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Aging manual for Pacific halibut: procedures and methods used at the International Pacific Halibut Commission (IPHC)

by

Joan E. Forsberg

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INTERNATIONAL PACIFIC HALIBUT COMMISSION
P.O. BOX 95009
SEATTLE, WASHINGTON 98145-2009, U.S.A.
www.iphc.washington.edu

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Abstract

Since its inception, the International Pacific Halibut Commission (IPHC) has collected Pacific halibut otoliths for use in age determination. This manual provides an overview of generalized age determination procedures for fish otoliths as well as detailing otolith preparation, storage, and aging techniques used at the IPHC.

General background on otoliths and age determination

In fisheries management or research, accurate age determinations for the fish species in question are critical for estimating growth and mortality rates as well as population age structure (Pentilla and Dery 1988, Chilton and Beamish 1982). Otoliths were first used for age determination by a researcher named Reibisch in 1899. Otoliths, also called "ear-bones" or "ear-stones", are calcareous structures found in the head of most fish. All teleost fishes have three pairs of otoliths: the *asteriscae*, *lapillae* and *sagittae*. The sagittae are much larger in size than the other otoliths and are the pair most often used in age determination. Each sagittal otolith is enclosed in a fluid-filled sac called the *sacculus* within the otic capsule of the head. The otic capsules are situated on either side of the posterior portion of the brain. Otoliths are not true bone; they are acellular and avascular, unlike skeletal bone. Rather, otoliths are composed of calcium carbonate in the crystalline form of *aragonite*, in a protein matrix. Otoliths act as sound receptors and also play a role in balance and orientation. Otolith size and shape, particularly of the sagittae, varies greatly among species. Size and shape of the sagittae are related to their function, namely sound detection in the fish (Popper and Lu 2000).

As the fish grows, so does the otolith. The otolith begins as a very small spherical body in the ear of the larval fish and with the growth of the fish, increases in size by the deposition of concentric *lamellae* or layers of material around the outside. Deposition is much greater in two planes than in the third, producing a flattened structure in the adult (Fig. 1).

In addition, seasonal changes in the fish's growth rate are reflected in the otolith. Material is deposited on the otolith from the *endolymph*, the fluid that surrounds the otolith. The deposits are formed in bands of alternating optical density, which appear either opaque or translucent under reflected light. The alternating zones on the otolith are due to differences in the amount of protein (called *otolin*) in the zones and shape of the aragonite crystals; aragonite crystals form longer and narrower at higher temperatures, shorter and

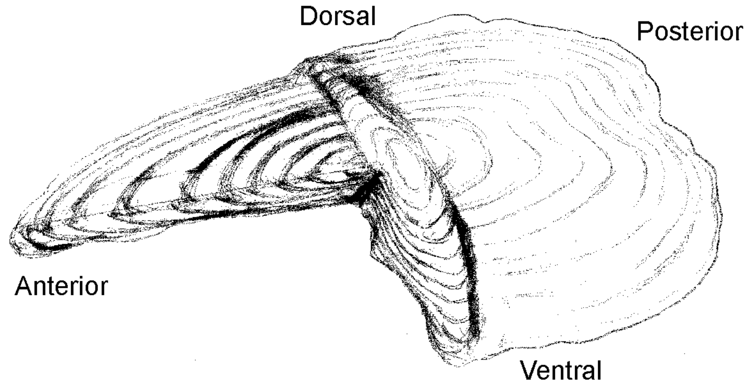


Figure 1. Cutaway three-dimensional illustration of a halibut otolith showing deposition of concentric lamellae.

wider at lower temperatures (Hagen 1997). A year's growth consists of both an opaque and translucent zone. The opaque zone is formed during the period of faster growth, which typically occurs in the summer and is made up of longer aragonite crystals. The translucent zone is formed during slower growth, which typically occurs in the winter, and is composed of shorter crystals of aragonite and contains comparatively more protein. The opaque and translucent zones are also often referred to as the summer and winter zones respectively. Winter-spawning fish such as halibut are assigned an arbitrary January 1 "birth date" by international convention. Therefore, the translucent or winter zones of halibut otoliths are counted to determine the age of the fish in years. The winter growth zones are also referred to as *annuli* (singular: annulus) or *hyaline zones*.

Within the opaque (summer) and translucent (winter) zones on the otolith are *daily rings*. Daily rings are, as the name implies, laid down daily and are composed of two alternating zones with different optical properties, as in annual zones. The differing appearances of the two zones in daily increments are due to the orientation of organic fibers in relation to the aragonite crystals and the relative widths of the zones. The alternating deposition of zones of different appearance and composition is related to both external (temperature, food, light, salinity, etc.) and internal (e.g., calcium metabolism and interaction of various hormone feedback systems) factors (Simkiss, 1974). Daily rings are only visible under high magnification and are not used in the production aging of Pacific halibut. In most species, daily rings are legible only through the first year. After the first year, daily growth rings are too compressed to differentiate. Weekly, bi-weekly and monthly patterns (as well as daily and annual) can also be seen in some species.

The otolith reflects growth rate changes over the years as well as seasons within a year. As the fish grows older, the relative width of the otolith zones decreases. In the first few years, otolith growth is rapid, resulting in broad opaque zones. As the fish ages, the opaque zones become narrower until they are almost the same width as the translucent zones.

Otolith terminology can be confusing; different agencies or researchers can use different terms for the same structures. The terms "opaque" and "translucent" are a particular problem, since the optical properties of these zones depend on whether illumination is reflected or transmitted. The opaque zone appears white under reflected light, but dark with transmitted light, since light doesn't pass through it. The translucent zone appears dark under reflected light but since light passes through it, it appears bright with transmitted light. Since reflected light is used in Pacific halibut aging techniques, in this manual *translucent* refers to the zone of slow growth and *opaque* to the zone of faster growth. See glossary in Appendix I for complete list of terms.

Other hard structures in fish have similar alternating patterns caused by seasonal changes in growth rates and may be used for age determination along with or instead of otoliths. These other structures include vertebrae, scales, fin rays, opercular bones, and cleithra. Certain structures show growth patterns more clearly in a given species. One advantage to using otoliths is their stability. Scales can be lost and replaced; a regenerated scale has fewer annual rings than the total age of the fish. Moreover, calcium can be resorbed from scales and other calcareous deposits in the body under certain physiological conditions, resulting in the loss of some previously deposited growth rings. On the other hand, calcium is not resorbed from otoliths, so otoliths provide a "permanent" record of growth.

Some additional sources of information on general aging procedures are listed in Appendix II.

The International Pacific Halibut Commission (IPHC) has used sagittal otoliths for aging halibut since 1914 (Fig. 2). Otoliths can provide other information as well as age; at one time, otolith radius, length, and weight (Table 1) were used to estimate the size of individual halibut (Clark et al. 2000). Other properties such as shape have been used to distinguish between stocks of fish in other species. At IPHC, otolith shape and a combination

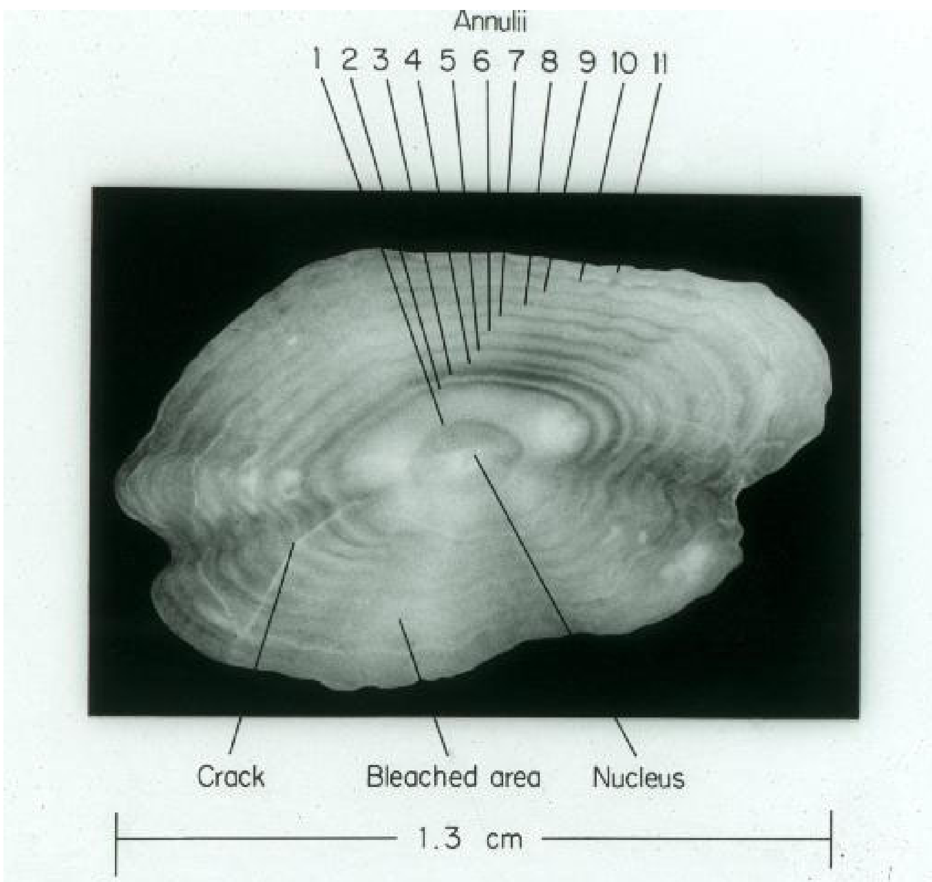


Figure 2. A left (blind) side halibut otolith with annuli marked.

Table 1. Otolith data collected and entered into database since 1963.

Year	Otolith data entered
1963-1967	Age, otolith radius
1968-1977	Age, otolith length
1978-1992	Age, otolith weight, otolith length
1993-1994	Age
1995	Age, otolith weight
1996-1998	Age, otolith weight, otolith length
1999-	Age, edge code

of otolith weight, length, and age were used in an attempt to discriminate sex in commercially caught halibut, for which sex data is unavailable (Forsberg and Neal 1993). Currently, only age data from otoliths is utilized; age data is incorporated into the annual stock assessment.

This manual describes the current otolith collection, storage, and age determination methods, criteria for making age determinations, and quality control measures at IPHC as well as outlining past procedures for the same. The accompanying otolith photographs should be useful both in training new readers and as a reference for experienced readers.

Halibut otolith collection and storage

The IPHC collects halibut otoliths from the following sources: commercial catch (market samples), setline and trawl surveys (general series), and recaptured tagged halibut. Currently, we routinely collect only the left, or blind side, sagittal otolith from commercial and survey samples. Only the left otolith is used for age determination. Both sagittae are collected from tagged halibut that were part of an oxytetracycline (OTC) age validation study, but only the left is aged. It was determined in early investigations into aging of Pacific halibut that the left otolith is easier to read and ages could be made with higher confidence than when the right, or eyed side, otolith is used. Figures 3 and 4 show the two methods used to extract halibut otoliths.

The Alaska Department of Fish and Game (ADF&G) has collected otoliths from sport-caught halibut from 1990 to present and also aged these samples until 1998. During the same period, yearly exchanges of a subset of Alaskan sport-caught halibut otoliths were made between ADF&G and IPHC. Sport halibut otoliths continue to be collected but are not currently being aged by either ADF&G or IPHC.

Otoliths collected in the field are either stored dry (tagged fish otoliths) or in 50% glycerin solution (commercial sample and survey). The glycerin solution used at IPHC consists of equal parts water and glycerin plus a small amount of *thymol*, a preservative, which is added to deter fungal and bacterial growth (the "recipe" for 50% glycerin solution can be found in Appendix III). Tag recovery otoliths are stored in individual envelopes until they are sent to the Seattle office, where they are set out on trays and covered in glycerin solution to clear. Accompanying data for each recovered tagged-fish is recorded on the envelope. Market sample and setline survey otoliths are collected in special boxes that have cells that keep the individual otoliths separate and are filled with glycerin solution. The accompanying specimen data are recorded on forms. Up until 1996, setline survey otoliths were stored dry in envelopes in the field and not put in glycerin solution to clear for, sometimes, many months. ADF&G sport-caught halibut otoliths are stored dry in envelopes. Prior to aging, the otoliths were immersed in glycerin solution and allowed to clear for several weeks.

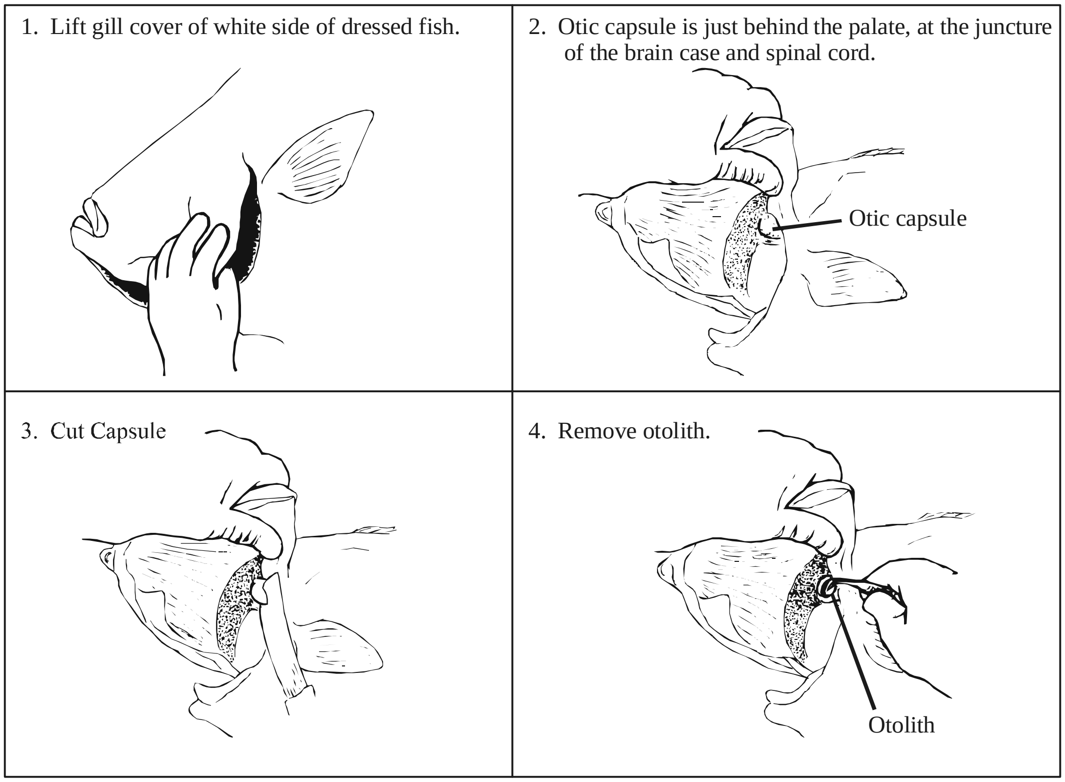


Figure 3. Extraction of left side otolith from dressed halibut.

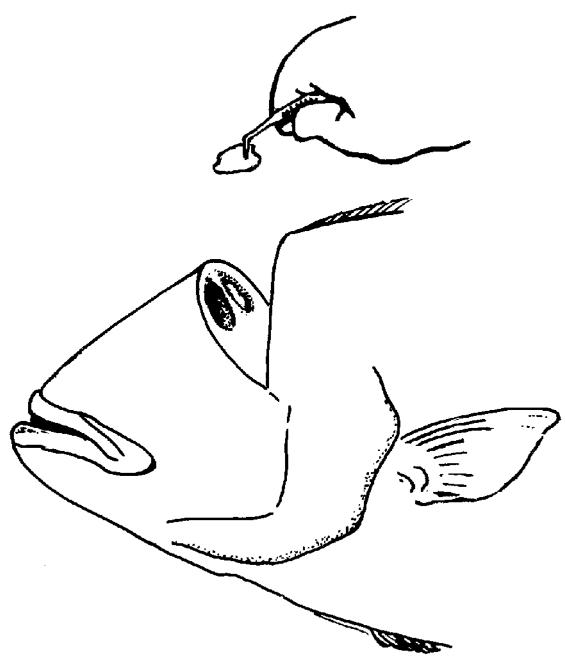


Figure 4. Extraction of left otolith from undressed halibut showing position of head cut.

All otoliths are put in vials (either glass or plastic) and covered in 50% glycerin solution with thymol for permanent storage. Market sample, setline survey, and tag otoliths are stored in vials measuring 19 mm by 65 mm with about 25 otoliths to a vial. Vials are stored in custom-made cardboard boxes with dividers to keep the vials separated and in order. Otoliths within the vials are stacked in order by otolith number, one on top of the other, with numbered paper disks just slightly smaller than vial diameter placed between to identify the individual otoliths. The stacking method works very well for whole otoliths from legal-sized halibut, but very small or broken otoliths can slip between the paper disks and the side of the vial and become mixed up. The very small otoliths from past IPHC juvenile trawl surveys were stored in small, individual vials. Trawl survey

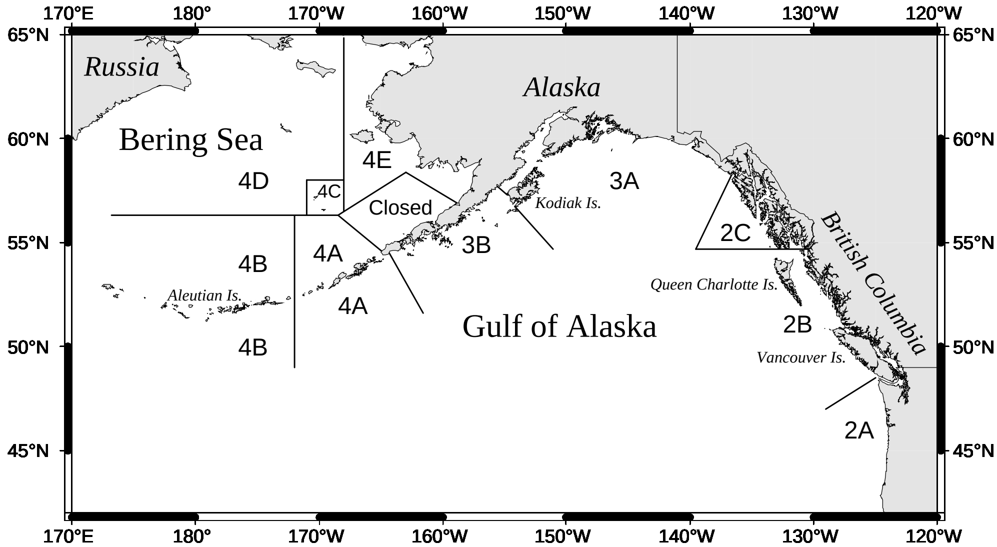


Figure 5. IPHC regulatory areas.

otoliths collected on National Marine Fisheries Service (NMFS) research cruises are stored in individual vials (with 50% glycerin solution) in Styrofoam boxes.

Market Sample otoliths

Currently, commercial samples are taken annually at a pre-set rate calculated to provide a target number of otoliths for each IPHC regulatory area. Regulatory areas are shown in Figure 5. Current annual otolith target numbers are 1000 for Area 2A; 2000 for each of Areas 2B, 2C, 3A, 3B, 4A and 4B; and 2000 for Areas 4C and 4D combined (Wade et al. 2001). Otoliths are not collected from Area 4E. Samples are taken throughout the entire nine-month fishing season. Vessels are sampled randomly and proportionally by weight.

