl et al., 1996).

Female Atka mackerel reach 50% maturity by 3.6 years (McDermott and Lowe, 1997). According to AFSC Age and Growth Program data, Atka mackerel from the Gulf of Alaska are larger at age than those from the Aleutian Islands (Fig. 30). Von Bertalanffy growth parameters were $L_\infty$=475.8 mm, k=0.3874/yr, and $t_0$=–0.267 yr (*n*=963) and $L_\infty$=426.5 mm, k=0.419/yr, and $t_0$=0.044 yr (*n*=1765) for Atka mackerel collected during trawl surveys in the Gulf of Alaska and Aleutian Islands, respectively, from 2003 through 2007 (Fig. 30). To date, the oldest Atka mackerel aged by the Age and Growth Program was 15 years, and the largest recorded was 600 mm FL. In the Aleutian Islands, there is strong evidence of a longitudinal gradation in average length at age; fish in the west are smaller at age than in the east (Lowe et al., 1998).

### Age determination history

The AFSC Age and Growth Program began age determination of Atka mackerel in 1979 due to increased

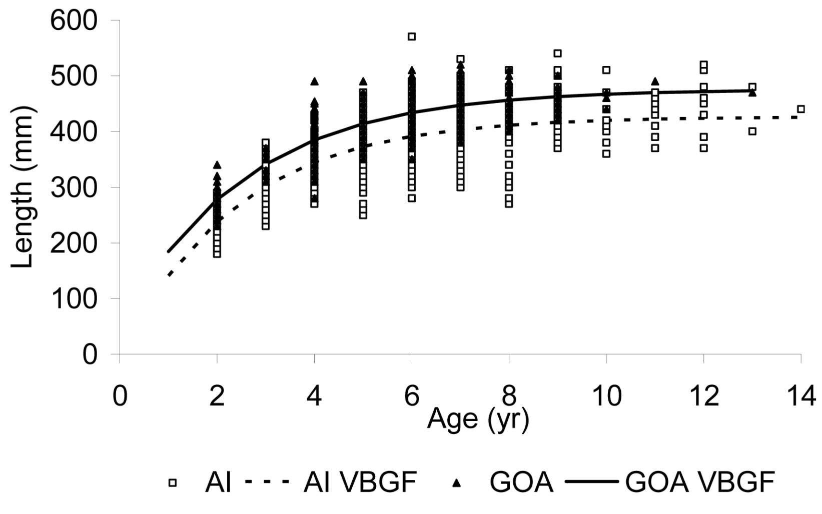

**Figure 30**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for Atka mackerel (*Pleurogrammus monopterygius*) collected during trawl surveys in the Gulf of Alaska (GOA; *n*=963) and Aleutian Islands (AI; *n*=1765) from 2003 through 2007. One year has been added to all age estimates to account for invisible first annual mark (see text).

fishery landings. At that time, very few Atka mackerel age determination studies were published (Bel'chuk, 1938; Gorbunova, 1962), and they did not adequately describe aging methodology. Though the hypural bone was used as an aging structure in one of these early studies (Bel'chuk, 1938), the AFSC Age and Growth Program selected the sagittal otolith because of its relatively clear pattern of alternating translucent and opaque growth zones.

Soon after production age determination of Atka mackerel began at the AFSC, fishery managers recognized that AFSC otolith-based length-at-age data were poorly correlated with published Russian data. Atka mackerel aged by the AFSC appeared to be a year younger than Russian fish of similar length. Adding a year to all Atka mackerel otolith-based age estimates resulted in better comparisons and revealed a large Atka mackerel year class that coincided with the 1977 phenomenon when large year classes were observed in many Alaskan fish species (Hollowed and Wooster, 1995). Because of this, U.S. fishery managers added a year to otolith-based age estimates for stock assessment purposes. Support for this decision came later from larval and juvenile studies. When otoliths from 180 mm FL spring-captured fish were aged, only one strong annual mark was found along the otolith margin (Fig. 31); however, these fish had already lived through two winter seasons (Anderl et al., 1996). We determined that if an annual mark is deposited during the first year of life, its position may be too close to the otolith core to be distinguishable. To maintain consistency with previous protocols, the AFSC Age and Growth Program continues to report ages to data users without a first annual mark, with the understanding that one will be added to all Atka mackerel age estimates during stock assessment.

To date, the AFSC Age and Growth Program has aged over 18,000 Atka mackerel otoliths. Atka mackerel otoliths are relatively easy to interpret, and estimates of precision between age readers tend to be very high. Atka mackerel age estimates have been corroborated by Anderl's work described above and by edge type analysis (Kimura et al., 2007), described in further detail in Chapter 6 of this manual.

**Figure 31**

Otolith from a 180 mm Atka mackerel (*Pleurogrammus monopterygius*) collected in May 1985. Only one annual mark is visible on the surface, but this fish has already lived through two winter seasons. Final age estimate is 2 years (one year added to account for invisible first annual mark).

## Current age determination methods

**Surface examination.** Atka mackerel otoliths are relatively small and easy to interpret when compared with the otoliths of other Alaskan groundfish species. An average 1-year-old otolith is only about 5 mm long. Whole otoliths are examined using a dissecting microscope at 25× magnification with reflected light. Atka mackerel otolith surface patterns are sometimes clear enough that an experienced age reader can assign age estimates up to 6 years without needing to examine the corresponding break-and-burn pattern. The clearest growth pattern is typically found on the posterior (rounded) side of the otolith, especially along the ventral knob (Fig. 32). The first visible annual mark, which is formed during the second year, encompasses a wide surface area when compared with subsequent annual marks. The dorsal-ventral diameter of the first visible annual mark measured through the core is typically about 1.4 mm.

In some cases, the anterior otolith half (pointed end) is the clearer choice for surface age determination. However, for old or difficult otoliths, examining the corresponding break-and-burn pattern (Chapter 3) becomes necessary to determine a final age estimate.

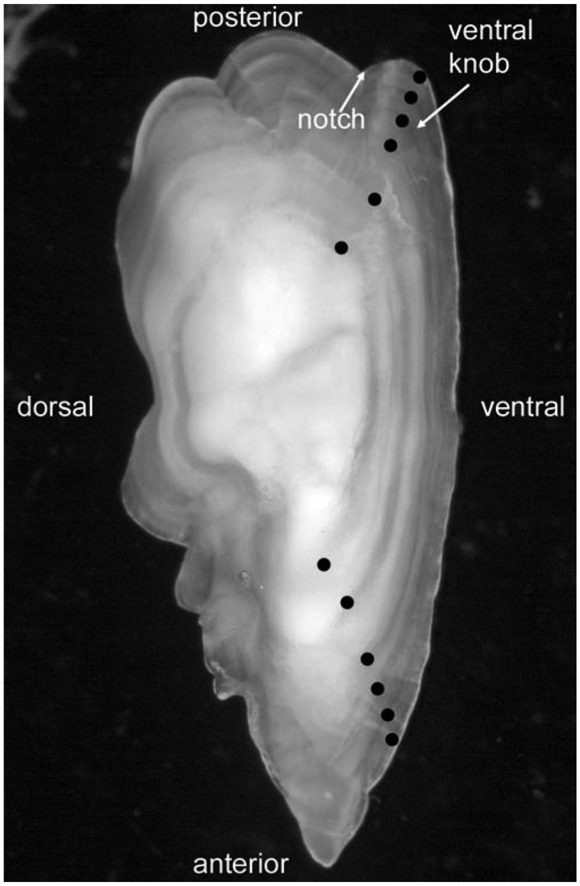

**Figure 32**

Atka mackerel (*Pleurogrammus monopterygius*) otolith with a clear surface pattern. Final age estimate is 7 years (one year added to account for invisible first annual mark). Collected from a 430 mm female in September 2007. Viewed with reflected light.

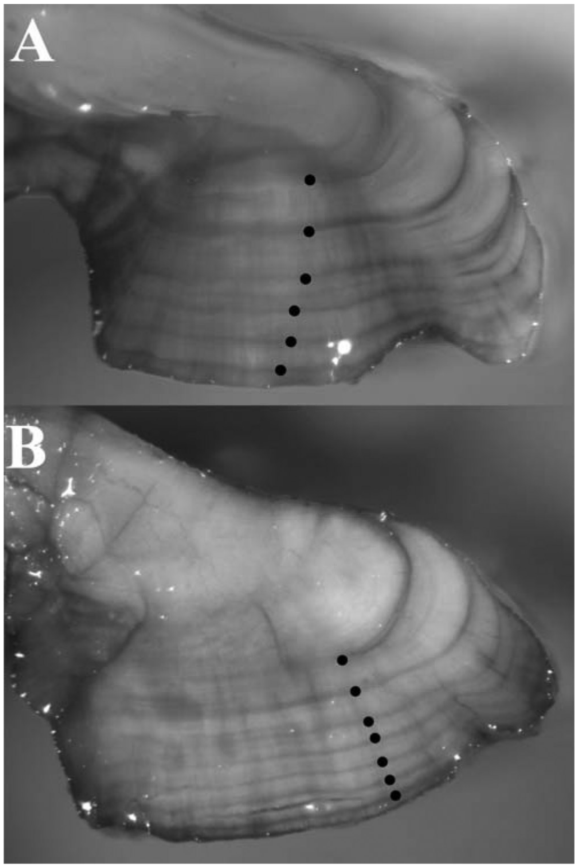

**Figure 33**

Clear break-and-burn cross sections of Atka mackerel (*Pleurogrammus monopterygius*) otoliths from (**A**) 430 mm female with a final age estimate of 7 years and (**B**) 410 mm male with a final age estimate of 8 years. (One year was added to each age estimate to account for invisible first annual mark.) Opaque growth at edge not counted in age estimates because both fish were collected in September 2007. Viewed with reflected light.

**Break-and-burn examination.** To produce a break-and-burn pattern, the otolith is gripped firmly in one's fingers and snapped along the transverse axis using the thumbnails. The broken end is toasted quickly over an alcohol flame until it is golden in color. Atka mackerel otoliths quickly char, so only a few passes over the flame are necessary. The break-and-burn cross section is then viewed with a dissecting microscope and reflected light.

Atka mackerel otoliths produce some of the clearest break-and-burn patterns of all the groundfish species aged at the AFSC (Fig. 33). Annual marks on a break-and-burn cross section are commonly read from the core to the proximal surface of the ventral region. Sometimes the more compressed dorsal region is clearer and can help interpret a questionable ventral pattern (Fig. 34). In most cases, annual marks are continuous and distinct all the way from the ventral edge to the sulcus. Even if the area adjacent to the sulcus is not clear, the banding pattern is often strongest near the ventral edge, which may be sufficient to determine an otolith age estimate (Fig. 35). In the later years, a notch commonly develops on the proximal surface between the outside ventral edge and the sulcus, sometimes creating a disruption in the continuity of older annual marks (Fig. 36).

Recognizing the regular spacing between annual marks is integral to Atka mackerel age determination.

The widest opaque growth zones occur within the first 2 to 5 years. Faint annual marks are not uncommon in Atka mackerel otoliths, and checks can disrupt the growth pattern (Fig. 37). However, because the general growth pattern is so regular across specimens, it is sometimes obvious where an annual mark should be.

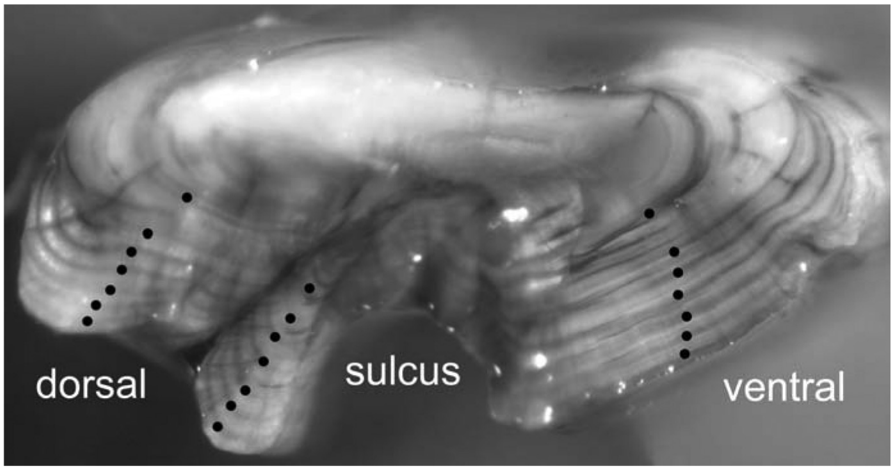

**Figure 34**

Break-and-burn otolith cross section collected from a 660 mm female Atka mackerel (*Pleurogrammus monopterygius*) on 27 October 2008. Both the dorsal and ventral sides were used to arrive at a final age estimate of 8 years (one year added to account for invisible first annual mark). Viewed with reflected light.

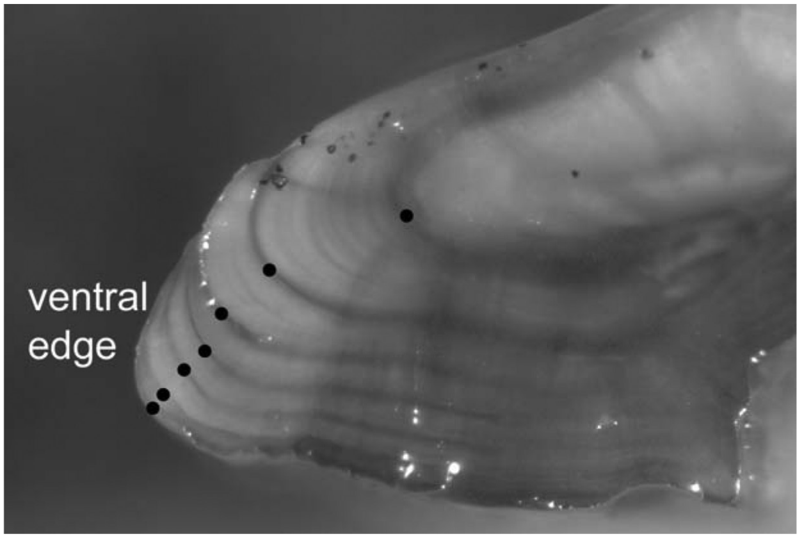

**Figure 35**

Break-and-burn cross section of an otolith collected from a 410 mm female Atka mackerel (*Pleurogrammus monopterygius*) on 25 June 2007. Final age estimate is 8 years (one year added to account for invisible first annual mark). Clearest pattern is along ventral edge. Viewed with reflected light.

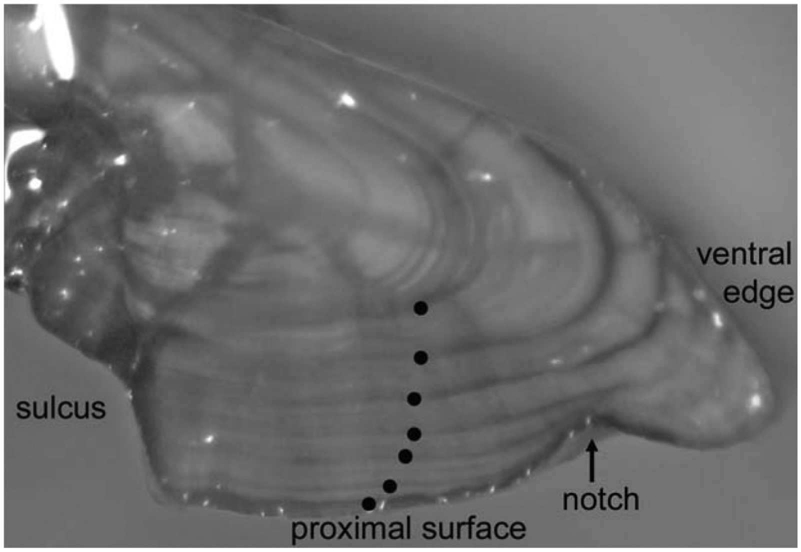

**Figure 36**

Break-and-burn cross section of an Atka mackerel (*Pleurogrammus monopterygius*) otolith, showing proximal surface notch which sometimes disrupts continuity of annual marks from the ventral edge to the sulcus in older fish. Final age estimate is 8 years (one year added to account for invisible first annual mark). Collected from a 420 mm male in July 2007. Viewed with reflected light.

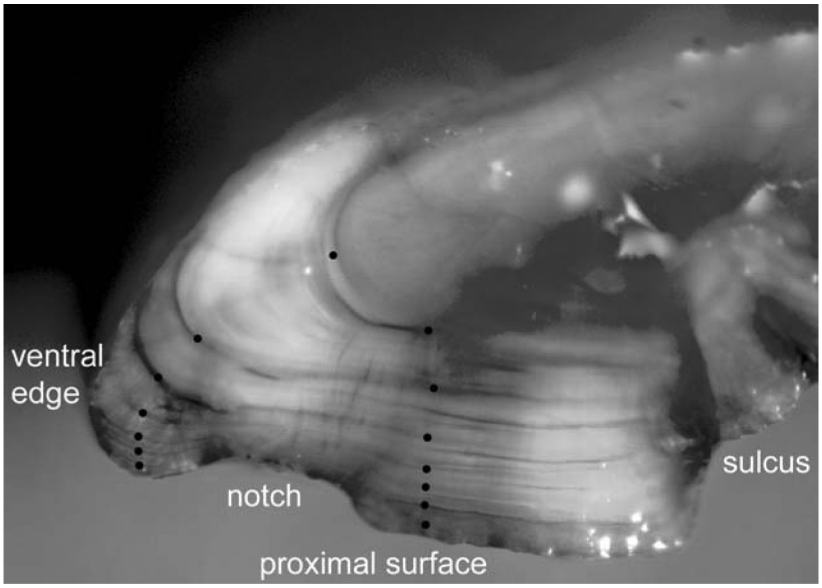

**Figure 37**

Break-and-burn cross section of an Atka mackerel (*Pleurogrammus monopterygius*) otolith. Growth pattern has multiple checks and is difficult to interpret, especially area between proximal surface notch and sulcus. Clearest reading axis is ventral edge. Final age estimate is 8 years (one year added to account for invisible first annual mark). Collected from a 400 mm male in the Aleutian Islands on 15 September 2007. Viewed with reflected light.

## Sablefish (*Anoplopoma fimbria*)

**by Delsa M. Anderl**

### Biology

Sablefish (*Anoplopoma fimbria*) supports a very important and lucrative fishery in the North Pacific and is one of the most studied fish along the North American Pacific coast. Sablefish range from Baja California through the Bering Sea to Kamchatka and Japan (Hart, 1973) and are capable of traveling great distances (Kimura et al., 1998). Tagging data suggest there may be at least two populations of sablefish: an Alaskan population and a West Coast population, with some mixing occurring from British Columbia to the Oregon coast (Kimura et al., 1998). Of the two groups, the Alaskan population appears more mobile. In the Gulf of Alaska, sablefish perform a two-way migration from the continental slope to seamounts located 270–465 km offshore (Maloney, 2004). Furthermore, fish tagged at ages 0–2 in southeast Alaska have been recaptured in British Columbia, the western Aleutian Islands, and the eastern Bering Sea (Maloney and Sigler, 2008). Migratory movement and depth preference are dependent on age; as a result, sablefish in the age-5 and age-6 year classes are most vulnerable to the target fishery (Maloney and Sigler, 2008).

Sablefish in the northeast Pacific Ocean spawn during winter in offshore waters. Eggs hatch in the water column and young larvae then make their way to surface waters. Soon afterwards, they migrate to inland waters and shallow bays (Kendall and Matarese, 1987). They spend about a year in these nursery grounds and then return to the open ocean sometime in their second year. By age-3 or -4, most fish are in offshore waters (Maloney and Sigler, 2008). Young sablefish are fast growers, reaching an average length of 600 mm at age-5 before growth begins to slow down (Fig. 38). Von Bertalanffy growth parameters were $L_\infty$=719.8 mm, k=0.2178/yr, and $t_0$=–3.633 yr (*n*=290) and $L_\infty$=728.0 mm, k=0.2273/yr, and $t_0$=–2.947 yr (*n*=884) for sablefish collected during trawl surveys in the Bering Sea/Aleutian Islands region and the Gulf of Alaska, respectively, in 2007 (Fig. 38). The maximum age reported to date by the Age and Growth Program for sablefish is 94 years.

### Age determination history

Of all the groundfish species studied by the AFSC Age and Growth Program, sablefish is one of the most difficult species to age. The complex growth patterns observed in sablefish otoliths may be related to their extensive geographic and depth migrations. Sablefish were first aged at the AFSC from whole otolith surface patterns. Around 1982, mark-recapture studies demon-

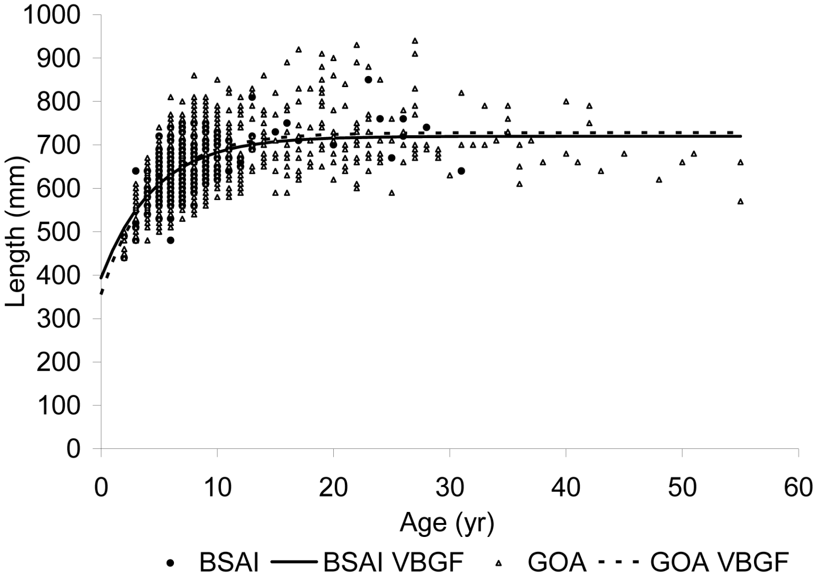

**Figure 38**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for sablefish (*Anoplopoma fimbria*) collected during trawl surveys in the Bering Sea/Aleutian Islands (BSAI; *n*=290) and Gulf of Alaska (GOA; *n*=884) in 2007.

strated that sablefish is a long-lived species and that the break-and-burn method results in more accurate age estimates than using the surface method alone (Beamish and Chilton, 1982; Boehlert and Yoklavich, 1985). The AFSC later confirmed these findings with a radiometric age validation study (Kastelle et al., 1994).

Since 1993, the AFSC Age and Growth Program has aged over 24,000 sablefish otoliths. The average inter-reader CV is 10.8% (*n*=8052), reflecting the relative difficulty of sablefish age determination. The pattern complexity of this long-lived species often requires re-aging some specimens. Otolith length and fish length of sablefish are not always strongly related with age (Fig. 39); this differs from many of the other groundfish studied at the AFSC.

Otoliths collected from sablefish during tagging studies (Heifitz et al., 1998; Rutecki and Varosi, 1997; Maloney and Sigler, 2008) have provided the Age and Growth Program with a rare opportunity to validate our sablefish aging criteria. Most of the images in this chapter are of known-age specimens that were tagged as juveniles (ages 0–2) and later recaptured as adults. Ages at the time of tagging were determined from non-overlapping length frequencies (Maloney and Sigler, 2008).

## Current age determination methods

Sablefish otoliths have several morphologically distinct shapes (Fig. 40). Recognizing each shape and its associated pattern is necessary for accurate age estimates, because sablefish aging criteria are dependent on both otolith shape and growth pattern. Nomenclature helpful in describing sablefish otolith reference points is found in Figure 41. At the AFSC, sablefish otoliths (surfaces and break-and-burn cross sections) are examined using a dissecting microscope and reflected light.

**Surface examination.** Examining the otolith surface pattern should always be the first step in sablefish age

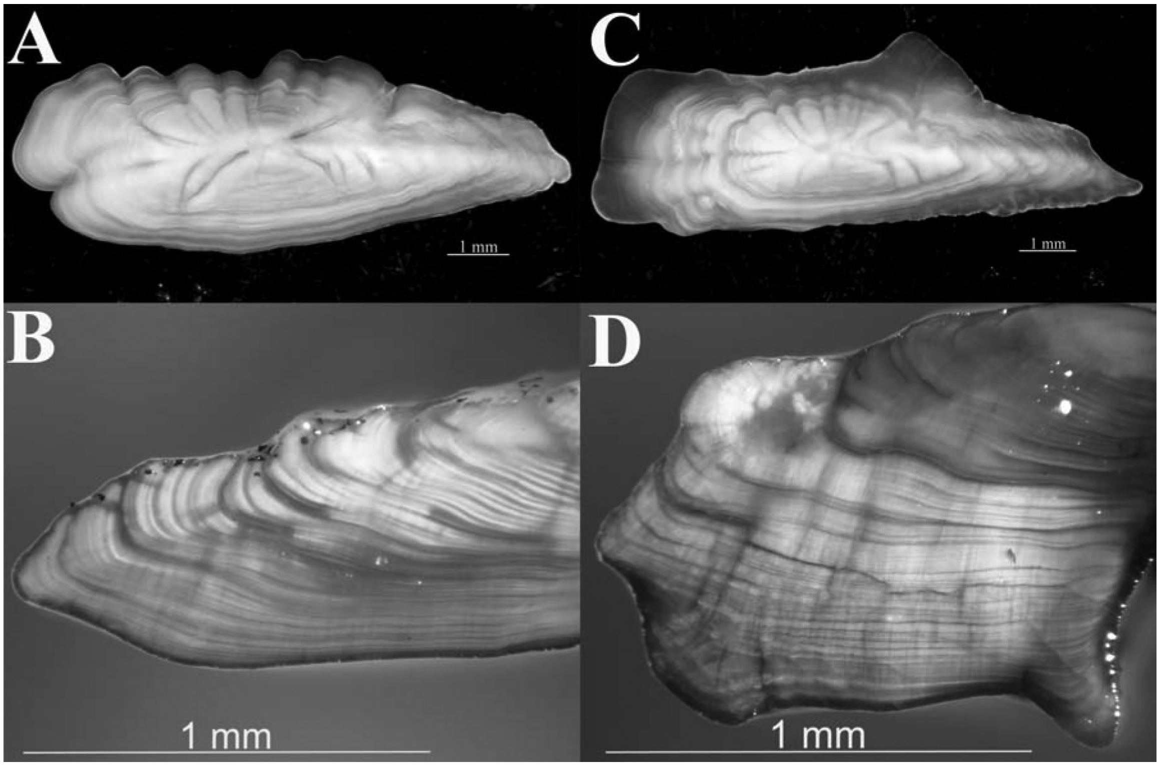

**Figure 39**

Otoliths collected from two different 600 mm sablefish (*Anoplopoma fimbria*), both captured in August. (**A**) Otolith surface and (**B**) break-and-burn preparation from a sablefish aged 4 years. (**C**) Otolith surface and (**D**) break-and-burn preparation from a sablefish aged 23 years. Otolith thickness and differences between otolith surface patterns are good indicators of break-and-burn age estimates. The otolith in (**A**) is relatively thin with distinct, dark annual marks. The otolith in (**C**) is relatively thick and has a broad, dark area observed on the surface following the first several annual marks, indicating the otolith is old. Viewed with reflected light.

