Radiometric Ageing — New Age Validation Technique Established in Resource Ecology and Fisheries Management Division

Information about the age structure of a fish stock is important for accurate stock assessment. Age data are used to estimate fish growth and mortality rates and to generate age-structured stock assessment models. Because inaccurate age data can lead to the over- or underharvesting of an exploited fish stock, accurate age data are critical to determining appropriate harvest and management strategies. To examine and improve the accuracy of age data, a new facility for the radiometric ageing of marine organisms has been established as part of the Age and Growth Task of the Resource Ecology and Fisheries Management (REFM) Division at the Alaska Fisheries Science Center (AFSC).

The conventional method of determining the ages of North Pacific groundfish is to analyze the yearly growth rings (annuli) in the fishes' otoliths (ear bones). The otoliths, composed of calcium carbonate (aragonite crystals) in a protein matrix, contain growth rings which appear as concentric circles laid down in a fashion similar to that seen in tree rings. In fishes from the North Pacific Ocean, the otoliths reflect the seasonality of growth, showing one period of fast growth and one period of slow growth each year. Thus, an otolith provides a record in an annular pattern of a fish's growth over time.

The REFM Division's Age and Growth Task predominantly uses otoliths to estimate the ages of North Pacific groundfish. However, different hard parts, such as scales, fin spines, vertebrae, or other bones also can be used for reading the ages of certain fish species. The Age and Growth Task reads 20,000 to 30,000 otoliths annually. Each otolith is viewed individually under a dissecting microscope, and the growth rings are counted (Fig. 1).

The patterns of concentric rings formed in the otoliths are often difficult to decipher. The seasonality of the growth patterns can be masked or disturbed by environmental or behavioral factors affecting the fish. Only in the best otoliths is there a clear pattern providing an easy ring count. Otoliths are three-dimensional structures, not always growing equally in all dimensions. Therefore, a number of interpretative options often exist when counting the growth rings, increasing in proportion to the age of the fish (Figs. 2 and 3).

Additional complexities occur due to a choice of sample preparation methods. Growth rings may be counted microscopically from the exterior of the otoliths using specialized lighting equipment. Rings also may be counted from the interior of the otolith using several methods of cutting or cross-sectioning the otoliths. Usually, a consistent method is used for each species. Depending on the method of sample preparation, all of the rings may not be visible in the otoliths, especially if the fish are old (Fig. 3). Therefore, the science of counting otolith rings is not exact but relies heavily on the interpretation made by individual otolith readers. For example, what one otolith reader may interpret as 10 supposed annuli, another reader may interpret as 30.

To ensure the best possible age data available, a conventional age estimated by interpretation of otolith growth rings must be confirmed for accuracy. Hence, the goal of age validation research is to