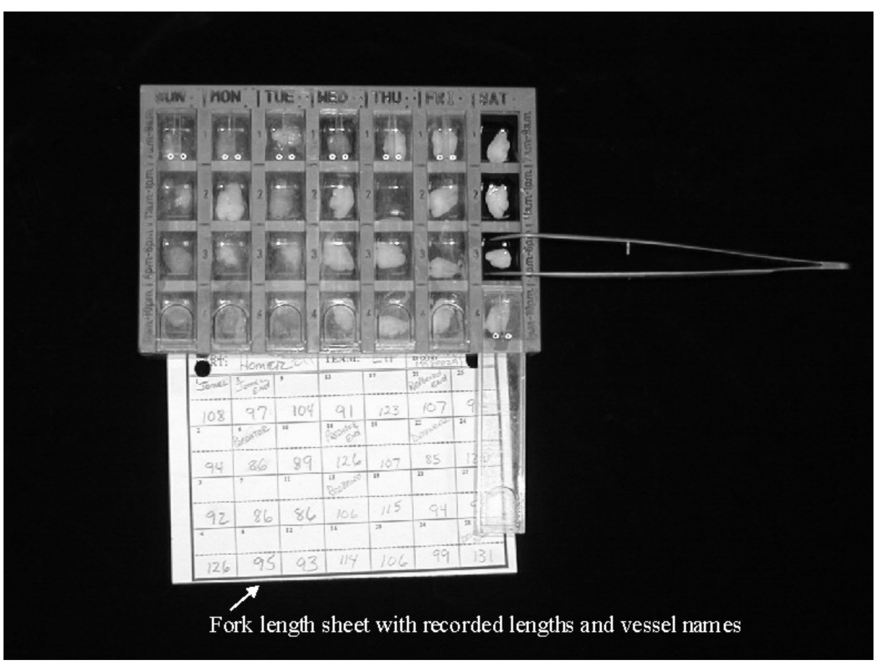


Figure 6. Medication organizer used to collect market sample otoliths.

Figure 7. Boxes and plastic trays used to hold market sample and recovered tag otoliths for aging.

Details on commercial catch sampling procedures can be found in Gilroy et al. (1995) and Quinn et al. (1983). Otoliths are stored in medication organizers in the field (Fig. 6) and covered immediately with glycerin/water/thymol solution. Due to a limited number of medication organizers, market sample otoliths are transferred on arrival in the Seattle office to uncovered trays held in wooden boxes (Fig. 7). The open trays are more convenient for aging as otoliths are more accessible, however trays must be handled carefully to avoid spilling or mixing of otoliths. Otoliths on the uncovered trays are especially prone to getting mixed up if the tray is jostled or tipped before the otoliths have been covered with glycerin solution. All market sample otoliths are aged, even when collections exceed the target numbers for the different areas.

Survey ("General Series") otoliths

Setline survey otoliths are stored in custom-made plastic containers (referred to by staff as "*Barto Boxes*" after the box's designer). The *Barto Box* consists of a black plastic bottom made up of 100 cells with a clear Plexiglas lid that tightens with screws (Fig. 8). Setline survey otoliths are kept in the *Barto Boxes* until final storage.

Survey halibut are also sampled at a predetermined rate set to achieve the collection of 2000 otoliths per regulatory area each year. When oversampling occurs for a given area, if possible only a subset of otoliths is aged in order to bring the number aged down to the target number of 2000. For example, if 2500 otoliths were collected, every 5th otolith would not be aged. Sometimes all otoliths from an area must be aged, even if over the target number. In order to subsample to a target number, the total

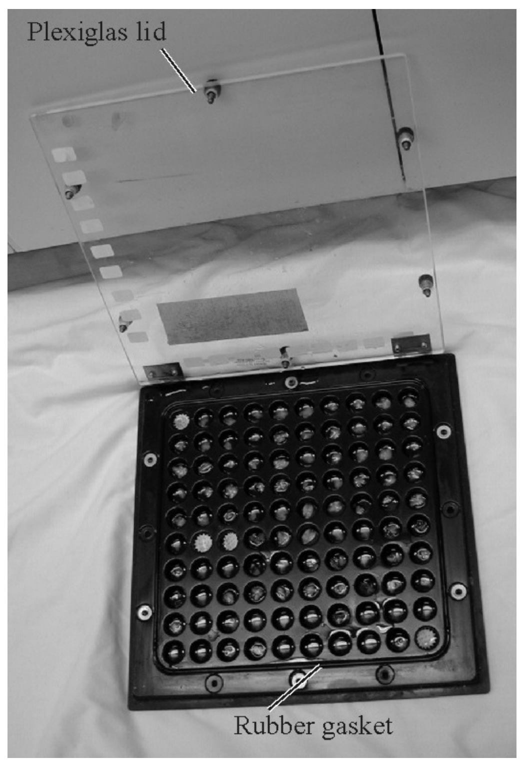


Figure 8. *Barto Box* for collection and short-term storage of longline survey otoliths.

number of otoliths collected in an area must be known. In 1999, survey otoliths from certain regulatory areas began to be included in the stock assessment. Ages must be available for stock assessment by mid to late October. In order to meet this deadline, aging must begin in August. If survey collections in an area are not complete by August, reading must begin, so subsampling is foregone and all otoliths for that area are read. Details on recent age-reading schedule changes can be found in Forsberg et al. (2001).

History of Pacific halibut age determination

The first ages for Pacific halibut were determined by reading scales. McMurrich (1913) looked at the scales of three halibut. Of 13 sampled, only three had scales suitable for aging. Former IPHC director W.F. Thompson was the next person to determine ages of Pacific halibut, using sagittal otoliths. (**Note:** since only sagittal otoliths are used for aging halibut, from here on, "*otolith*" will be synonymous with "*sagittal otolith*" in this report, unless specified otherwise.)

In an unpublished thesis written during the 1940s, another former IPHC director, Henry Dunlop, compared ages from halibut scales and otoliths. Otolith-derived ages averaged higher than ages obtained from examining scales. He stated that scale markings were sometimes fairly distinct on young fish, but only toward the center of scales of older fish. Scale markings are generally less distinct than those of otoliths and halibut scales are also very small and difficult to work with, therefore Dunlop recommended using otoliths exclusively for age determinations. Dunlop describes a halibut otolith in the following passage:

> *"It displays a series of irregularly concentric broad opaque, and narrow transparent zones which alternate from the center to the margin. The zones extend around the circumference of the otolith, conforming to the shape and size of the otolith at the time each was laid down. They are deposited successively from the center to the margin as the otolith increases in size during the life of the fish. Opaque and transparent zones correspond respectively to seasons of rapid and retarded growth, roughly to summer and winter."*

Dunlop noted that some otoliths are quite irregular and difficult to interpret. He pointed out that opaque zones might at times be hidden from the surface view. The practice in the 1930s was to count only the distinct zones and not the "obscure" zones. He stated that *"due to this practice and the possibility that some opaque zones may not show, age determinations must be regarded as minimum values"*.

Since ages are determined by counting annuli, knowledge of the time of year the annulus is completed is vital to assigning individuals to the correct age-class.

Dunlop described a *marginal increment analysis* (MIA) that was performed to determine the time of year of annulus formation. By measuring the width of the opaque zone outside the last complete annulus on a series of otoliths collected throughout the year, he found that the translucent zone or annulus was completed between February and May for the study samples. He also found that there was a great deal of variation in the time of year translucent growth began: new opaque zones begin in some before the transparent margins become distinct in others and at no time are the margins of all otoliths transparent.

Dunlop cautioned that although the MIA results showed that the annulus is completed in the winter and spring, it doesn't prove that one is laid down *every* year.

Clearing

In 1915 and 1916, W. F. Thompson experimented with different preparations in an attempt to make otoliths more "readable". He found that freshly collected otoliths were easier to read than those that had dried out after removal from the fish. Otoliths *in situ* contain water; this water evaporates after the otolith is removed from the fish and the otolith takes on a whiter, more opaque appearance and contrast between zones is reduced. Thompson compared the results of soaking otoliths in pure glycerin, 50% glycerin, water, and ethanol. Ethanol and 50% glycerin produced the best result in terms of restoring contrast between opaque and translucent zones. He also found that otoliths that dried out completely before being soaked cleared less well than otoliths placed in clearing medium immediately after collection.

Thompson also tried sectioning and polishing techniques and compared transmitted and reflected light on different preparations (Figures 9 and 10). However, the standard preparation and method used for production aging of halibut has been surface reading of whole otoliths that have been cleared in 50% glycerin solution and are illuminated by reflected light.

Aging Procedures

Currently, two age determination methods are used by IPHC readers: 1) surface and 2) break and burn. Pacific halibut otoliths are all surface-aged at IPHC. We look at the distal surface of the otolith for surface reads; the proximal surface has a deep groove (the *sulcus acousticus*) and annuli are obscured (Fig. 11). However, annuli *can* be seen from the proximal surface in the otoliths of young halibut (five years and under) since the otoliths are still relatively thin and the sulcus groove is less prominent. In fact, young otoliths are often viewed from the proximal side as a check of the age observed on the distal surface. In cases

Figure 9. Transverse (dorso-ventral) cross-section of halibut otolith under reflected light.

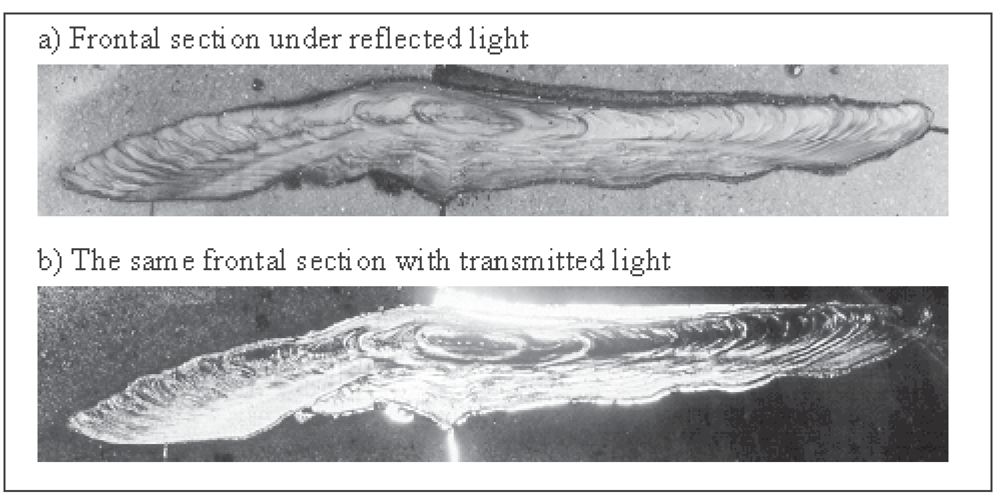


Figure 10. Frontal (antero-posterior) section of halibut otolith.

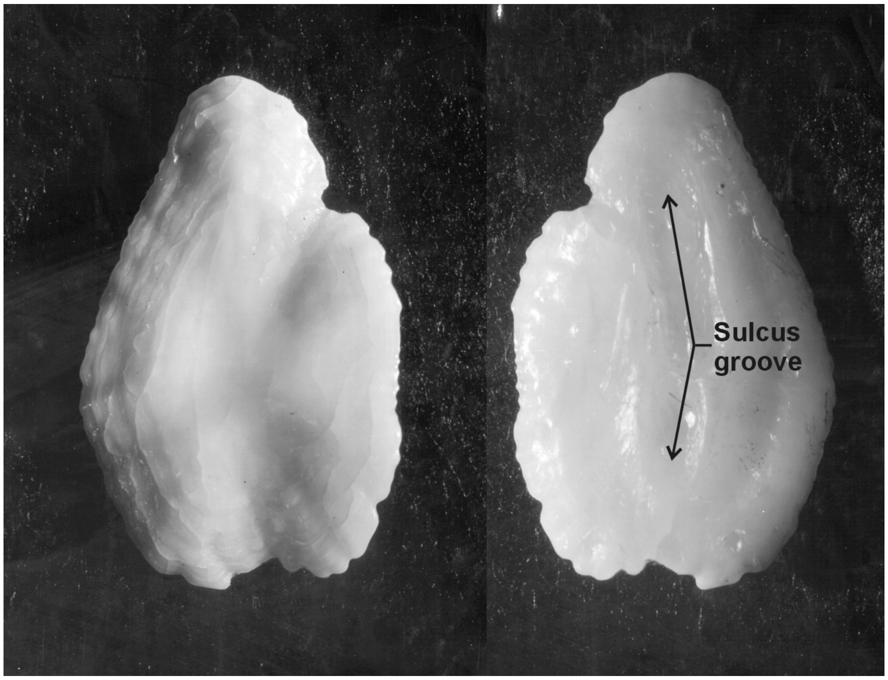


Figure 11. (L-R) Distal and proximal surfaces of a left sagittal halibut otolith.

where we are not confident of the surface age, (e.g., thick or steep edge, opaque or cloudy surface, odd growth pattern, etc.) a break and burn age determination is made. For both methods, the narrow winter zones (annuli) between the nucleus and the edge are counted to determine the age in years.

A dissecting microscope is used for both methods. Otoliths are observed at 5X to 50X power with reflected light from a fiber-optic light source (Fig. 12).

Data provided to the reader at the time of aging includes date and regulatory area of capture. In the past, fork length was included as well, but since there is a rough relationship

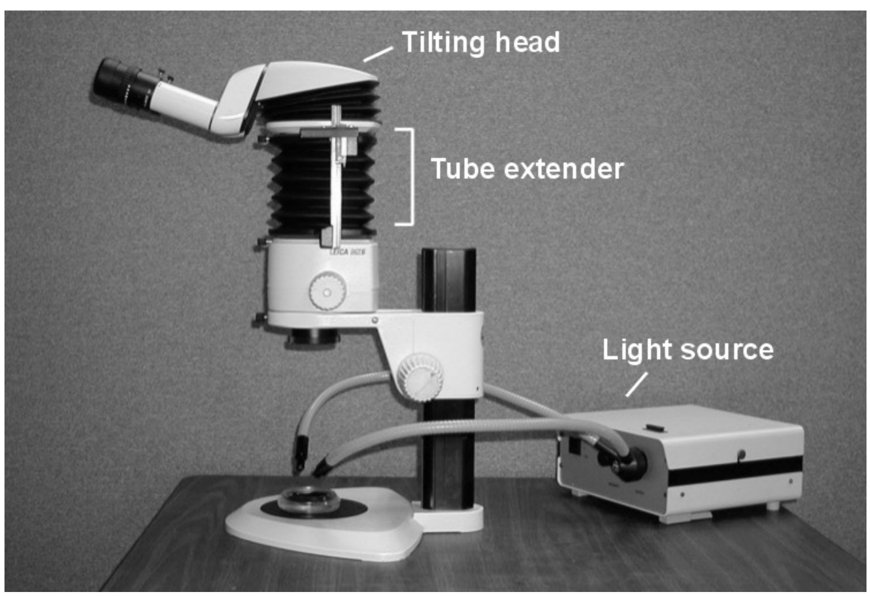


Figure 12. Stereo dissecting microscope and fiberoptic light source used for aging halibut otoliths. Tilting head and tube extender are ergonomic features.

of 10 cm length per year, it was decided that fork lengths should be withheld from agers to avoid potential bias from knowledge of this relationship. We record age(s), edge growth code (which indicates whether opaque edge growth was present, counted as last year's, or new), as well as remarks (such as crystallized, thick, questionable age, etc.). Only the ages and, as of 1999, the edge codes are entered. Only one age is used for assessment, however, an otolith may be read multiple times by different readers and several ages may be entered.

Surface

Surface ages are made under reflected light with the otolith immersed in water on a piece of black cloth in a container (to minimize glare from the light source and maximize contrast, respectively). Otoliths are rinsed to remove glycerin before reading, since the mixing of the two liquids with different refractive properties makes focusing on the otolith difficult. Adding a drop of liquid dish detergent to the water in the viewing container helps keep any glycerin not rinsed from the otolith from mixing with the water (Steve Wischniowski, IPHC, PO Box 95009, Seattle, WA 98145-2009, personal communication). The translucent zones are counted and since date of capture is provided at the time of aging, the edge is "interpreted" i.e., a decision is made whether to add an additional year to the annulus count or not. Figure 13 shows the preferred axes for making an annulus count on an otolith surface. Magnification for surface readings ranges from 5-10X.

The practice in the 1930s was to count only the "distinct" zones, and since some faint or overlapping annuli might have been missed, assigned ages were minimum values. Since information such as date of capture was not provided when ages were determined, all opaque zones were counted, including the edge, and the degree of development of the edge indicated. The width of the outermost opaque zone was recorded as a fraction, indicating its approximate width in relation to that of the next adjacent complete zone. Fish were later assigned to the appropriate age group according to dates of capture (Dunlop, unpublished thesis).

Break and Burn

Since deposition on the various surfaces of the otolith changes as the fish grows older, some annuli may not be visible from the distal surface. Viewing the otolith in cross-section allows viewing of such "hidden" annuli that may only be visible on the proximal surface. "Burning" increases the contrast between the opaque and translucent zones; the opaque zones turn light brown while the translucent (or hyaline) zones, due to their higher concentration of protein, turn dark brown when

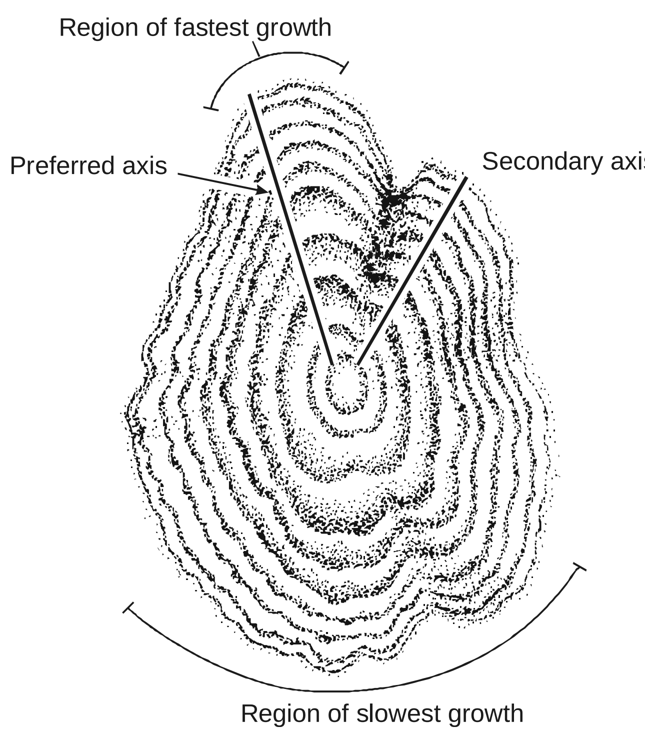


Figure 13. Preferred reading axes for halibut otolith surfaces.

heated. The following are the criteria a reader uses to decide whether an otolith should be broken and burned.