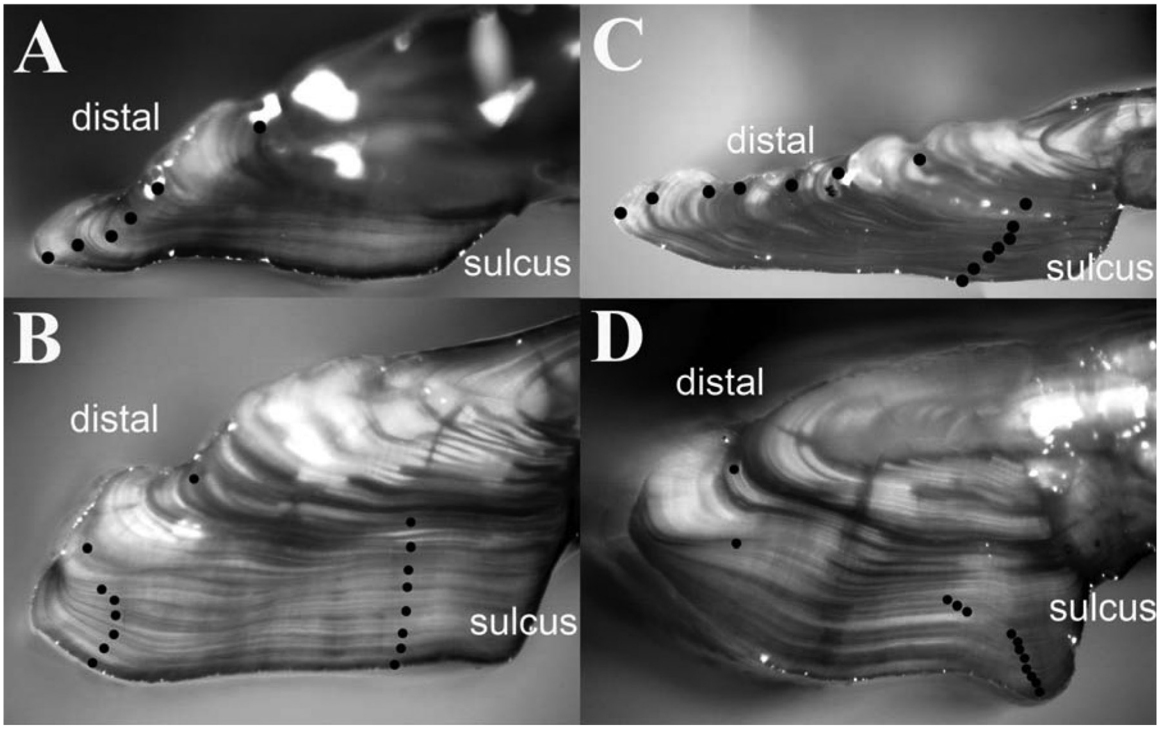

**Figure 40**

Four of several morphologically distinct shapes observed in sablefish (*Anoplopoma fimbria*) otoliths from part of the Alaska Fisheries Science Center Age and Growth Program's known-age collection. The shapes of otoliths in (**A**) (630 mm female) and (**C**) (670 mm female) are associated with fast-growing fish; the preferred reading axis is along the distal margin. The otolith in (**B**) (590 mm male) has translucent growth zones converging along the distal margin. Following each translucent growth zone to the sulcus, when possible, is helpful for identifying true annual marks. The otolith in (**D**) (730 mm male) has the greatest amount of growth along the sulcus. Although it can sometimes be difficult to view compressed annual marks in this region of the otolith, the sulcus is the best reading axis for this morphological shape.

determination. An initial surface age estimate helps with interpretation of the break-and-burn pattern, especially if the pattern has many checks. An intense dark band along the posterior margin, composed of multiple compressed annual marks, is usually a sign that the otolith is old. An otolith's thickness relative to its dorsal-ventral width is typically an indicator of age. However, if an otolith is thick, but the surface pattern looks young, the fish could be young but unusually fast-growing.

The first several annual marks form distinct grooves on the distal surfaces of sablefish otoliths. Break-and-burn cross sections should be tipped slightly, as demonstrated in Figures 42 and 43, to better see the groove patterns. The grooves are followed from the distal surface through the cut surface of the cross section, making it possible to identify the first three to four annual marks (Figs. 42 and 43). Checks are weaker in appearance and do not coincide with grooves, and thus tipping the cross section allows the age reader to discern between checks and annual marks. A check is commonly observed between the first two annual marks.

**Break-and-burn examination.** A few young otoliths with clear surface patterns can be assigned age estimates by examining only the whole otolith surface pattern. However, the overwhelming majority of sablefish otoliths are aged using the break-and-burn method (Chapter 3). The break-and-bake method does not produce enough contrast between translucent and opaque growth zones, and in some cases it appears to accentuate checks. Sablefish otoliths are too small to be effectively cut with a low-speed saw, so cross sections are made by gripping the otolith in one's fingers and snapping it in half along the transverse axis using the thumbnails. An alternative method is to cut through the core with a scalpel. The cut face of the cross section is slowly toasted over an alcohol flame. Sablefish

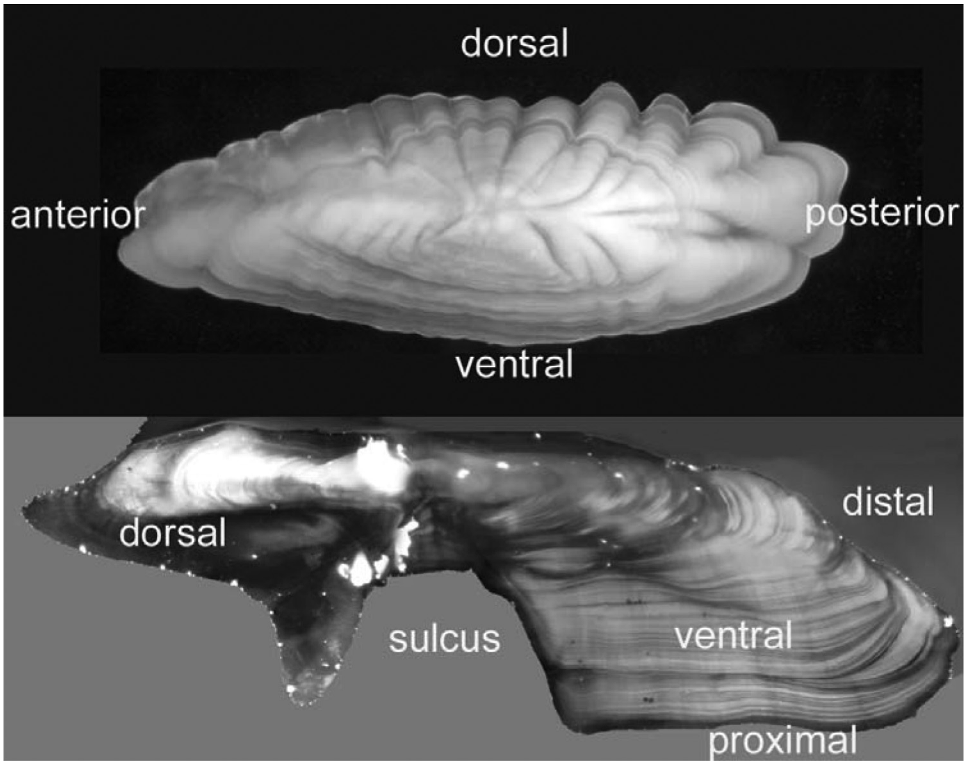

**Figure 41**

Terminology commonly used to describe sablefish (*Anoplopoma fimbria*) otoliths. Upper panel: whole otolith viewed from the distal surface. Lower panel: break-and-burn cross section. Sablefish otoliths are typically aged on the ventral side of break-and-burn cross sections. Viewed with reflected light.

otoliths are usually aged on the ventral side of break-and-burn cross sections. As described above, surface pattern grooves are important in properly aging sablefish otoliths, especially in identifying early annual marks. Tipping the break-and-burn cross section to observe these grooves aids the age reader in evaluating complicated growth patterns (Fig. 43).

Figure 44 is an example of a relatively easy break-and-burn pattern, despite having multiple checks. This otolith is from an 8-year-old from the known-age collection. The last five annual marks are clear on the surface and correspond to five clear growth zones on the break-and-burn cross section. The first three annual marks are more problematic, requiring tipping the break-and-burn cross section to follow individual grooves on the distal surface. The morphological shape of this otolith cross section is relatively common.

Another common morphological shape is one in which the distal edge abruptly curves towards the sulcus (Fig. 45). A transition zone begins after the fourth annual mark, followed by more compressed growth after the sixth annual mark. The area of compressed growth is usually also evident on the otolith surface as a dark band along the margin.

A more extreme case of the pattern described above is where the axis of maximum growth is along the sulcus and growth at the distal margin is greatly reduced (Fig. 46). This pattern is usually reflected on the surface by a dark band of compressed annual marks. Cross sections from this type of otolith must be evenly cut and should be burned slowly and carefully to get the best contrast between highly compressed annual marks.

Another common but problematic pattern is when annual marks converge at a common focal point along the distal margin, and identification of individual annual marks is difficult (Fig. 47). In most cases, when growth patterns are clear but discrepant on two or more reading axes, the area adjacent to the sulcus often (though not always) results in a more accurate age estimate.

Transition zone patterns can also be difficult. There is usually a dark band made up of compressed translucent growth zones observed within the transition zone. The age reader must decide whether the translucent growth zones are components of a single annual mark or if each translucent growth zone is an individual annual mark (Fig. 48).

Figures 49 and 50 show otoliths with less common growth patterns. The oldest sablefish otolith examined to date by the Age and Growth Program had an estimated age of 94 years and a surprisingly clear pattern considering its age (Fig. 51).

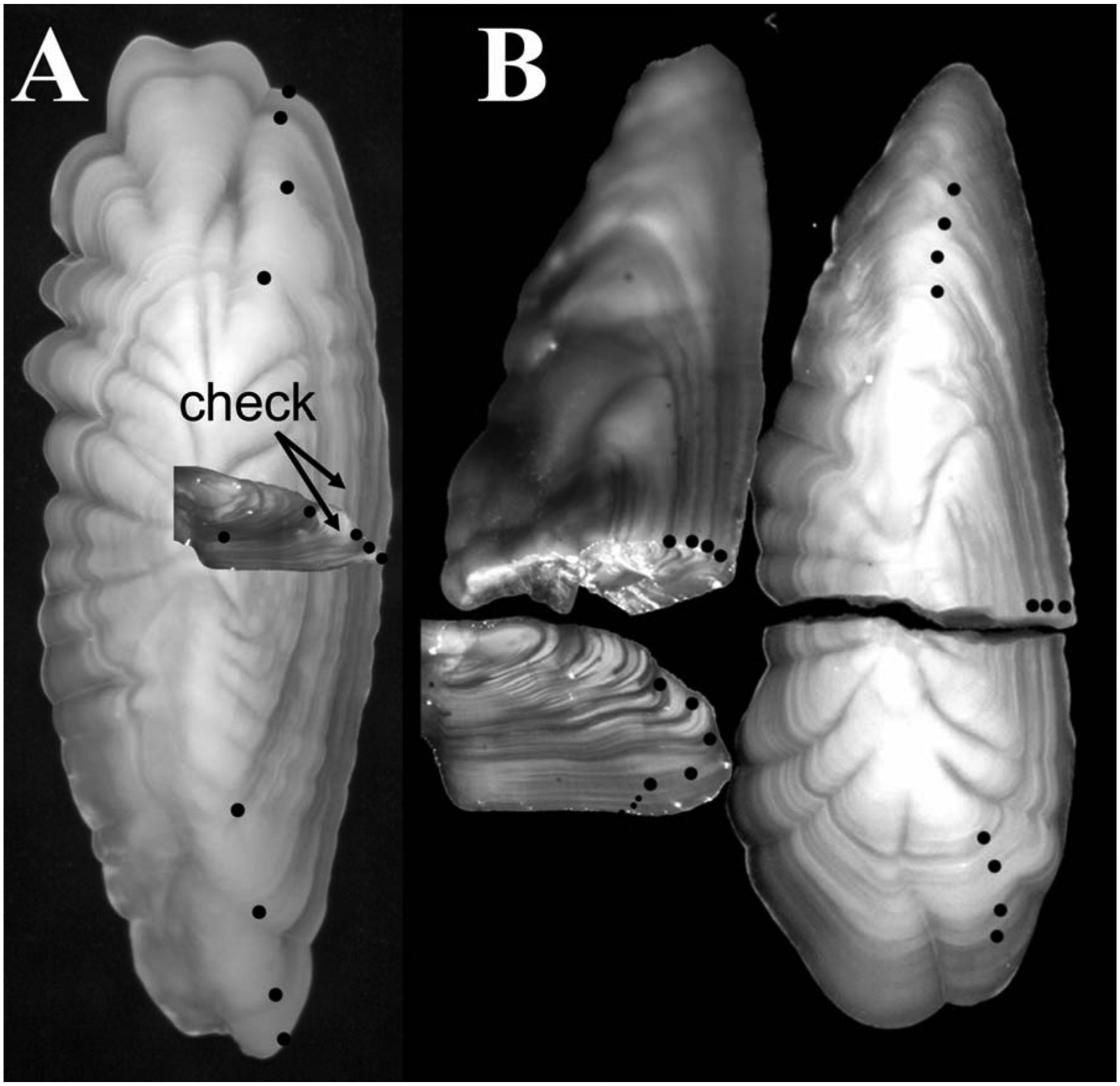

**Figure 42**

Composite image of sablefish (*Anoplopoma fimbria*) otoliths illustrating clear surface groove patterns that can be used to identify early annual marks in break-and-burn cross sections. (**A**) The translucent growth zone observed between the first and second annual marks on the cross section (inset) corresponds to a weak one on the otolith surface and therefore is considered a check. (**B**) Early annual marks appear as strong grooves on the break-and-burn distal surface. These are easier to see when the otolith is tipped (left) and correspond to strong marks on the whole otolith surface (right). Without first examining the surface pattern for corresponding grooves, identifying early annual marks in the two-dimensional cross section is difficult. Viewed with reflected light.

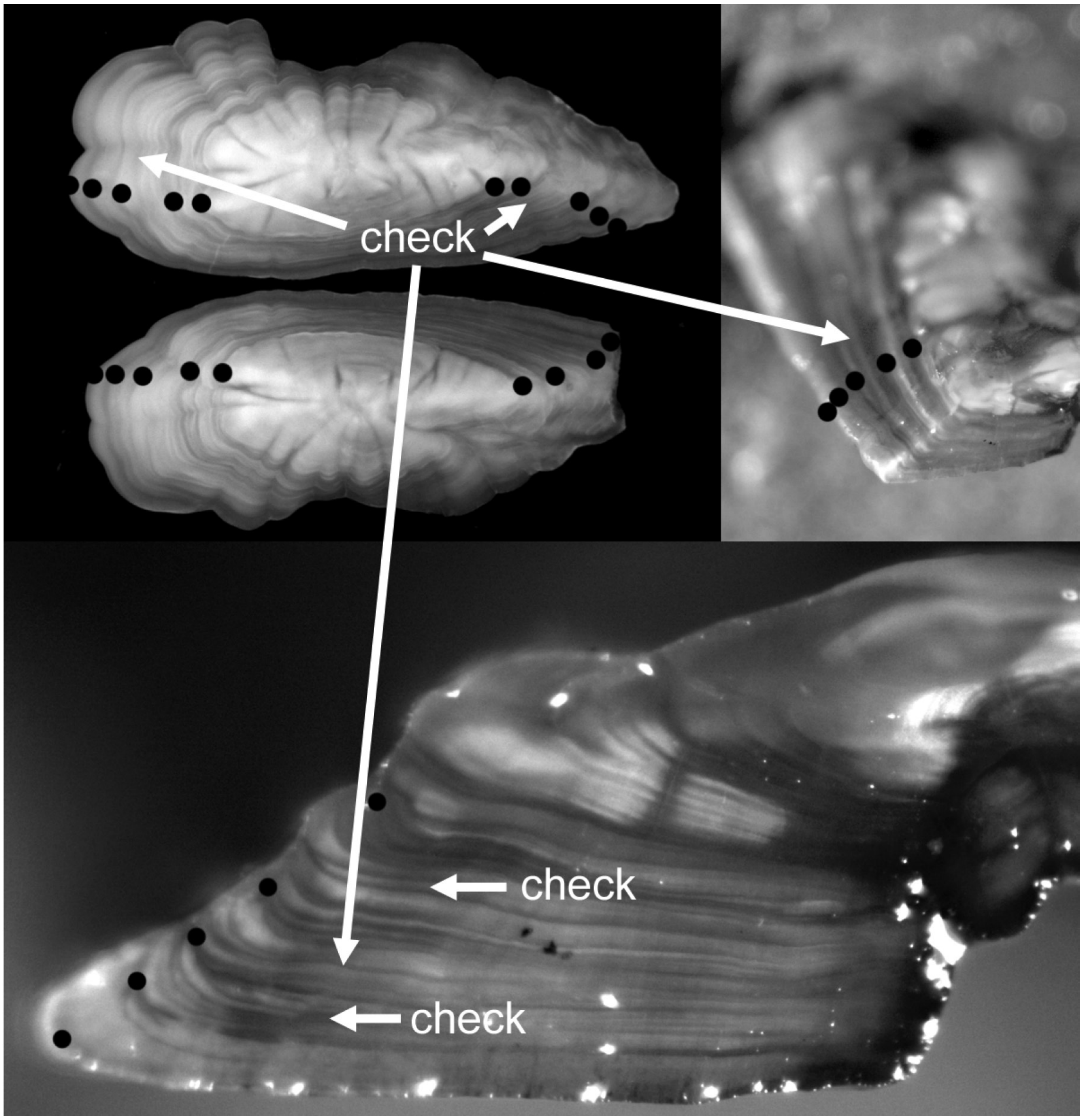

**Figure 43**

Sablefish (*Anoplopoma fimbria*) otolith with multiple checks that could easily be over-aged without careful examination. Collected from a known-age 5-year-old, 555 mm female recaptured in May. Prominent check observed between the second and third annual marks on the otolith surface and break-and-burn cross sections. Tipping the otolith cross section as shown (upper right panel) enhances the grooved annual marks and discerns between checks and annual marks. Viewed with reflected light.

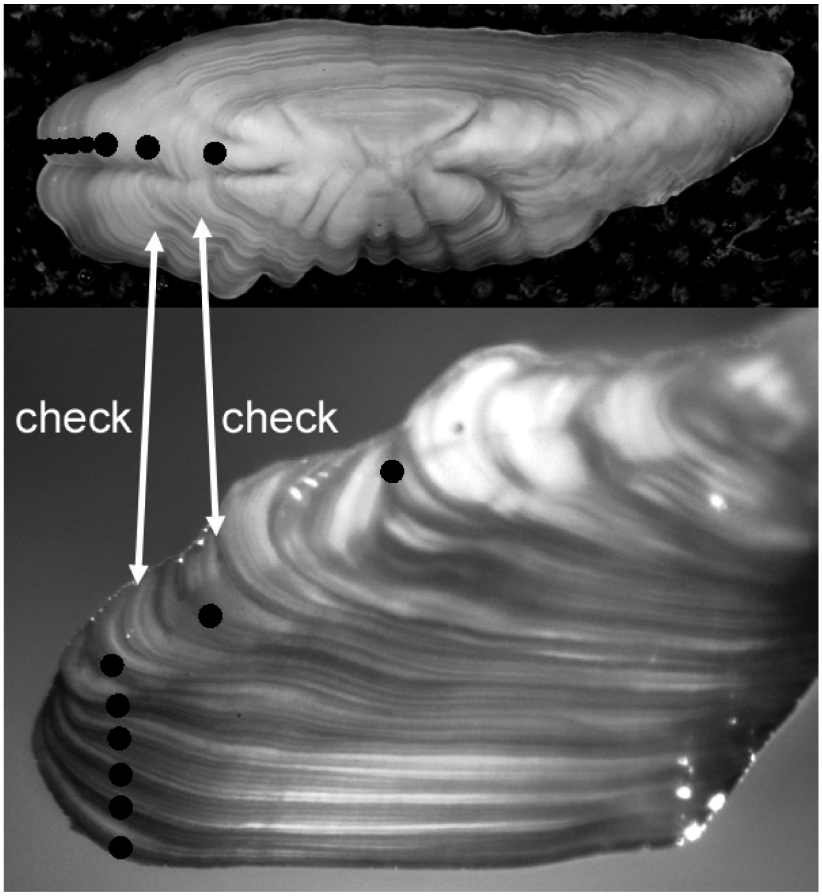

**Figure 44**

Known-age 8-year-old sablefish (*Anoplopoma fimbria*) otolith from a 710 mm female recaptured in April. Distinct growth pattern in the last 5 years. First 3 years are problematic; two translucent growth zones could be mistaken for annual marks but are likely checks because they are traced to weak zones on the whole otolith surface pattern. Viewed with reflected light.

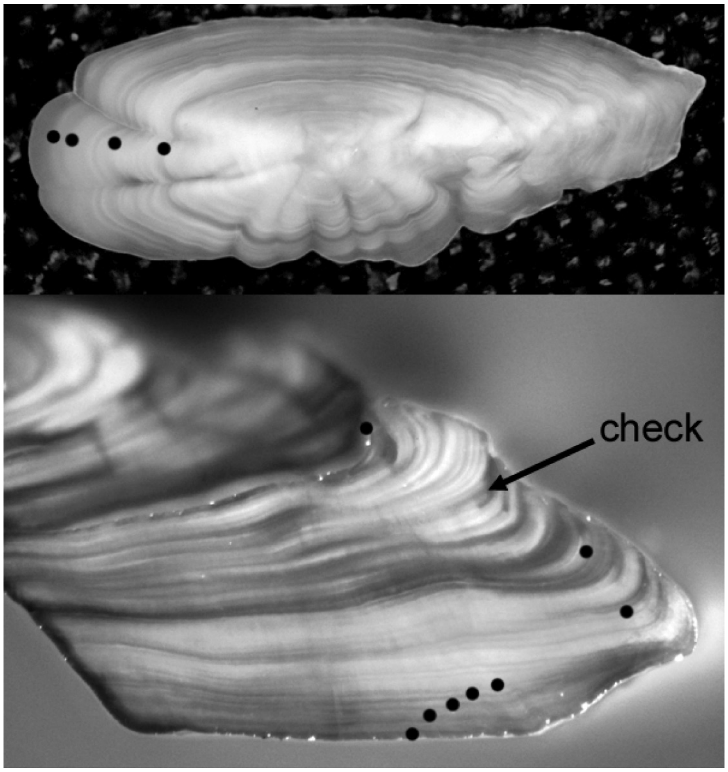

**Figure 45**

Known-age 8-year-old sablefish (*Anoplopoma fimbria*) otolith from a 590-mm male recaptured in March. Common growth pattern with sharp transition from fast to slow growth. Fast growth occurs during the first 3–4 years. After deposition of the fourth annual mark, growth between annual marks slows down significantly (usually associated with a wide dark band along the otolith surface margin). Viewed with reflected light.

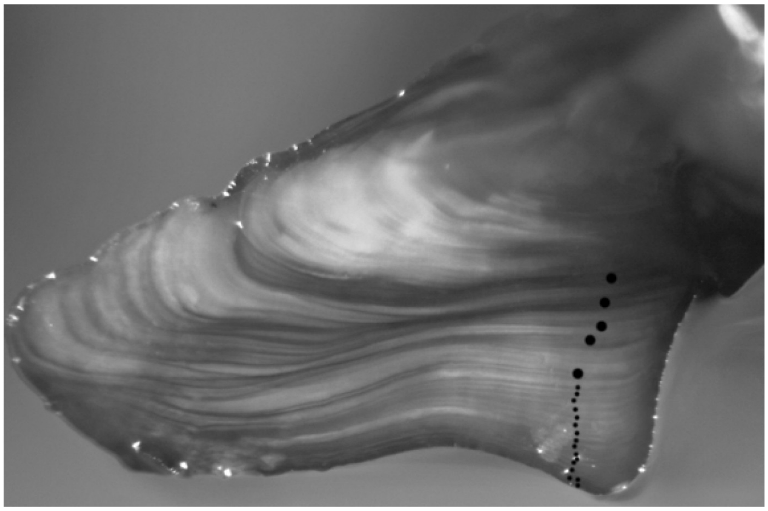

**Figure 46**

Otolith from a 670 mm male sablefish (*Anoplopoma fimbria*). Age estimate is 21 years. Highly compressed pattern. Axis of maximum growth is along sulcus. Distal margin is not useful for age estimation. Viewed with reflected light.

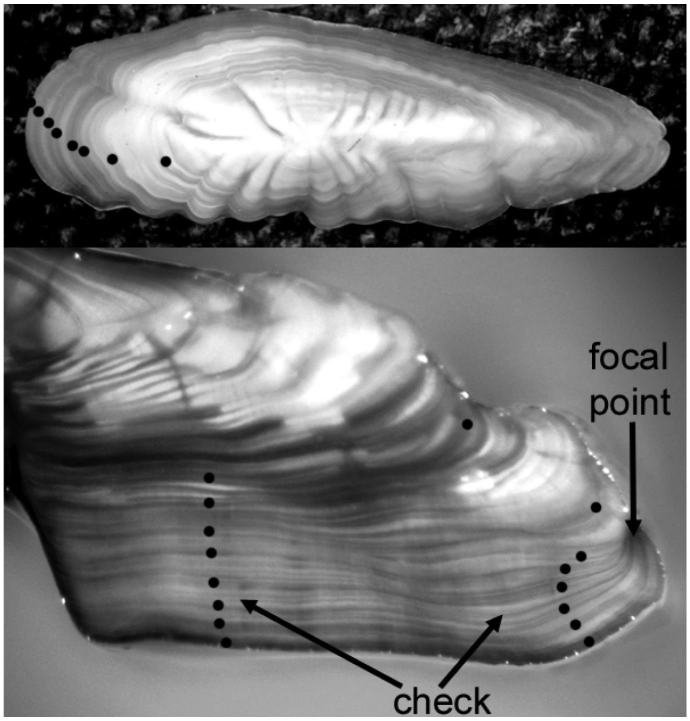

**Figure 47**

Known-age 8-year-old sablefish (*Anoplopoma fimbria*) otolith from a 590 mm male recaptured in July. A common growth pattern, where some annual marks merge at a focal point along the distal edge, fanning out into clear, regular growth zones toward the sulcus. The check between the fifth and sixth annual marks is strong along the distal edge but dissipates closer to the sulcus. Viewed with reflected light.

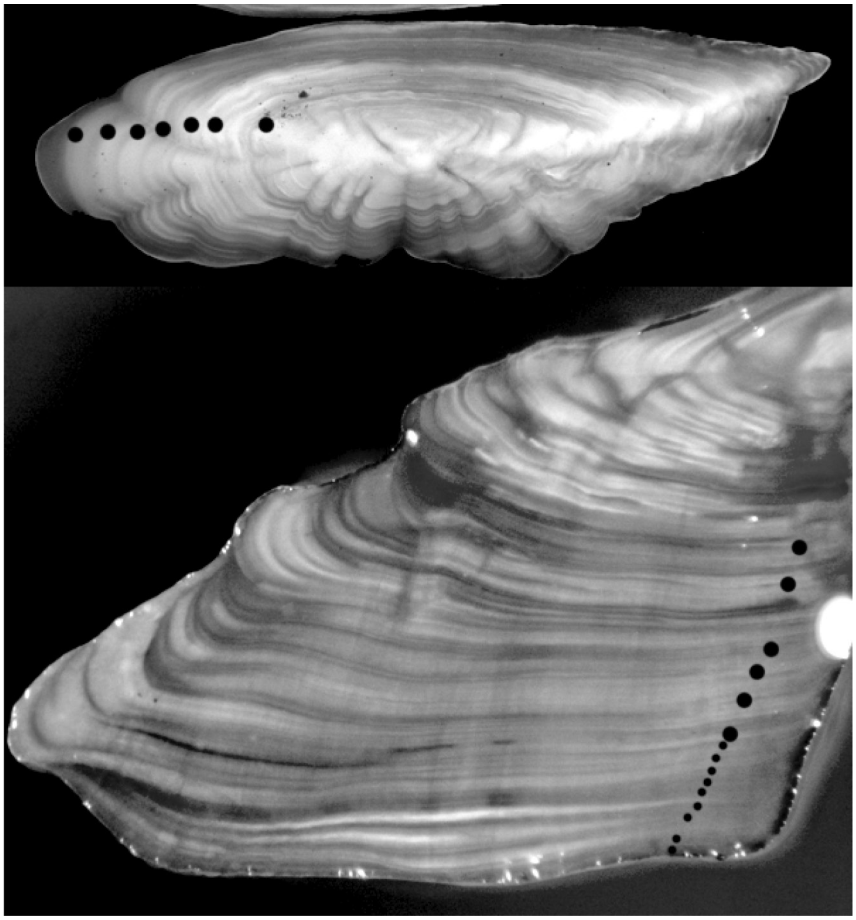

**Figure 48**

Known-age 15-year-old sablefish (*Anoplopoma fimbria*) otolith from a 700 mm male recaptured in April. The transition from fast to slow growth begins at 7 years where a thick, dark band made up of three thinner translucent growth zones is observed. Spacing between these bands is highly compressed compared to the surrounding pattern. True age indicates they should be considered individual annual marks. Viewed with reflected light.