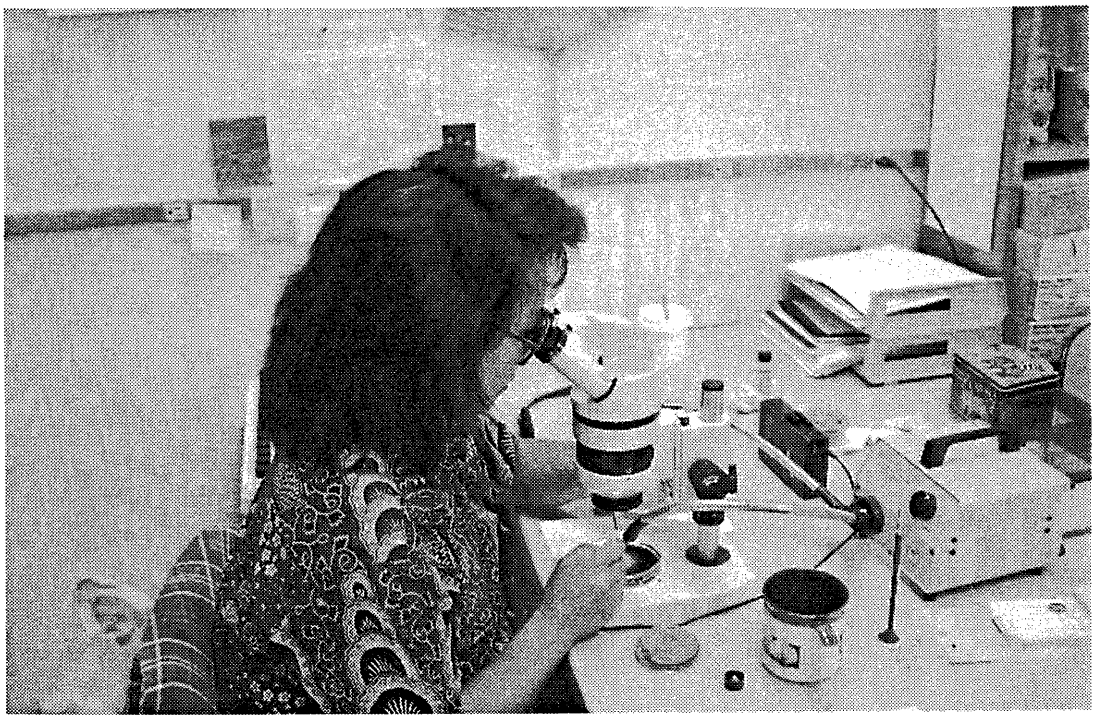

Figure 1. Biologist Delsa Anderl employs the conventional method of ageing fish by using a dissecting microscope with a special light source to read otolith growth rings.

confirm that the conventional methods used are generating accurate ages. This usually means finding another, independent (nonconventional) method of determining a fish's age. When the estimated age from an annular count agrees with the estimated age derived from an independent method, a degree of accuracy can be attributed to the annular count.

Fisheries science historically has used a number of age validation methods, often requiring a specialized collection of otoliths. Mark and recapture projects, which use external tags with an injection of a bone-marking chemical such as oxytetracycline, require large tagging efforts followed by even larger recovery efforts years later. In some species, those with a swim bladder for example, the survival rate is very poor after tagging. On rare occasions, a natural mark in the otolith from a known source such as El Niño can be used for age validation. However, such natural marks are not commonly seen in the large volumes of samples routinely collected by the AFSC. Otolith marginal incremental analysis can be used when samples can be collected frequently (every month) throughout a year. This method determines the time or season of otolith growth by studying periodic samples and looking for new deposition. Unfortunately, the cost of ship time prohibits this type of collection. The best validation is from reading otoliths of "known-age fish," but collections of known-age fish from their natural environment are extremely rare.

A new method of fish age validation and the focus of research at the Age and Growth Task's new radiometric facility uses the radionuclides Ra-226 and Pb-210, which are naturally occurring in seawater. These radionuclides can be measured in any sample routinely collected by the AFSC. This method is analogous to radiometric dating methods employed in geology and anthropology which use long-lived radionuclides. The major difference is that the half-lives of Ra-226 and Pb-210 are relatively short (Fig. 4), thereby allowing the dating of marine organisms 10-100 years old.

The radionuclides Ra-226 and Pb-210 are part of the natural decay chain of U-238 (Fig. 4). Each step in the chain has a specific half-life associated with it. Radium-226 is found in all seawater and thus is available to fish via food or by osmoregulation. In a fish's metabolism, the Ra-226 is a calcium

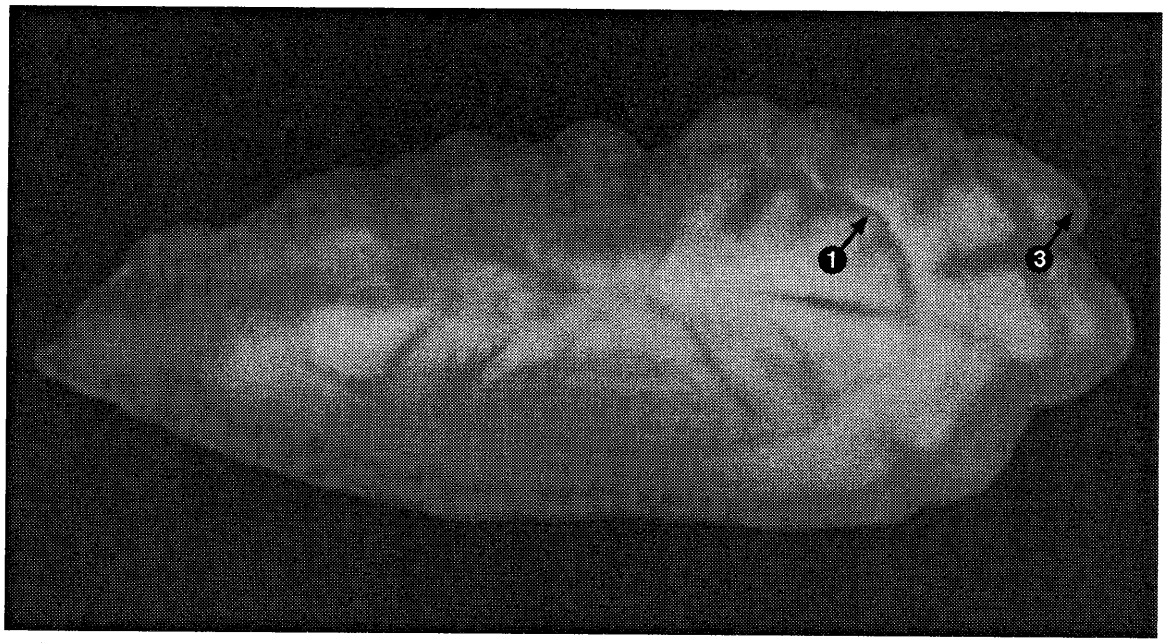

Figure 2. A three-year-old sablefish otolith. Arrows indicate the first and third yearly growth rings.