Break and burn criteria:

1. High surface age
2. Thick otolith, steep edges
3. Opaque/surface pattern obscured
4. Difficult surface pattern
5. Discrepancy in readings between different sites on the otolith
6. Choice surface-reading site broken off

Our break and burn technique is similar to that described in Chilton and Beamish (1982). The reader usually outlines the first annulus in lead pencil after making the surface reading and while the otolith is still under the microscope. The otolith is rinsed in water and dried with a paper towel then the otolith surface is scored through the nucleus (using the pencil mark as a guide) with a razor blade and then snapped in two by hand along the dorso-ventral axis. The dorso-ventral axis is also called the "transverse" plane and sections resulting from a cut or break along this axis are referred to as transverse sections. A section not commonly used for halibut otoliths is the frontal section, in which the otolith is cut along the antero-posterior axis. Figure 14 illustrates the axes and "geographical" regions of a halibut otolith. If the break is not through the nucleus or is very uneven, the reader

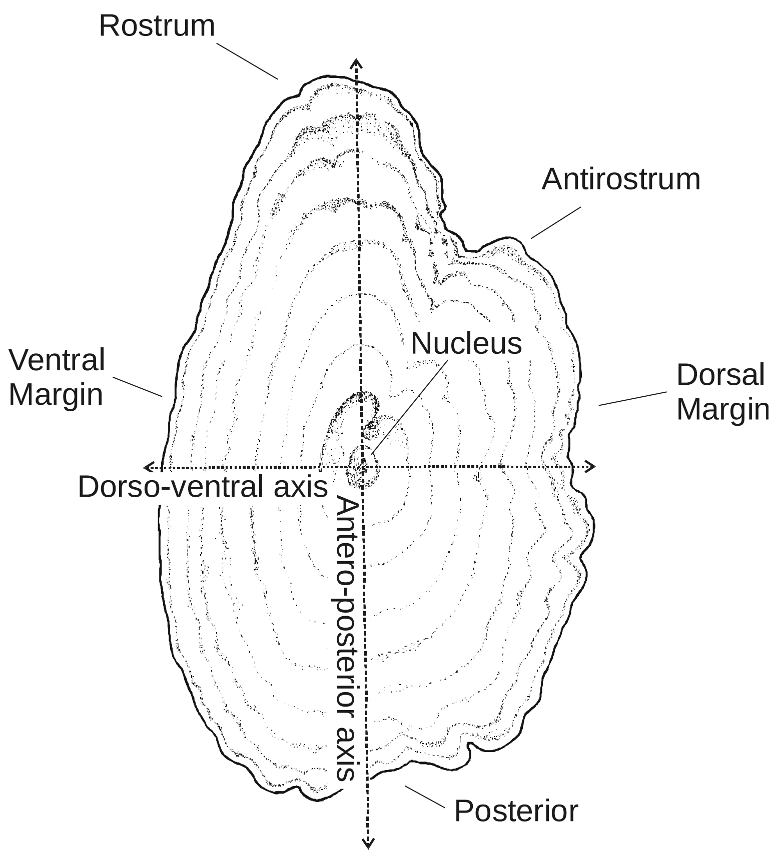


Figure 14. Distal surface of left sagittal Pacific halibut otolith (terminology from Härkonën 1986).

must use fine-grit sandpaper to sand the section down to the nucleus using the pencil mark as a guide. If the section is sanded, it must be cleaned in water and dried with a paper towel before burning to avoid sandpaper grit burning onto the surface. Some readers find that a straighter break can be achieved by laying the scored otolith over a straightened paper clip (as illustrated in Figure 15) with the scoring parallel to the paper clip. The posterior end is held down with the left index finger while the right index finger pushes down on the anterior end. The clip seems to spread the pressure across the dorso-ventral axis so that the anterior section is more likely to break off in one piece. The IPHC also has a low speed Isomet saw (Fig.16), which is occasionally used for cutting especially thick otoliths in half. To "burn" the otolith section, the broken surface is held over the flame of a small alcohol burner (Fig. 17). Some readers hold the section with the broken surface facing the flame, as in Figure 17. Others prefer to hold the section with the broken surface facing the reader, sulcus side down, since this way the reader can monitor the progression of the burn. Still another burning method is to hold the section with the broken surface up and the posterior end in the flame.

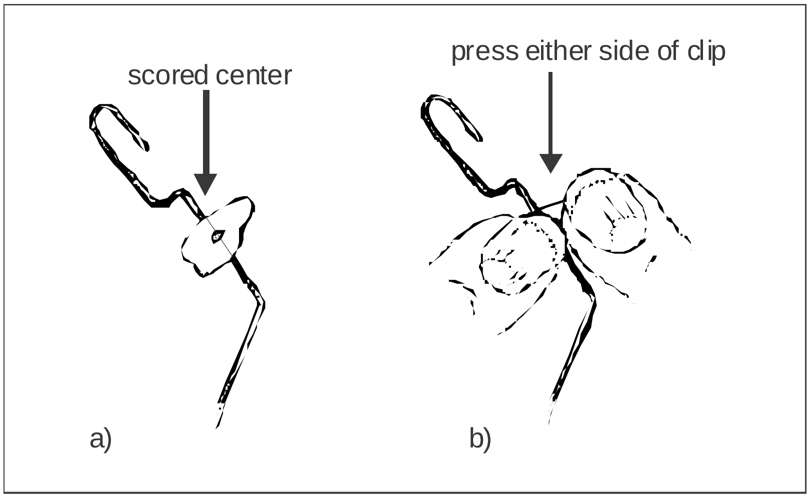


Figure 15. Breaking an otolith in half using the "paperclip method".

Note: keep as far away from the burner as possible while burning; otoliths can explode while heating. Some agencies have readers wear goggles or place the burner behind a plastic shield. Surface moisture can cause pieces to break off while burning, so sections should be dried before burning.

After burning one of the halves (keeping one unburned to repeat a surface reading), the sections are held in position by mounting in Plasticene™ or other type of modeling clay, then coated with mineral oil before viewing (Fig. 18). (Figures 18-74 are located on pages 21-36 of this report). Burnt sections are viewed at higher magnification than are surfaces (about 30-50X). Figure 19 shows the preferred sites for reading on a burnt section. The pencil marking the first annulus on the surface is still visible after burning and by tipping the section, the

Figure 16. Low speed Isomet saw used for cutting otoliths.

reader can see the pencil mark and match it to the first annulus in cross section. Usually, we burn only the posterior half, leaving the anterior portion of the otolith to repeat the surface reading if necessary. The anterior half may also be burned if the posterior half is damaged during breaking, burns poorly, or explodes during burning. We used vegetable oil for coating the burnt sections until 1999, when we switched to mineral oil on the advice of colleagues in another age lab. Glycerin and vegetable oil react to make a jelly-like sludge on the otolith, so burnt sections retrieved for a second reading had to have the "jelly" scraped off before viewing.

Figure 17. Burning a halibut otolith section over an alcohol flame.

Most of the readers at IPHC prefer to complete a series of surface readings, noting the otoliths that need to be broken and burned on the age forms, then do the necessary burns afterwards. This method is more efficient than interspersing surface reading with burns as they come up. After surface-aging a "batch" (usually one *Barto Box* of survey otoliths or group of sequential market samples from one port), the otoliths noted for burning are removed and set out in order on another tray, ready for rinsing and burning. Since most brands of soft modeling clay eventually dissolve in mineral oil, the clay becomes rather gooey and can ooze up onto the reading surface, necessitating removal and cleaning of the section. Burnt sections must also be cooled before mounting in soft modeling clay or the clay will melt and the section will tip over, getting melted clay on the reading surface.

As a result of such problems with soft modeling clay, some IPHC readers recently began using hardened mounts for holding burnt sections. Such mounts are made of Sculpey™ or Fimo™ brand bakeable modeling clay, which is scored with otolith-sized grooves in rows of five or ten, then baked in a regular oven according to package directions until hard (Fig. 20). Baked clay mounts do not dissolve in oil and sections can be mounted while still hot, however the fixed groove size limits the amount one can physically manipulate sections to optimize pattern visibility. Colored Silly Putty™ is used by some age labs and doesn't seem to dissolve in oil, but otoliths must still be cooled before mounting or the putty will melt and stick to the sections. For readers who do break and burns in batches, using soft clay requires an intermediate cooling step, i.e., sections must be set down on a metal or other heat-resistant surface to cool before mounting. Sections to be cooled must be laid out in a line or in an indented metal tray to keep the otoliths in order. Using a hardened mount eliminates this cooling step and the chance of sections getting out of order before or during transfer to the mounting medium.

There are alternatives to burning otoliths over a flame to achieve the heightened contrast burning produces. Other agers have used muffle furnaces and toaster ovens. In 1999, an IPHC reader compared the break and burn technique with the "break and bake" method (Barto 1999). Break and bake involves breaking the otolith through the nucleus in the same manner as is done for break and burn, but the sections are baked in a toaster oven at 500 degrees F for 10-15 minutes. In the study, the two halves of the otolith were given either the bake or burn treatment. Treatment was alternated between anterior and posterior halves. There was no significant difference in ages produced by the two methods, however the variance was somewhat lower for ages of otoliths that underwent the bake treatment. Readers now use either method, depending on sample size of otoliths to be sectioned and burned. Break and burn is more time efficient for small numbers of otoliths, whereas baking is more efficient when there are a large number of otoliths to be burned, since many can be baked at once. For baking, we use metal trays that are divided into 50 indented cells, which keep the otoliths from getting mixed up (Fig. 21).

There is a problem of burnt otolith sections "fading" over time in some species (Chilton and Beamish 1982). Fading of the hyaline zones can occur over a period of hours or years, but is not evident in Pacific halibut burnt otoliths. An otolith section that had been burned in 1993 was re-examined in 2000 and compared with a photograph of the section taken in 1993. No fading had occurred. One problem we have experienced with burnt sections at IPHC is with sections getting mixed up in the storage vials. At the current rate of break and burn determinations, this problem is not critical as long as care is taken in positioning the otolith sections on the paper discs while filling the storage vials, and even greater care taken during extraction of stored otoliths from the vials. However, if break and burn rates increase, alternate storage procedures will be necessary.

The break and burn method was first used with Pacific halibut otoliths in 1980. Readers from the Pacific Biological Station (PBS) in British Columbia aged a sample of halibut otoliths by break and burn and their ages were compared with IPHC surface ages for the same otoliths. The break and burn ages averaged higher than surface ages. Another exchange was made in 1987 and IPHC readers were trained in the break and burn technique by PBS staff. Break and burn ages were not routinely used at IPHC until 1992. At that time, some or all otoliths were aged twice, three times if the first two ages were different. If any paired readings differed by two or more years, a break and burn determination was made. In 1994, readers began breaking and burning otoliths that met the criteria described above, namely those that were thick, had steep edges, had a difficult pattern, were incompletely cleared, or had a high surface age. Numbers of break and burn determinations have increased over time, as readers became more familiar with and confident in the technique, and due to increasing numbers of otoliths meeting the break and burn criteria (Forsberg et al. 2001).

The 1987 comparison between IPHC surface ages and PBS burn ages indicated separation in the age methods at and above a surface age of 17, with the break and burn method producing a higher age for a given otolith. At that time, ages of 17 and over were grouped for stock assessment purposes so surface aging continued to be the standard aging method used at IPHC. A more recent comparison of surface and break and burn ages for a set of otoliths has indicated that the ages obtained begin to differ after a surface age of 14 (Calvin Blood, PO Box 95009, Seattle, WA 98145-2009, unpublished data). Current break and burn criteria may be amended in the future so that all otoliths above a certain surface age will be broken and burned.

Age ranges

The majority of halibut in both the setline survey and market samples are between 10 and 14 years old (>65% for market samples). Due to the size selectivity of longline gear and the 82-cm minimum commercial size limit, less than 1% of halibut in the market samples are 5 years old and younger.

Fewer than 10% of halibut in both commercial and survey samples are over the age of 20. The maximum age observed to date for Pacific halibut is 55 years. Two fish have been assigned this age: a 161-cm female caught in 2000 and a 118-cm male caught in 1992. Both fish were part of IPHC setline survey collections and both were captured in Area 4; additionally, both maximum ages were obtained by the break and burn method. While surface counts can reach into the 30s, most assigned ages of 30 and over are determined by break and burn.

Occasionally strong year classes are observed and can be followed in the sampled catch for several years. An example of a strong year class in recent times is the 1987 year class, which has accounted for the largest proportion (in numbers) of the commercial catch for the last four years (1997-2000).

Areas of difficulty

Edge interpretation

Many of the otolith samples are collected in May and June, and often the translucent or winter zone has not yet been deposited, or is still in the process of forming on the otolith edge. There is a problem of deciding whether opaque edge growth on a particular otolith is new (from the current spring or summer) or from the previous summer. Some fisheries agencies have readers record an *annulus count* and an edge code rather than an *interpreted age*. IPHC readers record interpreted ages. For example, an otolith collected in February with 12 annuli and a full opaque zone on the edge would have an interpreted age of 13, whereas the annulus count for the same otolith would be 12.

As a general rule for otoliths collected through June, IPHC readers include the opaque edge in the annulus count if the edge growth is greater than half the width of the previous opaque (summer) zone in fish older than 10 years, or almost the same width as the previous opaque zone in fish younger than 10 years. The opaque edge is not counted in younger fish unless it is about the same width as the previous summer's zone because young halibut start their growth season earlier in the year than older fish, and may already have close to half the previous year's width of new growth by late May or early June (Fig. 22). Edge growth is interpreted in the same way for both surface and burnt sections although it is sometimes more difficult to view on burnt sections, as the edge is sometimes obscured by over-burning. Figures 23 and 24 are photos of otolith surfaces with different amounts of opaque edge growth.

Sometimes fish collected in fall or even late summer may have translucent growth on the edge. We usually interpret this as early deposition of the coming winter's annulus rather than assuming the translucent edge is from the previous winter, which would imply zero summer growth. The otolith in Figure 25 has a translucent zone on the edge. If the otolith came from a fish caught in the spring, the translucent edge would be counted to give an age of 11. However, if the same otolith was from a fish caught in late August or September, the translucent edge would be ignored and the assigned age would be 10.

New opaque growth also appears on different parts of the otolith at different times. Growth appears first on the ventral edge of the rostrum, which is also the region of fastest growth, last on the posterior end of the otolith. Often one can find two or three edge types on different regions of a single otolith. The eleven-year-old otolith in Figure 26 has eleven annuli plus new opaque growth along the ventral rostrum, while along the dorsal antirostrum there are only ten annuli followed by a full zone of opaque growth.

Occasionally otoliths with eroded edges are encountered (Fig. 27). What causes this erosion is unclear; one possibility is a reaction with the storage medium, but eroded otoliths are found within samples of normal otoliths that have been in the same medium. Whether or not to age eroded otoliths is up to the discretion of the reader.

Difference in edge interpretation between readers is a possible cause of one-year age discrepancies. IPHC began entering edge codes in addition to ages in 1999. In analyzing duplicate surface readings that differed by a year, it appeared that only five percent of those discrepancies were explained by edge interpretation (Forsberg 2001). However, edge code assignment is subjective and dependent on the area of the otolith where the reader made their final count. Edge codes currently used by IPHC readers are listed in Table 2.

Patterns

Table 2. Edge codes used by IPHC readers.

Code	Description
1	The edge (last summer's opaque growth) is included in the annulus count
2	There is a hyaline zone on the edge and no new opaque growth
3	There is new opaque growth that is not included in the age
4	There is a hyaline zone at the edge that is not included in the age

Some common pattern problems include: checks, faint rings, double-ringing, overlapping rings, and irregular spacing. A *check* is a zone of slow growth that is not a true annulus. Checks are often difficult to distinguish from true annuli, but they tend to be discontinuous (i.e., cannot be followed all the way around the otolith) and inconsistent with the general growth pattern of the rest of the otolith. Checks occur most frequently during periods of rapid growth and in younger ages (Penttila and Dery 1988). Checks may be caused by metabolic changes such as larval settling, the onset of maturity or spawning, migration, a decrease in feeding, or temperature changes. Hagen (1997) found two checks in larval halibut otoliths that correspond to hatching and first feeding. In some species, checks are common to a particular year class and form a natural "tag" (MacLellan and Saunders 1995). Induced checks are used to "barcode" otoliths of hatchery bred Pacific salmon before release. The young fish are subjected to controlled intervals of alternating high and low temperature, which causes checks to form in a predetermined pattern unique to a particular release batch.

While in some species the first annulus is difficult to interpret, it is usually not a problem in Pacific halibut. Sometimes the first annulus is round (Fig. 28) or the nucleus is composed of *multiple primordia* (Fig. 29) or is unusually small. In the latter case, it is probably because the fish was spawned at the late end of the spawning period (which is November to March), and therefore had a shorter growing season its first year. Figures 30 and 31are examples of unusual otoliths.

Checks and faint rings

Figures 32 and 33 are examples of surface checks. Figures 32 and 34 are examples of otoliths with faint rings. These rings are fainter than surrounding annuli, but their relative position to other annuli suggests that they are annuli rather than checks. The otolith in Figure 32 also has a check, which is darker than the faint ring, but due to spacing it is interpreted as a check. For otoliths that have very uniform annular spacing, such as Figure 32, a dark, continuous ring may be called a check if it doesn't fit the spacing pattern. In an otolith that had irregular spacing, this same ring would be interpreted as an annulus. Figure 35 shows an example of a checky burnt section.

Figure 18. Otolith burnt sections in soft modeling clay.

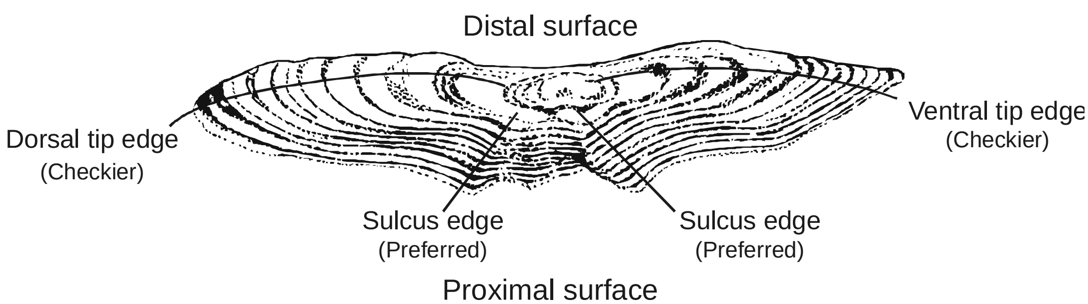


Figure 19. Preferred reading axes for halibut otolith burnt sections.

Figure 20. Otolith burnt sections in hardened clay mounts.

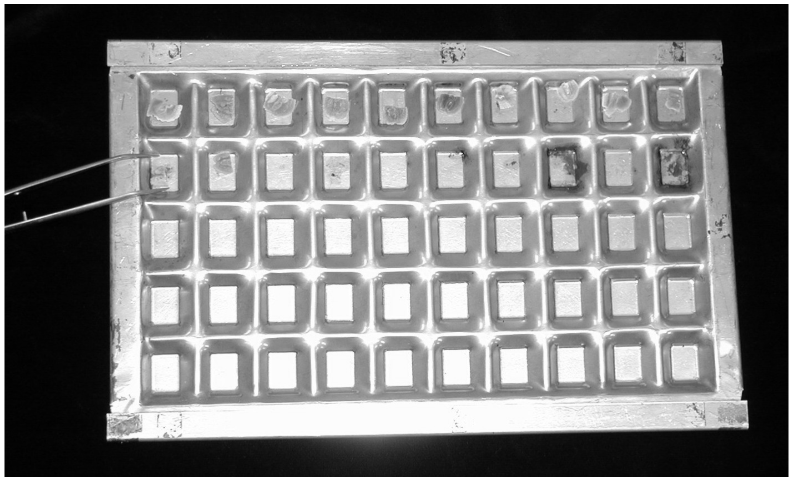


Figure 21. Metal tray used for baking otolith sections.

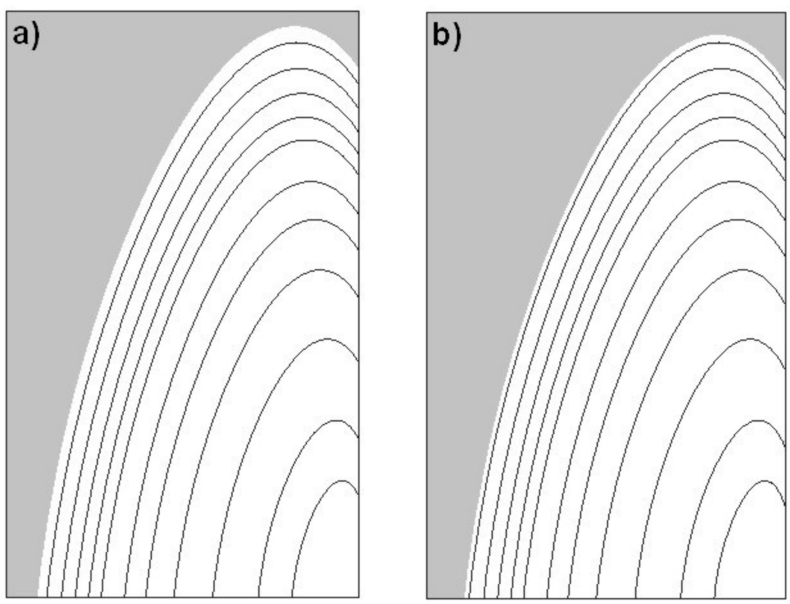


Figure 22. Diagrammatic otolith with 11 annuli. If both were collected in May, otolith a) would be assigned an age of 12, since there is almost the same amount of opaque growth on the edge as in the previous opaque zone, too much to be current year's opaque growth. Otolith b) would be assigned an age of 11, since the opaque edge growth is less than half the width of the previous opaque zone.