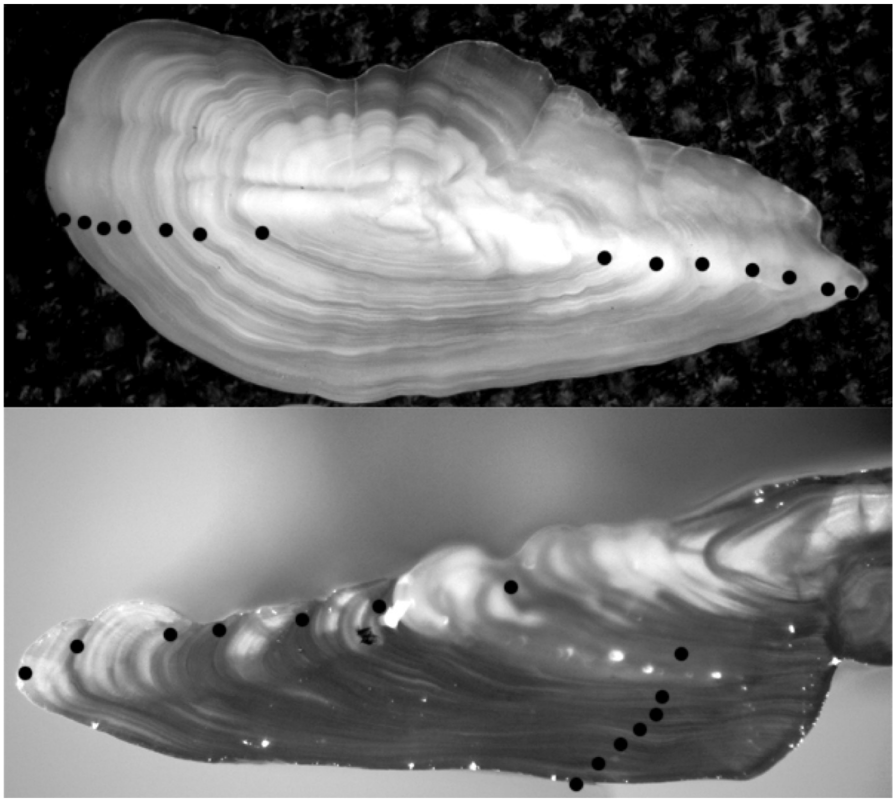

**Figure 49**

Known-age 7-year-old sablefish (*Anoplopoma fimbria*) otolith from a 670 mm female recaptured in June. Otoliths with this morphology always have broad, compressed annual marks and multiple checks. Viewed with reflected light.

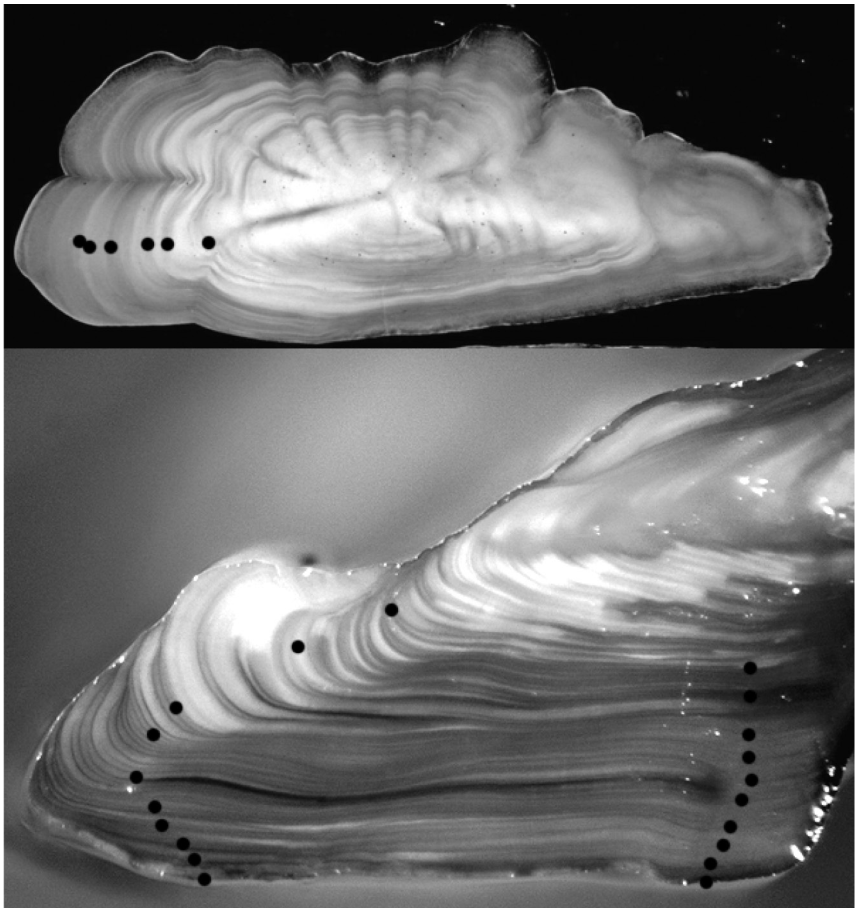

**Figure 50**

Known-age 10-year-old sablefish (*Anoplopoma fimbria*) otolith from a 620 mm male recaptured in April. Early years are difficult to interpret. The third annual mark is easier to identify after associating the cross-section bands with the surface and groove patterns, but the locations of the first and second annual marks on the break-and-burn cross section remain questionable. Viewed with reflected light.

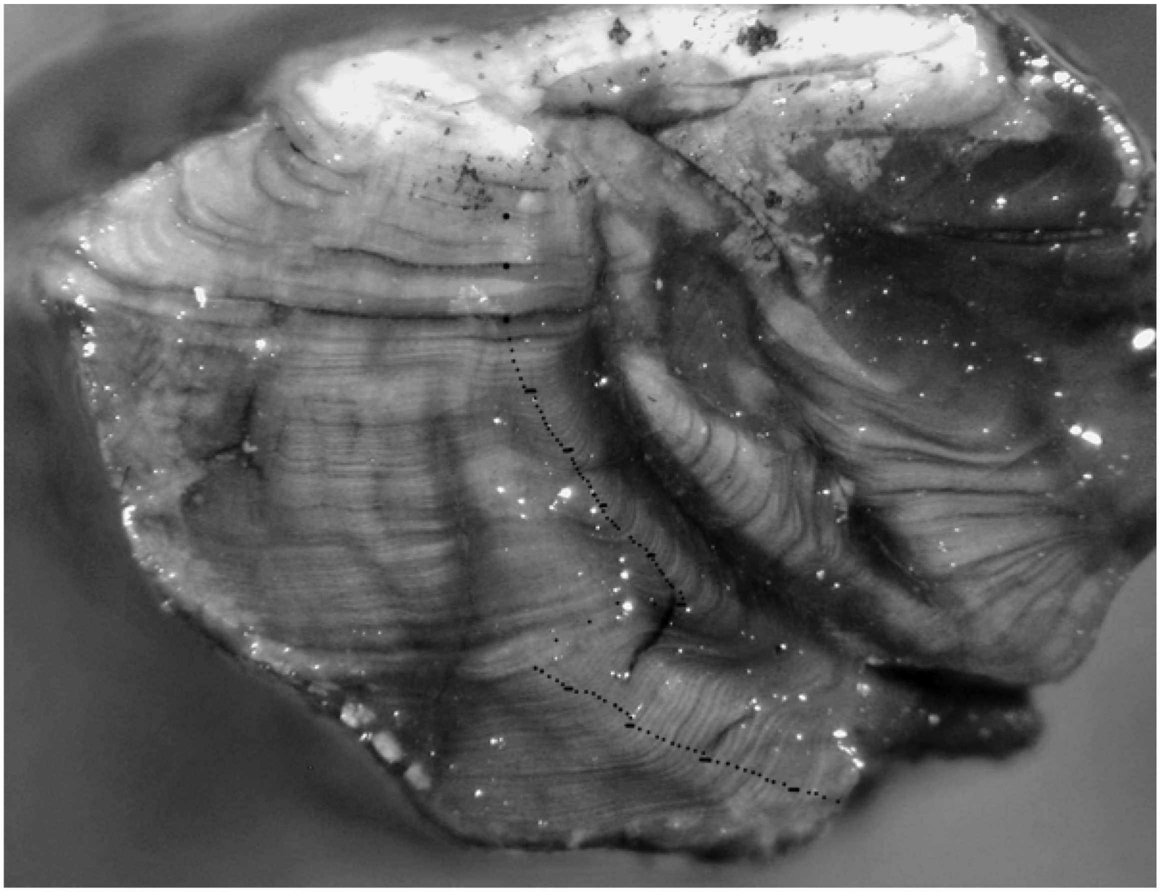

**Figure 51**

Oldest sablefish (*Anoplopoma fimbria*) otolith aged by the AFSC Age and Growth Program. Collected from an Aleutian Islands female sablefish captured and tagged in 1988 and recaptured one year later at 770 mm during a NMFS longline survey in the same vicinity where it was tagged. Estimated age is 94 years. Every tenth annual mark is indicated by a small black bar. Viewed with reflected light.

## Rockfish (*Sebastes*) species

**by B. J. Goetz, Charles E. Piston, and Christopher M. Gburski**

### Introduction

The AFSC Age and Growth Program ages five species of rockfish (genus: *Sebastes*) on a production basis, and an additional four species on an occasional basis (Table 2). Most rockfish species have similar otolith growth pattern characteristics, and aging criteria can be applied uniformly with few exceptions. Although some annual marks are apparent on otolith surfaces, it is typically difficult to estimate age using only surface patterns. The standard method for aging all size classes of rockfish species is the break-and-burn technique (Chapter 3). The otolith break-and-bake method has been attempted but was unsuccessful. Thin-sectioning is another option if the burning process does not produce satisfactory patterns.

### Rockfish age determination methods

**Otolith preparation.** Most rockfish otoliths are prepared for age determination using the break-and-burn method (Chapter 3). Otoliths are cut transversely through the core using a low-speed saw, with the sulcus facing the saw blade to obtain an even cut. Even when using the lightest saw weight, rockfish otoliths may snap before the cut has been completed, often resulting in a large ledge that must be sanded smooth.

Each rockfish species requires a slightly different burning technique which varies by age reader and alcohol burner. Age readers should experiment to find the best way to burn an otolith. One method that works well for most rockfish species is to hold the cut surface of the otolith about one inch from an alcohol flame, slowly passing it back and forth while it gradually browns. If this does not work well, the otolith can be burned with the cut surface facing away from the flame. After the entire otolith browns, the otolith half is turned so that the cut surface is down and rapidly passed through the flame four to five times to darken the center.

**Table 2**

Rockfish (*Sebastes* spp.) species aged by the Alaska Fisheries Science Center (AFSC) Age and Growth Program, ranked by age determination difficulty.

| Very difficult | Difficult | Moderate | Moderately easy |
|---|---|---|---|
| *S. borealis* | *S. aleutianus*<br>*S. melanostictus* | *S. alutus* | *S. ciliatus*<br>*S. polyspinis*<br>*S. proriger**<br>*S. variabilis*<br>*S. variegatus**<br>*S. zacentrus** |

* limited number aged by AFSC Age and Growth Program

An optional polishing step either before or after burning can enhance the pattern, especially if foreign matter on the otolith has left dark crusts obscuring the pattern. Prior to coating the burned surface with mineral oil, the surface must be dry, free of any preservative, and sufficiently cooled. At the AFSC, all rockfish otoliths are examined using a dissecting microscope and reflected light.

**Readability.** Readability varies among rockfish species and is a subjective assessment of pattern clarity, ease of burning, and other factors related to the interpretation and identification of annual marks (Table 2). Pattern difficulties most frequently involve the first three annual marks or interpretation of growth at the edge.

**Reading axis.** When looking at break-and-burn cross sections, there are a number of landmarks which help to orient the age reader (Fig. 52). The sulcus separates the dorsal and ventral sides of the otolith. On the ventral side, more checks are visible and annual marks have a greater tendency to split than on the dorsal side. The easiest patterns to interpret are often seen on the dorsal side of the sulcus (Fig. 52). Therefore, we tend to concentrate on the dorsal side of break-and-burn cross sections during age determination. The fastest growing axis is the dorsal tip. Since splitting is often more prominent along the distal surface of the dorsal side, translucent growth zones should be followed to the sulcus to identify annual marks.

**First and second annual marks.** Typically, the first and second annual marks on rockfish otoliths are difficult to interpret. It is quite easy to fail to pass through the core when cutting, resulting in a missed or vague first annual mark. Often the second annual mark is clearer than the first. The expected diameter of the first annual mark (measured from clear otoliths) can be used to decide whether a first year needs to be added to patterns with an obscure or vague center. We have not yet validated the aging criteria used to determine the first two years in rockfish, but in order to maintain precision we continue to apply expected diameter measurements of the first annual mark as criteria until more information becomes available.

One useful technique to help resolve particularly ambiguous early growth patterns is to compare difficult otolith patterns to clear otolith patterns. Viewing a clearer break-and-burn cross section alongside the problematic pattern may help identify the most probable annual marks. Age readers should note otoliths with clear early growth patterns and refer to them when viewing otoliths with more difficult patterns.

**Growth increment spacing.** Rockfish otolith growth slows down gradually with age, resulting in relatively regular growth patterns. The expected spacing between annual marks helps age readers decide how to interpret faintly burned areas. Additionally, vague patterns frequently appear near the edge. Relatively clear patterns with wide, dark areas comprised of multiple annual marks along the edge are common. By examining the spacing between previous annual marks, one can determine (usually within one year) how many annual marks fit within the dark area.

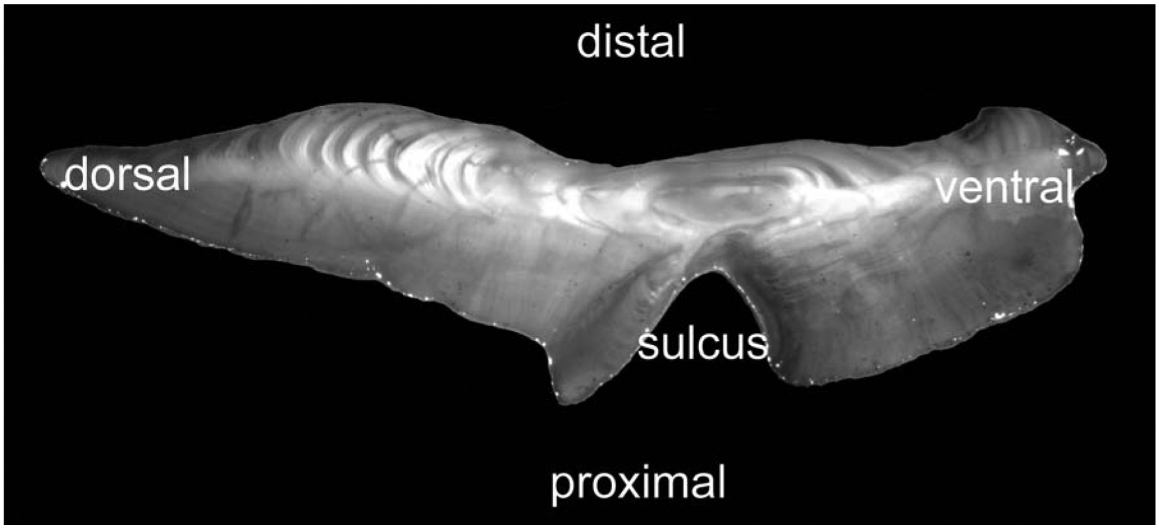

**Figure 52**

Break-and-burn preparation showing general terminology used when examining rockfish (*Sebastes* spp.) otoliths. Viewed with reflected light.

## Pacific ocean perch (*Sebastes alutus*)

### Biology

Pacific ocean perch (*Sebastes alutus*) is the most abundant and commercially important rockfish in Alaskan waters. This species occurs along the outer continental shelf and upper slope regions of the North Pacific Ocean and Bering Sea (Quast, 1970; Westrheim, 1970), from southern California northward through the Gulf of Alaska and the Bering Sea, westward along the Aleutian archipelago, Kamchatka Peninsula, and Kuril Islands (Phillips, 1957; Moiseev and Paraketsov, 1961), and to the east coast of Honshu Island, Japan (Barsukov, 1964).

Pacific ocean perch larvae are pelagic and transform to a bottom-dwelling state within the first year (Carlson and Haight, 1976). Juveniles predominantly reside in benthic habitats with complex vertical structures, such as boulders, macrophytes, sponges, and corals (Carlson and Haight, 1976; Carlson and Straty, 1981; Love et al., 1991; Rooper and Boldt, 2005; Rooper et al., 2007). Juveniles migrate from shallower continental shelf waters to deeper waters with increasing age, eventually residing over the continental slope at adulthood (Carlson and Haight, 1976; Carlson and Straty, 1981).

Pacific ocean perch are semi-demersal and spend most of their time near the bottom but migrate vertically day or night (Skalkin, 1964; Lyubimova, 1965; Brodeur, 2001) to feed opportunistically on mesopelagic zooplankton. Adults undertake a seasonal migration, inhabiting shallower depths (150–300 m) in the summer and migrating to deeper water (300–420 m) in the fall (Lyubimova, 1963; Paraketsov, 1963; Westrheim, 1970; Gunderson, 1971; Love et al., 2002). This pattern is likely related to summer feeding and winter spawning. Spawning takes place in the fall, and eggs are internally fertilized about two months later (Love et al., 2002). Larvae are released from March through May (Paraketsov, 1963).

Pacific ocean perch are slow-growing and long-lived. Age at 50% maturity is 10.5 years in the Gulf of Alaska (Hanselman et al., 2007). According to AFSC Age and Growth Program data, there are slight differences between the Bering Sea and Aleutian Islands (BSAI) stock and the Gulf of Alaska (GOA) stock in length at age, with those from the BSAI growing more slowly and to larger sizes than those from the GOA (Fig. 53). Von Bertalanffy growth parameters were $L_\infty$=428.3 mm, k=0.1259/yr, and $t_0$=–1.243 yr (*n*=1918) and $L_\infty$=412.2 mm, k=0.1847/yr, and $t_0$=–0.255 yr (*n*=2203) for Pacific ocean perch collected during trawl surveys in the BSAI region and the Gulf of Alaska, respectively, from 2004 through 2007 (Fig. 53). The maximum age reported by the AFSC Age and Growth Program for Pacific ocean perch to date is 105 years.

### Age determination history

The AFSC Age and Growth Program has aged over 50,000 Pacific ocean perch. This species is moderately

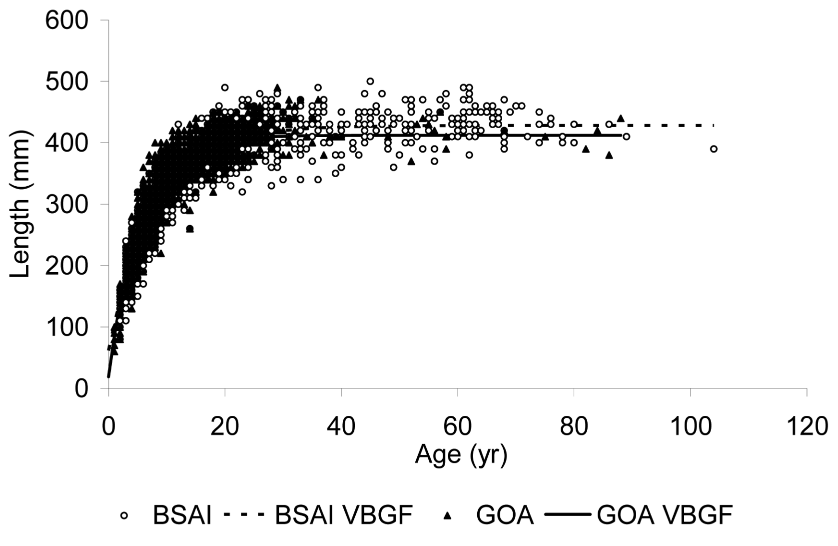

**Figure 53**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for Pacific ocean perch (*Sebastes alutus*) collected during trawl surveys in the Bering Sea/Aleutian Islands (BSAI; *n*=1918) and Gulf of Alaska (GOA; *n*=2203) from 2004 through 2007.

difficult to age, reflected in estimates of precision (CV=6.37%; *n*=8087). Prior to 1982, otolith surface examination was the primary method for rockfish age determination at the AFSC. Today, all Pacific ocean perch otoliths are processed using the break-and-burn method (Chapter 3). During the early to mid-1980s, periodic Pacific ocean perch otolith exchanges among members of the Committee of Age Reading Experts (CARE) verified that the general aging criteria used by all participating agencies were similar. Pacific ocean perch age estimates have been validated using radiometric methods (Kastelle et al., 2000) and bomb radiocarbon analysis (Kastelle et al., 2008b); these methods are described in further detail in Chapter 6 of this manual.

## Current age determination methods

When compared with other rockfish species aged by the AFSC Age and Growth Program, interpretation of Pacific ocean perch otoliths is moderately difficult. Annual marks are not well defined on Pacific ocean perch otolith surfaces. The first four annual marks are often vague on very young otoliths and clearer on older specimens. Growth patterns are interpreted using a dissecting microscope with reflected light at magnifications ranging from 6–96×, depending on the size of the otolith.

The first annual mark of Pacific ocean perch otoliths is typically larger than those of all other rockfish species aged by our program, with the exception of shortraker rockfish (*S. borealis*). The average diameter of the first annual mark is approximately 1.65 mm, determined from clear Pacific ocean perch otoliths.

It is common for age readers to cut otoliths off-center so that the first annual mark's shape is distorted. If the first clear translucent growth zone is much larger than the average first annual mark diameter, an extra year is added to the age estimate even if it is not clear on the break-and-burn preparation. Examples of Pacific ocean perch otoliths are shown in Figures 54 through 59.

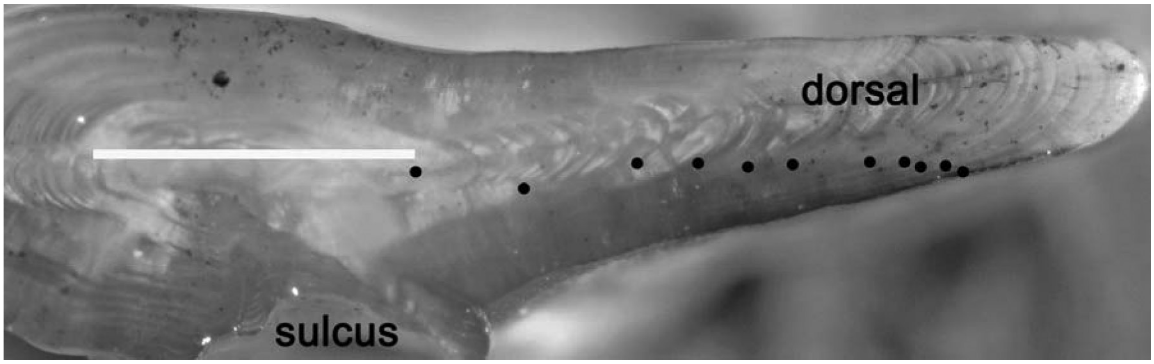

**Figure 54**

Break-and-burn otolith cross section from a 370 mm female Pacific ocean perch (*Sebastes alutus*) caught on 9 July 2006 in the Bering Sea. The pattern is relatively vague, especially in the first 3 years. Age estimate is 11 years. Bar represents the expected diameter of the first annual mark (1.65 mm). Viewed with reflected light.

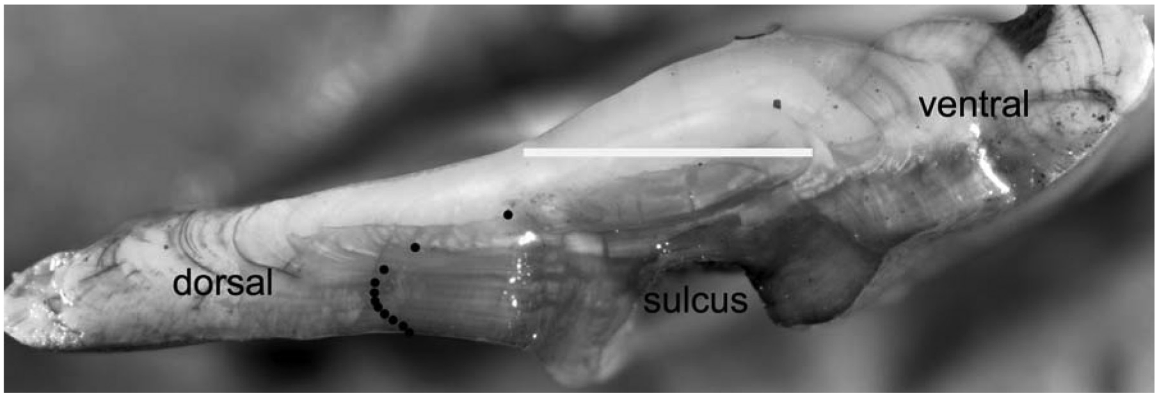

**Figure 55**

Break-and-burn otolith cross section from a 340 mm female Pacific ocean perch (*Sebastes alutus*) caught on 13 July 2006 in the Bering Sea. The first annual mark is relatively clear. Age estimate is 11 years. Bar represents the expected diameter of the first annual mark (1.65 mm). Viewed with reflected light.

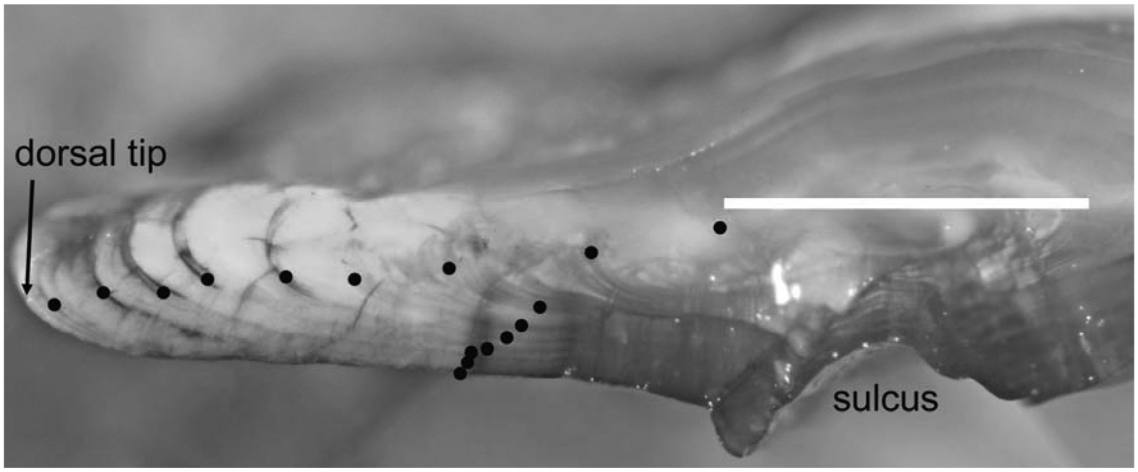

**Figure 56**

Break-and-burn otolith cross section from a 320 mm female Pacific ocean perch (*Sebastes alutus*) caught on 6 July 2006 in the Bering Sea. Pattern is relatively clear. Opaque growth at the dorsal tip not counted because it is attributed to the 2006 growth year. Age estimate is 9 years. Bar represents the expected diameter of the first annual mark (1.65 mm). Viewed with reflected light.