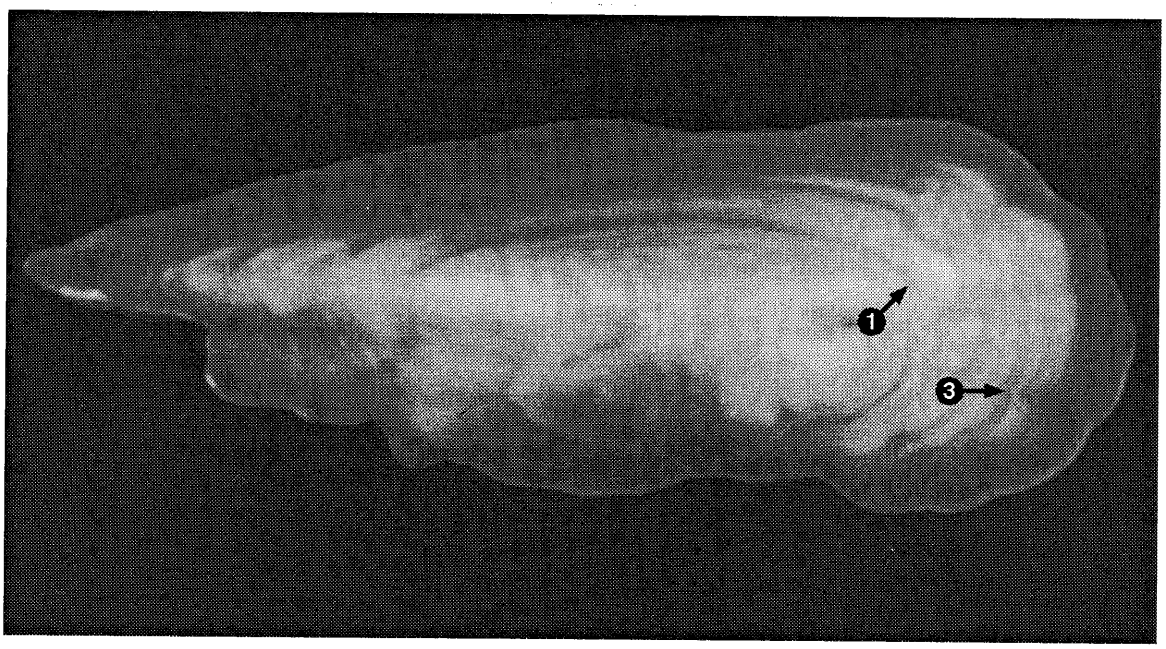

Figure 3. A twenty-year-old sablefish otolith. Arrows indicate the first and third yearly growth rings. Note the lack of clarity outside the third growth ring.

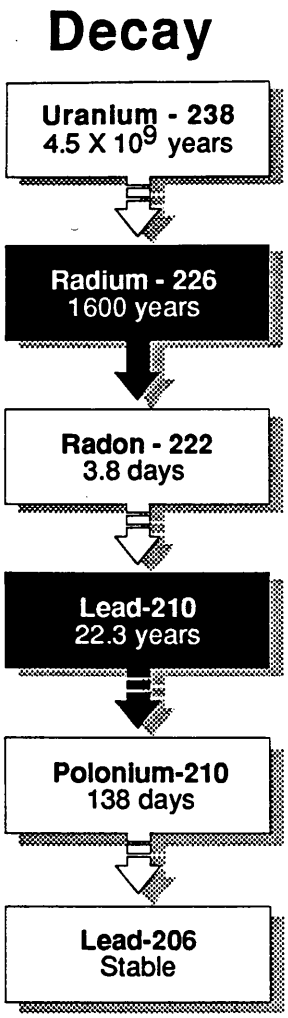

Figure 4. Decay chain of U-238. Dashed arrows indicate short-lived intermediary nuclides that are not shown. Half-lives are shown for the critical nuclides in the decay process.

analog, so it is incorporated during growth into the otolith where it decays, forming the remainder of the chain (i.e., the radionuclides further down the chain). In the crystalline structure of the otolith, the amount of Pb-210 increases with time. Therefore, the amount of Pb-210 relative to Ra-226 is a function of time and can be used to estimate the age of the fish. This radiometric age is compared to the conventional age determined from reading the otolith growth rings. If the two types of ages agree, then the conventional age is considered validated.