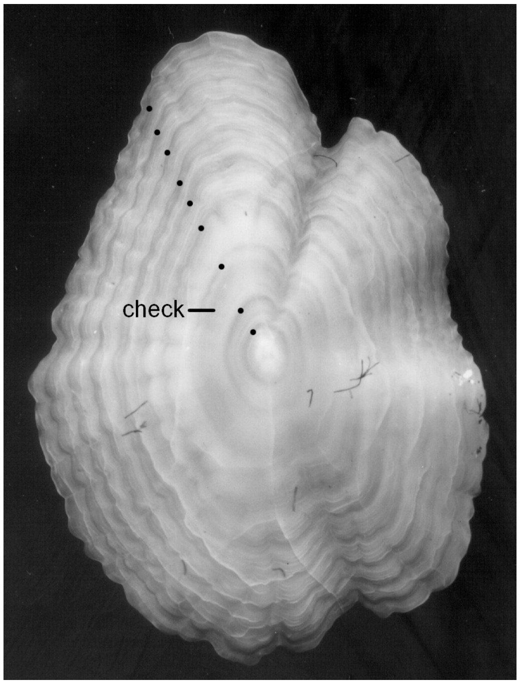


Figure 23. Otolith with nine annuli and a full opaque zone on the edge.

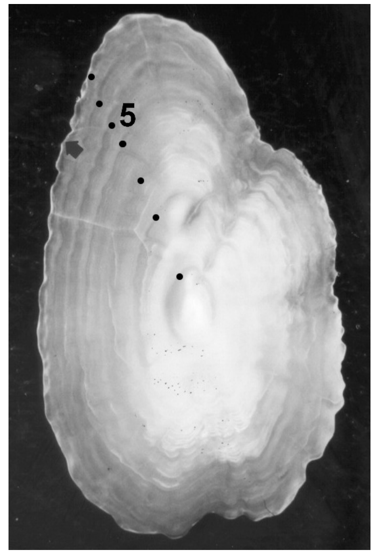


Figure 24. Seven annuli with a bit of new opaque growth.

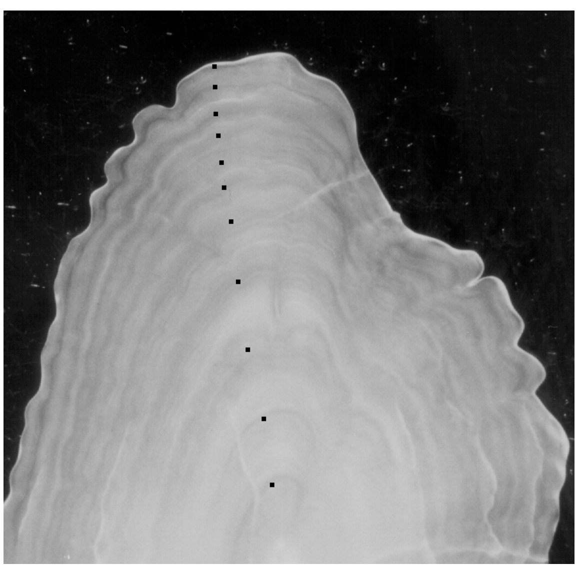


Figure 25. Otolith with 11 translucent zones. If the otolith came from a fish caught in August or later, the translucent zone on the edge would not be counted as an annulus.

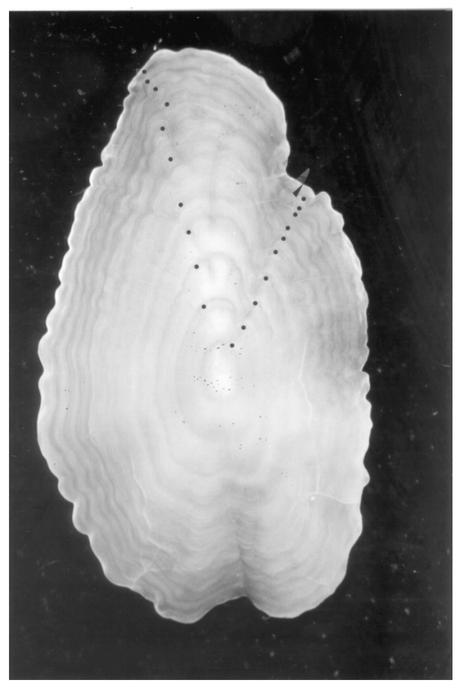


Figure26. Ventral side: 11 annuli plus new growth, dorsal side: 10 annuli plus full season of opaque growth.

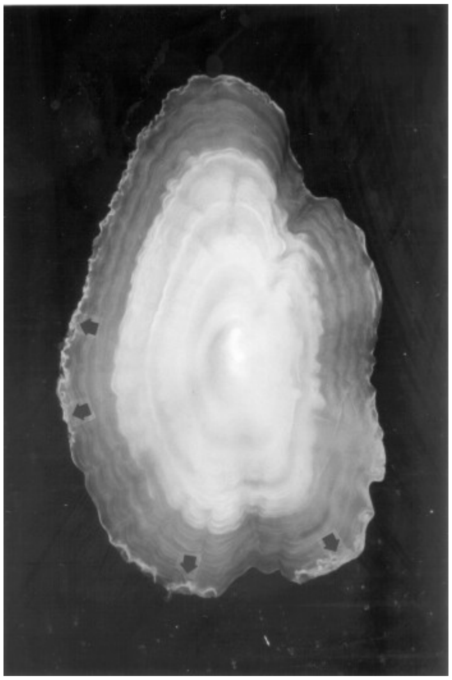


Figure 27. Nine-year-old with eroded edge. Otolith is from a male halibut, captured in Area 3B.

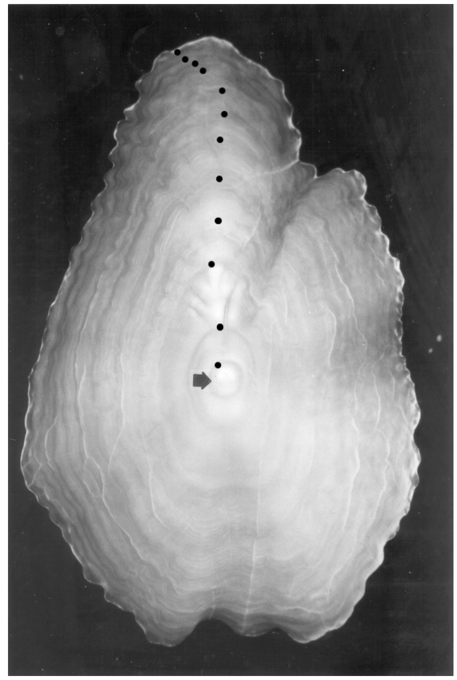


Figure 28. Twelve-year-old, first annulus is round. Otolith is from a female captured in Area 2C.

Figure 29. Multiple primordia.

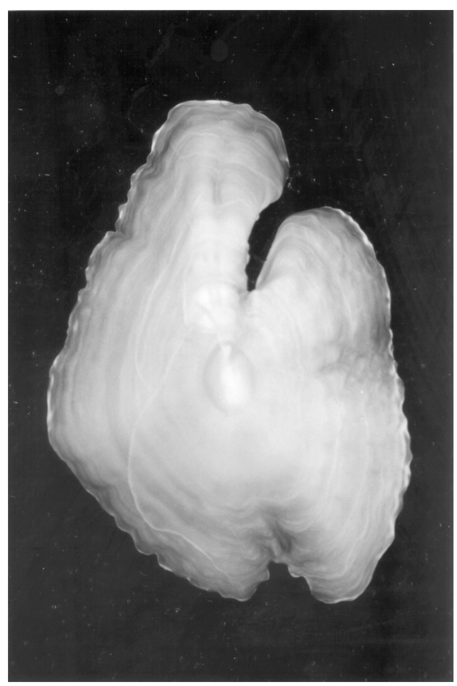


Figure 30. Unusual shape; deep notch between rostrum and antirostrum.

Figure 31. Spike projecting from surface.

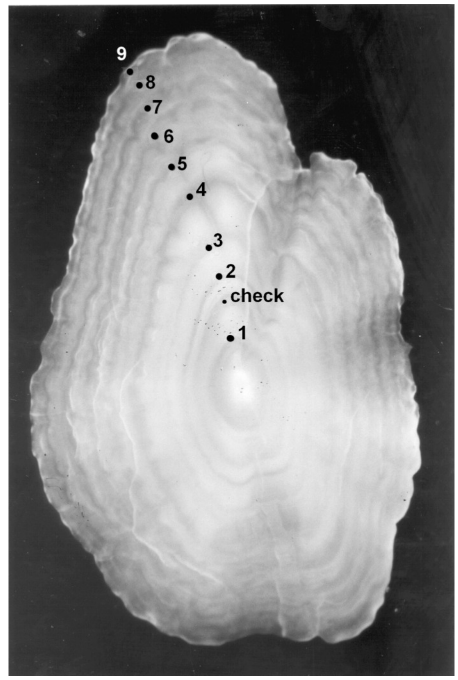


Figure 32. Age 9+, check between years 1 and 2, year 3 faint.

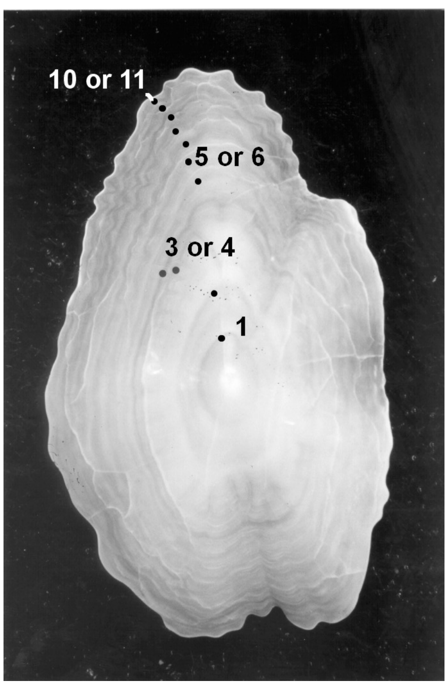


Figure 33. Check or year 3 and 4 close? Although spacing is irregular before and after the mark in question, this mark appears to be discontinuous and would be interpreted as a check.

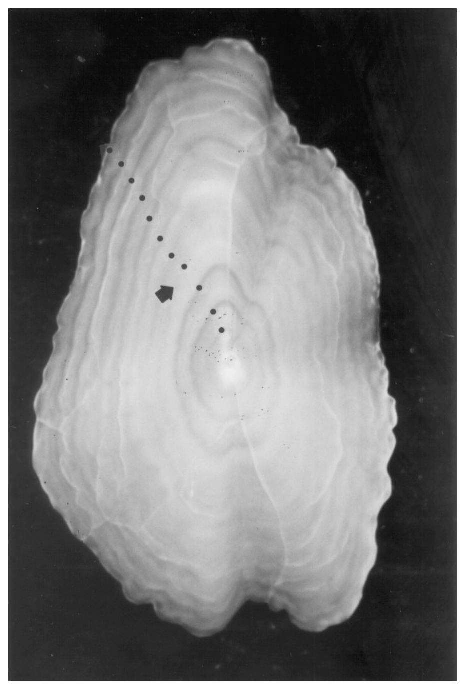


Figure 34. Year 4 is faint in this 11-year-old otolith, but note how spacing is regular before and after faint ring. Calling this ring an annulus rather than a check fits the pattern.

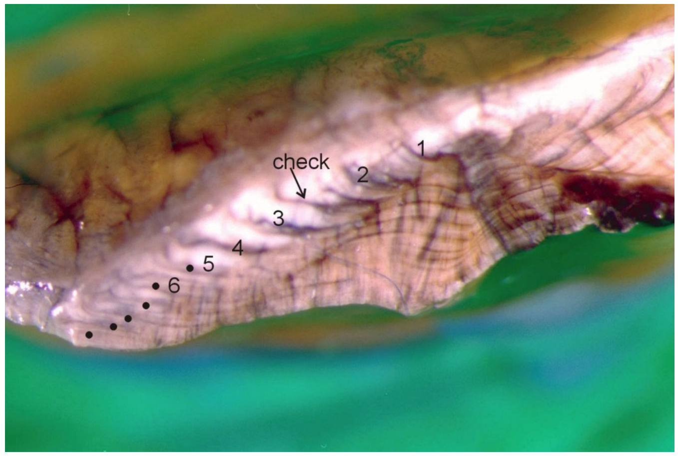


Figure 35. Ten-year-old otolith with a check between 2 and 3. Also slightly over-burned. Otolith is from a female halibut captured in Area 3B.

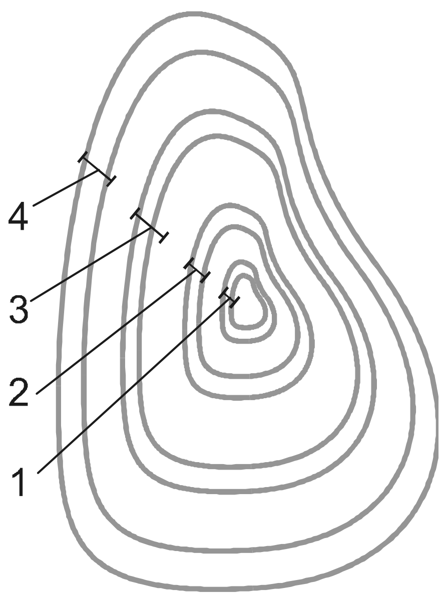


Figure 36. Diagrammatic representation of a lumped pattern.

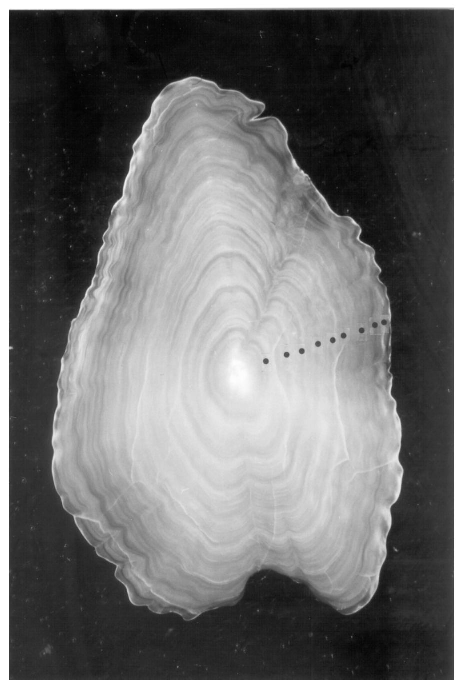


Figure 37. Nine-year-old "lumped". A higher count can be made on the ventral side by "splitting". Otolith is from a female halibut captured in Area 3A.

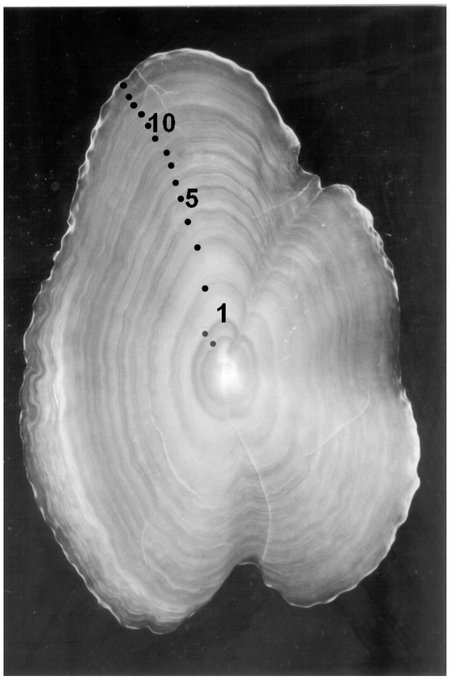


Figure 38. Age 14+, year 1 is a "double ring". Otolith is from a female halibut captured in Area 3A.

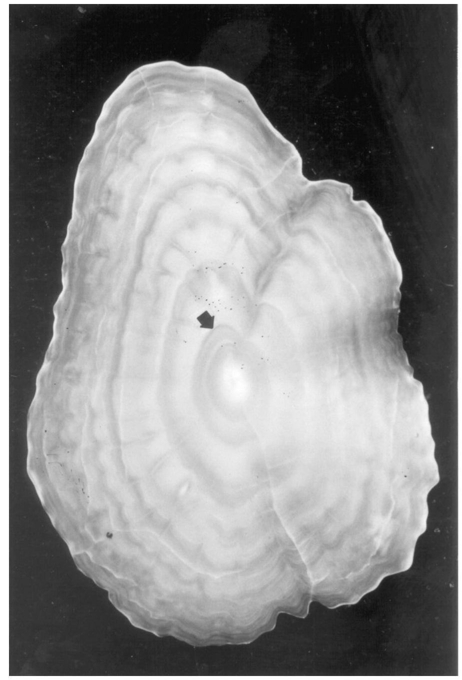


Figure 39. Another "double ring" for the first year.

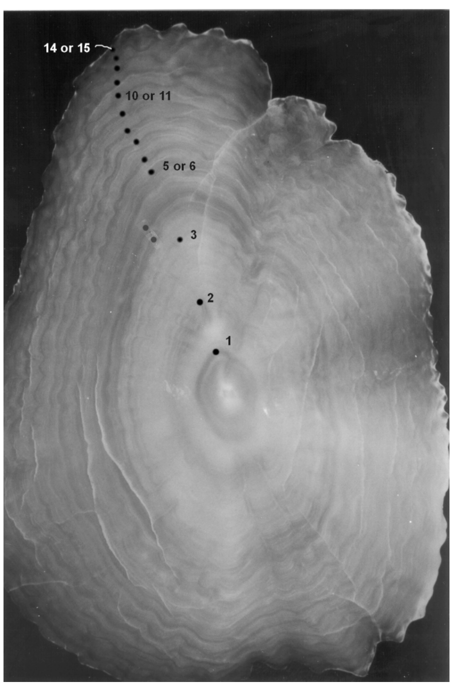


Figure 40. 14 or 15? Years 4 and 5 close or is year 4 a double ring? Since spacing becomes markedly and uniformly narrower after the rings in question, we would count both rings. Otolith is from a female halibut captured in Area 2C.

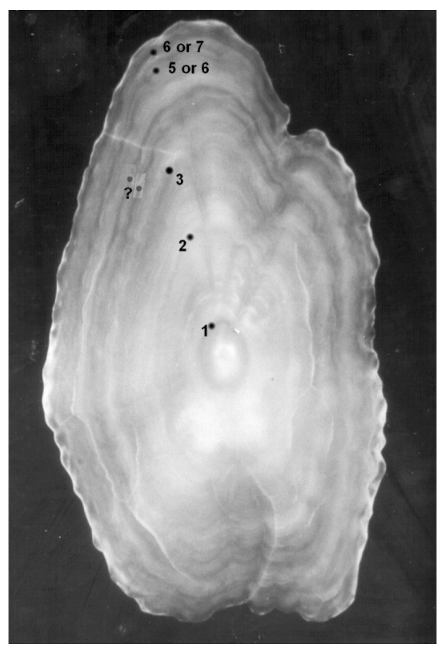
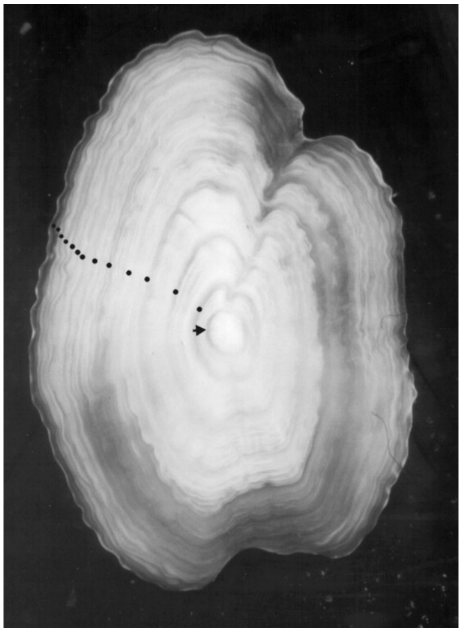


Figure 41. Six or seven? This otolith would be aged as a 6 with year 4 considered to be a split or double ring. This is a young otolith with a regular pattern when the check is ignored. Also, checks are common in the early years. Otolith is from a female halibut captured in Area 2C.