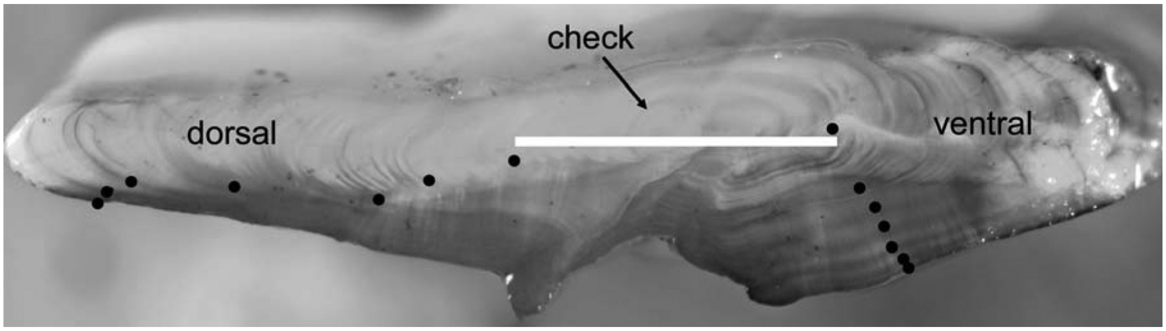

**Figure 57**

Break-and-burn otolith cross section from a 340 mm female Pacific ocean perch (*Sebastes alutus*) caught on 16 May 2006 in the Bering Sea. Relatively clear pattern, but a vague translucent growth zone observed around the core was not counted because it is much smaller than the expected first annual mark diameter (white bar, 1.65 mm). Opaque growth at the dorsal tip was counted since it extends to the sulcus and this otolith was collected in the spring. Pattern on the ventral side of the sulcus is relatively clear, corroborating the age estimate of 7 years. Viewed with reflected light.

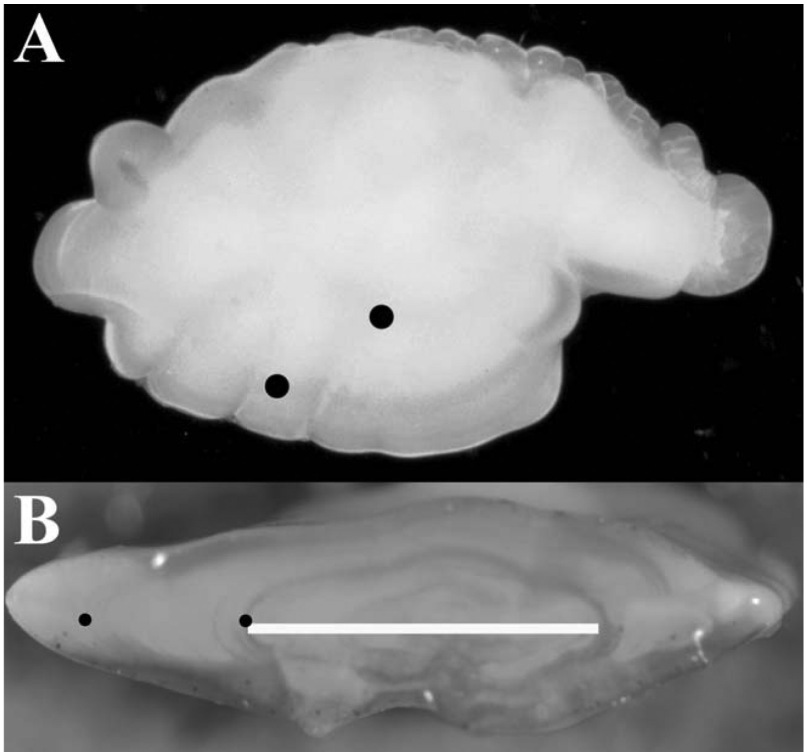

**Figure 58**

(**A**) Vague otolith surface pattern and (**B**) clear break-and-burn cross section from a 150 mm female Pacific ocean perch (*Sebastes alutus*) caught on 25 July 2007 in the Gulf of Alaska. Small translucent growth zone inside the first annual mark is not counted. Opaque growth on edge is not counted because it is attributed to 2007. Age estimate is 2 years. Bar represents the expected diameter of the first annual mark (1.65 mm). Viewed with reflected light.

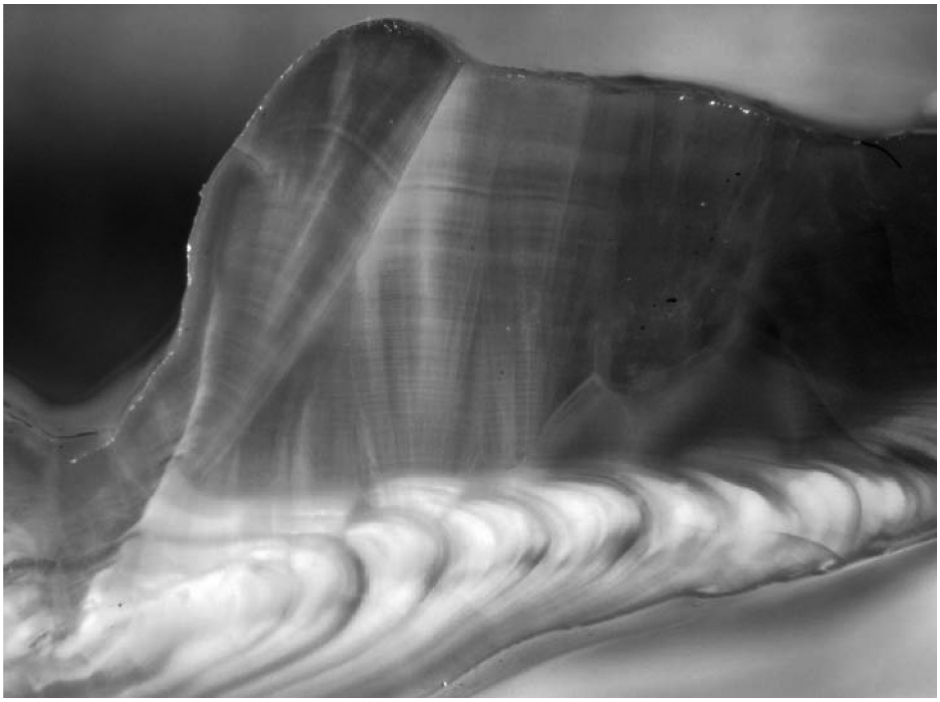

**Figure 59**

Pacific ocean perch (*Sebastes alutus*) break-and-burn otolith cross section. Estimated age is 100 years. Viewed with reflected light.

## Northern rockfish (*Sebastes polyspinis*)

### Biology

Northern rockfish (*Sebastes polyspinis*) is a commercially important species in Alaskan waters and is the second most abundant species caught in the Gulf of Alaska (Clausen and Heifetz, 2002). The distribution of northern rockfish encompasses the waters off the extreme northern part of British Columbia through Alaska to eastern Kamchatka and the northern Kuril Islands (Allen and Smith, 1988; Ito et al., 1999). Northern rockfish is most abundant in Alaskan waters, from the western end of the Aleutian Islands to Portlock Bank in the central Gulf of Alaska (Clausen and Heifetz, 2002). This species forms large schools over rough, hard bottom and is caught over steep slopes at depths of 75 to 125 m using bottom trawl gear (Clausen and Heifetz, 2002). Northern rockfish is a target species in the Gulf of Alaska, whereas it is taken primarily as bycatch in the Aleutian Islands and Bering Sea.

Little is known about the early life history of northern rockfish, particularly the larval and juvenile stages. This species is ovoviviparous with internal fertilization. Gulf of Alaska research surveys indicate parturition (larval release) occurs in the spring and summer. Older juveniles (>200 mm) are found on the continental shelf inshore of adult habitat (Heifetz et al., 2007).

Northern rockfish mature around 8 years, or 310 mm (Chilton, 2007). According to AFSC Age and Growth Program data, northern rockfish from the Gulf of Alaska grow faster and reach a larger maximum length than the Aleutian Islands stock (Fig. 60). Von Bertalanffy growth parameters were $L_\infty$=413.0 mm, k=0.1788/yr, and $t_0$=0.108 yr (*n*=1035) and $L_\infty$=351.4 mm, k=0.1602/yr, and $t_0$=–0.778 yr (*n*=1050) for northern rockfish collected during trawl surveys in the Gulf of Alaska and Aleutian Islands, respectively, from 2004 through 2007 (Fig. 60). Despite differences in life history, these stocks are genetically homogenous[3]. The oldest northern rockfish aged to date by the Age and Growth Program was 88 years and 410 mm.

### Age determination history

The AFSC Age and Growth Program began age determination of northern rockfish in 1988. Inter-reader precision is relatively high (CV=4.1%; *n*=2852) when compared with some of the other rockfish species aged by the AFSC. Northern rockfish age estimates have been validated using radiometric methods (Kastelle et al., 2000), described in further detail in Chapter 6 of this manual. A bomb radiocarbon study is also currently in progress at the AFSC to provide further support for our age estimates.

### Current age determination methods

Otolith surfaces are not sufficient for accurate age determination of northern rockfish; thus, otoliths are

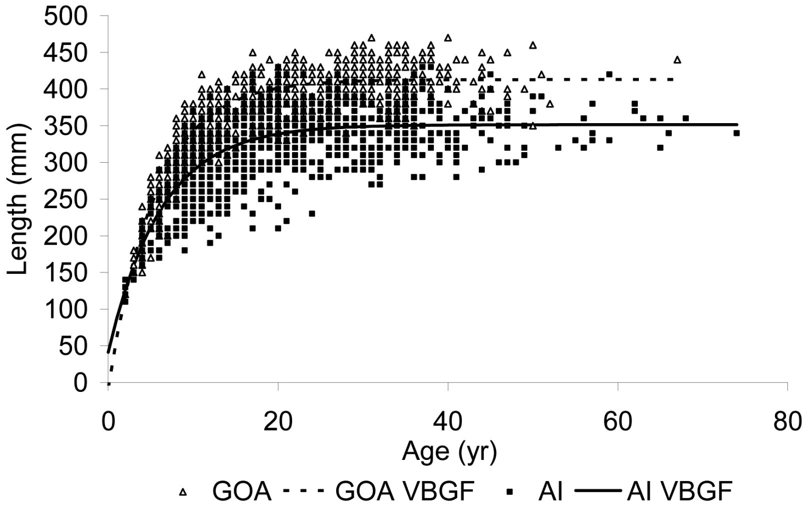

**Figure 60**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for northern rockfish (*Sebastes polyspinis*) collected during trawl surveys in the Gulf of Alaska (GOA; *n*=1035) and Aleutian Islands (AI; *n*=1050) from 2004 through 2007.

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[3] Gharrett, A. J., A. K. Gray, D. Clausen, and J. Heifetz. 2003. Preliminary study of the population structure in Alaska northern rockfish, *Sebastes polyspinis*, based on microsatellite and mtDNA variation. Unpubl. contract report. Fisheries Division, School of Fisheries and Ocean Sciences, Univ. of Alaska, Fairbanks, Juneau, AK 99801,16 p.

prepared using the break-and-burn method (Fig. 61). Growth patterns are interpreted using a dissecting microscope with reflected light at magnifications ranging from 6–96×, depending on the size of the otolith. With practice it is easier to get a clear break-and-burn pattern in northern rockfish otoliths than in more difficult species like Pacific ocean perch. The growth patterns of northern rockfish otoliths are very similar to those of dusky rockfish (*S. variabilis*) otoliths.

The first annual mark is typically smaller in northern rockfish otoliths than in Pacific ocean perch otoliths. The approximate diameter of the first annual mark is 1.0 mm. The expected size of the first annual mark has been determined from specimens with extremely clear growth zones and subsequently verified while preparing samples for bomb radiocarbon validation experiments. A problem that commonly arises during otolith preparation is failure to section the otolith directly through the core, which results in distortion of the shape of the first annual mark. If the first well-defined translucent growth zone is much larger than 1 mm, a missed first annual mark is assumed to be inside it.

Digital images of northern rockfish otoliths with annotated age estimates are shown in Figures 62 though 66.

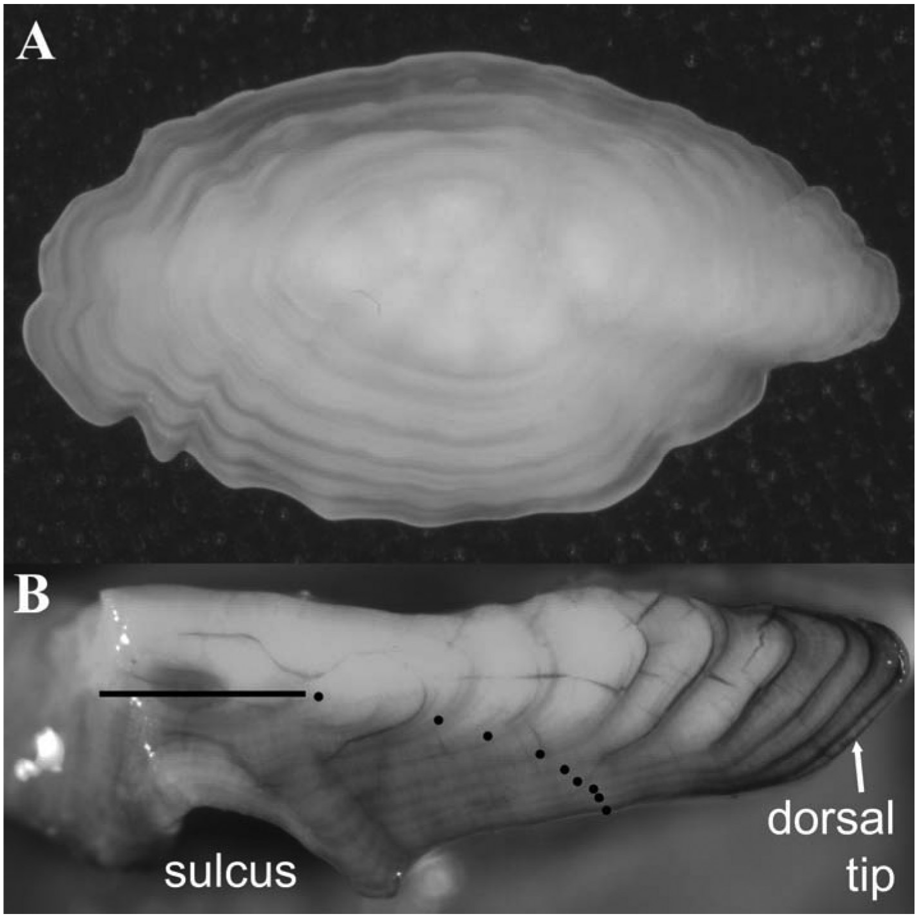

**Figure 61**

(**A**) Otolith surface and (**B**) break-and-burn cross section from a 200 mm female northern rockfish (*Sebastes polyspinis*) collected 15 July 2006 in the Aleutian Islands. Otolith surface alone is insufficient for age determination. During break-and-burn preparation, the otolith was not sectioned directly through the core. Opaque growth zone evident on the dorsal tip was not included in the age estimate because it was likely deposited during 2006. Age estimate is 9 years. Expected diameter of first annual mark (1 mm) indicated by black bar. Viewed with reflected light.

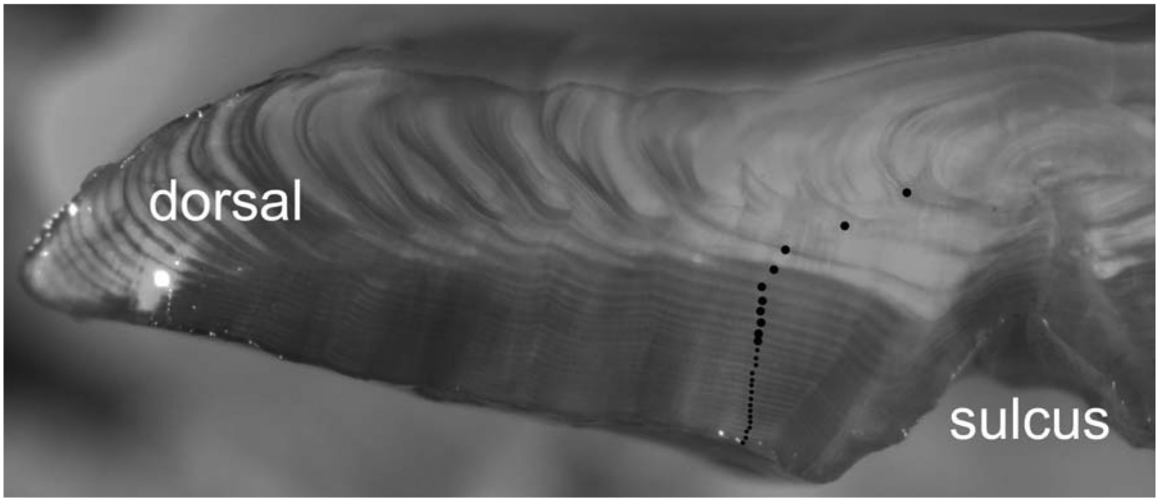

**Figure 62**

Break-and-burn otolith cross section from a 390 mm female northern rockfish (*Sebastes polyspinis*) collected on 10 June 1996 in the Gulf of Alaska. Clear early annual marks. Age estimate is 26 years. Viewed with reflected light.

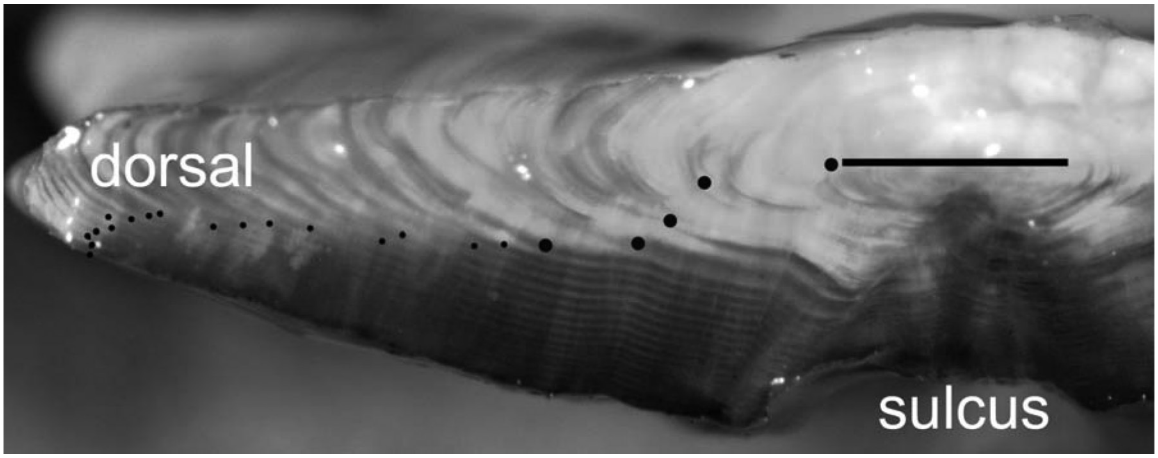

**Figure 63**

Break-and-burn otolith cross section from a 370 mm female northern rockfish (*Sebastes polyspinis*) collected on 5 August 1987 in the Gulf of Alaska. Vague first annual mark. Relatively clear growth pattern after second annual mark. Age estimate is 22 years. Expected diameter of first annual mark indicated by black bar (1 mm). Viewed with reflected light.

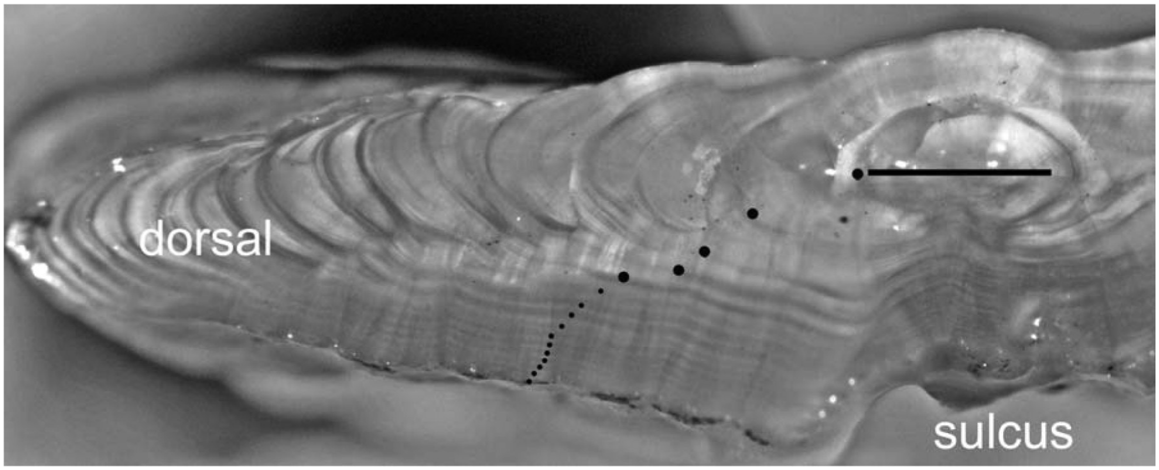

**Figure 64**

Break-and-burn otolith cross section from a 390 mm female northern rockfish (*Sebastes polyspinis*) collected on 7 August 1987 in the Gulf of Alaska. Relatively clear growth pattern. Age estimate is 16 years. Expected diameter of first annual mark indicated by black bar (1 mm). Viewed with reflected light.

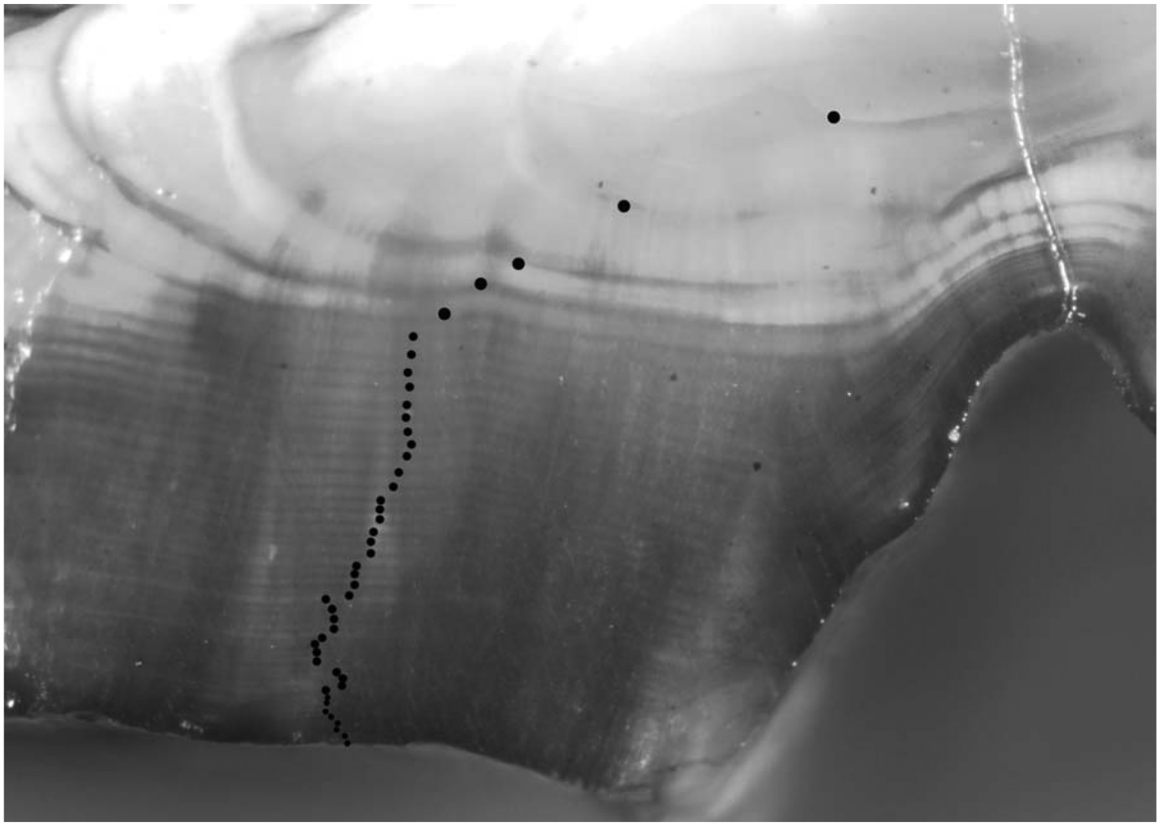

**Figure 65**

Clear break-and-burn otolith cross section from a 370 mm female northern rockfish (*Sebastes polyspinis*) collected on 14 July 2006 in the Aleutian Islands. Age estimate is 46 years. Viewed with reflected light.

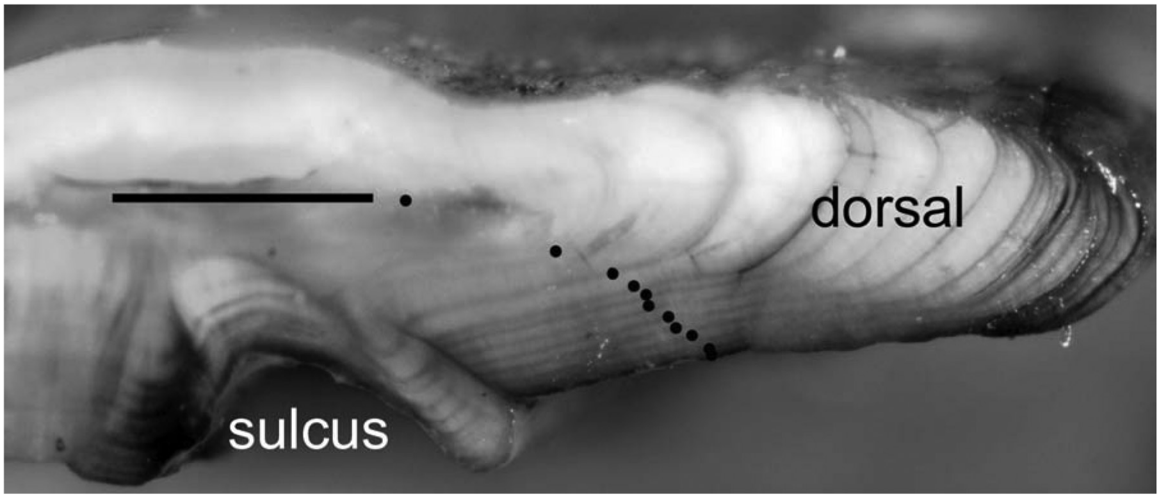

**Figure 66**

Break-and-burn otolith cross section from a 250 mm male northern rockfish (*Sebastes polyspinis*) collected on 15 July 2006 in the Aleutian Islands. Relatively clear growth pattern. Checks observed near dorsal tip. Vague early annual marks. Age estimate is 11 years. Expected diameter of first annual mark indicated by black bar (1 mm). Viewed with reflected light.

## Rougheye rockfish species complex

### Biology

Genetic analysis has shown that the rougheye rockfish complex consists of two species: rougheye rockfish (*Sebastes aleutianus*), which ranges from the eastern Aleutian Islands off Unalaska Island and the eastern Bering Sea at Pribilof Canyon to southern Oregon; and blackspotted rockfish (*S. melanostictus*), which ranges from Japan through the Kuril Islands, Aleutian Islands, and the Bering Sea south to California (Gharrett et al., 2005; Orr and Hawkins, 2008). At-sea field identification of these two species is problematic, and misidentification of a third species, shortraker rockfish (*S. borealis*), can lead to mixed-species samples. Although both species have a high market value, they are primarily caught as bycatch in trawl and longline fisheries targeting other species (Shotwell et al., 2007; Spencer et al., 2008).

