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**Aging manual for Pacific halibut:
procedures and methods used at the
International Pacific Halibut
Commission**

by

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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

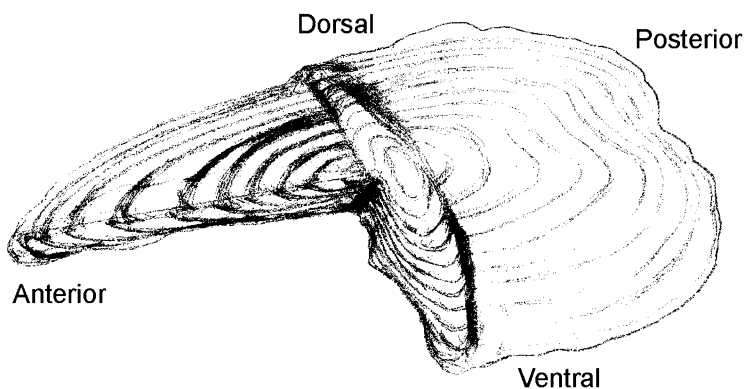


Figure 1. Cutaway three-dimensional illustration of a halibut otolith showing deposition of concentric lamellae.

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

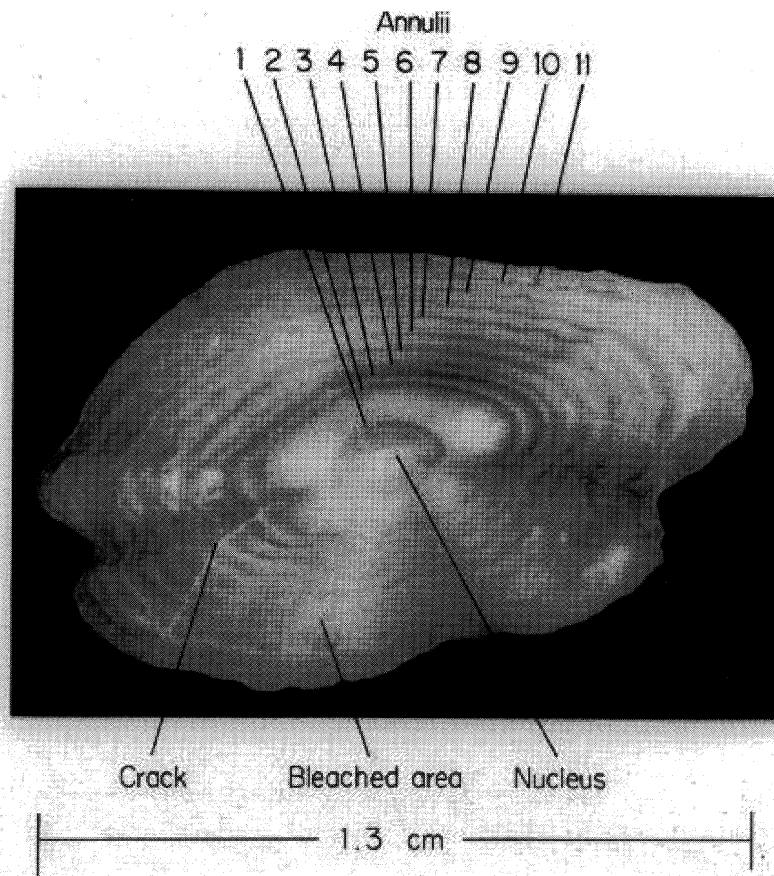


Figure 2. A left (blind) side halibut otolith with annuli marked.

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.

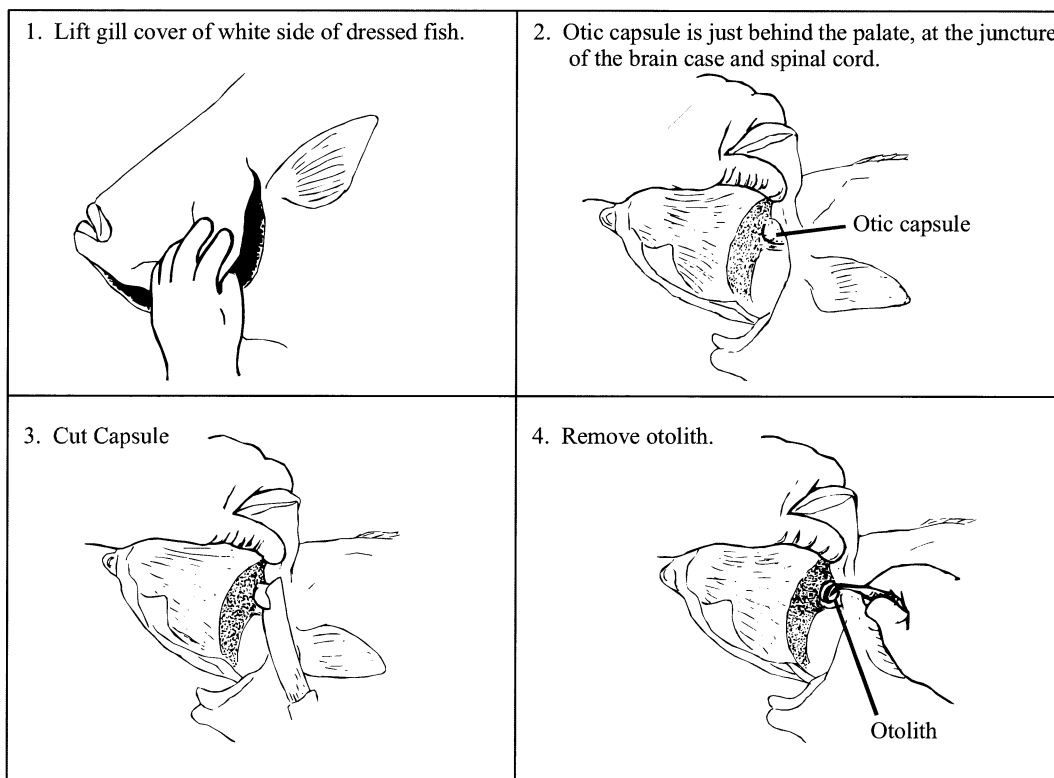


Figure 3. Extraction of left side otolith from dressed halibut.