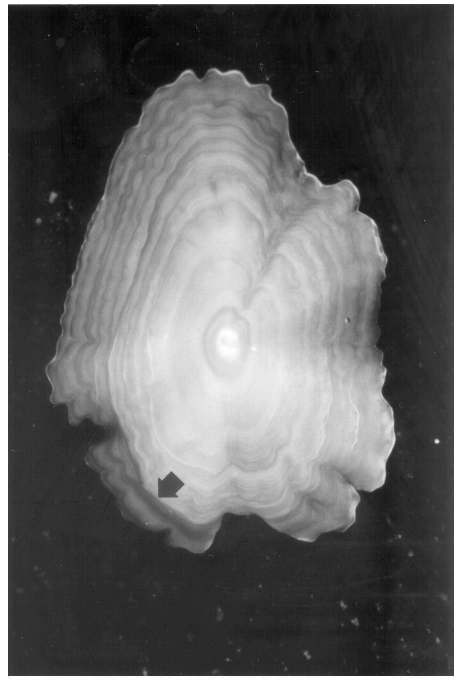


Figure 42. A 14-year-old otolith. The first year is round and close to the second year, but more distinct and not as closely spaced as the double-ring first years in the otoliths of Figures 38 and 39. Otolith is from a male halibut captured in Area 3B.

Figure 43. Steep "shelf" at edge of fifth annulus at posterior end overlaps several subsequent annuli in that spot. Otolith is from a male halibut captured in Area 3B.

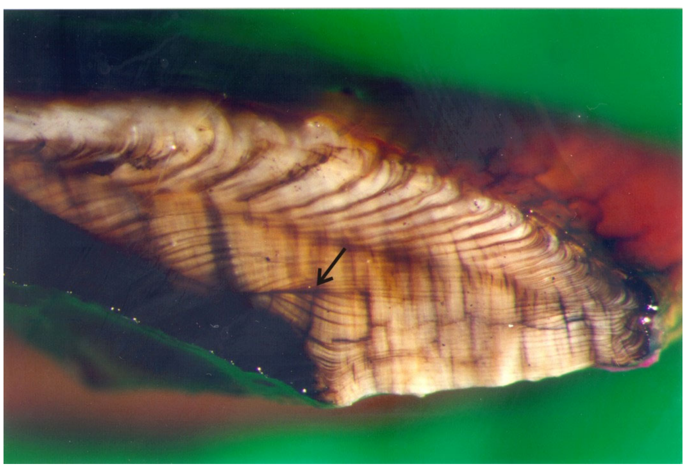


Figure 44. Shifted pattern changes direction where indicated by arrow. Otolith is from an Area 2C female halibut aged at 24 years by surface, 40 years by break and burn.

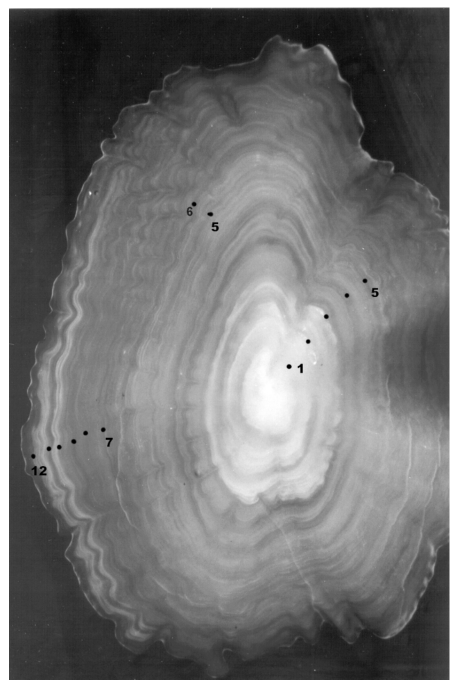


Figure 45. 12-year-old otolith with checks. A full count can't be made in one continuous transect. Partial counts are made in several spots and added together. Otolith is from a female halibut captured in Area 3A.

Figure 46. Twenty-year-old; also difficult to read along a continuous transect. Otolith is from a male halibut captured in Area 3B.

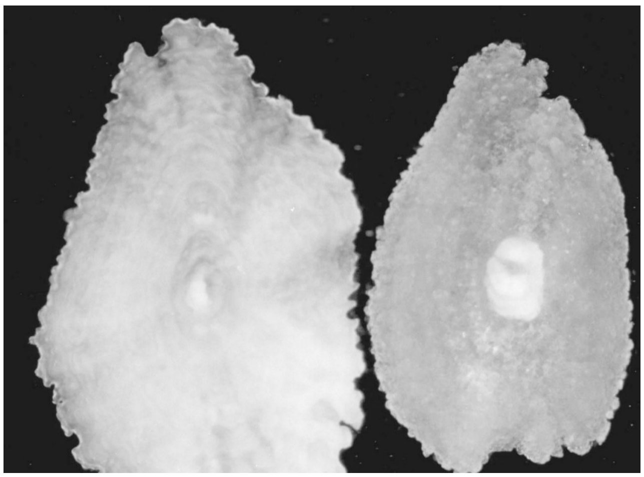


Figure 47. Translucent (left) and granular (right) forms of crystallization.

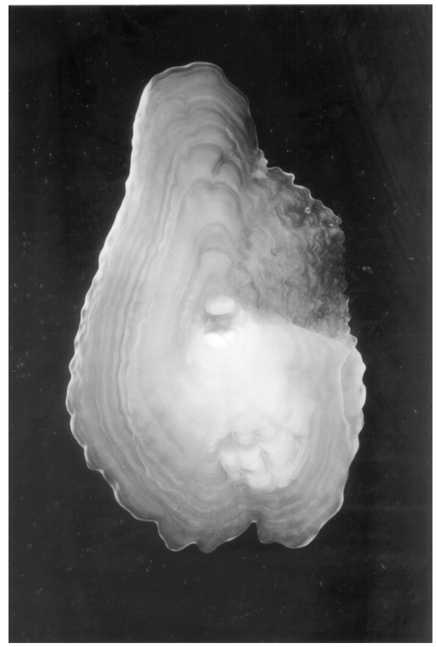


Figure 48. Part crystallized, multiple primordia.

Figure 49. Crystallized outside, inside "normal" aragonite form.

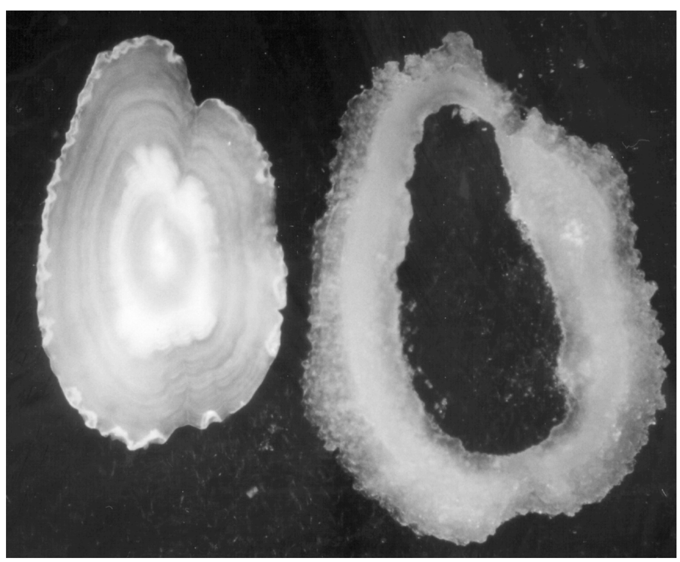


Figure 50. Broken off crystallized ring.

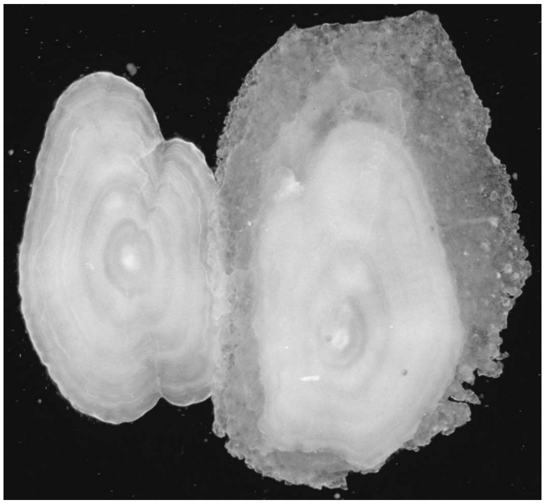


Figure 51. Left and right sagittae from the same fish. The left otolith had a crystallized ring that broke off, leaving a center that looks like a whole otolith.

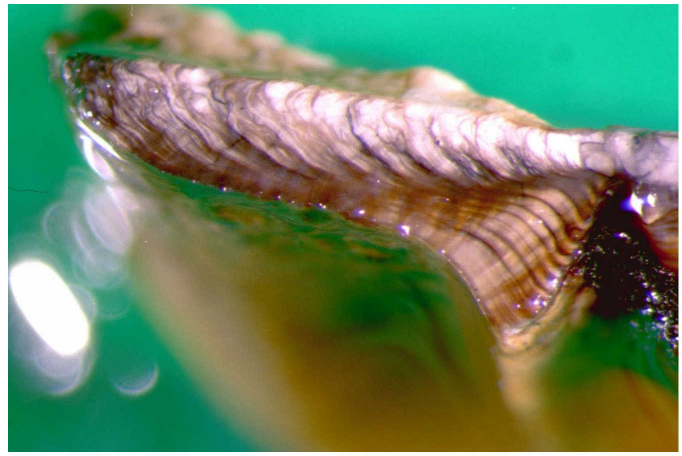


Figure 52. Burn of a translucent type crystallized otolith.

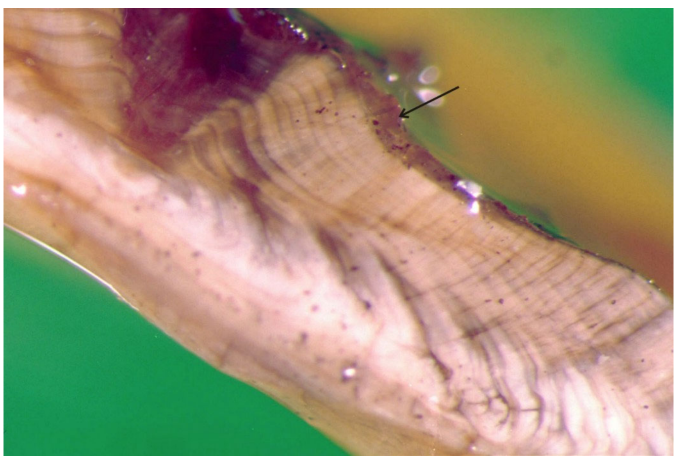


Figure 53. Crystallized on sulcus edge. Otolith is from an Area 3B female halibut which was aged at 16 years by surface, 15 by break and burn.

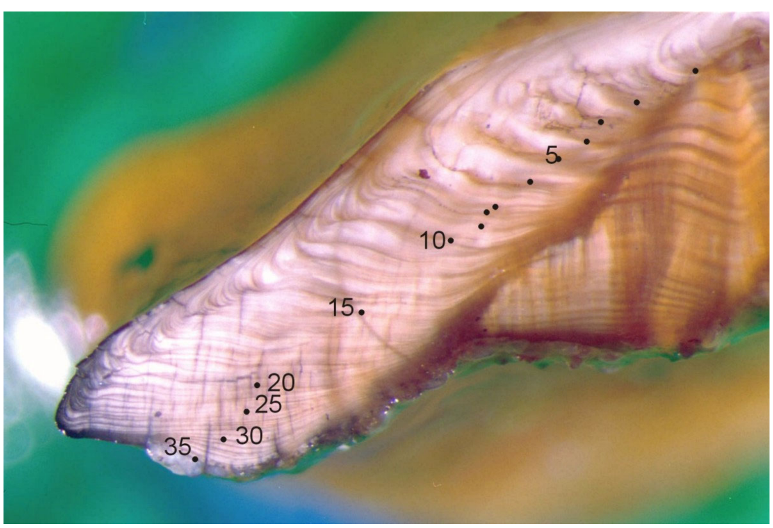


Figure 54. Crystallized on most of proximal surface except at dorsal tip. Otolith is from a male halibut captured in Area 2C.

Figure 55. Distal surface view of a right (eyed side) otolith.

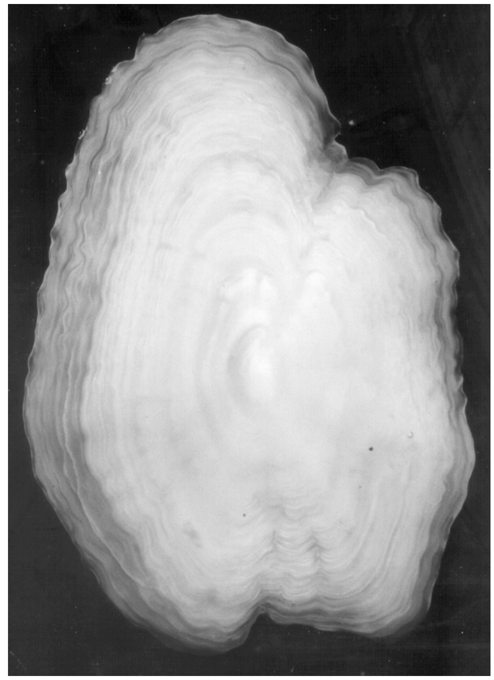


Figure 56. Otolith of a thirteen-year-old female halibut which has not cleared. Surface reading is difficult. Fish was captured in Area 3A.

Figure 57. Two-year-old with surface scum.

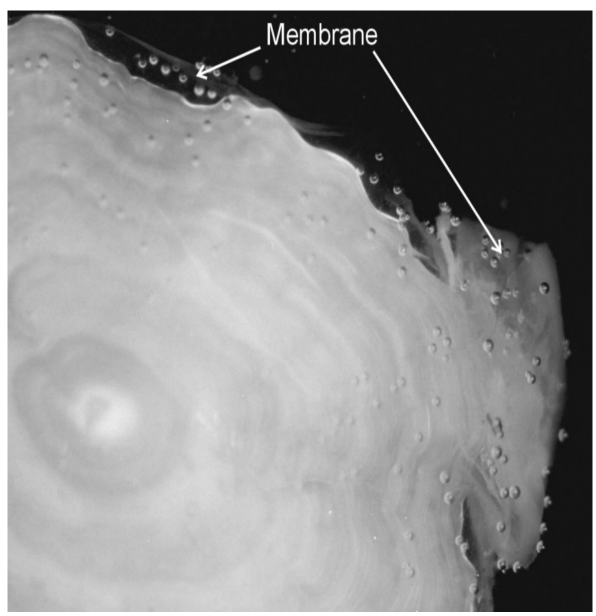


Figure 58. Otolith with adhering membrane.

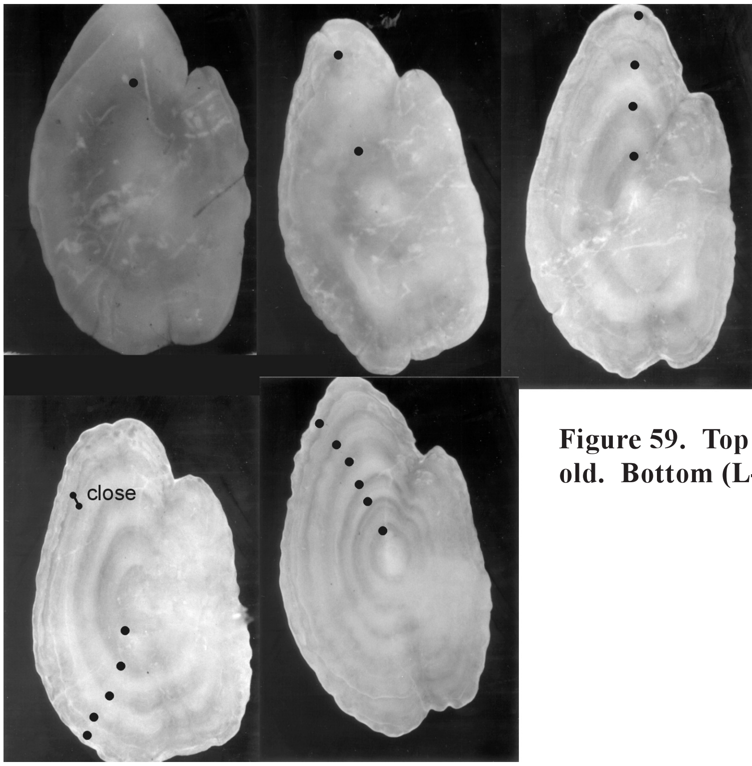


Figure 59. Top (L-R): 1-, 2- and 4 year-old. Bottom (L-R): 5- and 6-year-old.

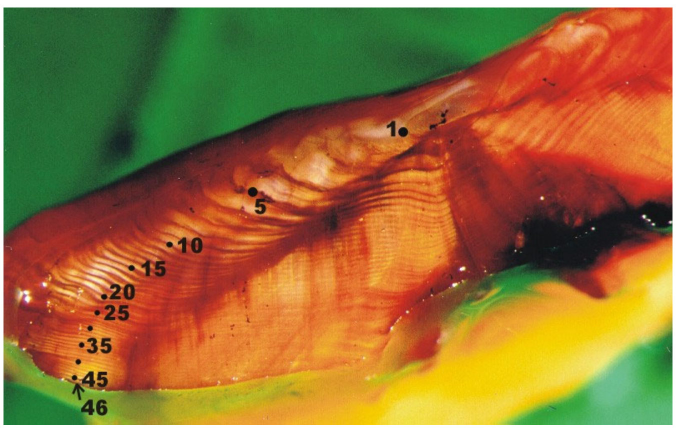


Figure 60. Otolith of a 46-year-old female halibut captured in Area 4B.

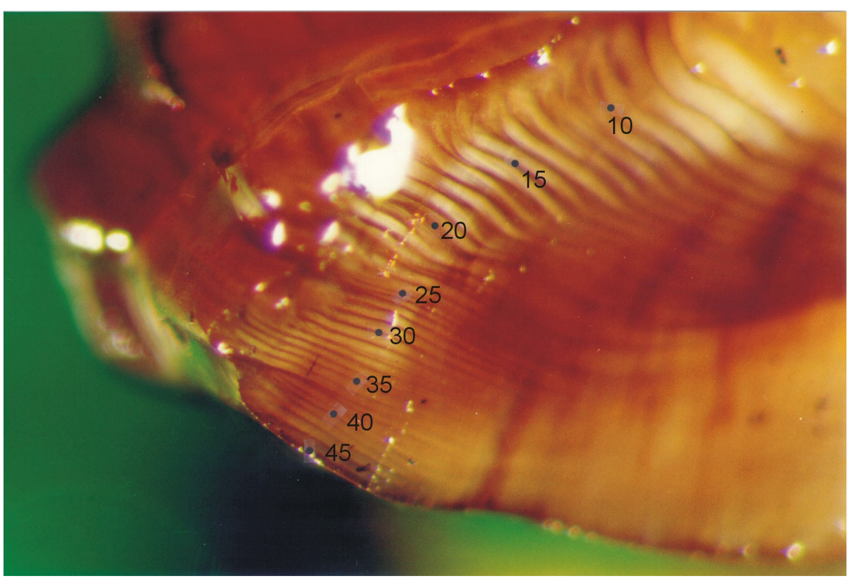


Figure 61. Closeup of same 46-year-old in Figure 60.