Rougheye rockfish are typically found at shallower depths (45 to 439 m) than blackspotted rockfish (84 to 490 m). Submersible observations have shown that adults have a relatively even distribution over steep, rocky areas (Krieger and Wing, 2002). Very little is known about the early life history of these species as larvae and juveniles must be identified genetically. Larval release may take place from December through April (McDermott, 1994), and the larvae are thought to be pelagic. The length of the larval period is unknown.

### Age determination history

Rougheye rockfish complex otoliths were first aged by the AFSC Age and Growth Program in 2003. While aging these samples, we observed different otolith growth patterns; some had very distinct, clear early growth patterns whereas others had multiple checks and indistinct patterns that were difficult to interpret. Genetic and morphological studies subsequently confirmed that the complex consists of two species (Gharrett et al., 2005; Orr and Hawkins, 2008). Since 2006, samples have been identified at sea as either rougheye rockfish or blackspotted rockfish. However, AFSC survey personnel have indicated that accurate field identification of these species still lacks precision, and for now we assume that collections are comprised of mixed species despite at-sea species designation. Otoliths have recently been collected together with fin clips for genetic identification and to determine whether otolith morphology can be used to distinguish between the two species; this work is currently underway.

To date, the program has aged over 6000 rougheye rockfish complex otoliths, with a maximum age estimate of 132 years and an average CV of 7.8%. Rougheye rockfish age estimates have been validated using radiometric methods (Kastelle et al., 2000), described in further detail in Chapter 6 of this manual.

### Current age determination methods

Rougheye rockfish complex otoliths are typically prepared for age determination using the break-and-burn method (Chapter 3). Growth patterns are interpreted using a dissecting microscope with reflected light at magnifications ranging from 6–96×, depending on the size of the otolith.

To date, we have not received any positively identified rougheye or blackspotted rockfish otoliths, so we cannot comment on species-specific differences in otolith growth. We have noted specimens with particularly clear early growth or patterns showing multiple checks. Growth patterns are similar to very old Pacific ocean perch, and some of the oldest rougheye rockfish complex otoliths have required thin-sectioning (Chapter 3) to assign age estimates.

The approximate diameter of the first annual mark is slightly over 1.0 mm, roughly the same as northern rockfish. If the first visible annual mark is much larger than the expected size of 1.0 mm, an extra year is added to the age estimate. Images of rougheye rockfish complex otoliths are shown in Figures 67 through 70.

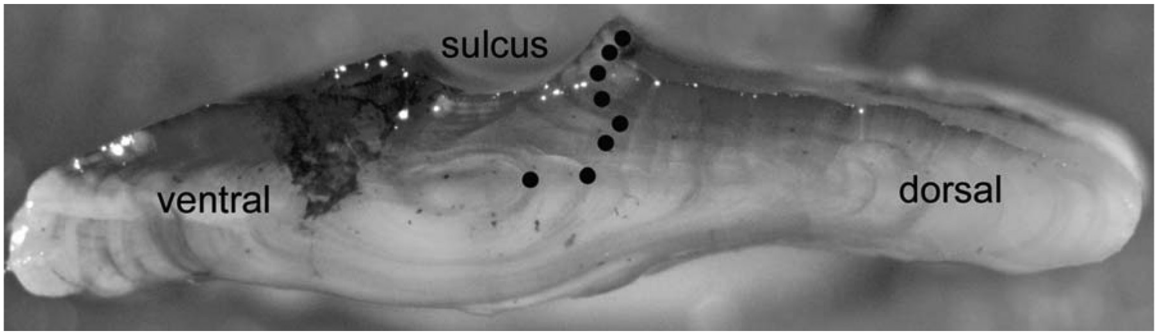

**Figure 67**

Break-and-burn preparation of a rougheye (*Sebastes aleutianus*) or blackspotted (*S. melanostictus*) rockfish otolith. Rare, relatively clear growth pattern. Age estimate is 8 years. Collected from a 330 mm female in the eastern Bering Sea in June 2002. Viewed with reflected light.

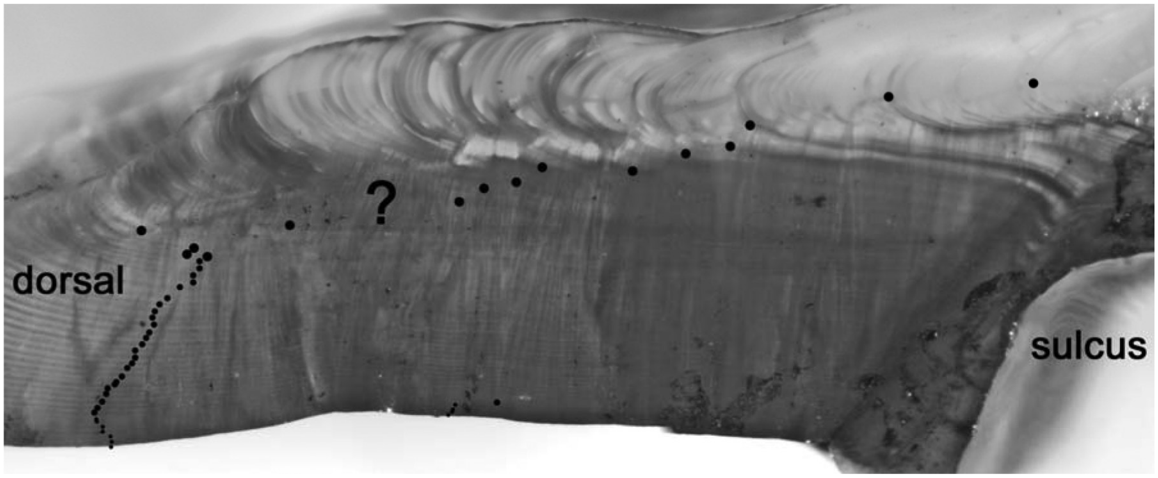

**Figure 68**

Break-and-burn preparation of a rougheye (*Sebastes aleutianus*) or blackspotted (*S. melanostictus*) rockfish otolith. Collected from a 660 mm male in the Aleutian Islands on 11 June 2006. A relatively clear growth pattern, especially the early annual marks and edge, but the area immediately after the tenth annual mark is vague (indicated by question mark). Age estimate is 47 years. Viewed with reflected light.

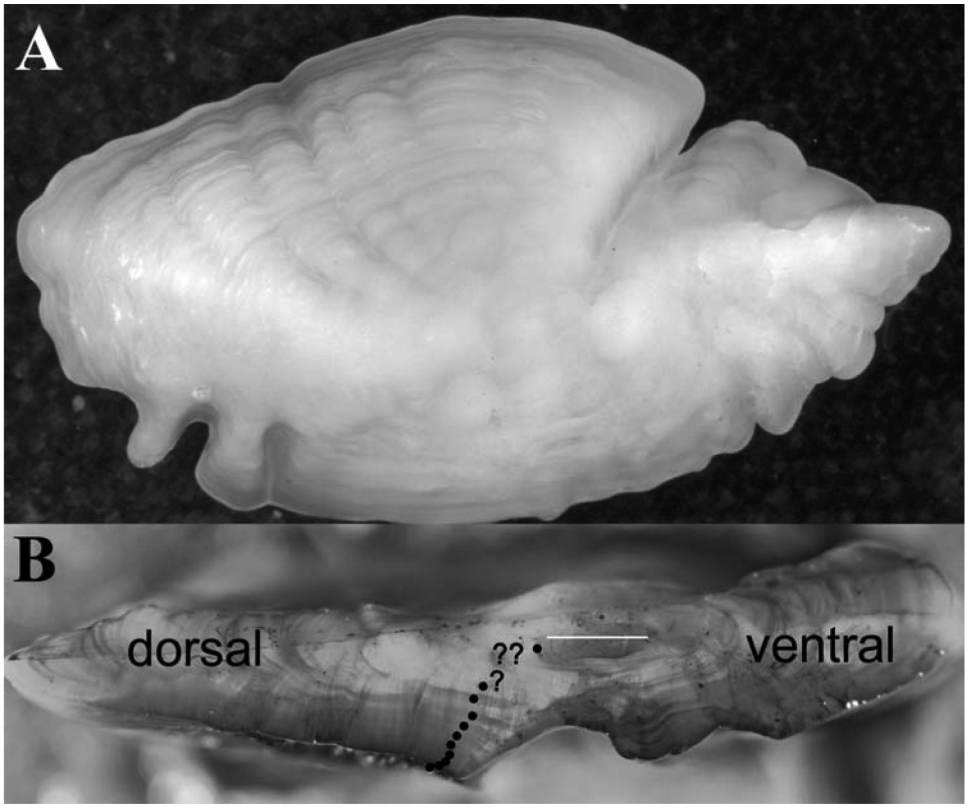

**Figure 69**

(**A**) Otolith surface and (**B**) break-and-burn patterns from a rougheye (*Sebastes aleutianus*) or blackspotted (*S. melanostictus*) rockfish. Collected from a 400 mm male in the Aleutian Islands on 10 June 2006. Vague growth pattern, especially the second and third annual marks (indicated by question marks). The area on the dorsal side adjacent to the sulcus is the best reading axis for this otolith. Age estimate is 11 years. Expected diameter of first annual mark indicated by white bar (1 mm). Viewed with reflected light.

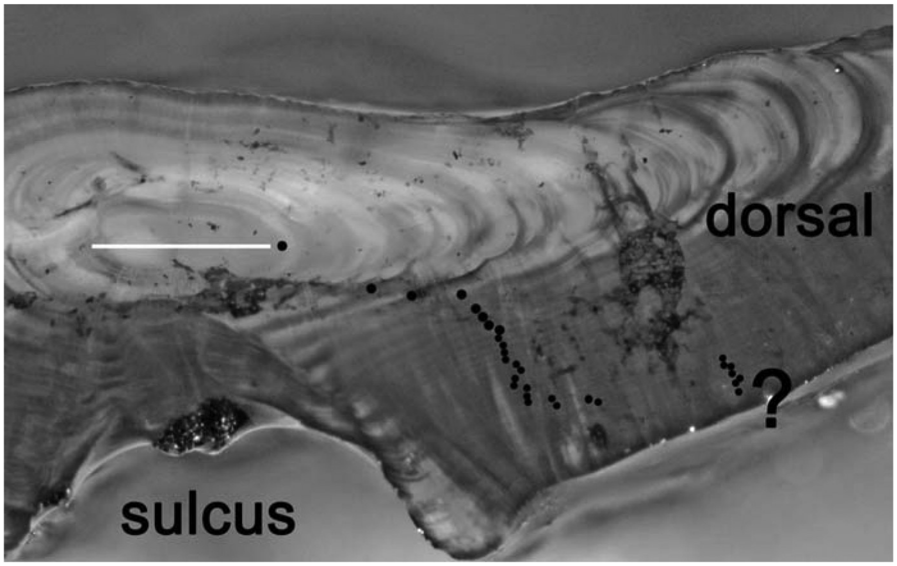

**Figure 70**

Break-and-burn preparation of a rougheye (*Sebastes aleutianus*) or blackspotted (*S. melanostictus*) rockfish otolith. Collected from a 500 mm female in the Aleutian Islands on 1 July 2006. Pattern is clear to thirtieth annual mark; edge is vague (indicated by question mark). Age estimate is 38 years. Expected diameter of first annual mark indicated by white bar (1 mm). Viewed with reflected light.

## Greenland halibut
## (*Reinhardtius hippoglossoides*)

**by Delsa M. Anderl**

### Biology

In the North Pacific, Greenland halibut (*Reinhardtius hippoglossoides*), also known as Greenland turbot, is most abundant in the waters of the eastern Bering Sea, followed by the Aleutian Islands (Alton et al., 1988). This species is also found in the North Atlantic, where it was a target of intensive directed fisheries during the latter half of the last century, although a decline in spawning stock size led to reduced commercial catches in recent years (Alton et al., 1988; Bowering and Nedreaas, 2000). Greenland halibut was an important commercial species in the eastern Bering Sea until catches declined in the early 1980s, primarily due to reductions in fishing quotas prompted by concerns over poor recruitment (Ianelli et al., 2010). The use of two common names dates from 1968 when concerns were voiced by the Pacific halibut (*Hippoglossus stenolepis*) fishing industry that the name Greenland halibut could mislead consumers to believe they were the same species. The name change to Greenland turbot was never successful in the Atlantic fisheries, so both common names are used interchangeably when referring to this species (Scott and Scott, 1988).

Greenland halibut spawning in the Bering Sea and Aleutian Islands occurs in winter, most likely from December to January (Sohn, 2009). Larvae appear to have a long pelagic duration and may be subject to extensive drift pathways prior to juvenile settlement (Sohn, 2009). In the Bering Sea, juveniles reside in waters of the continental shelf (<200 m) and are thought to move to deeper waters over the continental slope (200 to >1000 m) around age-4 or age-5 (Alton et al., 1988).

This species grows very quickly during the first three years of life, reaching lengths up to 160–200 mm after the first year and 320–400 mm by the third year (Kodolov and Matveychuk, 1995). Females grow to larger sizes than males (Fig. 71). Von Bertalanffy growth parameters were $L_\infty$=864.88 mm, k=0.10/yr, and $t_0$=–1.00 yr (*n*=307) and $L_\infty$=1024.26 mm, k=0.09/yr, and $t_0$=–0.68 yr (*n*=383), respectively, for male and female Greenland halibut collected during trawl surveys in the Bering Sea from 1990 through 2008 (Fig. 71). Males mature between 5 and 9 years (500 and 700 mm) and females mature between 5 and 10 years (500 and 800 mm) (Kodolov and Matveychuk, 1995). The maximum age reported by the AFSC Age and Growth Program to date for Greenland halibut is 32 years.

### Age determination history

Greenland halibut otoliths are morphologically different from other fish otoliths studied by the AFSC Age and Growth Program. Greenland halibut otoliths

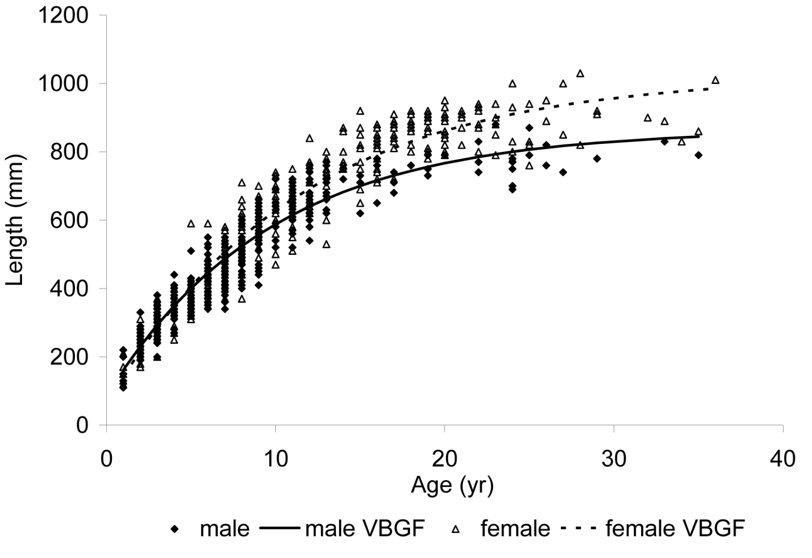

**Figure 71**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for male (*n*=307) and female (*n*=383) Greenland halibut (*Reinhardtius hippoglossoides*) collected from the Bering Sea from 1990 through 2008.

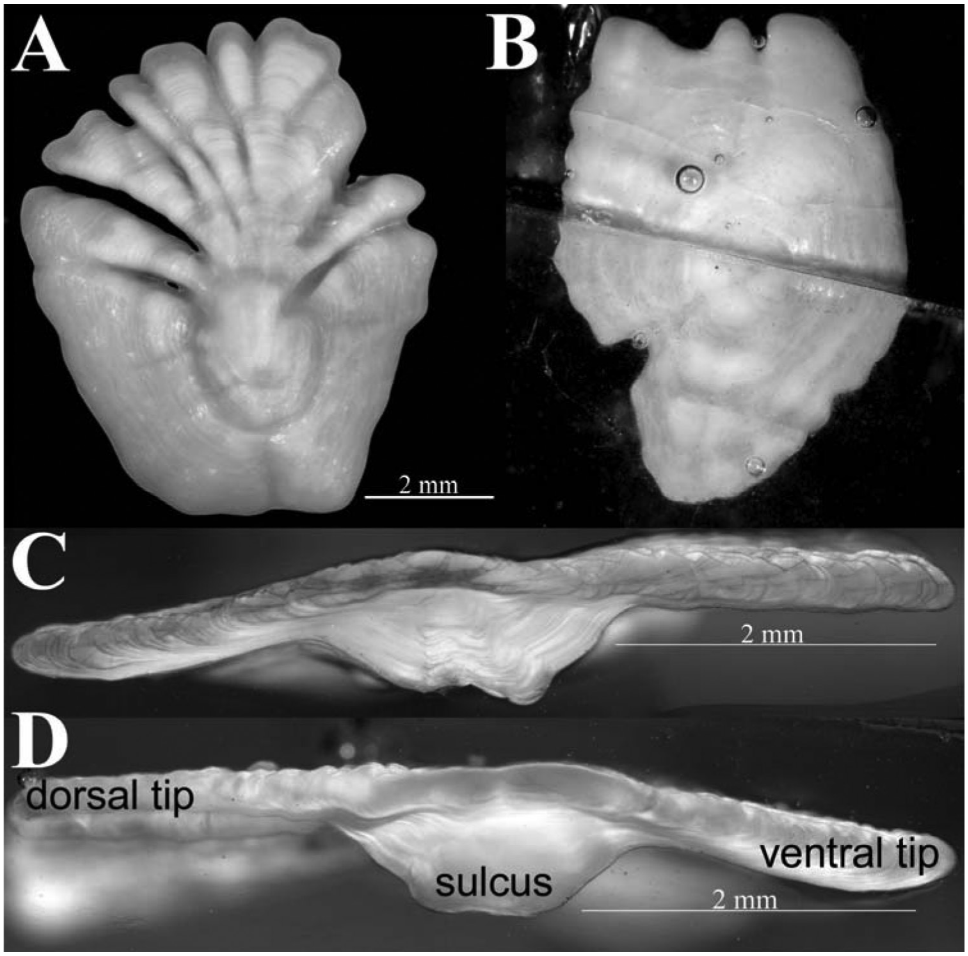

**Figure 72**

Otoliths from a 460 mm Greenland halibut (*Reinhardtius hippoglossoides*) captured on 4 July 1994. (**A**) Whole right (eyed-side) sagitta. (**B**) Embedded and sectioned left (blind-side) sagitta, cut slightly oblique to the transverse plane. (**C**) Stained cross-section pattern of the anterior half of left sagitta. (**D**) Stained cross-section pattern of the posterior half of left sagitta. Age estimate is 4 years. Note the clarity of the whole otolith surface pattern compared to the cross-section pattern which has multiple checks. Viewed with reflected light.

have extended finger-like appendages radiating from the core that are easily broken, as well as a dome-shaped sulcus bulging proximally from the core (Fig. 72). These fragile structures hinder age readers from using the standard break-and-burn technique (Chapter 3) for Greenland halibut age determination.

Our program first attempted to age Greenland halibut in the early 1990s by analyzing only the surface pattern, as commonly practiced by Atlantic age determination laboratories. Surface patterns typically have a myriad of checks, and identifying an annual pattern can be difficult, especially near the otolith margin. For many years, our program, as well as some of the Atlantic laboratories, suspected that the surface method of Greenland halibut age determination was biased and underestimated true age.

An age validation study in the Northwest Atlantic using bomb radiocarbon and oxytetracycline tagging confirmed that otolith surface and simple cross-section methods underestimate age in larger, older Greenland halibut (Treble et al., 2008). Bomb radiocarbon analysis estimated longevity to be at least 27 years for this species (Treble et al., 2008).

The importance of Greenland halibut in the North Pacific and concerns regarding its declining population prompted our laboratory to embark on a study to develop viable aging criteria. This study found that staining transverse otolith cross sections resulted in increased precision for older specimens (Gregg et al., 2006). Our findings led our laboratory to adopt this method as our primary means of Greenland halibut age determination.

Greenland halibut otolith growth patterns are difficult to interpret, and inter-reader precision tends to be low (CV=11.05%; *n*=1115). To support the validity of our age estimates, a bomb radiocarbon study is in progress at the AFSC. This validation method is described in further detail in Chapter 6 of this manual.

## Current age determination methods

Our program primarily uses resin-embedded, stained otolith cross sections for Greenland halibut age determination. However, preparing cross sections is time-intensive and may not always be the best method for young otoliths with relatively clear surface patterns. Age readers should first examine each otolith surface pattern and decide whether the surface pattern is sufficiently clear to estimate age. Some otoliths have very clear surface patterns but checks within their cross-section patterns (Fig. 72). Correlating surface and cross-section patterns is a valuable method to prevent over-aging the cross-section pattern (Fig. 73). In general, otoliths from fish 300 mm or smaller are better aged from surface patterns because they are generally no older than 3 years. Furthermore, making precise cuts through the core of very small embedded otoliths is very difficult and likely to result in poor cross-section patterns.

The orientation and nomenclature used to describe Greenland halibut otoliths are shown in Figures 72 and 74. Both the blind-side and eyed-side otoliths are relatively flat with a dome-shaped bulge at the core. In older fish, this dome-shaped core is a dominant characteristic of the blind-side otolith's proximal surface. The centric orientation of the core and the prominent domed sulcus found in the blind-side otolith makes it the otolith of choice for cross-sectioning. The recommended cutting plane is transversely through the core and sulcus (Fig. 72).

**Surface examination.** In general, the first annual mark, with a diameter of about 2 mm, is easy to identify on the otolith surface, as is the second annual mark. Typically the second and third annual marks are around 3 and 4 mm in diameter, respectively. The surface pattern contains checks thereafter, and successive annual marks can be difficult to identify.

If the otolith is older than 4–5 years and does not have an exceptionally clear pattern, it should be cross-sectioned. However, prior to embedding, the surface pattern of the intact otolith should be examined and used to develop an age determination strategy for the cross section. Greenland halibut is not an easy species to age and requires more time investment in pattern analysis. As illustrated in Figure 72, viewing the cross section without the surface pattern may be ambiguous and misleading. Otoliths with widely spaced translucent growth zones are generally from younger, fast-growing fish, while otoliths with tightly spaced translucent growth zones, especially those that are dense along the margin, are usually from older, slow-growing fish.

**Cross-section examination.** To prepare otolith cross sections for age determination, the left (blind-side) otolith is embedded in polyester resin and cut in half using a low-speed saw. The cut is made through the transverse plane and adjusted to ensure that the saw blade bisects the core and passes through the thickest part of the domed sulcus (Fig. 72). The cut face is polished with 800 grit wet-dry sand paper to remove saw marks.

Staining techniques have been adapted from Richter and McDermott (1990). Polyester resin blocks containing cut otoliths are submerged in a solution of 1% Aniline Blue WS in 1% acetic acid. The stain solution should be stored at 20–23°C. Staining times vary but average around 13 minutes, after which the otoliths are rinsed with fresh water and wiped clean to ensure that residual acid and stain are removed. Both halves of the stained cross section are examined under a dissecting microscope at 12–50× magnification, using reflected light. Mineral oil is applied to the polished surfaces to eliminate surface glare.

To the trained eye, the first and second annual marks are readily identifiable if the otolith was sectioned through the core. Typically observed inside the first annual mark is a well-defined, mushroom-shaped check (Fig. 75). The first annual mark sometimes appears to be merged with or just slightly bordering this check. If the early growth pattern is difficult to interpret, the first three annual marks are identified using average measurements from clear specimens. The expected diameters of the first three annual marks are 1.5–2.5 mm, ~3 mm, and ~4 mm, respectively. Occasionally, the first and second annual marks are difficult to separate from surrounding checks, but the third annual mark may be clearly identified using expected measurements (Fig. 76). If the core is not visible due to a bad cut, extra care must be taken in identifying early annual marks.

The remaining annual marks are identified by examining both sides of the sulcus. If the cross section includes the domed sulcus, annual marks may be seen crossing the sulcus connecting the dorsal and ventral patterns (Fig. 77). Age estimates are typically made using the domed sulcus as a reading axis. The dorsal and ventral tips often have too many checks to be useful for determining a precise age estimate, although they may provide supplementary information helpful in interpretation of the later annual marks.

A recurring pattern in many Greenland halibut otoliths is a transition zone around the sixth and seventh annual marks (Fig. 77). It is not currently known why this transition zone occurs in some otoliths, what percentage of otoliths it is clearly identified in, and whether it is associated with biological changes. In difficult otoliths, the transition zone can be a useful reference point.

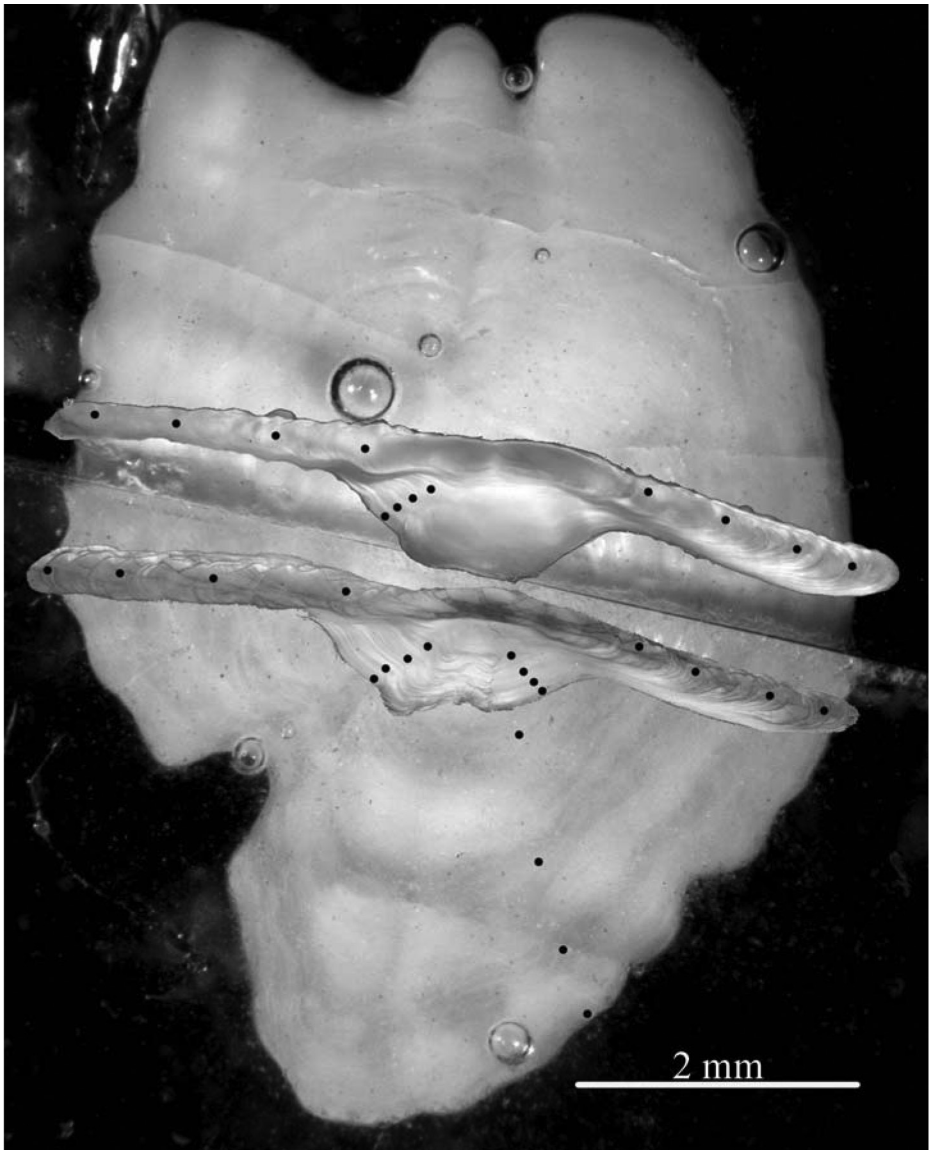

**Figure 73**

Composite image of the left sagitta in Figure 72. Stained cross-section patterns of each half are superimposed onto the corresponding surface image to elucidate the annual pattern. Together, the cross sections and surface patterns yield an age estimate of 4 years. Viewed with reflected light.