Determining a radiometric age is not without its own set of challenges. The level of radionuclides in otoliths is very low. This means that any measurement of Ra-226 or Pb-210 will have an error associated with it. Therefore, radiometric analysis is best suited to answer the question of whether a fish is 10 or 30 years old, not 10 or 11 years old. Also, radiometric ageing has three major assumptions that must be considered individually for each species as part of a validation exercise.

1)The otoliths must be a closed system with respect to the loss or gain of any radioisotopes in the decay chain (other than initial Ra-226 incorporation and natural decay processes).

2)The initial activity ratio of Pb-210/Ra-226 in the otoliths should be much smaller than one, ideally close to zero, and known or measured.

3)The specific activity (disintegrations per minute per gram (dpm/g)) of the radioisotopes in the material incorporated into the otoliths must be constant.

The procedures used in radiometric age validation are described in Figure 5. First, the otolith annuli are read (as described earlier) to provide a conventional age. Because a gram of otolith material is needed for a radionuclide measurement, a number of otoliths (typically 10 to 100) from the same collection and with similar ages are combined for each analysis. Most commonly, only the center of the otoliths are used. The outer perimeter is newly deposited material and has no relation to the radionuclides in the center of the otolith. Therefore, the centers of the otoliths are extracted mechanically by grinding and polishing off the exteriors of the bones. This is a tedious procedure in which outer material is removed down to a specified and consistent ring in each otolith, leaving only material from the first year or two. In this step, the otoliths also are ultrasonically cleaned and acid rinsed to remove any contamination. The sample material is then processed chemically to extract specific radionuclides for measuring later. Polonium-210, a product of Pb-210, is isolated from the sample and is measured by actually count-

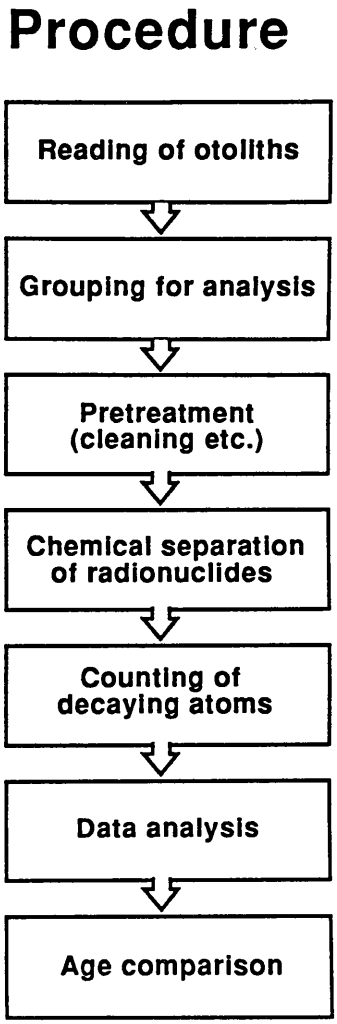

Figure 5. Outline of procedures used to analyze samples in the radiometric laboratory.

Figure 6. The author pictured with an Alpha spectrometer system and its supporting computer.

ing the number of decaying atoms over time, up to 3 weeks in some cases. The counting utilizes an alpha spectrometer (Fig. 6). Radon-222, a product of Ra-226, is collected from the samples and is measured in a gas scintillation counter. The Pb-210 or Ra-226 is not actually measured, but their abundance is deduced through measuring their products further down the decay chain. Any background or other contamination must be carefully measured and dealt with in consideration of the very low level of radionuclides present in the otoliths. The utmost care must be observed in the laboratory to reduce contamination. All glassware must be kept clean and free of contamination, and the electronic measuring equipment must be monitored for excessive background levels. To complete the process, a final data analysis provides an estimated radiometric age.

Radiometric analysis is a relatively new method of age validation in fisheries science. Only four other laboratories worldwide pursue this type of research. The long monitoring times and the problems associated with contamination make radiometric age validation difficult. The long monitoring times preclude using the equipment for any other measurements, which can be a problem in a laboratory geared to process many samples. However, because of the difficulties associated with traditional age validation methods and the need for a reliable alternative validation technique, the Task decided to establish a dedicated radiometric laboratory in house.