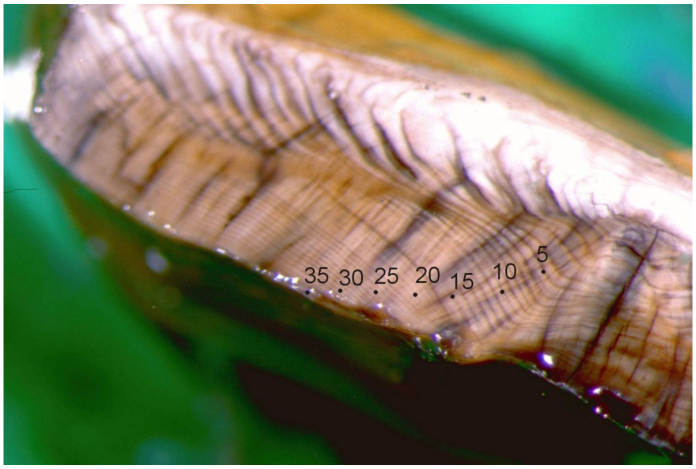


Figure 62. Thirty-six-year-old otolith with steep edge. Otolith is from a female halibut captured in Area 2B.

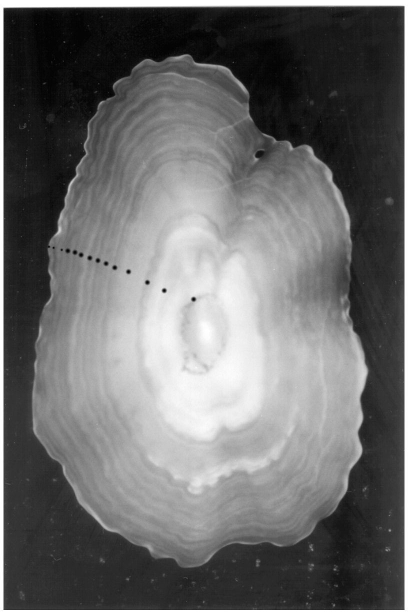
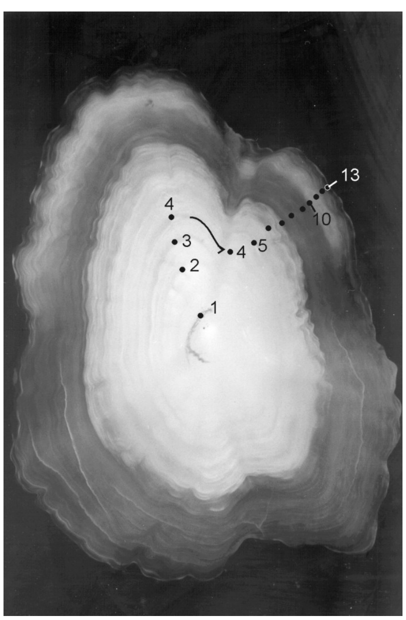


Figure 63. Surface (left) and burn (right). Surface age estimated at 14, with strange pattern. Burn age = 15. Otolith is from a male halibut captured in Area 3B.



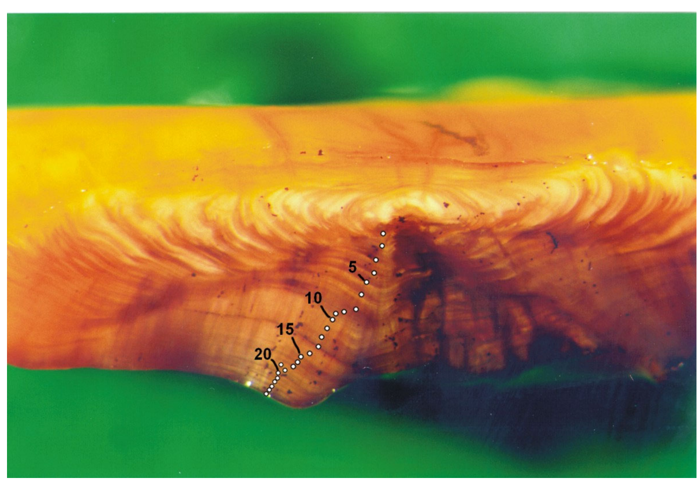
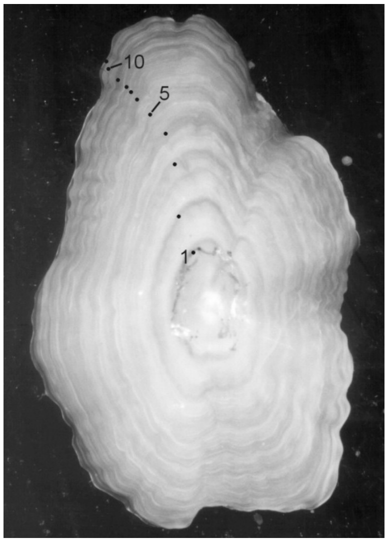


Figure 64. Surface (left) and burn (right). Surface age estimated to be 13 with noisy pattern. Burn age = 25. Otolith is from a female halibut captured in Area 3B.



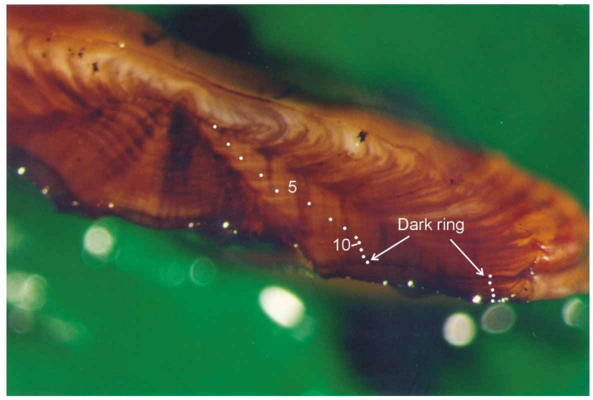
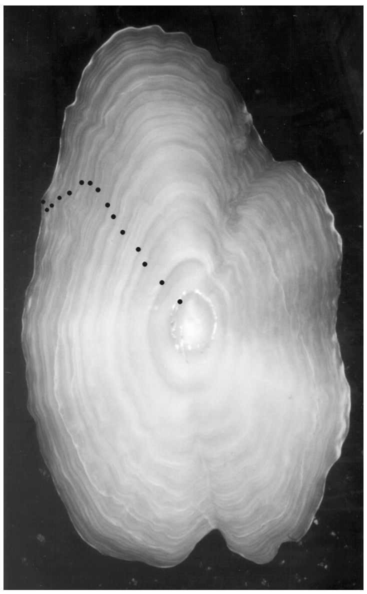


Figure 65. Surface (left) and burn (right). Steep otolith. Surface age estimated at 11, burn age at 18. Otolith is from a male halibut captured in Area 2B.



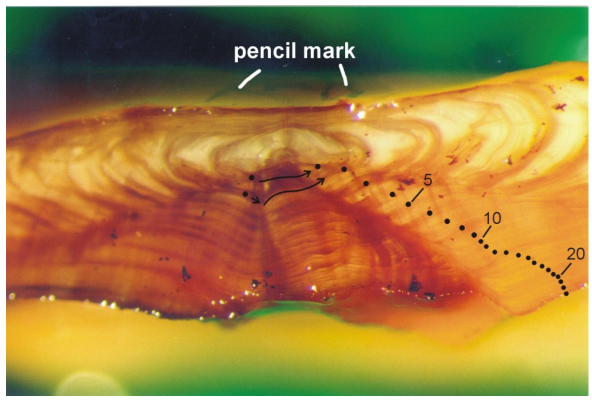
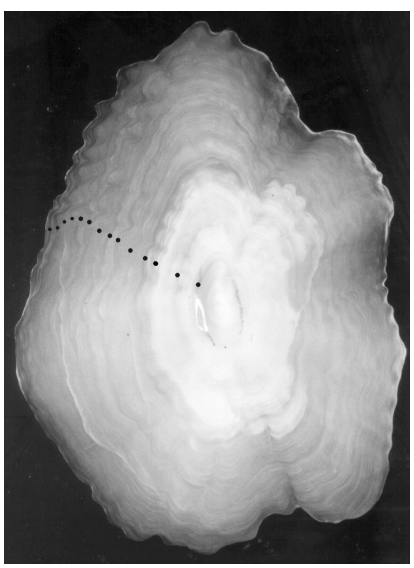


Figure 66. Surface (left) and burn (right). Surface age =15, difficult pattern. Burn age = 23. Note pencil mark on surface. Otolith is from a male halibut captured in Area 3B.



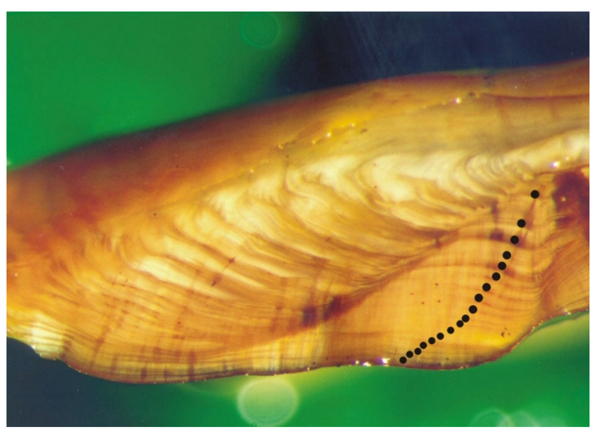


Figure 67. Surface (left) and burn (right). Surface pattern noisy. Surface age estimated at 14, burn age at 18. Otolith is from a male halibut captured in Area 3B.

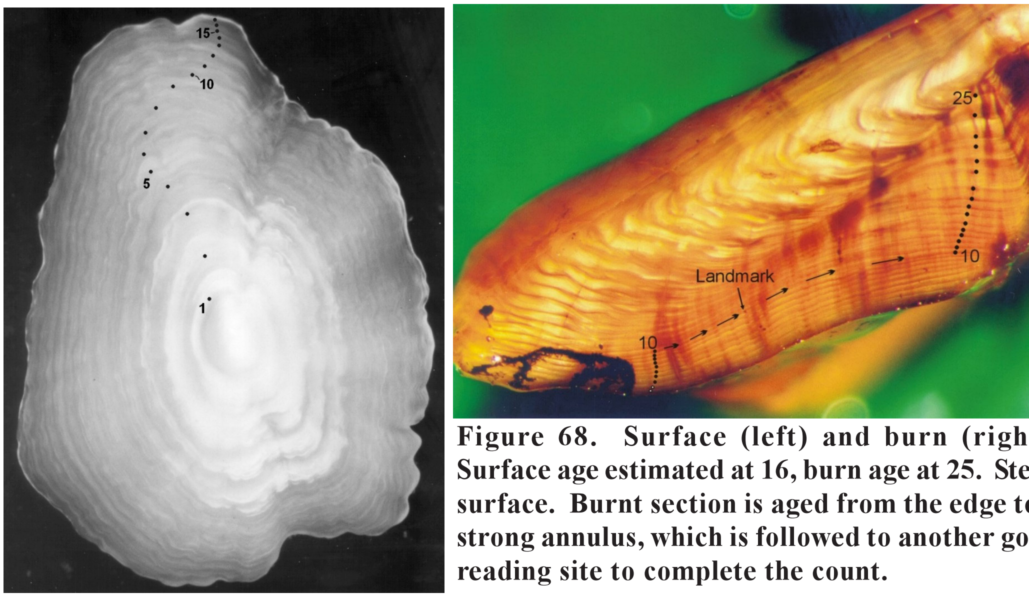


Figure 68. Surface (left) and burn (right). Surface age estimated at 16, burn age at 25. Steep surface. Burnt section is aged from the edge to a strong annulus, which is followed to another good reading site to complete the count.

Figure 69. Slightly overcooked, but contrast is actually enhanced. This is the posterior half of the otolith in Figure 68.

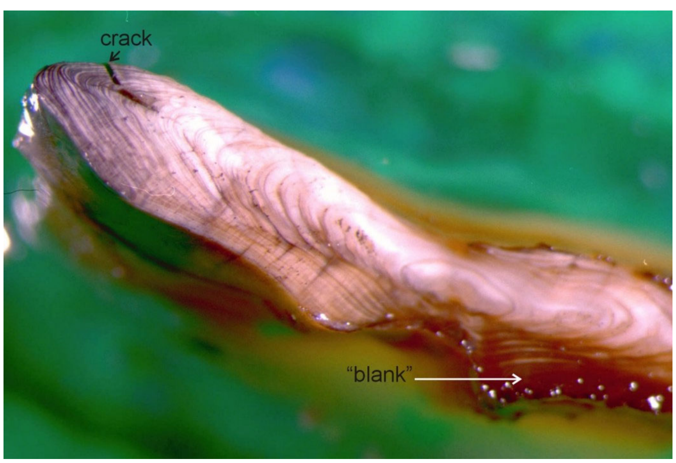


Figure 70. Dorsal tip is over-burned and cracking. In the bottom right of the photo, there is a blank spot to the right of the ventral sulcus area.

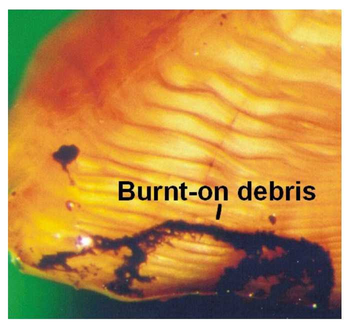


Figure 71. Enlargement of the burnt section in Figure 68 showing burnt-on debris.

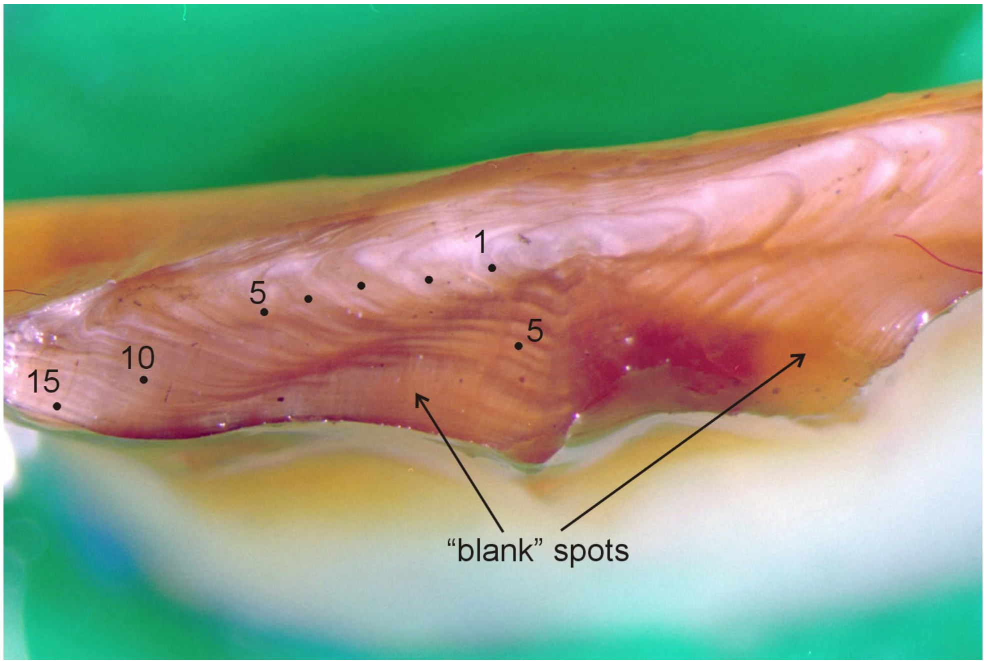


Figure 72. Pattern fades in and out on this burnt section. Reader must read around the blank spots. Otolith is from a female halibut captured in Area 3B.

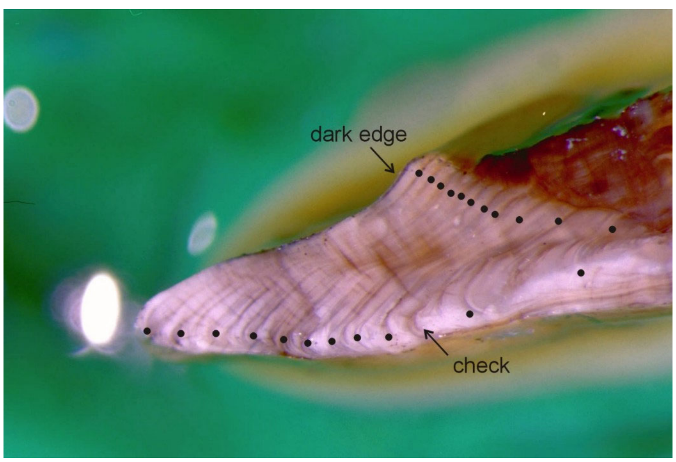


Figure 73. Edge is dark along the sulcus of this 11-year-old halibut, but is an artifact of the burn. This fish was caught in early summer, too early to see an early annulus. New opaque growth is visible on the ventral tip. Otolith is from a female halibut captured in Area 3B.

Figure 74. Otolith was not broken through nucleus. First annulus is year two. (marked on photo). Otolith is from a male halibut (captured in Area 3B) which was aged at 11 years.

Lumping and splitting

The term *lumping* refers to the practice of classifying units of closely spaced translucent rings that are separated by comparatively wider opaque spaces as single annuli (Fig. 36). On the other hand, *splitting* involves counting all discrete, continuous, translucent rings as annuli, regardless of how closely or irregularly they are spaced (Fig. 37). Otolith size and thickness are considered in deciding whether to lump or split. For example, young fast-growing otoliths are often checkier, so the reader tends to lump when there are groups of closely spaced rings separated by wider opaque zones. On otoliths that are thick, or have closely spaced rings throughout the otolith, the reader is more likely to split, since this could be the otolith of an older, slow-growing fish. An irregular pattern may be a reflection of poor growth in some years.

Double or split rings

Continuous, well-defined rings that are very close together within an otolith that has an otherwise uniform annular spacing are referred to as "double rings" or "split rings". The otoliths in Figures 38 and 39 would be interpreted as having a double ring for the first year, since spacing is very even throughout the rest of the otolith. The double ring phenomenon is commonly seen in the first year. The otoliths in Figures 40, 41 and 42 are less clear-cut, since spacing is somewhat less regular than in the otoliths in Figures 38 and 39.

Overlapping rings

Figure 43 is an example of an otolith in which some annuli, not necessarily at the edge, are hidden from surface view by adjacent annuli that are *overlapping*. On the otolith in the example, the fifth annulus forms the edge of a "shelf" in the ventral-posterior quadrant. This shelf overhangs and obscures the three following annuli.

Irregular or shifting pattern

Another difficulty associated with both surface and burnt section reading occurs when the pattern shifts (e.g., Fig. 44) or when the reader can't make a reading out one straight transect from nucleus to edge (Figs. 45 and 46). Instead, the reader reads out several annuli, then follows the last clear annulus to another site where a few more annuli can be read. This process is repeated until a count can be made all the way to the edge.

Crystallized otoliths

The term "crystallized" refers to otoliths that are composed of *vatarite*, a structural variant of aragonite. The physiological reasons for crystallization and the implications of this condition to the fish are unclear. Crystallization may be present in one of the otolith pair but not the other. There are different degrees of crystallization in terms of appearance and pattern readability. Crystallized otoliths have a rougher surface texture than do normal otoliths. Some are almost completely transparent with no visible banding patterns (these look something like partially dissolved sugar cubes; this form is referred to as *granular*). Figure 47 shows two types of crystallization, the transparent granular form and the less noticeable *translucent* form. Sometimes otoliths start out with normal aragonite crystals, then at some point in the fish's life, vatarite starts to be deposited. A partially crystallized otolith can have aragonite deposition in some areas and vatarite in others (Fig. 48), or have an aragonite center but vatarite edges (Fig. 49). In the latter case, this outer, crystallized "ring" can fall off when the otolith is collected (Fig. 50), leaving a normal-looking center which can be mistaken for a whole otolith. Sometimes such partial otoliths are discovered in the market samples when the age is suspiciously low for fish of at least 82 cm in length.