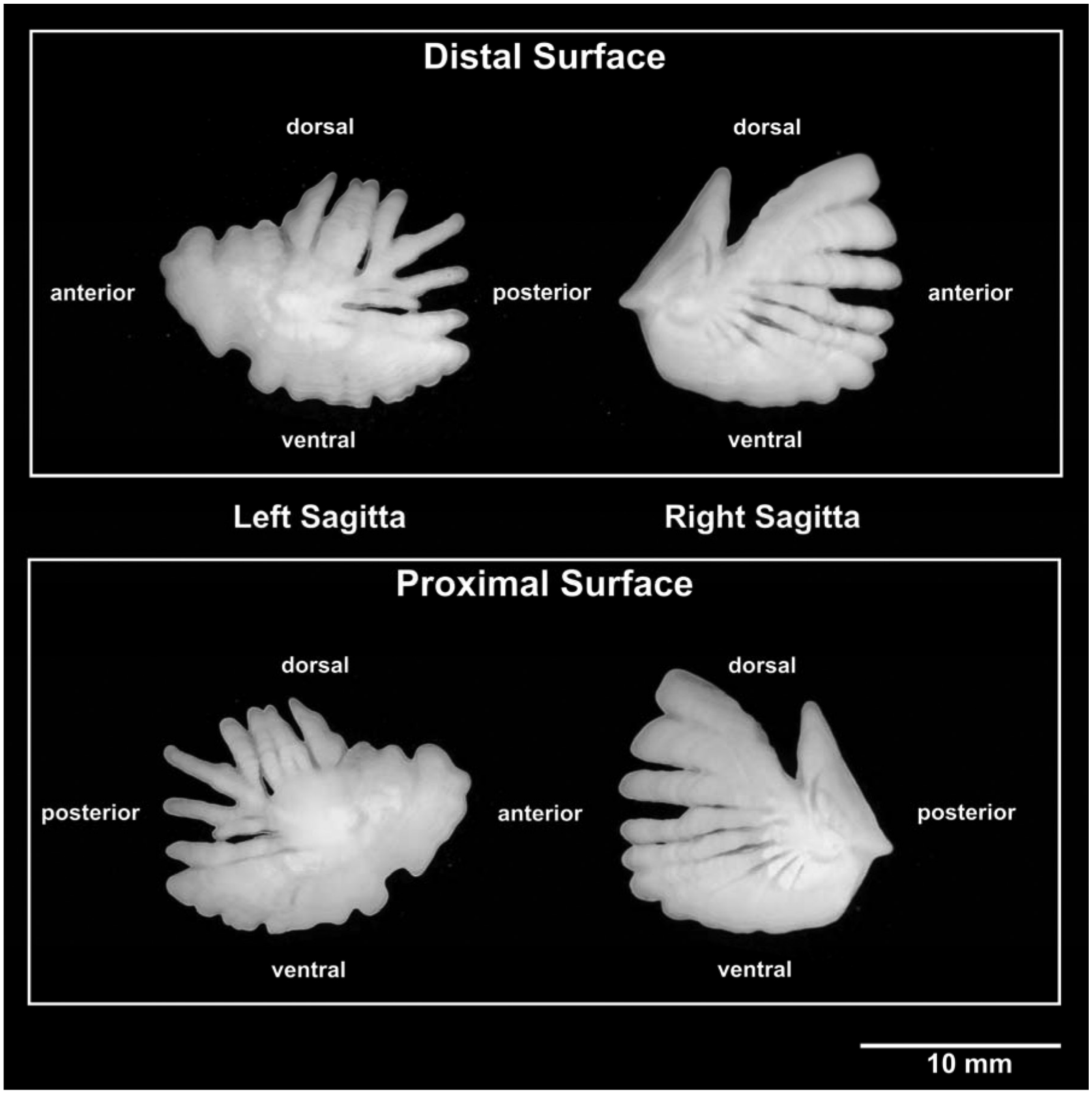

**Figure 74**

Orientation of whole Greenland halibut (*Reinhardtius hippoglossoides*) sagittal otoliths. Viewed with reflected light.

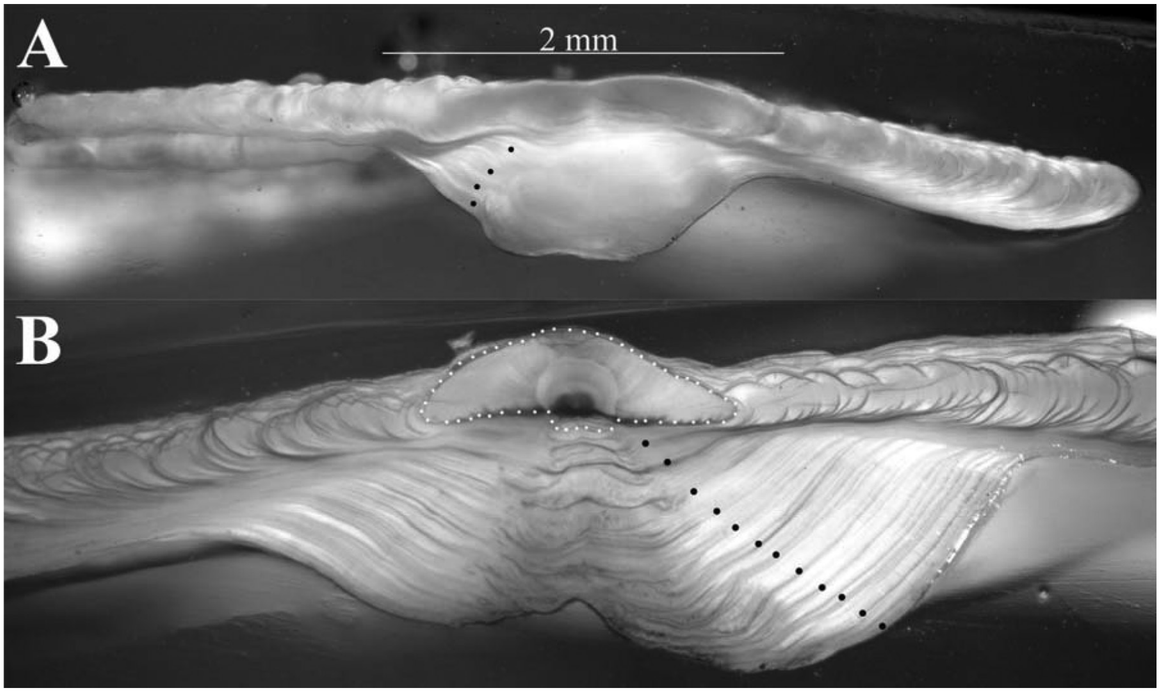

**Figure 75**

Stained otolith cross sections from two different Greenland halibut (*Reinhardtius hippoglossoides*). (**A**) Cut is not directly through the core, resulting in problems identifying the early annual marks. Age estimate is 4 years. (**B**) Cut is made directly through the core, and early annual marks are easier to identify. Mushroom-shaped check inside first annual mark is outlined by white dots. Age estimate is 12 years. Viewed with reflected light.

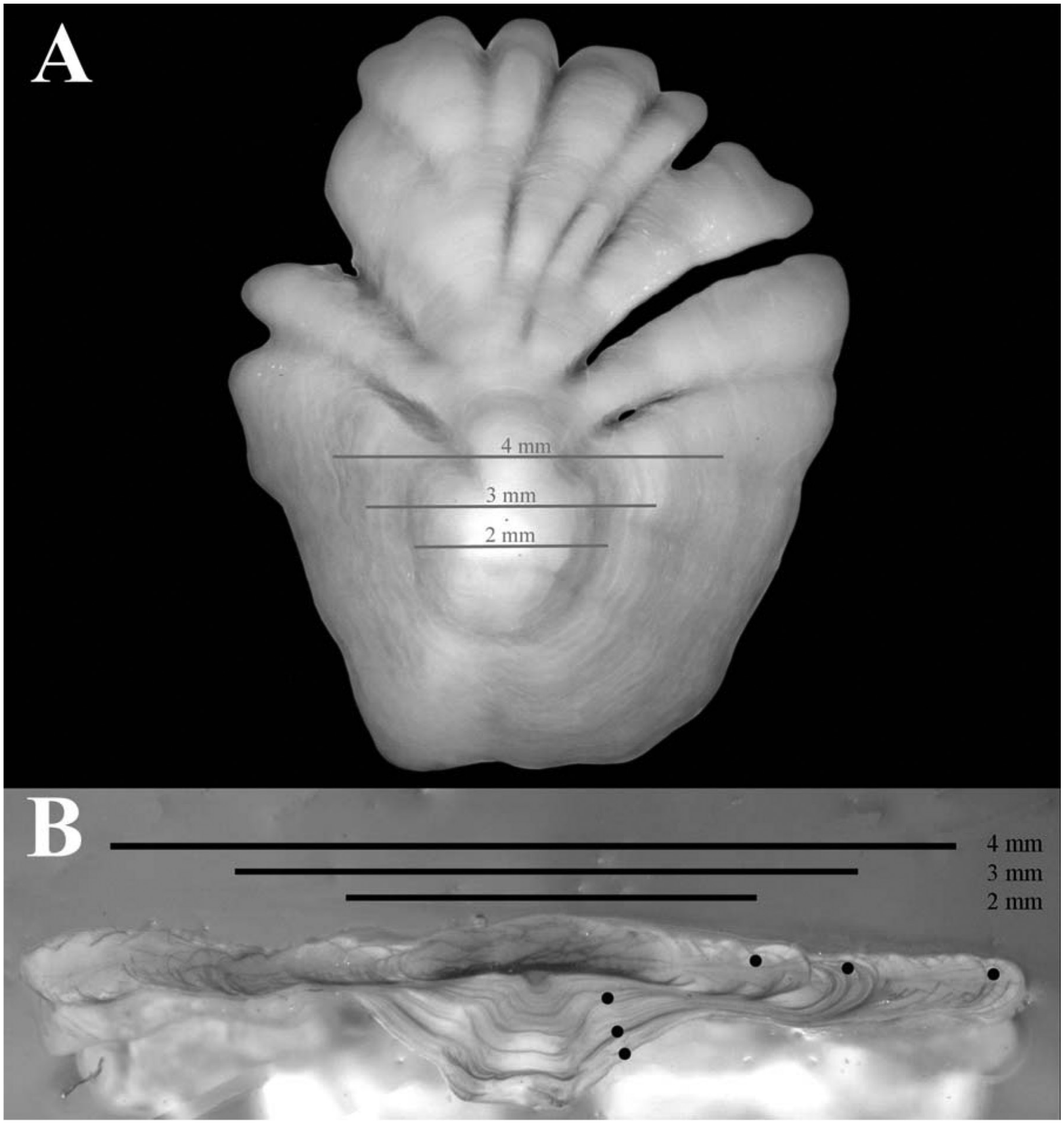

**Figure 76**

Measurements help identify early annual marks in otoliths from two different Greenland halibut (*Reinhardtius hippoglossoides*). (**A**) Otolith with a clear surface pattern. (**B**) Stained cross section where measurements were used to reduce ambiguity in identification of the first, second, and third annual marks. Viewed with reflected light.

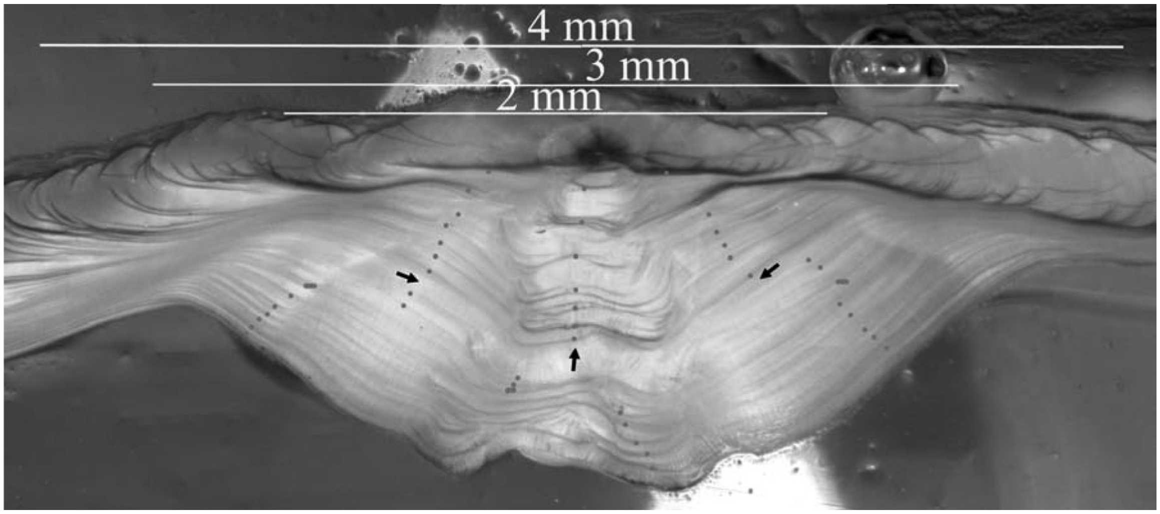

**Figure 77**

Stained otolith cross section from a 590 mm male Greenland halibut (*Reinhardtius hippoglossoides*) collected in July 1994. Annual marks can be followed from the dorsal side to the ventral side of the otolith through the sulcus. Checks are observed after the seventh annual mark. The annual pattern is clearer within the domed sulcus. A transition zone commonly observed in Greenland halibut otoliths is visible after the seventh annual mark (indicated by arrows). Age estimate is 16 years. Viewed with reflected light.

## Arrowtooth flounder (*Atheresthes stomias*)

**by John D. Brogan**

### Biology

Arrowtooth flounder (*Atheresthes stomias*) is a large, piscivorous flatfish species ranging from central California to the eastern Bering Sea (Hart, 1973). Arrowtooth flounder is one of the most abundant fish species in the Gulf of Alaska (von Szalay et al., 2010).

Research conducted along the Washington coast has demonstrated that arrowtooth flounder is a group-synchronous batch spawner (Rickey, 1995). Although there are differences in specific spawning times for different areas along the Pacific coast, spawning in Alaskan waters generally occurs from December through February (Witherell, 2000; Blood et al., 2007).

In the Gulf of Alaska, female arrowtooth flounder attain 50% maturity at 7 years or 460 mm (Stark, 2008). Males reach 50% maturity at a length of 420 mm (Zimmerman, 1997). According to AFSC survey data, females grow more slowly and much larger than males, and arrowtooth flounder from the Bering Sea exhibit slightly faster growth rates than those from the Gulf of Alaska (Fig. 78). Von Bertalanffy growth parameters were $L_\infty$=816.2 mm, k=0.1253/yr, and $t_0$=–0.262 yr (*n*=593), and $L_\infty$=836.3 mm, k=0.0931/yr, and $t_0$=–0.843 yr (*n*=729), for arrowtooth flounder collected during trawl surveys in the Bering Sea in 2004 and Gulf of Alaska in 2005, respectively (Fig. 78). The maximum age reported to date by the AFSC Age and Growth Program for arrowtooth flounder is 32 years.

### Age determination history

The AFSC Age and Growth Program began age determination of arrowtooth flounder in 1975. In 1991, we discovered that specimens collected as arrowtooth flounder (*Atheresthes stomias*) included a different species, Kamchatka flounder (*A. evermanni*). Over 9000 positively identified arrowtooth flounder have been aged since the early 1990s. The majority of specimens have been aged using the break-and-burn method; however, surface examination has also been used for young specimens with clear growth patterns. Arrowtooth flounder otoliths are generally more difficult to interpret than some of the other flatfish species aged at the AFSC, reflected by somewhat lower estimates of inter-reader precision (CV=8.4%; *n*=2487). To date, there are no published studies on arrowtooth flounder age validation.

### Current age determination methods

**Surface examination.** Whole arrowtooth flounder otoliths are examined at 6× magnification using a dissecting microscope and reflected light. The average distance from the anterior tip to the posterior tip of a 1-year-old blind-side otolith is 4.0 mm. The clearest

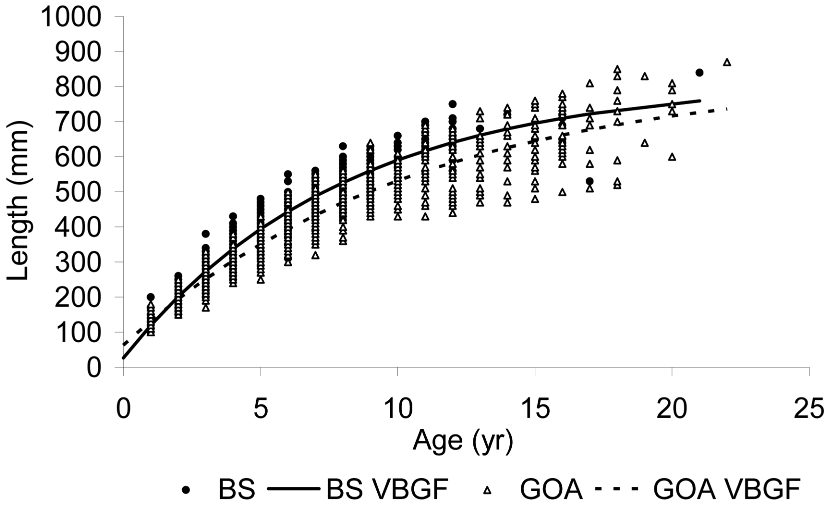

**Figure 78**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for arrowtooth flounder (*Atheresthes stomias*) collected during trawl surveys in the Bering Sea (BS; *n*=593) in 2004 and Gulf of Alaska (GOA; *n*=729) in 2005.

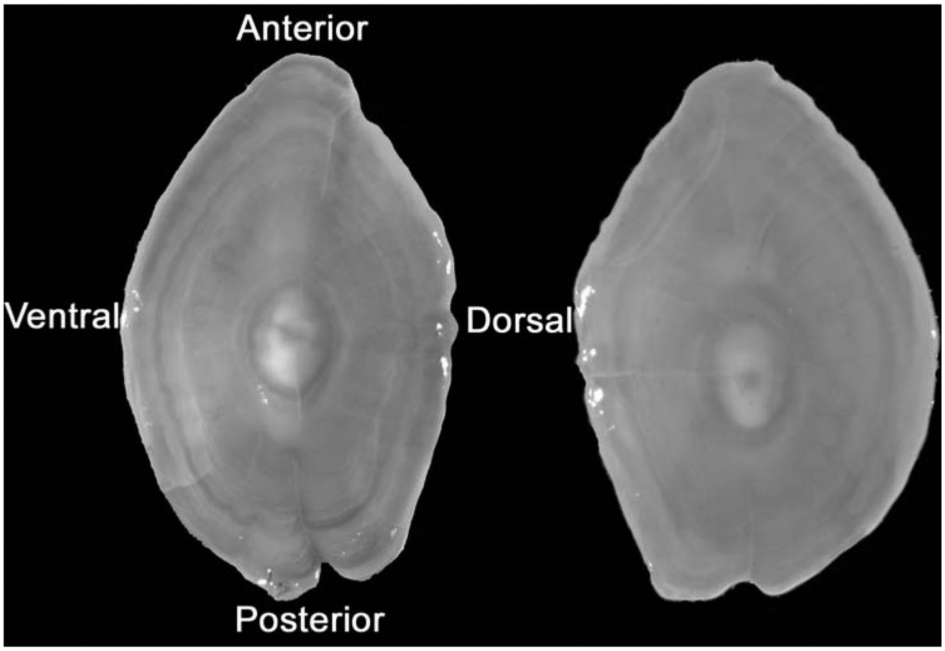

**Figure 79**

Distal surfaces of blind-side (left) and eyed-side (right) arrowtooth flounder (*Atheresthes stomias*) otoliths.

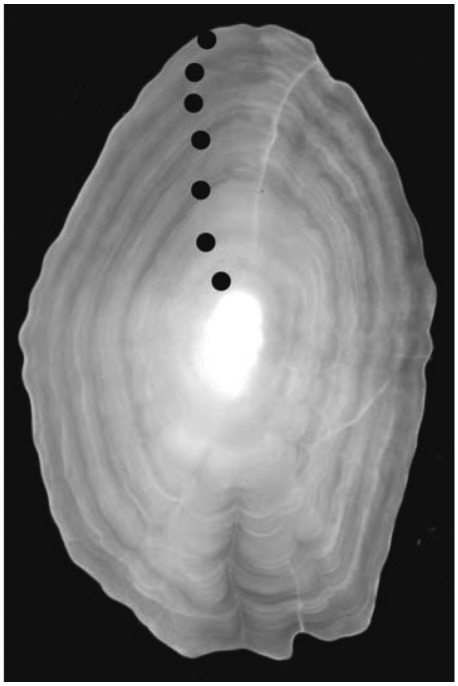

**Figure 80**

Arrowtooth flounder (*Atheresthes stomias*) otolith. Faint second annual mark close to the first. Age estimate is 7 years.

growth patterns are typically found on the anterior side of either the distal or proximal surface (Fig. 79). Arrowtooth flounder otoliths can be difficult to age on the surface; however, an experienced age reader can assign age estimates up to 7 years for otoliths with clear surface patterns (Fig. 80).

Using expected measurements can be helpful in identifying the early annual marks. The first annual mark encompasses a wide surface area and has an average dorsal-ventral diameter of about 1.5 mm. The first and second annual marks can be very close together, and it can be easy to mistake the second annual mark as a check. Figure 80 shows this type of pattern, with a faint second annual translucent growth zone very close to the first. Because it forms a complete ring around the otolith, it should be counted as an annual mark.

When the surface pattern is difficult to interpret or there are questions about the surface age estimate, the specimen should be aged using the break-and-burn method or the break-and-bake method (Chapter 3). The criteria applied for age determination are the same for both methods.

**Break-and-burn examination.** As with surface examination, the blind-side otolith is preferred for the break-and-burn method. The first three translucent growth zones should be traced on the surface of the otolith with a pencil prior

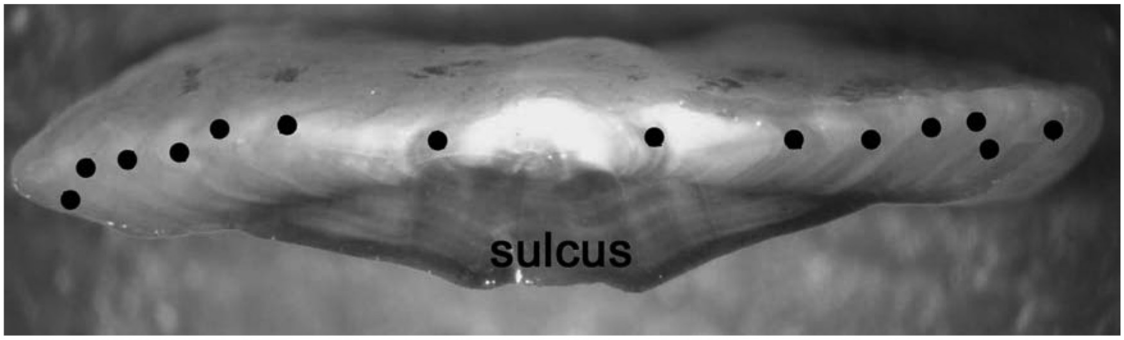

**Figure 81**

Clear break-and-burn cross section of an arrowtooth flounder (*Atheresthes stomias*) otolith. Dots indicate annual marks and preferred reading axes for arrowtooth flounder otoliths. Age estimate is 7 years.

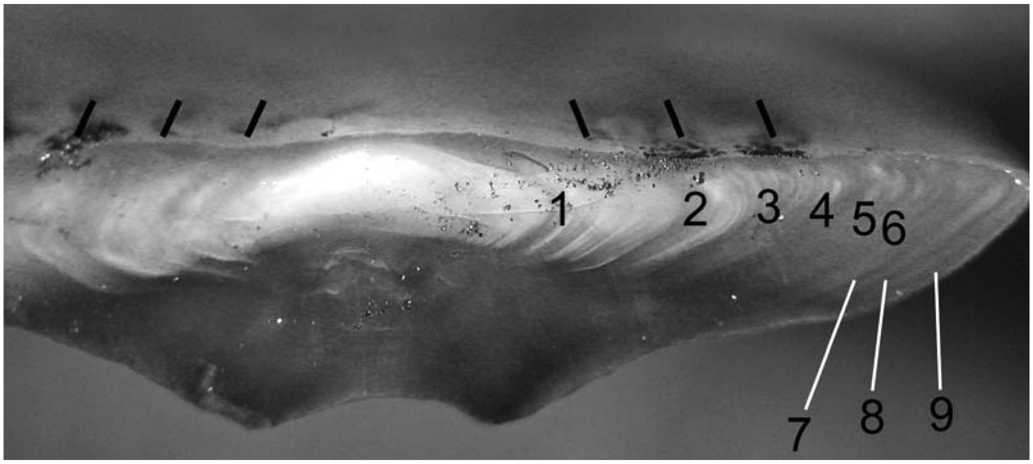

**Figure 82**

Arrowtooth flounder (*Atheresthes stomias*) otolith break-and-burn cross section with some irregular spacing. Tracing the translucent growth zones on the distal surface with a pencil (indicated by black ticks) helped identify the first three annual marks. Age estimate is 9 years.

to cutting and burning because the early years of the break-and-burn pattern often contain too many checks to definitively identify annual marks. Also, because the otoliths are small, precise cutting through the core is sometimes difficult, and a traced first year will help determine whether the core was missed during sectioning.

Typically, the posterior otolith half is burned, although sometimes the anterior half is also burned to clarify questionable annual marks. If the cut surface is too rough, the otolith is sanded using 600 grit sandpaper. The otolith can be burned by quickly passing it over an alcohol flame a few times; however, it is easy to over-burn arrowtooth flounder otoliths, causing them to crumble. An easy way to prevent over-burning is to use the break-and-bake method, which results in a uniform pattern. It is best to bake arrowtooth flounder otoliths for 7–8 minutes at 500°F.

Cross sections are examined at 25× magnification using a dissecting microscope and reflected light. Unlike most other flatfish otoliths, arrowtooth flounder otoliths do not typically have distinct annual marks near the sulcus. Arrowtooth flounder otoliths are usually read from the core to the dorsal and ventral tips as illustrated in Figure 81. When possible, annual marks should be followed from the distal edge toward the sulcus. If spacing is irregular in the first few years, the pencil marks on the otolith surface will help to determine annual marks. For example, Figure 82 shows a

9-year-old otolith with a questionable second annual mark. The second annual mark looks discontinuous, but it can be followed to the sulcus, and this fact, combined with the pencil marks, clearly shows that it is an annual mark and not a check.

In older arrowtooth flounder specimens, the annual marks are narrowly spaced after the third or fourth year. In some of these cases, it can be helpful to use the sulcus and dorsal and ventral tips as reading axes. Figure 83 shows a 15-year-old otolith with irregular banding patterns. The tip of the cross section is a clearer reading axis than the sulcus; however, there can still be trouble interpreting the edge of this specimen. When reading the tip, the tenth through twelfth translucent growth zones appear to be a single annual mark with some splitting. However, closer examination reveals that these translucent growth zones are likely three separate annual marks observed throughout the otolith.