Past radiometric research conducted by the Age and Growth Task successfully validated that the methods and interpretative options used to age sablefish (*Anoplopoma fimbria*) were producing accurate ages. Task members divided sablefish otoliths from 481 samples into four age groups and validated ages up to 34 years, which is the maximum age regularly seen in commercial or recreational catches of sablefish. The Task currently is conducting radiometric age validation for six rockfish species: Pacific ocean perch (*Sebastes alutus*), shortraker (*S. borealis*), rougheye rockfish (*S. aleutianus*), dusky rockfish (*S. ciliatus*), northern rockfish (*S. polyspinis*), and shortspine thornyhead (*Sebastolobus alascanus*). Rockfish from the North Pacific Ocean typically can live up to 80 or 100 years, but conventional ageing methodologies need to be validated for each species. Twelve rockfish samples aged conventionally from 3 to 60 years old currently are being analyzed. The Age and Growth Task hopes to collaborate with other agencies and expand research to other species in the future.

By CRAIG KASTELLE of the Resource Ecology and Fisheries Management Division.

AUKE BAY LABORATORY (ABL)

Marine Resource Assessment Activities

Preliminary Stock Assessment and Fisheries Evaluation (SAFE) Reports for the Gulf of Alaska were completed for sablefish (*Anoplopoma fimbria*) and for slope and pelagic shelf rockfish. The reports were submitted to the Gulf of Alaska Groundfish Plan Team and will eventually be sent to the North Pacific Fisheries Management Council (NPFMC) to help determine values of acceptable biological catch (ABC) in the 1995 commercial fisheries.

By DAVID CLAUSEN.

Range of Atlantic Salmon in Alaska Expands

When the Auke Bay Laboratory (ABL) reported an Atlantic salmon (*Salmo salar*) taken in Alaska in 1990, we predicted that the species would eventually have a range similar to that of chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*O. mykiss*). From 1990 through 1994, the Atlantic salmon found in Alaska have been collected by Alaska Department of Fish and Game (ADF&G) port samplers. Most of the Atlantic salmon have been taken in the southern districts of Southeast Alaska, with a few unconfirmed reports in northern Southeast Alaska and near Kodiak Island.

This quarter, we confirmed the presence of Atlantic salmon in the Shumagin Islands in the western Gulf of Alaska. A 76-cm Atlantic salmon was caught in a set net on the western side of Nagi Island in the Shumagins. Scales from the fish were much like that of coho salmon, showing 2 years of freshwater or pen growth and rapid marine growth this past season.

By BRUCE WING.

Atlantic Salmon Reported in Alaskan Salmon Fisheries

Year	1990	1991	1992	1993	1994
Number	1	5	1	24	20

United States/Canada Program Highlights

ABL scientists sampled chum salmon (*O. keta*) at Fish Creek, Alaska, in Portland Canal on 12-17 September, with assistance from staff from the ADF&G Ketchikan office. Salmon River chum salmon spawning areas and escapements were also surveyed with ADF&G staff. (Fish Creek is a tributary of the Salmon River.) Canadian and U.S. chum salmon populations remain a conservation concern under the Pacific Salmon Treaty.

Stock baseline sampling and stream colonization studies were carried out in Glacier Bay, Alaska, from 29 August to 8 September aboard the NOAA ship *John N. Cobb*.

The Taku Point field camp was turned over to the U.S. Forest Service, ending more than a decade of salmon research on the Taku River, a transboundary United States/Canada river.

ABL staff sampled sockeye salmon (*O. nerka*) and pink salmon (*O. gorbuscha*) spawning escapements in the Kenai Fjords National Park 11-15 September for stream colonization studies and stock identification baseline data. Assistance was provided by the National Park Service and the ADF&G.

By JAMES OLSEN.

Exxon Valdez Field Studies End For Season

Field activities of the *Exxon Valdez* studies ended in late August with resampling of restored mussel bed sites. Samples will be analyzed during the remainder of the fall. Rearing of pink salmon formerly exposed (April 1994) in oiled gravels will continue in net pens at Little Port Walter until spawning next summer.

By MALIN BABCOCK.

Derelict Trawl Web Studies Begin

In early September, the Entanglement Task completed the first phase of a study on Kayak Island in Southeast Prince William Sound to determine the fate and redistribution of derelict trawl web once stranded ashore. ABL scientists attached radio tags to 35 pieces of trawl web ranging in size from 200 g to over 20 kg. In addition to tagging the trawl webs, staff recorded the net characteristics (i.e., mesh size) and global positioning of each piece of trawl web encountered. Two remote tracking stations (RTS) have been established on Kayak Is-