Figure 51 shows a pair of otoliths; the left otolith looks normal, as does the center of the right otolith. The left otolith probably had a crystallized ring (like the right side does) that fell off during collection.

Fully crystallized otoliths are not aged, due to poor pattern clarity or complete lack of visible zones. Partially crystallized otoliths are aged if at least part of the rostrum or antirostrum is normal all the way to the edge (e.g., the otolith in Figure 48 could be aged, while those in Figures 49, 50 or 51 could not). Some crystallized otoliths of the translucent form have much improved contrast when broken and burned (Fig. 52). A study is planned to investigate whether some crystallized otoliths can be used for the age composition if aged by the break and burn method.

Sometimes crystallization is not visible from the surface. Figures 53 and 54 show burnt sections with crystallization on the proximal surface of the otolith. Such otoliths can be aged as long as the crystallization doesn't obscure increments along at least one preferred reading axis. For example, the burnt section in Figure 54 is readable along the dorsal tip edge.

Right or "eyed-side" otoliths

In flatfish, the right and left sagittae are not mirror images as they are in round fish. Most agers use only the blind side otolith for aging since right side otoliths are more difficult to age; the annuli are less distinct and there are more checks. The nucleus is also offset posteriorly compared with the left side (Fig. 55), which makes viewing the zones in the posterior section difficult. Breaking a right otolith through the nucleus by hand is more difficult and may necessitate using a saw. Eyed or right side otoliths that are inadvertently collected in the survey or market samples are not currently used for aging at IPHC. Occasionally, however, the IPHC receives otoliths collected by sport or commercial fishers who are curious about a particular fish's age. If the person only collected the right otolith, it is aged as accurately as possible.

Broken otoliths

During the years when otolith measurements were used to estimate fish size, otoliths that were broken at the time of collection or during transit to the Seattle office were rejected for aging purposes. Since collection and use of actual fork length measurements was resumed, broken otoliths are no longer discarded unless broken in so many pieces that they can't be "reassembled" for viewing. Often otoliths broken into three or four pieces are aged. Partial otoliths are also routinely collected and aged as long as the nucleus is present. Sometimes a partial otolith contains the nucleus but is missing the anterior tip (i.e., the preferred reading axes for surface aging). In this case, the reader may not have confidence in the surface age, so a break and burn determination is done as well.

Area differences

Otoliths of halibut from different parts of their range can have different patterns. Otoliths of fish from the Bering Sea have very distinct opaque and translucent zones with few checks. Those of fish from parts of Area 2B and 2C often have comparatively wider translucent zones and more checks, especially in the first five or so years. Some areas require more break and burn determinations (e.g., Bering Sea/Aleutians, where we get a lot of thick, old otoliths), while very few otoliths from Oregon/Washington require breaking and burning (generally young and fast growing).

Timing of zone deposition differs over the halibut's range as well. Opaque (summer) growth in otoliths of halibut in the southern part of their range begins earlier in the year than in those of the Bering Sea and Aleutians. In a study comparing timing of opaque zone formation in the otoliths of 94 fish species, Beckman and Wilson (1995) found that opaque zone formation occurred later in the year and over a more extended time period.

Poor clearing

This problem is much less prevalent now that survey otoliths are stored in 50% glycerin-thymol solution right in the field. Otoliths allowed to dry out (i.e., stored dry for several months) before immersion in glycerin solution often never clear completely (Fig. 56) and there are opaque areas on the otolith where annuli are not clearly visible. This is more of a problem for surface readings. Opaque otoliths burn just as well as well-cleared ones.

Surface deposits ("scum")

Otoliths immersed in glycerin solution sometimes develop a "scum" or cloudy deposit on the surface (Fig. 57). This organic residue can be scraped off, distinguishing the problem from an opaque or poorly cleared otolith. The scum usually develops on otoliths that were not clean when put in the glycerin solution. Even with thymol in the glycerin solution, membrane, blood and other tissues adhering to an improperly cleaned otolith (Fig. 58) break down and cause the scum coating on the otolith. Sometimes mold will grow on the otolith. Readers from Oregon Department of Fish and Wildlife (ODFW) believe that the thymol itself causes the otoliths to become cloudy (Bob Mikus, Oregon Department of Fish and Wildlife, 2040 SE Marine Science Drive, Newport, Oregon 97365, personal communication). The scum deposit can be removed by immersing the otolith in a weak (10%) hydrochloric acid (HCl) solution for about 5 seconds. If the otolith is left in too long, the otolith itself starts to dissolve. At IPHC, we use *muriatic acid*, which is 32% HCl and is sold in hardware stores as a cleaner for tile and masonry. It is much cheaper than anhydrous HCl and requires less dilution.

Sex differences

Otoliths from male and female halibut are different in appearance, particularly at ages above 13 or so. Otoliths of male halibut tend to be smaller in length and weight than those of females of the same age. Shape is also different, with male otoliths being more elongate and thicker in relation to size. Annular spacing is narrower in males, and older male otoliths often have steep edges (Forsberg and Neal 1993). For these reasons, otoliths from males *seem* harder to age, however percent agreement isn't much different between males and females for both surface and break and burn ages (Table 3). However, since many of the male otolith characteristics are also break and burn criteria, proportionately more male otoliths are broken and burned. For example, of the survey otoliths collected in 2000, twenty-five percent of otoliths from males were broken and burned compared with

Table 3. Percent agreement by sex.

	Percent Agreement			
	+/- 0 year	+/- 1 year	Coefficient of variation (CV)	Number of ages
Surface males	67.5	91.9	2.7	10466
Surface females (1992-93 survey ages)	62.6	92.2	2.7	10912
Break/burn males	60.0	90.8	2.4	363
Break/burn females (1996 survey ages)	59.8	90.6	2.4	360

14% for females. This may explain the seemingly greater difficulty in aging otoliths from males.

Young halibut

Otoliths from young halibut (4 and younger) pose their own challenges to the reader. Edge interpretation can be a problem. Sometimes the edge appears almost "clear" from the distal surface but in fact, is not hyaline material (i.e., not more proteinaceous), but opaque growth that appears translucent because the otolith is thinner on the edge. The first few annuli are also relatively thicker with fuzzier margins that gradually blend into the opaque zones. As mentioned in the surface-reading section, sometimes the reader views a young otolith from the proximal side as well, to double check the interpretation of the distal side. This is particularly helpful in deciding whether a "clear" edge is true hyaline growth or just a thin deposit of opaque material. Figure 59 shows a series of young halibut otoliths. Note how the one- and two-year-old otoliths appear "fuzzy" compared to the four-, five- and six-year-old otoliths. Young halibut otoliths are also very easy to over-burn and should be held further away from the flame.

Sinistral halibut

"*Sinistral*" halibut have the eyes on the left side of the body. Over 99% of Pacific halibut are *dextral*, having the eyes on the right side of the body. In the rare cases where sinistral halibut are sampled, the right otolith is used for aging instead of the left. In a sinistral halibut, the right otolith looks like the mirror image of a normal left otolith.

Interannual variation in difficulty and edge growth

The degree of difficulty of pattern interpretation and timing of annulus formation can change from year to year. Readers exchange their observations on edge growth when they begin aging a new year's samples. The collection "season" for otoliths begins in mid-March for commercial samples, late May and on for survey otoliths. Observations of edge type trends in samples collected early in the season helps the readers evaluate edge type in otoliths collected in the "problem" time period of June. For instance, if new opaque growth was seen in a high proportion of otoliths collected from Area 3A in April, readers observing a full opaque zone in 3A otoliths collected mid to late June would interpret the edge as new growth. Without the observations from the earlier samples, the reader would be much less confident in interpreting the edge of June otoliths.

Difficulties unique to break and burn readings

While breaking halibut otoliths allows viewing of annuli on the proximal surface in cross section, the burning not only increases contrast of true annuli, but of checks as well. The first five to eight years on a burnt halibut otolith are more difficult to interpret than the outer rings. Often on very old otoliths, IPHC readers count from the outer edge inwards to a "landmark" (a bit of debris, crack, etc.) on the area of the section where the checky inner rings start. The reader then interprets the inner rings (which may take a few counts) then adds the inner and outer ring counts together for the final age. Counting from nucleus out or from edge inward is a matter of reader preference. Figures 60 and 61 are photos of the otolith of a 46 year-old female. Note how clear and well-defined rings 10 through 46 are compared to rings one through five. Figure 62 is an otolith of another fairly old fish, being relatively clear along the sulcus edge, but very checky along the distal tip edge. This is an example of an otolith that fit the break and burn criteria of steep edge; only 22 annuli could be seen from the surface and the section view shows the steep edge (left side of photo) with later years' growth occurring only on the proximal surface.

Figures 63 through 68 are "before" and "after" photos of otoliths for which readers had low confidence in the surface ages, requiring break and burn.

Otoliths that are held in the flame too long become very brittle and start to separate into layers where the protein matrix has burned away. The color changes to light gray in the opaque zones and dark gray in the translucent zones. Slightly overcooking sometimes enhances contrast (Fig. 69) but when burned beyond that point, the contrast fades (Fig. 70) and the otolith eventually turns pale gray and crumbles into ash.

The burnt section in Figure 66, particularly in the right side of the photo, is an example of "under-burning"; this otolith should have undergone more burning to increase zonal contrast. It is not necessary to remove mineral oil from the reading surface of the section before re-burning, although a heavy coating of oil may spatter when heated. In the past, cedar oil was commonly used to coat burnt sections and cedar oil will burn into sooty debris when a section is re-burned. Use of cedar oil was discontinued by most aging labs since it was not only difficult to remove from burnt sections if re-burning was required, but it is a toxic substance. Although mineral oil burns away clean, sandpaper grit and other contaminants on the broken surface of an otolith can burn onto the section and obscure annuli (Fig. 71).

Sometimes burnt sections will have "blank" spots even though the otolith was burned well and shows good contrast on other parts (Figures 70 and 72). In such cases, the reader has to read around the blank spot as when reading a shifted pattern.

Sometimes the edge of the section will burn dark and look like an annulus (Fig. 73). If the reader is uncertain whether the dark edge is an annulus or just an artifact of the burning (e.g., if there is a dark edge in mid to late summer), the edge growth should be compared on other parts of the otolith.

Another problem unique to burnt sections is failure to cut through the nucleus when breaking the otolith (Fig. 74) which, if not recognized, would result in under-aging.

If for any of the above reasons a burn is of such poor quality that the section cannot be aged with confidence (annulus counts differing by five or more years are obtained on different sites on the otolith, and the reader can't determine which of the counts is most accurate), first repeat the burn with the second otolith half. If the second half also produces a poor burn, then neither surface nor burn age should be entered, however the reader should note the reason in the comments field of the age form.

Accuracy, precision and quality control

In terms of age determination, *accuracy* refers to how close the estimated age is to the true age of the fish while *precision* refers to how close in value two or more age readings of a given otolith are. Accurate ages are both precise and unbiased (where bias is deviation from the true age). Multiple age readings may be precise without being accurate. Precision may be tested by percent agreement, coefficient of variation (CV), and average percent error (APE). Accuracy is more difficult to measure in otoliths, unless the age of the fish is known (captive-reared). Accuracy is usually measured by validation of aging techniques.

Validation

Mark-recapture experiments can validate the annual periodicity of features designated as annuli, and therefore, the accuracy of the aging method. The fish is caught, injected with an agent that permanently marks the otolith (e.g., oxytetracycline or OTC), or immersed in a bone staining dye (e.g., alizarin crimson), marked externally (with a tag), then released. On recapture, the time of marking is known, as well as time at large, so if the number of observed annuli present beyond the mark corresponds with the number of years at large, the annual nature of annuli can be inferred. The annual periodicity of Pacific halibut growth zones was validated using the OTC method (Calvin Blood, PO Box 95009, Seattle, WA 98145-2009, unpublished data). Other validation methods include radiometric aging and the "*bomb radiocarbon chronometer*" method, the latter of which is currently being used to validate Atlantic halibut age methods (Campana, Unpub)[1].

Measuring precision

Duplicate readings of the same otoliths are usually the standard for measuring precision. Duplicate readings can be compared within and between readers and the degree of variation can be used to evaluate several things, such as difficulty of a sample or species, changes in precision between experienced readers over time, or improvement of a new reader. Reference sets and dual scopes are tools utilized for precision testing along with replicate readings. A reference set is a set of otoliths that is re-read periodically, usually once a year, by each reader to monitor within- and between-reader drift. Some drawbacks to the use of a reference set are: a) the reader can memorize the otoliths if the set is too small and interval between readings is too short; b) if the set is too large, it takes too much time away from the reader's other duties; c) the chance of otoliths getting put back in the wrong cells or containers increases with handling; and d) the otoliths themselves can be damaged by repeated handling (especially burnt sections). A dual scope or "discussion tube" has two sets of oculars, allowing viewing by two people at the same time (Fig. 75). Some agencies use a dual scope for resolving discrepancies between two readers; the age agreed upon by both readers becomes the final or *resolved* age.

Historical protocol for precision testing

Recent changes in numbers of multiple readings are summarized in Table 4. Details of precision testing are incomplete for earlier years, but the following is a rough outline of past duplicate aging procedures.

In the 1930s and 40s, otoliths were aged with the following information withheld: location, length, sex, and date of capture. Samples were aged "blind" a second time after a period of at least several days to several weeks. Disagreements were resolved by a third reading with knowledge of the first two readings. The cause of discrepancy was determined as well. Dunlop (unpublished thesis) reported that total agreement was around 90% among the youngest age classes to below 50% among the oldest. The first reading agreed with the resolved in 81.5% of cases. The second reading agreed with the resolved age 87.0% of the time, a tendency that persisted through all age classes and was thought to be due to increased clearing of the otoliths (i.e., the otoliths had absorbed more glycerin in the interval between readings). Dunlop also pointed out the importance of bias, not just percent agreement, in age determinations.

In the 1960s, two independent readings were made for each otolith and if the two ages agreed, this was the final age of the fish. If the first two readings disagreed, a third reading was made and if the third reading agreed with either of the first two, this was the final age. If the third reading differed from both the first and second, the otolith was aged

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[1]http://www.mar.dfo-mpo.gc.ca/science/mfd/otolith/english/current.htm

Table 4. Recent changes in multiple readings of commercial (market sample) and survey otoliths (QC = quality control).

Year	Commercial sample	Survey
1987-1989	Double blind + independent resolving of discrepancies (surface only)	
1990	10% QC, independent resolving of discrepancies	Double blind + resolved
1991	20% QC, independent resolving of discrepancies	Double blind + resolved
1992-1993	20% QC, break and burn discrepancies =2 yrs	Double blind + resolved + break/burn of discrepancies =2 yrs
1994-1995	Restructuring of age staff duties. Drop QC readings	Double blind + resolved + break/burn of discrepancies =2 yrs
1996	20% QC, 1 QC reader	20% QC, 2 QC readers
1997	500 QC per reader, 2 QC readers	500 QC per reader, 2 QC readers
1998	500 QC per reader, 3 QC readers	500 QC per reader, 2 QC readers
1999-2000	200 QC per reader, 3 QC readers	200 QC per reader, 3 QC readers

a fourth time. If agreement wasn't achieved in the fourth reading, the otolith was rejected as illegible and discarded (Hardman and Southward 1965).

In the years 1983 through 1985, and 1987 through 1989, all otoliths were read twice by two different readers, with the second reader having no knowledge of the first reader's age ("double blind"). A third independent reading was made by the senior reader of all discrepancies. This third reading was called the "resolved" age even though it was an independent reading. In 1986, there was only a single reader on staff, so otoliths were read only once.

In 1990, double blind readings were discontinued for market sample otoliths. Instead, a single reading was made for all samples, and 10-20% of all the otoliths (i.e., one in ten or one in five) were aged a second time by the senior reader. These second readings are called *quality control* (QC) ages. If the initial and QC ages disagreed, the senior reader

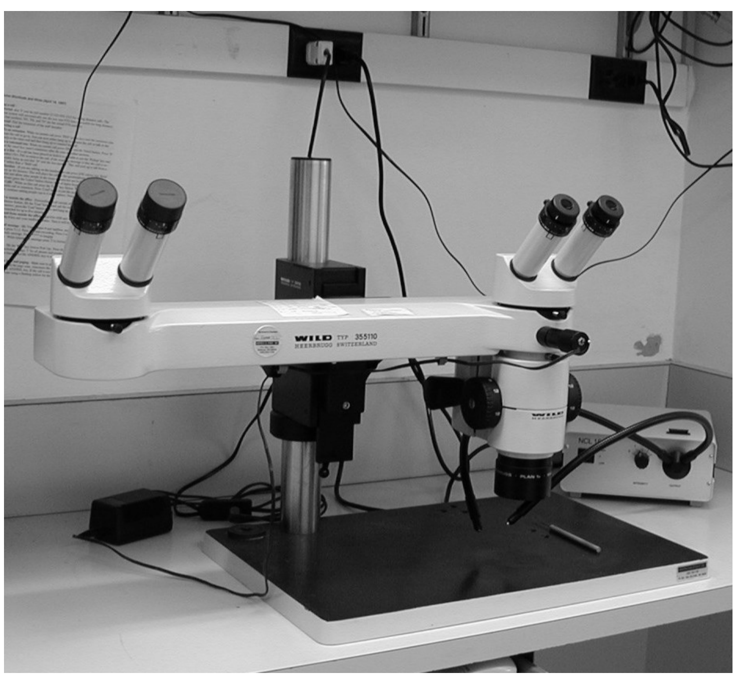

Figure 75. "Discussion tube" or "dual" stereo dissecting microscope used for training.

made a resolved age reading. Double blind and resolved readings were continued for survey otoliths.

In 1992, the senior reader began breaking and burning otoliths for which there was a two year or greater discrepancy between the initial and QC age (for market samples) or between the resolved and both double blind readings (for survey otoliths).

In 1994, due to restructuring of age room staff duties, QC readings on commercial samples were dropped in order to meet deadlines. In 1996, double blind reading of survey otoliths was discontinued and QC readings were made on 20% of all otoliths with no resolved readings for discrepancies. Since 1996, otoliths for QC readings have been selected arbitrarily in consecutive batches, although an attempt is made to spread the QC samples over time and area. The reason for the switch from reading every 5th or every 10th otolith for quality control to arbitrarily selected but consecutive samples was for easier data entry. For example, several whole market samples from different ports and regulatory areas or a whole or partial survey trip might be selected for QC.

There is still a field for resolved age in the database but it is automatically filled during data entry by either the initial or "human 1" age or break and burn age if there is one. Both initial and break and burn ages are superceded by a QC age. In 1997, due to the increase in number of survey otoliths, the number of QC otoliths was decreased to 500 from each reader. In 1999, this number was further reduced to 200 per reader, however, with three of the four readers performing QC ages, the overall decrease in number of QC readings was not as drastic. Since the 1980s, QC ages have been done and entered at a later date. Age data used in stock assessment must be available by October 31, so priority has been given to getting as many samples as possible aged once before this deadline.

While IPHC readers have never routinely reported ages obtained by resolving between two readers at the dual scope, agers have informally used the dual scope as both a training tool and to calibrate between experienced readers. For example, the senior reader and a newly trained reader will examine otoliths for which there is an age discrepancy of two years or greater between the two readers. During the years when double-blind aging was practiced, the senior reader would often look at otoliths together with the initial readers when there was a large difference in the double blind ages. In addition, experienced readers will occasionally look at an unusual or difficult otolith with a colleague and collaborate on an age, or jointly view an otolith with a difficult pattern to verify that both readers are designating the same features as checks, split rings, etc.