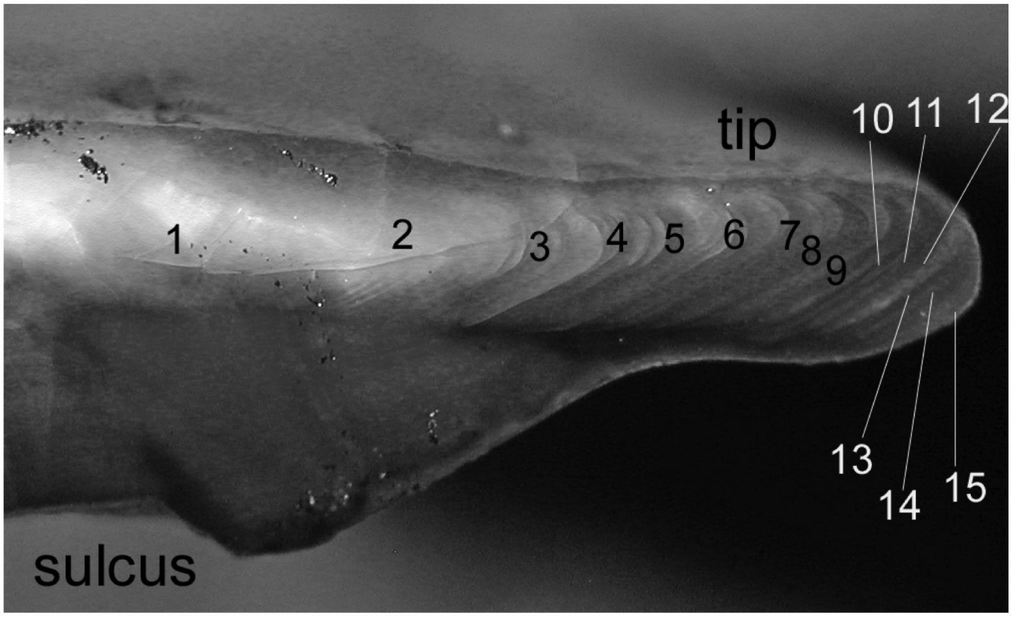

**Figure 83**

Arrowtooth flounder (*Atheresthes stomias*) otolith break-and-burn cross section with some ambiguity in the growth pattern near the edge. Age estimate is 15 years.

## Yellowfin sole (*Limanda aspera*)

### by Wes Shockley and Mary Elizabeth Matta

### Biology

Yellowfin sole are found in continental shelf waters of the North Pacific Ocean, ranging from the Chukchi and Bering Seas south along the continental shelves to the Sea of Japan and Vancouver Island, British Columbia (Bakkala, 1981; Wilderbuer et al., 1992). This species supports the largest commercial flatfish fishery in the United States, with 121,029 metric tons caught in the eastern Bering Sea in 2007 (Wilderbuer et al., 2008). Yellowfin sole experienced a dramatic decline in stock size due to overfishing in the late 1950s and early 1960s, but with reduced fishing pressure abundance increased in the 1980s and has since stabilized (Wilderbuer et al., 2008).

Yellowfin sole spawn between May and August (Fadeev, 1970; Wilderbuer et al., 1992). Juveniles reside in shallow bays and gradually move to deeper waters as they age (National Research Council, 1996). Adults overwinter along the continental shelf-slope break in waters 100–270 m deep and migrate to the inner shelf (<100 m) during the summer months for the purposes of spawning and feeding (Bakkala, 1993; Wilderbuer et al., 1992). In the eastern Bering Sea, size at 50% maturity is 203 mm for males and 288 mm for females, corresponding roughly to the ages of 7 and 11 years, respectively (Wilderbuer et al., 1992). According to AFSC Age and Growth Program data, females are larger at age than males after reaching maturity (Fig. 84). Von Bertalanffy growth parameters were $L_\infty$=352.46 mm, k=0.16/yr, and $t_0$=0.68 yr (*n*=883) and $L_\infty$=398.27 mm, k=0.13/yr, and $t_0$=0.37 yr (*n*=1149), respectively, for male and female yellowfin sole collected during trawl surveys in the Bering Sea from 2006 through 2008 (Fig. 84).

To date, the oldest specimen aged by the AFSC was a 39-year-old female collected in the Bering Sea. A strong, positive correlation (r=0.90) between bottom temperature and the width of yellowfin sole otolith growth zones has been observed (Matta et al., 2010).

### Age determination history

Of the flatfish species routinely aged by the AFSC Age and Growth Program, the growth patterns of yellowfin sole otoliths are typically the easiest to interpret, with a high level of agreement between readers (CV=2.9%; *n*=5643). Since 1982, over 22,000 yellowfin sole have been aged by the Age and Growth Program. In some cases otolith surface patterns have been used to estimate age for this species, but the majority of yellowfin sole otoliths have been aged using the break-and-burn method. Yellowfin sole age estimates have been corroborated by edge type analysis (Kimura et al., 2007) and synchronous growth patterns observed among individuals (Matta et al., 2010). A bomb radiocarbon study is currently in progress at the AFSC to provide a

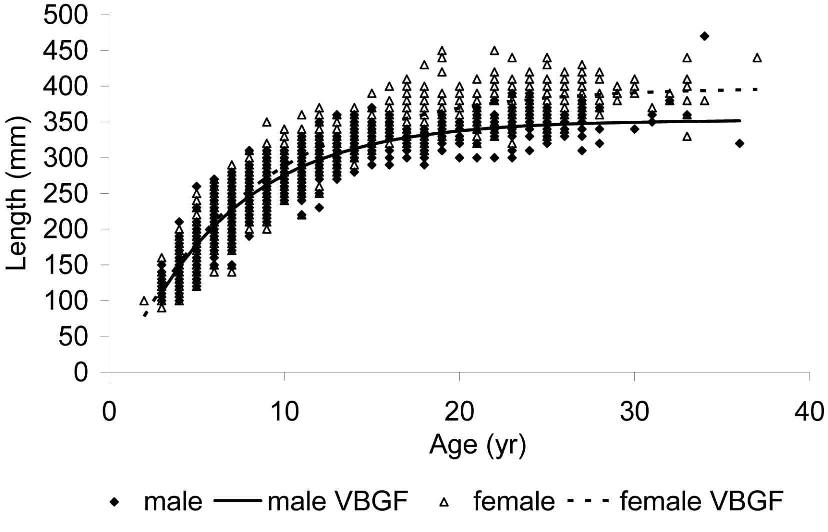

**Figure 84**

Length-at-age data fit with von Bertalanffy growth functions (VBGF) for male (*n*=883) and female (*n*=1149) yellowfin sole (*Limanda aspera*) collected during trawl surveys in the Bering Sea from 2006 through 2008.

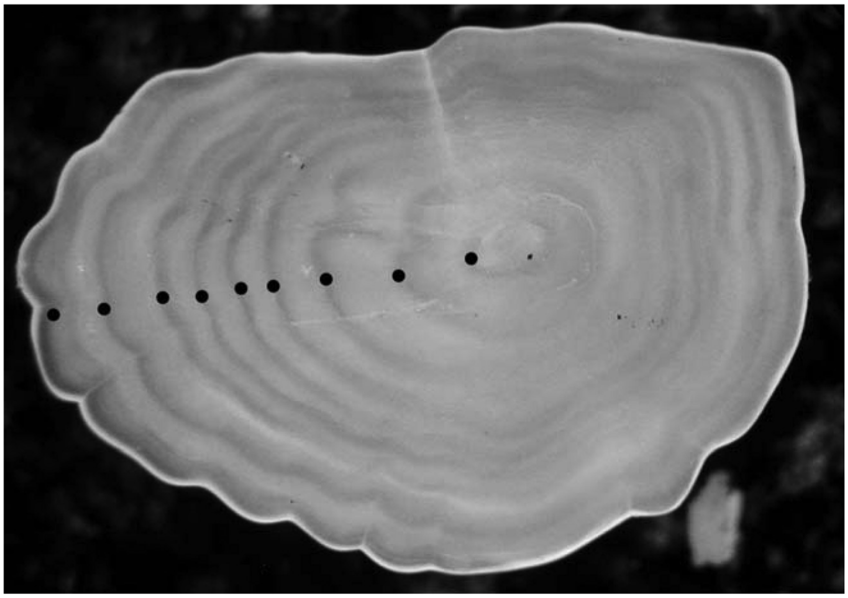

**Figure 85**

Clear proximal surface pattern of a yellowfin sole (*Limanda aspera*) otolith. Age estimate is 9 years. Viewed with reflected light.

stronger method of validation for this species. Validation methods are described in further detail in Chapter 6 of this manual.

## Current age determination methods

**Surface examination.** Yellowfin sole otoliths usually possess readily identifiable first annual marks, distinct translucent growth zones, and fairly regular spacing. Depending on the specimen, surface age determination (Fig. 85) is generally reliable for otoliths up to 12 years. Yellowfin sole otolith surfaces are examined using a dissecting microscope at 6–16× magnification with reflected light.

**Break-and-burn examination.** When the otolith growth pattern is difficult to interpret, the break-and-burn method is used (Chapter 3). Yellowfin sole otoliths are usually cut transversely through the core using a scalpel. Yellowfin sole otoliths should be burned longer than otoliths of most other flatfish species, as this clarifies the growth patterns for easier interpretation. Break-and-burn cross sections are examined at 32–60× magnification using a dissecting microscope with reflected light.

Age is determined by counting annual marks from the core to the otolith edge (Fig. 86). In yellowfin sole otoliths, the first annual mark is relatively small in diameter and is normally clear. However, the first annual mark can be difficult to detect in some break-and-burn patterns, as it sometimes can be diminished by the burning process (Fig. 87). Furthermore, the heat from the flame may cause the accreted otolith layers to physically separate, such that the material corresponding to the first year of growth may break away from the rest of the otolith (Fig. 88). The surface pattern should always be compared with the break-and-burn pattern to ensure that the first annual mark is not overlooked.

**Age determination problems.** While yellowfin sole age determination is typically easy relative to other groundfish species, they occasionally exhibit some of the problems of interpretation common to all otoliths. Crystallization is probably the most frequently encountered impediment to age determination of yellowfin sole otoliths. Crystallization can render a specimen difficult or impossible to age because it often obscures the edges and the most recently deposited annual marks.

Other problems one encounters in yellowfin sole otoliths are irregular spacing, splitting, and pre-annular checks. Particularly on older specimens, irregular spacing can lead to doubt as to whether the presumed annual marks are true or are the result of splitting, where two translucent growth zones are deposited within the same year (Fig. 89). Because spacing between annual marks may be a result of interannual differences in water temperature (Matta et al., 2010), age readers should approach otoliths displaying irregular spacing with caution. When close spacing of annual marks occurs, the areas adjacent to the sulcus are typically the best reading axes (Fig. 90). Pre-annular checks are a fairly common problem in yellowfin sole (Figs. 90 and 91). Pre-annular checks tend to be pronounced near the distal surface but insignificant or absent closer to the sulcus; they are therefore seldom a problem for age determination.

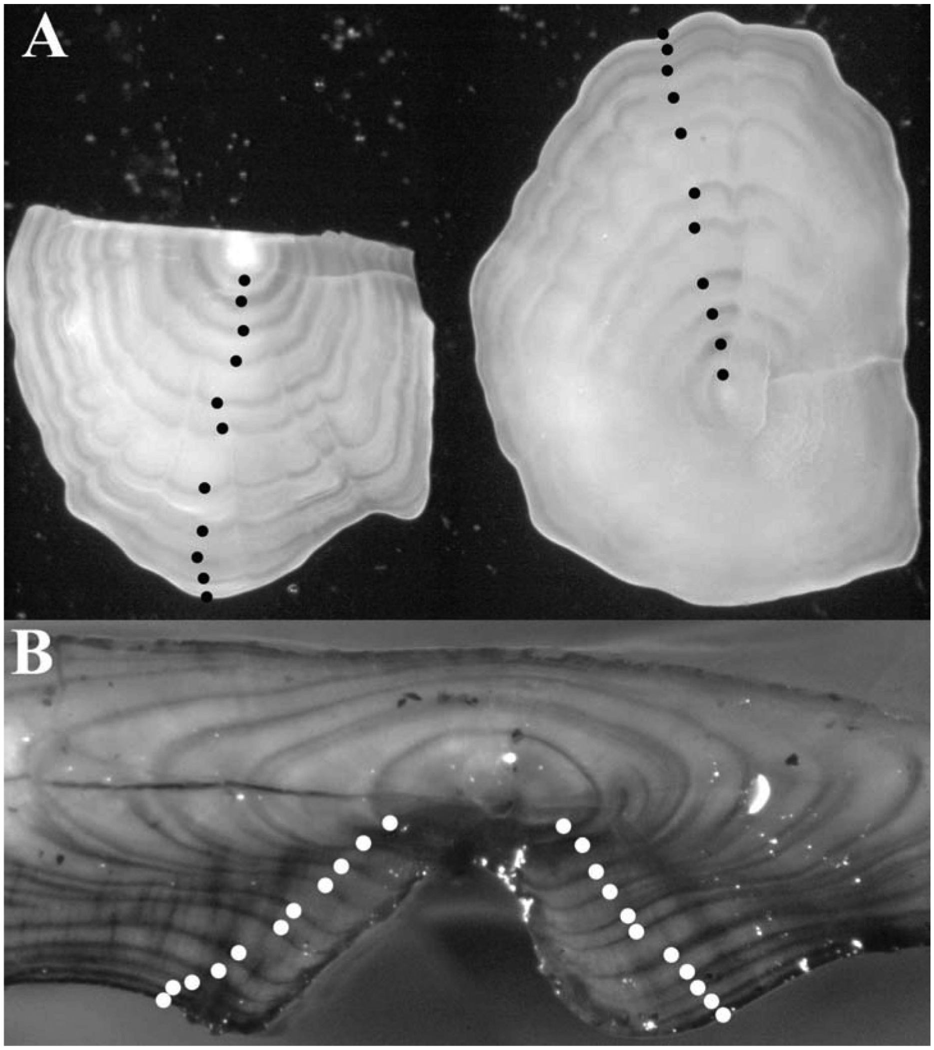

**Figure 86**

Clear yellowfin sole (*Limanda aspera*) otolith (**A**) surface pattern and (**B**) break-and-burn pattern. Collected from a 310 mm male on 24 February 2007. Age estimate is 11 years. Viewed with reflected light.

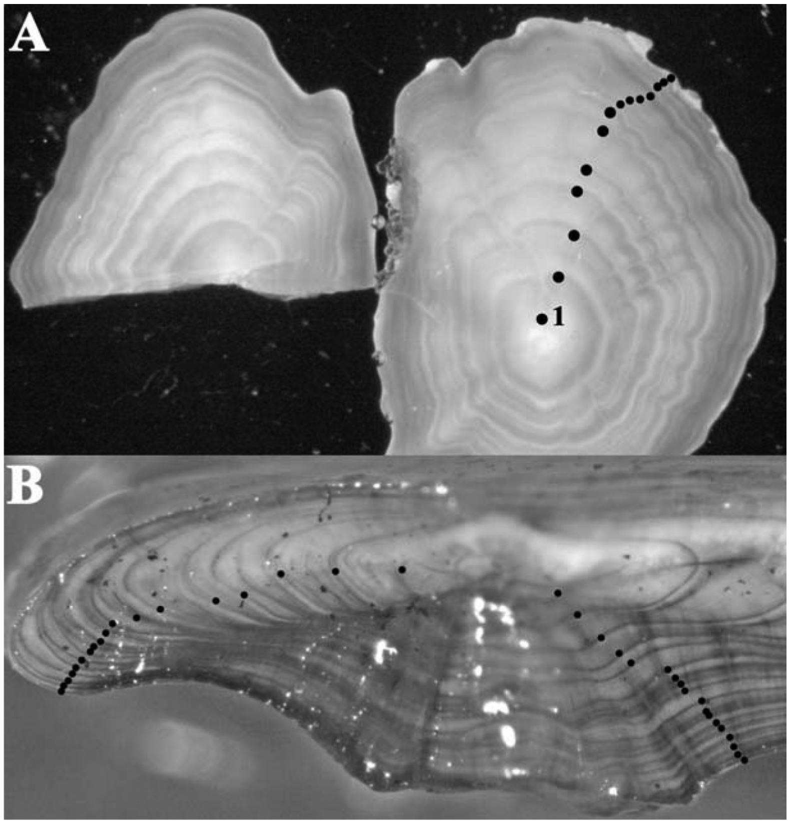

**Figure 87**

(**A**) Otolith surface and (**B**) break-and-burn growth patterns from a yellowfin sole (*Limanda aspera*) collected on 4 March 2007. Faint first annual mark, especially on the break-and-burn face. Only 14 annual marks can be viewed on the surface pattern, illustrating the importance of comparing preparation methods. Age estimate is 18 years. Viewed with reflected light.

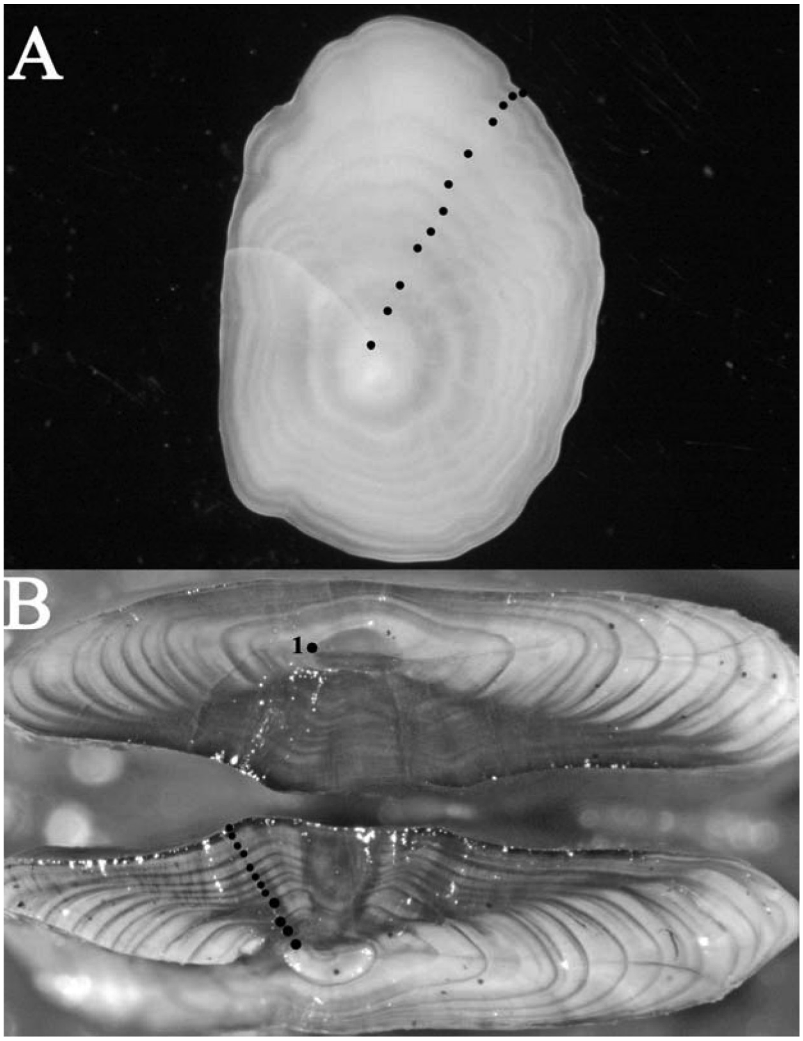

**Figure 88**

(**A**) Otolith surface and (**B**) break-and-burn patterns from a yellowfin sole (*Limanda aspera*) collected 9 March 2007. In the upper cross section, layers of material including the first annual mark broke away from the otolith during the burning process, leaving a pit behind. Age estimate is 12 years. Viewed with reflected light.

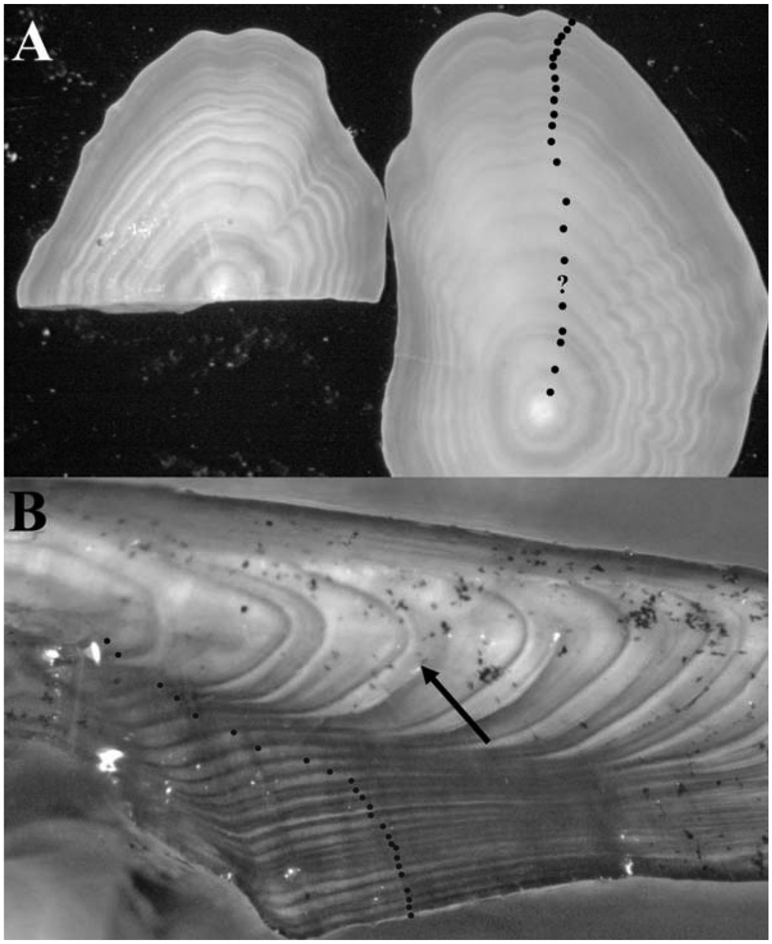

**Figure 89**

(**A**) Otolith surface and (**B**) break-and-burn patterns from a yellowfin sole (*Limanda aspera*) collected in April 2007 with irregularly spaced annual marks. Faint first annual mark and narrow spacing between the third and fourth annual marks compared to the wider spacing of the fifth, sixth, and seventh annual marks. Questionable sixth annual mark (indicated by question mark in (**A**) noted on the surface pattern is most likely a check, evident from the break-and-burn pattern (indicated by arrow). Age estimate is 25 years. Viewed with reflected light.

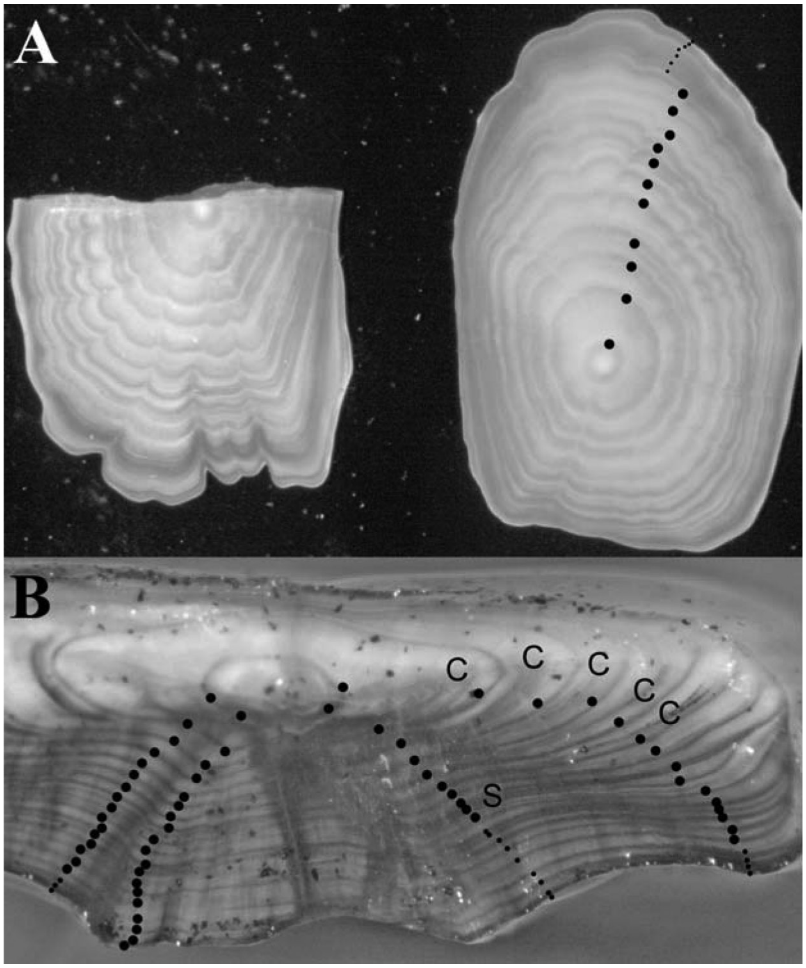

**Figure 90**

(**A**) Otolith surface and (**B**) break-and-burn patterns from a yellowfin sole (*Limanda aspera*) collected on 19 February 2007. Pre-annular checks (indicated by "C") and closely spaced annual marks (indicated by "S") are visible in the break-and-burn pattern. The clearest reading axes are along either side of the sulcus. Age estimate is 19 years. Viewed with reflected light.

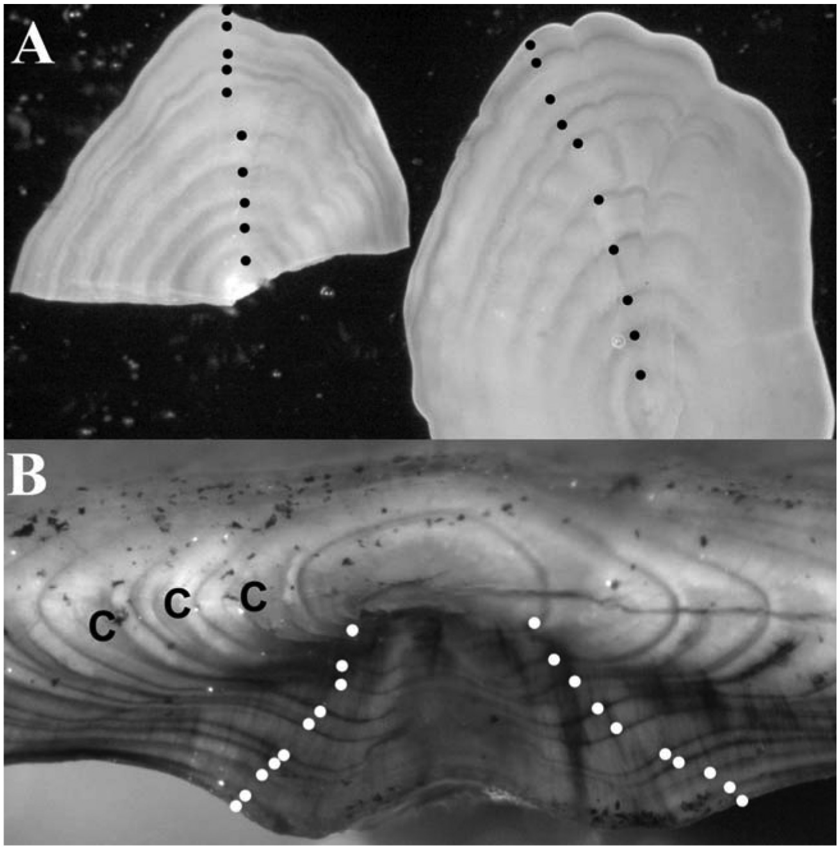

**Figure 91**

(**A**) Otolith surface and (**B**) break-and-burn patterns from a yellowfin sole (*Limanda aspera*). Pre-annular checks (indicated by "C") can be distinguished from annual marks by their faintness and lack of continuity. Collected from a 320 mm female on 17 February 2007. Age estimate is 10 years. Viewed with reflected light.

# Chapter 5: Precision and bias in age determination

**by Daniel K. Kimura, Delsa M. Anderl, and Mary Elizabeth Matta**

Fish age determination typically relies on the interpretation of perceived annual marks in hard structures. While age readers are expertly trained in pattern recognition and the use of species-specific aging criteria, some degree of subjectivity is typically involved. In age determination science, the term "accuracy" describes how well estimates of age (such as from counting annual marks) compare with true ages. "Precision" refers to the degree to which an age estimate is reproducible, either by the same age reader or among different age readers. For age data to be useful, high levels of both accuracy and precision are required. However, without known-age materials, accuracy is difficult to evaluate. Precision statistics do not necessarily reflect accuracy but may provide useful measures of the relative difficulty and repeatability of age determinations (Campana, 2001).

