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I. Age validation of Pacific halibut

II. Comparison of surface and break-and-burn otolith methods of ageing Pacific halibut

by
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Contents

Abstract

A mark-recapture study was initiated to validate halibut otolith growth rings as being annular in nature. In 1982 and 1983, the International Pacific Halibut Commission tagged and injected 1,791 Pacific halibut with oxytetracycline (OTC) off the coasts of Alaska and British Columbia and recoveries through 1989 have been analyzed. Both surface and break and burn methods of age reading were used to test criteria for age validation.

Regression analysis of estimated versus known time at large read by three age readers suggests slight over ageing using the surface technique. The most experienced age reader tested the break and burn technique and underestimated the time-at-large. Deviation from the known age was usually ±1 year using both techniques with a tendency toward a +1 year error in surface ageing and a -1 year error using break and burn ageing. Error was believed to be confined to edge interpretation, but may be related to an injection check, particularly in halibut injected in May. Ageing error did not increase with time-at-large. While some evidence exists for validation of the surface technique, there are too numerous discrepancies present which prevent us from declaring so. Further examination of the criteria used to describe annuli also is necessary to eliminate inconsistency between readers.

I. Age validation of Pacific halibut

Calvin L. Blood

Introduction

The Pacific halibut *(Hippoglossus stenolepis)* is an important commercial and sport fish species, ranging from the central California coast to the Sea of Japan. Throughout the waters of the United States and Canada the Pacific halibut is managed by the International Pacific Halibut Commission (IPHC or Commission). Accurate ageing of halibut is critical to evaluating population abundance, age structure, recruitment, growth, and mortality (Quinn et al. 1983). Age validation is necessary to ensure this accuracy, although validation has often been ignored by fishery scientists (Beamish and McFarlane 1983).

Scales and otoliths have been used since the early 1900s to age halibut. The earliest age determinations of halibut were made by interpreting the circuli on scales (McMurrich 1913). He concluded that halibut scales were subject to resorption and regenerative problems. The collection of scales from large numbers of fish to acquire a few perfect scales was impractical. Thompson (1915), influenced largely by the successful ageing of plaice (*Pleuronectes platessa*) otoliths by English, Scottish, and German investigators, used otoliths to determine the age of halibut and found them more suitable than scales.

Since the 1960s, tetracyclines have been found to be a useful tool as a time marker for validating annuli in bony structures of teleosts (Kobayashi et al. 1964; Casselman 1974). The mark provides a method of validating the interpretation of the annulus. Renewed interest in age validation has resulted in the use of oxytetracycline (OTC) as a time-marker for many fish species, including sablefish (*Anoplopoma fimbria*, Beamish et al. 1983), Leopard shark (*Triakis semifasciata*, Smith 1984), rock sole (*Lepidopsetta bilineata*, Fargo and Chilton 1987), yellowtail rockfish (*Sebastes flavidus*, Leaman and Nagtegaal 1987), and snapper (*Pagrus auratus*, Francis et al. 1992).

A comprehensive evaluation of the ageing of halibut otoliths has never been undertaken (Quinn et al. 1983). The earliest work on the routine surface ageing of halibut by the Commission was established by H. A. Dunlop in the 1930s (Quinn et al. 1983). Dunlop (unpub.)[1] observed that average age determined from otoliths by the surface ageing method was higher than that based on scales, and that this difference increased with age. He concluded that otoliths were more suitable, noting that the Petersen length-frequency method and the seasonal formation of the annuli were more consistent with otolith ages from 0-2 years. The only other supporting evidence for the validity of halibut age determination is a growth curve calculated from IPHC age readings which was not significantly different from an independent growth function calculated from tagged fish returns (McCaughran 1981). To validate the surface and burnt section technique of otolith ageing for Pacific halibut, IPHC initiated a mark-recapture study in 1982.

Materials and Methods

In 1982 and 1983, 1,791 halibut were tagged and injected with the antibiotic Medamycin® 100 (oxytetracycline hydrochloride) off the Queen Charlotte Islands, Gulf

[1] Dunlop, H. A. Unpublished report. Age Studies on the Pacific halibut *(Hippoglossus stenolepis* Schmitt). Int. Pac. Halibut Comm. P.O. Box 95009, Seattle, WA 98145.