Current protocol and standards

Until 1998, the IPHC had no formal precision testing procedures. Percent agreement for double blind readings was occasionally hand-tallied by the involved readers out of interest's sake, and the progress of newly trained readers was monitored by comparing their percent agreement with an experienced reader. We began using the "ager training program" used by the age determination unit at the Alaska Fisheries Science Center (AFSC, NMFS) in Seattle. This program calculates percent agreement, bias between reader and tester and coefficient of variation (CV) as in Kimura and Lyons 1991, as well as average percent error (APE) as in Beamish and Fournier (1981). Current standards for precision for IPHC readers were set in 1998.

Based on comparisons of past and current readers (from 1987 through 1998), the following standards were set for surface ages: maximum CV of 4.0, minimum percent agreement of 55 for total agreement and minimum of 80% agreement within one year. The agreements for surface ages are consistent with Blood's findings in 1993 (Calvin Blood, PO Box 95009, Seattle, WA 98145-2009, unpublished data).

Table 5 shows the percent agreement results of a 1995 break and burn comparison (William Clark, PO Box 95009, Seattle, WA 98145-2009, unpublished data). Based on the results, standards for break and burn ages were set as follows: maximum CV of 7.0, minimum agreement of 75% within one year, and minimum 33% for total agreement.

Bias should be equally distributed as well. If bias is strongly skewed one way or the other, it should be determined if the bias is occurring in all age groups or just one or two. The less experienced reader should re-read a subset of otoliths in the problem age group(s) and if the bias persists, the subset should be resolved with the more experienced reader at the dual scope. A future goal is to resolve at least a portion of the otoliths for which the initial and QC ages disagree by more than one year in this manner (i.e., with the two readers together at the dual scope).

Efficiency standards were also established in 1998. Readers are expected to make at least 100 surface readings or 40 break and burn preparations and readings in an eight-hour day when aging full time. It should be noted, however, that as the stocks of halibut go through periodic changes in growth rates, the difficulty of the otoliths also changes over time, and both levels of aging efficiency and percent agreement must be periodically adjusted.

In 1999, IPHC readers began reading a reference set of 212 otoliths once each year. Reference sets have been used at IPHC at various times in the past but the practice was discontinued, usually due to time constraints. The current reference set is composed of otoliths that were collected on the 1997 setline surveys. There are 23 to 24 otoliths from each of the nine sampled IPHC regulatory areas, selected to represent the 1997 age distributions for those areas. There are no burnt sections in the reference set for the following reasons: 1) the reader would be influenced by the fact that mostly otoliths from

Table 5. Percent agreement for break & burn ages (from 1995 survey otoliths).

Tester		Percentage			Reader deviation from tester				Bias from tester	
Age	#Aged	Agreement +/- 1	CV	APE	#-	#+	%-	%+	Bias (yr)	%Bias(yr)/Ave. Age
5	5	80.0	9.9	7.0	0	3	0.0	60.0	0.80	13.9
6	27	85.2	8.1	5.8	0	14	0.0	51.9	0.81	11.5
7	75	85.3	6.5	4.6	4	36	5.3	48.0	0.60	7.6
8	66	81.8	6.9	4.9	12	23	18.2	34.8	0.42	4.0
9	50	78.0	8.1	5.7	7	28	14.0	56.0	0.76	7.0
10	59	81.4	5.8	4.1	11	24	18.6	40.7	0.29	2.2
11	52	73.1	7.4	5.2	12	25	23.1	48.1	0.81	5.6
12	52	78.8	6.1	4.3	14	20	26.9	38.5	0.54	3.6
13	38	68.4	7.3	5.1	14	20	36.8	52.6	0.29	1.5
14	38	68.4	6.2	4.4	15	13	39.5	34.2	0.08	-0.1
15	22	63.6	6.2	4.4	11	7	50.0	31.8	-0.14	-1.5
16	18	72.2	5.5	3.9	8	6	44.4	33.3	-0.11	-1.2
17	10	80.0	5.7	4.1	5	3	50.0	30.0	-0.50	-3.6
18	20	70.0	5.5	3.9	7	7	35.0	35.0	0.10	-0.1
19	3	66.7	5.3	3.8	2	0	66.7	0.0	-1.33	-7.5
20	5	60.0	7.2	5.1	2	3	40.0	60.0	0.00	-0.7
21	2	50.0	7.2	5.1	2	0	100.0	0.0	-2.00	-10.0
24	1	100.0	3.0	2.1	1	0	100.0	0.0	-1.00	-4.3

Percent agreement +/- 1 year = 77.2	
Percent agreement +/- 0 year = 33.9	(184 tests)
Percent minus bias = 23.4	(127 tests)
Percent plus bias = 42.7	(232 tests)
Total number of comparisons	543
Total CV = 6.7	
Total APE = 4.7	

older fish are broken and burned; 2) the readers could "memorize" ages from burnt sections, especially if there were relatively few burnt otoliths in the reference set; 3) burnt sections are more fragile than whole otoliths and could deteriorate over time with handling. The same precision standards apply to the reference set ages, but the purpose of the reference set is to monitor within-reader variation over time. Within-reader percent agreement for Pacific halibut surface ages is about the same as agreement between two experienced readers for a given set of otoliths (Gilroy et al. 1995). In spite of the potential for otolith deterioration mentioned above, a reference set of burnt sections may be assembled in the future to monitor within-reader variation in break and burn readings.

Training

Training initially involves use of the dual scope or using a microscope-camera setup to view otoliths on a monitor (Fig. 76) with an experienced reader. Both systems can be used, or one may be preferred over the other. For example, two individuals with very different eyesight may have difficulty getting the image in focus for both viewers with the dual scope (focus can only be changed slightly with the second oculars). Simultaneous focussing isn't a problem with the microscope-camera system; however, the images on a monitor are not as sharp, especially near the edges.

In the second stage of training, the trainee reads about 1000 otoliths that were previously aged by an experienced reader. Ages are compared and problem areas are reviewed. The newly-trained ager then begins production aging. QC readings of the new reader's ages are made at a higher rate for the first year, from 20 to 100% depending on the number of otoliths aged by the new reader and the time available to the senior readers.

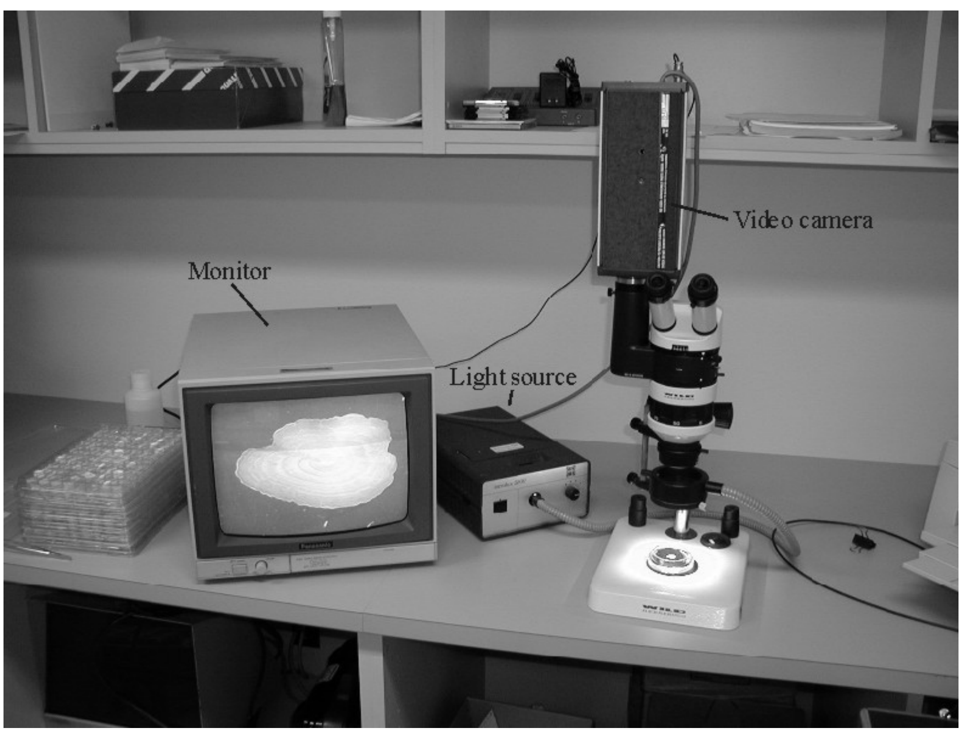

Figure 76. Dissecting microscope attached to video camera, frame grabber and monitor. Used for training or making measurements of otoliths.

Exchanges

Exchanges are important in that they reduce isolation between agencies. Readers who do not have occasional contact with agers from other agencies are at risk of drifting or stagnating in terms of criteria and methodology.

Pacific halibut otolith exchanges have been made with the following agencies: Tokai Regional Fisheries Research Laboratory, Japan, the Canadian Department of Fisheries and Oceans (DFO), and the Alaska Department of Fish and Game (ADF&G).

The Japanese exchange involved otoliths collected in the northwest Bering Sea by Japanese research and commercial vessels (Second Resource Research Laboratory, Tokai Regional Fisheries Research Laboratory 1964). A total of 243 halibut were otolithed and surface-aged. Independent readings were made by two readers from IPHC and two readers from Japan. Agreement was thought to be acceptable: age readings for fish under 66 cm had greater than 80% agreement, while for fish over 100 cm, agreement was less than 50%.

Exchanges were made with DFO's Pacific Biological Station (PBS) in Nanaimo, BC in 1980, 1987 and 1999.

In the 1980 exchange, IPHC surface ages were compared with DFO burn ages. DFO's ages were higher. The 1987 and 1999 exchanges were informal and showed better agreement. The 1999 exchange was limited to broken and burnt otoliths.

An exchange of Atlantic halibut (*Hippoglossus hippoglossus*) otoliths is being planned. Readers from DFO's Bedford Institute of Oceanography in Dartmouth, NS were trained by IPHC in the mid-1980s on both Pacific and Atlantic halibut otoliths. Otoliths of Atlantic and Pacific halibut are almost identical in appearance and criteria for aging are very similar as well (Trumble et al. 1993).

IPHC and ADF&G conducted halibut otolith exchanges between 1990 and 1998. The otoliths in the exchange samples were subsets of ADF&G's annual sport halibut otolith samples. Agreement and bias levels indicated that IPHC and ADF&G were applying the same criteria in determining ages (Meyer 2000).

Occasionally, agers from IPHC look at otoliths from other species. Since IPHC is the only agency currently reading Pacific halibut otoliths on a production basis, opportunities for halibut otolith exchanges with other agencies are limited. Otoliths of other fish species, particularly other flatfish, have a lot of general similarities with those of halibut and some of the exchange benefits can be gained through other-species exchanges. IPHC participated in an annual hands-on workshop and exchange of Dover sole (*Microstomus pacificus*) otoliths in 1999, 2000 and 2001 and frequently looks at small numbers of other-species otoliths at age reading workshops.

Workshops also provide an opportunity to discuss unusual phenomena, (such as the translucent edge observed on otoliths collected in mid to late summer in 1998 for several species, including halibut) and how to interpret them. They also provide an opportunity to discuss other data recorded besides age (e.g., edge type, certainty, and readability) and to review other agencies' protocols for assigning ages; i.e., recording age as an annulus count only versus incorporating birthdate/month of capture information. Information is also exchanged on new or different techniques and tools.

Current and Future Research

Marginal increment analysis (MIA)

Incidental observations on timing of annulus formation from the recent Pacific halibut age validation study (Calvin Blood, PO Box 95009, Seattle, WA 98145-2009, unpublished data) showed some dissimilarities to Dunlop's MIA results in the 1930s. Knowledge of the timing of annulus formation is necessary for interpreting edge growth and assigning otoliths to the correct age group. Halibut otoliths from winter, spring and summer were collected by fisheries observers in Canada and the United States in 1999 and 2000 for use in a more comprehensive MIA.

Right/left break and burn age comparison, right vs. left rate of crystallization and left otolith rates of crystallization by area

Early studies at the IPHC found that right otoliths were harder to read and it was felt that ages determined from right otoliths were less accurate. On the year 2000 setline and trawl surveys, both right and left otoliths were collected from a portion of the halibut caught. The paired ages, both surface and break and burn, will be compared. We have only compared surface ages between right and left-side otoliths in the past; right-side otoliths might be easier to read using the break and burn technique and the age from right side might be comparable to that of the left side using this method.

The survey otolith pairs collected in 2000 will also be part of a crystallization study in which we will: a) compare rates of right and left side crystallization; and b) compare rates of left side crystallization by area of capture.

Consistency of application of criteria via computer images and software

This study will compare between and within reader consistency in application of aging criteria. There is no permanent record of any particular age interpretation. A reader cannot replicate 100% of his or her own ages, let alone another reader's. In these cases of disagreement, whether with oneself or another reader, the reader(s) must be interpreting the growth patterns differently, and it may not be clear why, or where the discrepancy(s) occur(s). Even when two readers assign the same age to a given otolith, it cannot be assumed that they have "read" or interpreted the same marks as annuli. A series of scanned otolith photos and a computer paint program will be used. For each image, readers will mark what they are interpreting as annuli and save the marks in an overlay. Each reader would repeat the process after a period of time and the overlays would be compared within and between readers for discrepancies in application of aging criteria.

Break and burn percentage agreement

Double blind readings of broken and burnt otolith sections will be compared for percent agreement. The sample is a subset of the 1999 survey otolith collection. Percent agreement values will be incorporated into IPHC aging quality control standards.

Changes in assigned ages over time due to changes in application of criteria and equipment

Current readers will age a set of archived otoliths that were originally aged 20-25 years ago and current and original ages will be compared and examined for changes/shifts in the age distribution due to different aging practices. These otoliths will then be aged again using an old Bausch & Lomb microscope to test for differences in ages due to equipment.

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Appendix I

Glossary of Terms

Accuracy How close the estimated age is to the true age of the fish. An accurate age is both precise and unbiased. Measured by validation studies.

Annulus Commonly used to describe a continuous, concentric zone which forms once each year, usually the "winter" growth zone which marks the end of a year of growth. Synonyms: rings, bands, annual rings. The word actually is derived from the Latin "*anus*" for ring, not the Latin "*annus*" for year.

Asteriscae (singular: asteriscus) One of the three pairs of otoliths. The asteriscae are found in the lagena of the inner ear and are not used for age determination of Pacific halibut.

Check Translucent material in the opaque zone, which is not a true annulus and not counted as such. Such rings are distinguished by the width of the zone relative to annuli, location relative to annuli, and incomplete formation (doesn't go all the way around the otolith) or poor definition.

Crystallized otolith Calcareous portion of otolith is made up of vatarite (structural variation of aragonite) Age determinations are generally not possible due to inability to distinguish annuli.

Edge Outer periphery of the otolith.

Edge type opaque or translucent deposition occurring on the outer edge of the otolith, representing the most recent growth.

Hyaline zone See translucent zone.

Lapillae (singular: *lapillus*) One of the three otolith pairs, located in the *utricle* of the inner ear, and not used for age determination in Pacific halibut.

Nucleus Central portion of the otolith, bounded by the first annulus; sometimes used synonymously with the terms core, kernel, center, focus or primordium.

Opaque zone A zone that inhibits the passage of light. The zone is composed primarily of inorganic aragonite needles, which are long and thick relative to those formed in hyaline zones. With transmitted light, opaque zones appear dark; with reflected light, they appear bright. Synonyms: Summer zone, summer ring, white ring, light ring.

Precision Measure of how close in value two or more readings of a given otolith are.

Sagittae (singular: *sagitta*) Largest of the three pairs of otoliths in the inner ear of a fish. The left sagittal otolith is used for aging Pacific halibut. The sagittae are enclosed in a membranous sac called the *sacculus*.

Sulcus acusticus Or simply "sulcus". Longitudinal groove extending down the proximal surface of an otolith. The otolithic membrane is thicker over the sulcus acusticus, which lies against the *macula acusticus,* or sensory epithelium, of the sacculus. Motion of the otolith is picked up by the hair cells of the *macula acusticus* and directional and auditory information is relayed to the brain (Popper and Lu 2000).

Translucent zone A zone that allows the passage of light. This zone is composed primarily of organic material (a protein called *otolin*) with a reduced amount of inorganic material in the form of short, thin aragonite needles. With transmitted light, translucent zones appear bright; with reflected light, they appear dark. Synonyms: winter zone, winter ring, dark ring, annulus, hyaline zone

Validation A process by which an aging method is validated, i.e., annual periodicity of annuli. Some commonly used age validation techniques are:

- Mark-recapture experiments using bone-staining chemicals (e.g. alizarin crimson, oxytetracycline)

- *Bomb radiocarbon chronometer*: A validation technique for long-lived fish utilizing the high levels of radiocarbon in seawater from atmospheric testing of nuclear bombs in the 1950s and 1960s. The relative amount of radioactive Carbon 14 in the first year of the otolith is measured and compared with known seawater levels over time and used to estimate the birth year.
- *Radiometric aging*: A method for aging long-lived species with difficult otolith patterns. Also used for validation of other aging methods. Uses the relative proportions of radioisotope pairs in the otolith and known decay rates to estimate age.
- Examination of otoliths from known-age fish (i.e., captive reared)
- Marginal Increment Analysis (MIA) An indirect method of validation. Determines the time of year of annulus formation by comparing edge growth amount and type from otoliths sampled throughout the year.
- Following dominant year classes through time (another indirect method)

Appendix II

Additional resources

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Otolith Research Laboratory
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The Committee of Age Reading Experts (CARE): Pacific Coast Groundfish Ageing Technicians. (online manual on generalized age determination procedures for groundfish)
http://www.psmfc.org/care/

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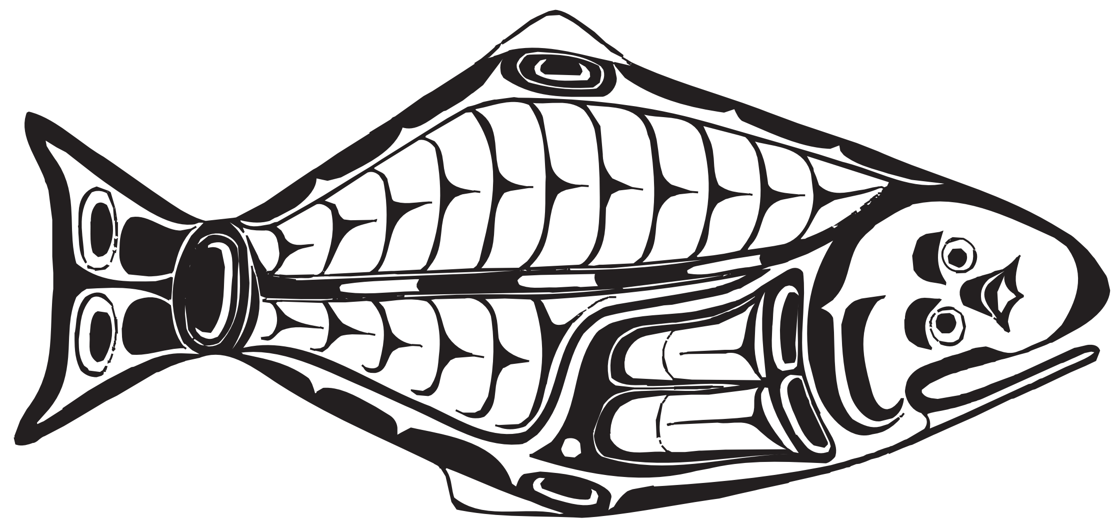
Appendix III

Instructions for preparing glycerin solution.

Ingredients:
½ gallon water
½ gallon pure glycerin
5.5 g thymol
20 ml ethanol or isopropanol

1. Crush thymol crystals into coarse powder
2. Dissolve thymol in alcohol by stirring/agitating mixture
3. When dissolved, add thymol/alcohol solution to glycerin* and shake to mix (we usually use large plastic jugs or carboys for mixing and storing the glycerin solution)
4. Add water to the glycerin mixture and shake to mix.

*Do not add the thymol solution to the water first: the thymol will precipitate out of solution.



HALIBUT CREST - adapted from designs used by Tlingit, Tsimshian and Haida Indians