This chapter briefly outlines the procedures used by the AFSC Age and Growth Program to quantify age determination precision and relative bias. These procedures are more thoroughly described in Kimura and Anderl (2005). Campana (2001) also provides a thoughtful review of methods used to measure precision and accuracy in fish age determination. Methods used to assess age estimate accuracy are discussed in Chapter 6 of this manual.

## Precision testing

Age determination at the AFSC is based on the discipline of precision testing. A random subsample of at least 20% of all specimens is tested by a second age reader (Kimura and Anderl, 2005). To generate the subsample for a given collection of specimens, a number from one to five is randomly chosen and every fifth specimen thereafter is selected for testing. The second reader (hereafter referred to as the tester) ages the subsample without any knowledge of the first reader's age estimates. The reader and tester generally use the same preparation method to age a given specimen, but occasionally the tester may prefer a different method based on the relative clarity of the preparation. To ensure that repeated readings are statistically independent, age readers do not have knowledge of any other ages estimated from the structures. This means that if the same age reader makes repeated readings, the readings are separated in time so that age determinations are not done from memory.

Specimens without agreement between age estimates (Kimura and Anderl, 2005) are re-examined, and any remaining discrepancies are resolved between reader and tester. A second independent test may be required for samples which fail to meet an acceptable level of precision, and in some cases the primary age reader must re-age the sample. The 20% test sample has been used by our program for over 20 years to calculate precision statistics and is the standard that maintains our aging criteria over time and among age readers.

We typically calculate percent agreement (PA), average percent error (APE; Beamish and Fournier, 1981) and the coefficient of variation (CV; Chang, 1982) to evaluate inter-reader precision for a given sample. High PA values and low CV and APE values indicate good precision between age estimates. All precision statistics are relayed to data end users at the AFSC.

Because of tradition and simplicity, no statistic will likely ever replace PA as the fundamental statistic in age precision studies. It is simple to calculate and is usually used to make gross comparisons between samples. However, it does not consider specimen age, a problem given that exact agreement generally decreases with increasing age for most species (Kimura and Lyons, 1991). Both APE and CV account for age, allowing easier comparisons among species or samples with dissimilar age ranges. These measures can give a general idea of the relative difficulty associated with estimating age in different species (Kimura and Lyons, 1991; Kimura and Anderl, 2005). Precision statistics for all species aged at the AFSC are available on our website (Alaska Fisheries Science Center, http://www.afsc.noaa.gov/REFM/Age/Default.htm).

## Measures of bias

At the AFSC, all samples are scrutinized for relative bias between reader and tester age estimates. The single most important tool used by our laboratory for comparing age readers is a simple cross-tabulation of age estimates, so that the $x$-axis is reader age and the $y$-axis is tester age (Kimura and Anderl, 2005). These tables are used to see precisely where the reader and tester differ in their age estimates. For example, the tester may generate consistently older age estimates than the reader only for fish older than 20 years. Thus, a cross-tabulation is an easy way to identify potential systematic biases that may occur disparately among age classes.

Bias is statistically assessed using Bowker's test for symmetry (Bishop et al., 1975). A significant test statistic indicates that the reader and tester are interpreting aging structures differently. Bowker's test is a convenient index of overall similarity between reader and tester but may not be adequate for determining differences when sample sizes are small or when age ranges are broad.

Age bias plots are also used to graphically show bias (Campana et al., 1995), either on a relative basis (such as describing the difference between two age readers) or on an absolute basis (such as describing the difference between true age and estimates of age). Typically, the true (or most credible) age is plotted on the *x*-axis. The mean test age and error bars corresponding to each of the true age categories are plotted on the *y*-axis. Deviance from a 1:1 equivalence line indicates the presence of aging bias.

When bias occurs at the AFSC, readers and testers typically examine discrepant specimens together to pinpoint potential systematic differences in aging criteria. Sometimes a third age reader is asked to read the specimens independently. Conversely, the tester may be required to age another 20% subsample, or the primary age reader may have to re-age the sample, depending on the situation. It is a standard practice of the AFSC Age and Growth Program to resolve any bias issues prior to releasing age data to end users.

# Chapter 6: Age validation methods used at the Alaska Fisheries Science Center

**by Craig R. Kastelle, Daniel K. Kimura, and Charles E. Hutchinson**

## Introduction

Validation of fish age estimates and aging methods has been recognized as a critical element of the Age and Growth Program at the AFSC. Growth zones can often be enumerated in hard structures like otoliths, but it is necessary to validate the annual nature of translucent and opaque growth zone deposition (Beamish and McFarlane, 1983). In recent history our program has undertaken a number of special studies using different age validation methods to confirm the accuracy of routinely estimated fish ages (Table 3). The validation method chosen depends upon the specific research question being addressed, the species' estimated age range, the availability of suitable specimens and ancillary biological data, and the resolution of the validation methods available. The goals in age validation studies can be as broad as confirming a general age range or as specific as an aging error of one year.

In this chapter, age validation methods are broadly categorized as "direct" or "indirect." However, the quality of an age validation study realistically exists on a continuum that depends not only on the method used but also on the quality of data employed (Kimura et al., 2006).

## Direct methods

### Known-age studies

The age validation studies that are considered to be the most reliable and direct are those that utilize known-age specimens released into the wild. In this method, juvenile fish of known age are marked and released into their natural environment with the expectation that some percentage of them will be caught in several years as adults. The number of growth zones seen in the aging structure can be compared to the known age of the fish to reveal aging error. This age validation method assumes that the act of initial tagging and releasing does not affect the fish's future growth rate or the aging structure's appearance. However, the stress of tagging or the long-term presence of an external tag could change the fish's ability to survive or grow in the wild.

Sablefish (*Anoplopoma fimbria*) is the only groundfish species studied at the AFSC for which known-age studies have been undertaken. From 1985 to 2005, juvenile fish were caught as confirmed age-0 to age-2 specimens, tagged, and released (Heifetz et al., 1998; Maloney and Sigler, 2008). Recaptured sablefish have been used to validate age estimates and aging criteria up to 18 years.

### Bomb-derived radiocarbon validation

Bomb-derived radiocarbon ($^{14}$C) has the ability to validate fish age estimates directly with much more resolution than most other methods (Campana, 2001). Bomb-derived radiocarbon was first used to validate fish age estimates by Kalish (1993) and since has seen extensive use across many species worldwide (Kalish, 1995; Campana, 1997; Kerr et al., 2004; Piner and Wischniowski, 2004; Treble et al., 2008).

Above-ground testing of atomic bombs during the 1950s and 1960s produced an increase of environmental $^{14}$C above the natural levels (Nydal, 1993). This $^{14}$C became part of the biota, and the increase can be measured in calcified marine biological structures and used as a time signal (Kalish, 1993). To apply this validation method, bomb radiocarbon is first measured in otoliths from fish with known birth years to establish a "reference chronology" of $^{14}$C (Kerr et al., 2004; Piner and Wischniowski, 2004). When otolith-derived age estimates require validation, $^{14}$C is measured in otolith cores and posited birth years are determined from the growth zone-based age estimates. The timeframe of the $^{14}$C increase in the reference chronology is then compared to that of the species being validated (i.e., the "test species"). If the increases in $^{14}$C of the test species and reference chronology are in phase, the test species' age estimates are considered validated and accurate.

### Radiometric validation

The radiometric age validation method was pioneered by Bennett et al. (1982) in a study on splitnose rockfish (*Sebastes diploproa*) and has since proven very useful at the AFSC. In early studies, general age ranges were validated, such as in sablefish (Kastelle et al., 1994). In more recent studies, the process has been refined to be more specific, such as in the case of walleye pollock (*Theragra chalcogramma*), for which age estimates of young fish were validated (Kastelle and Kimura, 2006). Typically, this method excels at answering general questions such as, "What is the maximum age range of this species – is it long-lived or short-lived?"

The radiometric age validation method relies on the disequilibrium between a daughter-parent pair of radionuclides, $^{226}$Ra and one of its progeny, $^{210}$Pb, both part

**Table 3**

Age validation studies for species aged at the Alaska Fisheries Science Center (AFSC). This list is not exhaustive for all species aged at the AFSC. Studies in process at the AFSC as of 2010 are listed as "In process."

| | Direct validation methods | | | Indirect validation methods | |
|---|---|---|---|---|---|
| Species | Known age | Radiometric | Bomb radiocarbon | Edge type analysis | Other methods |
| Alaska skate<br>(*Bathyraja parmifera*) | | | | Matta and Gunderson (2007) | |
| Atka mackerel<br>(*Pleurogrammus monopterygius*) | | | | Anderl et al. (1996)<br>Kimura et al. (2007) | Anderl et al. (1996) |
| Dover sole<br>(*Microstomus pacificus*) | | | Kastelle et al. (2008a) | | |
| Giant grenadier<br>(*Albatrossia pectoralis*) | | Burton (1999) | In process | | |
| Greenland halibut<br>(*Reinhardtius hippoglossoides* | | | Treble et al. (2008)<br>In process | | |
| Northern rock sole<br>(*Lepidopsetta polyxystra*) | | | | Kimura et al. (2007) | |
| Northern rockfish<br>(*Sebastes polyspinis*) | | Kastelle et al. (2000) | In process | Kimura et al. (2007) | |
| Pacific cod<br>(*Gadus macrocephalus*) | | | | | Roberson et al. (2005) |
| Pacific ocean perch<br>(*Sebastes alutus*) | | Kastelle et al. (2000) | Kastelle et al. (2008b) | Kimura et al. (2007) | |
| Rougheye rockfish<br>(*Sebastes aleutianus*) | | Kastelle et al. (2000) | | | |
| Sablefish<br>(*Anoplopoma fimbria*) | Heifetz et al. (1998)<br>Maloney and Sigler (2008)<br>Rutecki and Varosi (1997)<br>In process | Kastelle et al. (1994) | | Kimura et al. (2007) | Beamish and Chilton (1982)<br>Beamish and McFarlane (2000)<br>Beamish et al. (1983)<br>McFarlane and Beamish (1995)<br>Pearson and Shaw (2004) |
| Shortraker rockfish<br>(*Sebastes borealis*) | | Hutchinson et al. (2007)<br>Kastelle et al. (2000) | In process | | |
| Shortspine thornyhead<br>(*Sebastolobus alascanus*) | | Kastelle et al. (2000)<br>Kline (1996) | | | |
| Walleye pollock pollock<br>(*Theragra chalcogramma*) | | Kastelle and Kimura (2006) | | Kimura et al. (2006)<br>Kimura et al. (2007) | Kimura et al. (2006) |
| Yellowfin sole<br>(*Limanda aspera*) | | | In process | Kimura et al. (2007) | |

of the naturally occurring decay chain of $^{238}$U. During metabolism, $^{226}$Ra is deposited along with calcium in calcified structures such as otoliths (Swanson, 1985; Porntepkasemsan and Nevissi, 1990). When $^{226}$Ra is deposited in otoliths and remains immobile in the closed calcium carbonate structure, a disequilibrium is created between $^{226}$Ra and all of its progeny. Over time, the activity (decays/min) of shorter-lived daughter products increases due to radioactive decay of the parent. The difference between the half-lives of $^{226}$Ra and $^{210}$Pb is great, so the activity ratio of $^{210}$Pb/$^{226}$Ra is a function of the time elapsed since deposition. In a time span of about 100 years, the activity of both $^{226}$Ra and $^{210}$Pb will become equal as secular equilibrium is reached asymptotically. Therefore, this method is only effective for validating age estimates of specimens under 100 years old.

In the mid-1990s, the AFSC developed a small radiochemical facility geared specifically toward analyzing $^{210}$Pb and $^{226}$Ra. This pair of radionuclides has since been used to age species younger than 5 years (Kastelle and Kimura, 2006) and as old as about 75 years (Kastelle et al., 2000). Other daughter-parent pairs, such as $^{228}$Th and $^{228}$Ra, have been used to validate fish age estimates ranging from 1 to 10 years (Smith et al., 1991), but the capability does not exist at the AFSC. The $^{210}$Pb and $^{226}$Ra radionuclides are comparatively easy to extract from otoliths and analyze, and the applicable age range is appropriate for most species aged at the AFSC.

## Indirect methods

Several indirect age validation methods have been used on species routinely aged at the AFSC. These are considered to be weaker forms of validation and as such are usually considered along with other evidence. Many of these methods may be more appropriately called "corroboration" and not validation (Kimura et al., 2006). However, when several lines of corroborating evidence all lead to the same conclusion, strong confidence can be placed in the probable accuracy of the age estimates.

### Mark-recapture studies

Oxytetracycline (OTC) mark-recapture has been used successfully to indirectly validate sablefish aging criteria (Beamish and Chilton, 1982; Beamish et al., 1983; McFarlane and Beamish, 1995; Beamish and McFarlane, 2000). Fish, often as adults, are caught, tagged, injected with OTC, and released. When a fish is recaptured some years later, the otolith displays an OTC mark and the apparent number of otolith growth zones after initial tagging can be compared to the number of years at liberty. One caveat associated with this method is that age is usually unknown upon tagging fish with OTC. Thus, upon recapture, age must be estimated from counts of unvalidated growth zones observed prior to the OTC mark. In sablefish, as many as the first 50 years were left unvalidated and were only presumed accurate based on the similar appearance of growth zones before and after the OTC mark (Beamish and McFarlane, 2000).

Other indirect age validation methods using tag-recapture data have proven useful at the AFSC. Pacific cod age estimates were validated two ways with otoliths from spaghetti-tagged and recaptured fish (Roberson et al., 2005). First, the estimated length of each fish at time of release was back-calculated from measurements of otolith annual marks. The estimated length agreed with observed fish length at time of release, supporting the assumption of annual growth zone deposition. Second, otolith age and von Bertalanffy parameters, estimated from tagging data, were used to predict how much each fish grew in length during its time at liberty. Otolith aging criteria applied to the tagged and recaptured fish accurately predicted the observed growth increments. Thus, multiple lines of evidence led to the conclusion that Pacific cod age estimates are accurate.

### Edge type analysis

In edge type analyses, estimated fish ages are validated indirectly by determining whether an annual pattern is present through evaluation of the edge growth of aging structures. These types of studies can range from a qualitative assessment of edge type (i.e., translucent or opaque) to a quantitative marginal increment analysis, in which new growth is measured and assessed as a proportion of the previous year's growth. This method has been used to verify the existence of an annual growth pattern for eight species of groundfish aged by the AFSC Age and Growth Program (Table 3).

### Other validation methods

Following a strong year class over time, where it annually advances by one year, is another way to indirectly validate age estimates. This method was used with walleye pollock, where a strong 1978 year class was confirmed by the age-independent method of length mode analysis and subsequently tracked for 10 years in age frequency distributions (Kimura et al., 2006).

Timing of first translucent growth zone deposition in Atka mackerel (*Pleurogrammus monopterygius*) otoliths was validated using three methods: larval seasonal length data, counts of daily growth zones in larval otoliths, and edge type analysis (Anderl et al., 1996). Atka mackerel spawn and hatch in the fall, and this research confirmed that the first translucent growth zone was formed at an age of 1.5 years during the second winter or spring.

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# Appendix. Glossary

**by Mary Elizabeth Matta, Daniel K. Kimura, Delsa M. Anderl, and Craig R. Kastelle**

This glossary provides definitions for terms commonly used by the AFSC Age and Growth Program. Several sources were used to compile and verify accuracy of these terms, including Chilton and Beamish (1982), Summerfelt and Hall (1987), Penttila and Dery (1988), Kalish et al. (1995), CARE (2006), and VanderKooy (2009). In composing the definitions below, all attempts were made to maintain consistency with previous publications.

**Accuracy:** the closeness of an **age estimate** to the **true age**; contrast with **precision**

**Aging criteria:** standard rules that an **age reader** uses to recognize regular growth patterns and estimate age in a given species

**Age determination (**also **age estimation):** the process of assigning **age estimates** to fish

**Age estimate:** an age assigned to a fish in an effort to approximate its **true age**; this is typically accomplished by counting **annual marks** on an **aging structure** and applying an **edge interpretation** consistent with the **international birth date convention**; see also **age determination**

**Aging structure:** any calcified body part that grows consistently throughout the life of an organism and forms annual growth patterns that can be used to estimate age; in fish, includes **otoliths**, fin rays, spines, thorns, scales, opercles, cleithra, and vertebrae; the structure most appropriate for **age determination** depends upon the species

**Age reader:** within the context of age and growth studies, a person who generates **age estimates**

**Age reading:** the process of examining **aging structures** to determine **age estimates**

**Annual mark:** within an **aging structure**, a growth zone that forms yearly and can be used to estimate the age of fish; in general, consists of one **opaque growth zone** and one **translucent growth zone**; **age readers** at the AFSC often use this term to refer solely to the narrow **translucent growth zone**

**Annulus:** a concentric growth zone in an **aging structure**; by convention, **age readers** often assume this term is equivalent to "**annual mark**" even though "annulus" is derived from the Latin "anus," meaning ring, not from "annus," meaning year

**Anterior:** towards the head of an animal's body; in fish, synonymous with the term "cranial"

**Anti-sulcus:** the area on the **distal** side of an **otolith** opposite the **sulcus**

**Asterisci:** (pl.) one of the three pairs of **otoliths** found in the internal ear of **teleosts**

**Average percent error (APE):** a statistic used to measure **precision** within or between **age readers** or **aging structures**; see also **coefficient of variation**

**Back-calculation:** a method used to estimate fish lengths at previous ages by assuming a relationship between the size of an **aging structure** and fish length

**Bias:** a systematic error where an **age reader** consistently estimates older or younger ages than the **true age**; see also **relative bias**

**Bomb radiocarbon ($^{14}$C) age validation:** a method used to directly validate **age estimates** by tracking the increase in **otolith** $^{14}$C corresponding to atomic bomb testing in the 1950s and 1960s; one of the strongest methods of age **validation**

**Bowker's test of symmetry:** a statistical test used to detect **bias** between two **age readers' age estimates** using paired **age readings** from the same fish

**Break-and-bake method:** a method used to clarify **otolith** growth patterns, where the otolith is sectioned in half **transversely** and baked in an oven for several minutes; sometimes referred to as "toasting"

**Break-and-burn method:** a method used to clarify **otolith** growth patterns, where the otolith is sectioned in half **transversely** and the exposed surface is passed over an alcohol flame

**Check:** a non-annual **translucent growth zone** observed in the growth pattern of an **aging structure**; can be distinguished from **annual marks** by faint appearance and inconsistency throughout an aging structure; should not be counted in **age estimates**; may form due to physiological or environmental stress

**Clearing:** a technique of improving the visibility of growth zones, usually by immersion in a solution that reduces opacity such as water, ethanol, oil, or glycerin; see also **over-clearing**

**Coefficient of variation (CV):** a statistic used to measure **precision** within or between **age readers** or **aging structures**; see also **average percent error**

**Cohort:** within a population, a group of fish born within the same time period; see also **year class**

**Core:** within an **otolith**, the **primordium** and surrounding areas; in conventional **age determination**, generally refers to the part of the otolith deposited early within the first year of life, but in **radiometric age validation** studies, may refer to the part of the otolith deposited within the first several years of life; see also **nucleus**

**Corroboration:** a comparison of age data with other data sources to infer the **accuracy** of fish **age estimates**; the consistency or repeatability of **age estimates**; the strongest methods of age corroboration are sometimes referred to as age **validation**

**Crystallization:** abnormal biomineralization in fish **otoliths** where part or all of the otolith has a glass-like or crystalline appearance, often resulting in obscured growth patterns that cannot be aged; in **sagittae**, typically caused by replacement of the normal form of calcium carbonate (aragonite) with the less common form (vaterite)

**Daily rings:** growth increments that form on a daily basis within an **aging structure**

**Distal surface:** in situ, the part of an **otolith** facing away from the midline of the body; the surface opposite the **sulcus**; sometimes referred to as the "external surface;" see also **anti-sulcus**

**Dorsal tip:** in situ, the part of an **otolith** corresponding to the dorsal (back) side of the fish; best seen in **otolith transverse sections**

**Edge:** the outermost part of an **aging structure** corresponding to the most recent period of growth; an **edge interpretation** is required to arrive at an **age estimate**; synonymous with **margin**

**Edge interpretation:** a judgment concerning growth observed on the **edge** of an **otolith** made according to the **international birth date convention**; the date of capture and the amount and type of growth along the otolith edge are used to decide whether the edge should be included in the final **age estimate** (i.e., whether an additional year should be added to the **annual mark** count), thus assigning fish to the proper **year class**

**Edge type:** the characterization of the extent of **opaque** or **translucent** growth present on the **edge** of an **aging structure**; see **marginal increment analysis**

**Fishery observer:** a scientifically-trained person who collects data and biological samples from commercial fishing vessels and transmits the information to the National Marine Fisheries Service for the purpose of monitoring and managing fisheries

**Flatfishes:** any of the **teleost** species which are laterally compressed and have both eyes on the same side of the body, including halibuts, flounders, and soles

**Focus:** the center (origin) of a vertebral centrum; sometimes refers to the point of origin of an **otolith** or scale

**Growth function:** any parametric model that describes length or weight at age; the most commonly used form in fish biology is the von Bertalanffy growth function (VBGF)

**Hyaline growth zone:** synonymous with **translucent growth zone**; within an **aging structure,** a zone of low calcification that allows the passage of light; may be an **annual mark** or a **check**; the term "translucent" is preferred

**International birth date convention:** an international convention adopted by many **age determination** agencies, where all fish in the northern hemisphere are assumed to have a birth date of 1 January regardless of spawning season

**Lapilli:** (pl.) one of the three pairs of **otoliths** found in the internal ear of **teleosts**

**Longitudinal section:** any section along the long axis of an organism (or **aging structure**), perpendicular to the **transverse** plane; **sagittal sections** are a type of longitudinal section

**Margin:** the outermost part of an **aging structure**; synonymous with **edge**

**Marginal increment analysis:** an indirect method for **validation** of **age estimates**; the relative amount of new growth on the **edge** of **aging structures** is compared throughout the year to determine whether **annual marks** are laid down once per year; see **edge type**

**Mark-recapture:** a field method where fish that have been marked (tagged) and re-captured can be used to estimate abundance, analyze movements, and estimate growth parameters; when used in conjunction with a chemical marker such as **oxytetracycline**, can provide a form of **age estimate validation**

**Nucleus:** an ambiguous term usually used to refer to the **otolith primordium** or **core**

**Opaque growth zone:** within an **aging structure**, a zone of high calcification that restricts the passage of light; appears light in color when viewed with **reflected light** against a dark background; contrast with **translucent growth zone**

**Otoliths:** three pairs of calcified structures (**sagittae**, lapilli, and asterisci) that are located in the heads of **teleost** fishes; **age readers** often use this as a general term for the sagittae, which form annual growth patterns that can be used for **age determination**

**Over-clearing:** during the process of **clearing**, the point at which the **otoliths** have spent too much time in a clearing solution and the growth zones are no longer visible

**Oxytetracycline (OTC):** a chemical marker that binds to calcified tissues upon injection or immersion; used to **validate age estimates** from **otoliths** or other **aging structures** in **mark-recapture** studies

**Percent agreement:** a measure of **precision** used to compare **age estimates** within or between **age readers** or **aging structures**

**Posterior:** towards the tail of an animal's body; in fish, synonymous with the term "caudal"

**Pre-annular check (growth pattern):** a phenomenon in **otoliths** where, prior to deposition of an **annual mark**, there is a broad, indistinct, and poorly defined **translucent growth zone** or **check**; sometimes referred to as "shadowing"

**Precision:** the degree to which an **age estimate** is reproducible, either by the same **age reader** or between *age readers*; contrast with **accuracy**

**Primordium:** the initial structure of an **otolith**; see also **core**

**Production aging:** routine **age determination** carried out over time in order to develop a database of ages that can be used for stock assessment modeling

**Proximal surface:** in situ, the part of the **otolith** facing towards the midline of the body; the surface containing the **sulcus**; sometimes referred to as the "internal surface"

**Radiometric age validation:** a method used in age **validation** studies to determine age in fish by measuring the ratio of $^{210}$Pb/$^{226}$Ra or of $^{228}$Th/$^{228}$Ra in **otolith cores**

**Readability:** the relative ease with which an **age reader** can assign an age to a given **aging structure**; can be affected by the method of preparation used to estimate age of the structure as well as physical characteristics within the structure such as **crystallization**

**Reading axis:** within an **aging structure**, the preferred path of reading **annual marks** from the **core** or focus to the **edge**

**Reflected light:** light that is shone down onto an **aging structure** from above; the use of reflected or **transmitted light** affects the appearance of **opaque** and **translucent growth zones**

**Relative bias:** a systematic error between **age estimates**; can occur between **age readers**, when one person consistently attains either older or younger **age estimates** than another person; can also occur between **aging structures** or aging methods

**Ring:** a general term describing concentric growth zones; is not necessarily synonymous with "**annual mark**"

**Sagittae:** (pl.) for nearly all species, the largest of the three pairs of **otoliths** found in the internal ear of **teleosts**; the otolith pair most frequently used in **age determination** studies

**Sagittal section:** a section dividing the right and left sides; see also **transverse section**

**Splitting (growth pattern):** a type of **check** commonly observed in **otoliths** from certain species, where two or more closely-spaced **translucent growth zones** were deposited within the same year of growth and are equivalent to a single **annual mark**

**Sulcus:** a deep groove on the **proximal** side of an **otolith** containing a thickened part of the otolithic membrane; the areas adjacent to the sulcus are often used as **reading axes** in **transverse sections** of otoliths from various species

**Surface examination:** the practice of using the intact surface of an **aging structure** to determine age; sometimes adequate for generating **age estimates** but is generally less desirable for old specimens or difficult species; in **otoliths**, either the **distal** or **proximal surface** can be used for surface examination

**Teleost:** any of the higher bony fishes (Teleostei); excludes more ancestral groups such as bichirs and sturgeons

**Test sample:** a subset of specimens aged by a second **age reader**; at the AFSC, **production** species are typically tested at a rate of 20%

**Tester:** a second, usually more experienced **age reader**, who ages a subset of specimens for comparison with the primary age reader to estimate between-reader **precision** and to detect potential **relative bias** as a means of quality control

**Thin section:** an **aging structure** preparation method typically used at the AFSC for difficult or long-lived species such as certain rockfish species and skates; time-intensive but generally produces even reading surfaces with relatively high resolution compared to the **break-and-burn method**

**Translucent growth zone:** within an **aging structure**, a zone of low calcification that allows the passage of light; appears dark in color when viewed with **reflected light** against a dark background; sometimes referred to as a **hyaline growth zone**, although "translucent" is the preferred term; may be an **annual mark** or a **check**

**Transmitted light:** light that is projected through an **aging structure** from beneath; the use of transmitted or **reflected light** affects the appearance of **opaque** and **translucent growth zones**

**Transition zone:** within an **otolith**, an area of significant change in the growth pattern; usually corresponds to significant events during the life of the fish such as the process of sexual maturation; older otoliths are often observed with one or more transition zones

**Transverse section:** a section dividing the **anterior** and **posterior** ends of an **aging structure**; generally the preferred method of sectioning otoliths; see also **sagittal section**

**True age:** the actual age of an organism, a very difficult quantity to know with certainty; most commonly, **age estimates** are used to approximate true age

**Unburned face:** a term used by AFSC age readers to describe the untreated cut surface of a transversely sectioned **otolith**; for some otoliths, it can occasionally be used to determine age

**Validation:** any of a variety of strong methods used to confirm the **accuracy** of **age estimates**; see also **corroboration**

**Ventral tip:** in situ, the part of an **otolith** corresponding to the ventral (belly) side of a fish; best seen in **otolith transverse sections**

**Year class:** a group of fish of the same stock born within the same year; see also **cohort**

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