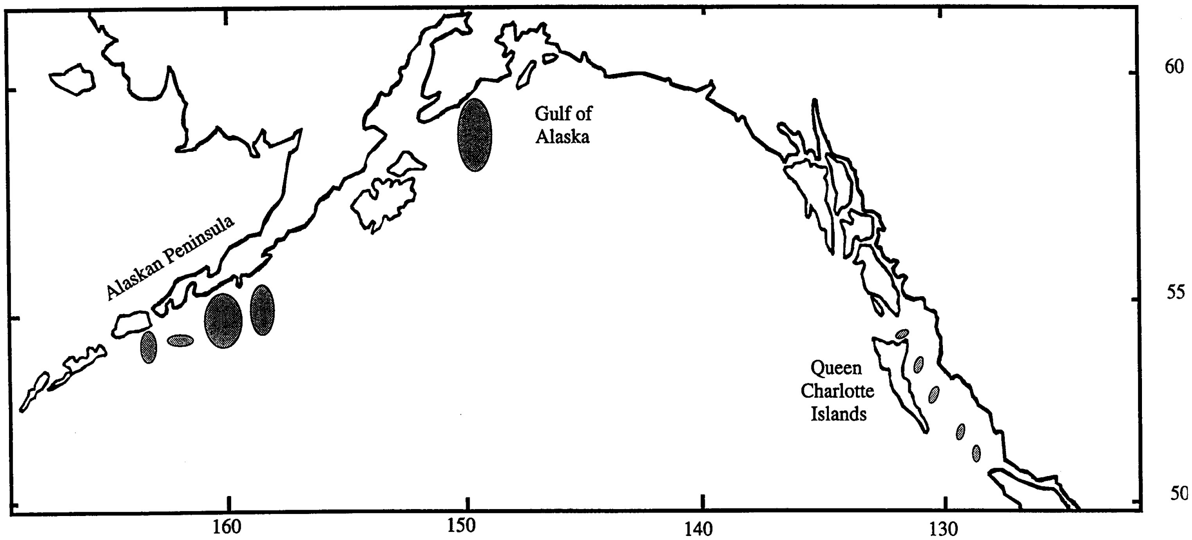

Figure 1. Sampling locations for 1982 and 1983 releases in the Queen Charlotte Islands, Gulf of Alaska, and Alaskan Peninsula.

of Alaska, and the Alaskan Peninsula (Figure 1). A control group of 1,386 halibut was also tagged but not injected. Each group was otherwise handled in a like manner. The areas were chosen because of the convenience of chartered commercial vessels participating in stock assessment surveys and/or identified as areas in which previous experiments had a high rate of tag recoveries. The 1982 releases were intended as a pilot project and equipment test. Priority was given to tagging and injecting with OTC in an effort to maximize returns and evaluate whether the OTC dosage was strong enough to produce an adequate mark. In the Queen Charlotte Islands, only young halibut, 40 to 75 centimeters, were tagged and injected. In the Alaskan Peninsula releases, halibut greater than 40 centimeters were tagged and injected. In this area, two-thirds of the tagged halibut were injected, and the other third was released as a control group. In the 1983 releases in both the Queen Charlotte Islands and the Gulf of Alaska, halibut greater than 40 centimeters were tagged and injected. The injected and control groups were roughly equal in size. Both longline and trawl gear were used to capture the halibut. Fish considered to be in good condition were tagged, injected, and released. Releases and recoveries are summarized by area in Table 1.

Although no laboratory studies were performed on Pacific halibut, a dosage of 50 mg kg^{-1} of body weight was recommended (McFarlane and Beamish 1987) as sufficient to leave a readable mark on the otolith without causing undue mortality, and was used for both years of release. Halibut were injected intraperitoneally with OTC through a continuous pipetting 10 cc syringe fitted with an 18 gauge regular bevel hypodermic needle (Figure 2). An intraperitoneal injection was chosen rather than an intramusculature one to reduce the possibility of discoloration of the flesh. Discoloration of the flesh may result in unnecessary trimming wastage at the injection site in a commercially-caught fish (Rossof, 1975). The control group received no injection of any type. Halibut were tagged through the dark (eyed)-side preoperculum with a numbered, plastic tag with a wire insert (Trumble et al. 1990). An unknown number of the 1982 releases from the Alaskan Peninsula were inadvertently tagged at the end of the operculum.