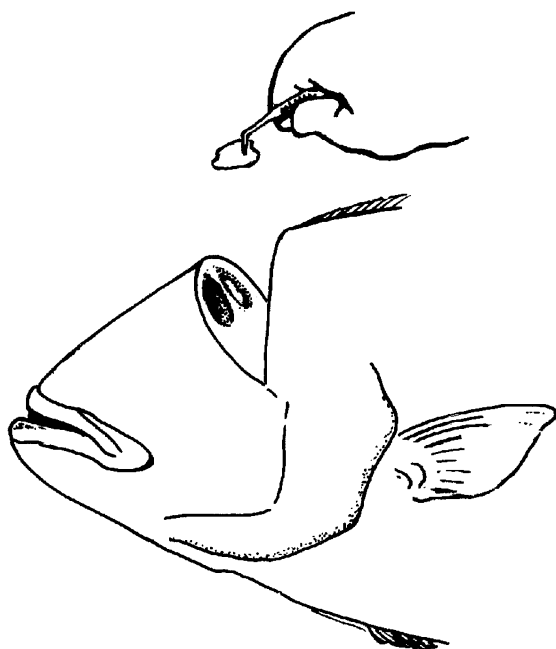


Figure 4. Extraction of left otolith from undressed halibut showing position of head cut.

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

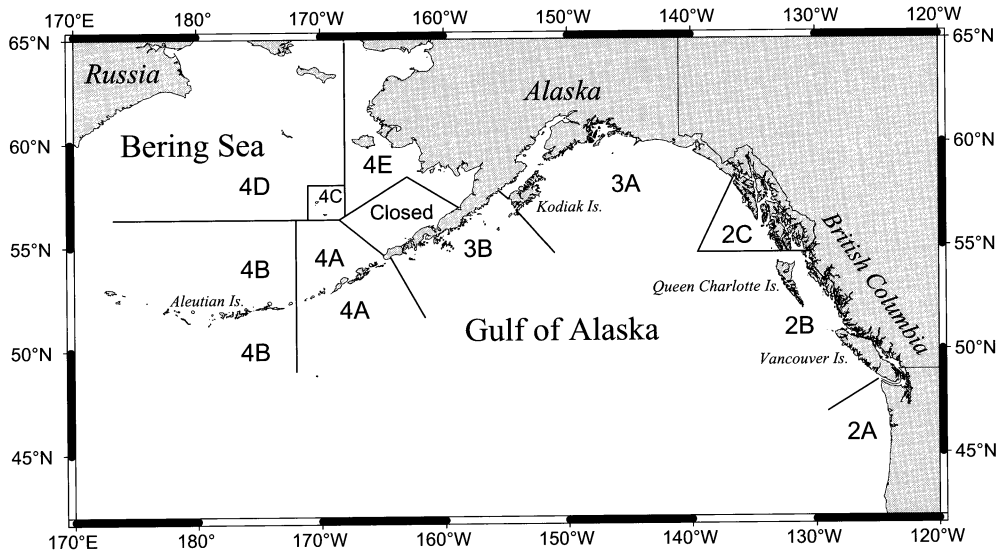


Figure 5. IPHC regulatory areas.

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.

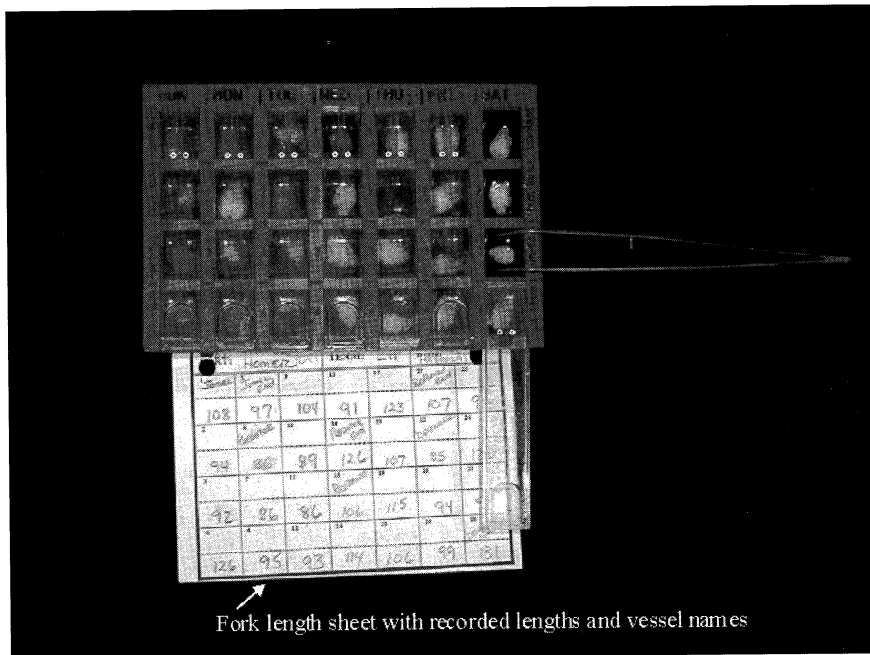


Figure 6. Medication organizer used to collect market sample otoliths.