An intensive dockside sampling program accounted for most of the recovered tags and otoliths. Tags and otoliths were temporarily stored in manila envelopes in the field. Upon return to the laboratory, otoliths were placed in trays and covered with a 50 percent

Table 1. Pacific halibut age validation study tag recoveries (recoveries with otoliths in parentheses) and rates of recoveries.

OTC GROUP				
Release Year	Area	Number Tagged	Total Recoveries	Recovery Rates
1982	Queen Charlotte Islands	111	16(8)	14.4%
	Alaskan Peninsula	459	20(9)	4.4%
1983	Queen Charlotte Islands	765	150(93)	19.6%
	Gulf of Alaska	456	70(29)	15.4%
Totals		1791	256(139)	14.3%

CONTROL GROUP				
Release Year	Area	Number Tagged	Total Recoveries	Recovery Rates
1982	Queen Charlotte Islands	-	-	-
	Alaskan Peninsula	287	18(7)	6.3%
1983	Queen Charlotte Islands	627	111(57)	17.7%
	Gulf of Alaska	472	93(42)	19.7%
Totals		1386	222(106)	16.0%

solution of glycerin and water and stored in darkness, as exposure to light diminishes the ability of the OTC deposited in the bone to fluoresce (Chilton and Beamish 1982). By 1989 tag returns had dwindled to less than five per year with the return of more fish unlikely, and the experiment was considered completed.

Surface Method

Halibut were aged by counting growth zones from surface readings. Only the left sagittal otolith was used to estimate the age of the fish. Otoliths were immersed in water on a black background, illuminated by reflected light, and observed through a dissecting microscope. An annual growth zone is the growth which forms during one year and consists of an opaque (summer growth) and a translucent (winter growth) zone. Opaque and translucent zones were easy to distinguish. Opaque zones are generally wider than translucent zones in young halibut and become closer in size as the halibut ages. Commonly, the translucent zone is referred to as the annulus and forms during a period of slow or no growth, often in the winter. The general criterion for distinguishing false annuli, or checks, from true annuli is whether the translucent growth zone can be seen continuously around the otolith surface. Checks denote a slowing of growth within the opaque zone and may reflect various environmental or physiological changes. Latitude is provided the reader to interpret the authenticity of the ring as an annulus in instances where areas of opaqueness obscure the translucent zones.

Edge interpretation is most difficult in late spring, particularly in young halibut. The edge is counted if the width of the outside ring is greater than half the width of the previous growth ring when the estimated age is 10 years or younger (Forsberg 2001). Otherwise the edge growth is considered the current year's growth. The edge is counted on halibut 11 years and older because the annuli are narrower in width and new growth generally does not appear until mid to late summer. Halibut otoliths vary little in shape and annuli can be identified from the nucleus out through several axes.

Otoliths recovered from OTC-injected fish were examined by three age readers. Age readers were provided the month of recovery, and were aware the time-at-large (TAL) period could range from zero to seven years. The TAL was calculated from the date of release to the date of capture. Otoliths were placed under an ultraviolet light source (long wave, 366 nm) for detection of the OTC mark. Otoliths were eliminated if the left otolith was not collected, or the left otolith was so broken it could not be reconstructed. Each age reader read all OTC otoliths, at one week intervals, for a total of two readings and recorded the estimated age and the number of annuli laid down distal to the OTC mark. The second readings were done without knowledge of the previous readings. All three readers had to identify an OTC mark for both readings or that otolith was eliminated from the sample. All three readers were experienced in otolith surface age determinations, with two readers having three years experience each and the third, eight years. The most experienced reader resolved age discrepancies and assigned a final age. No attempt was made to re-read the otoliths after assigning the resolved age. Each control otolith was read once by two independent readers and where ages did not agree, the most experienced age reader resolved the differences.

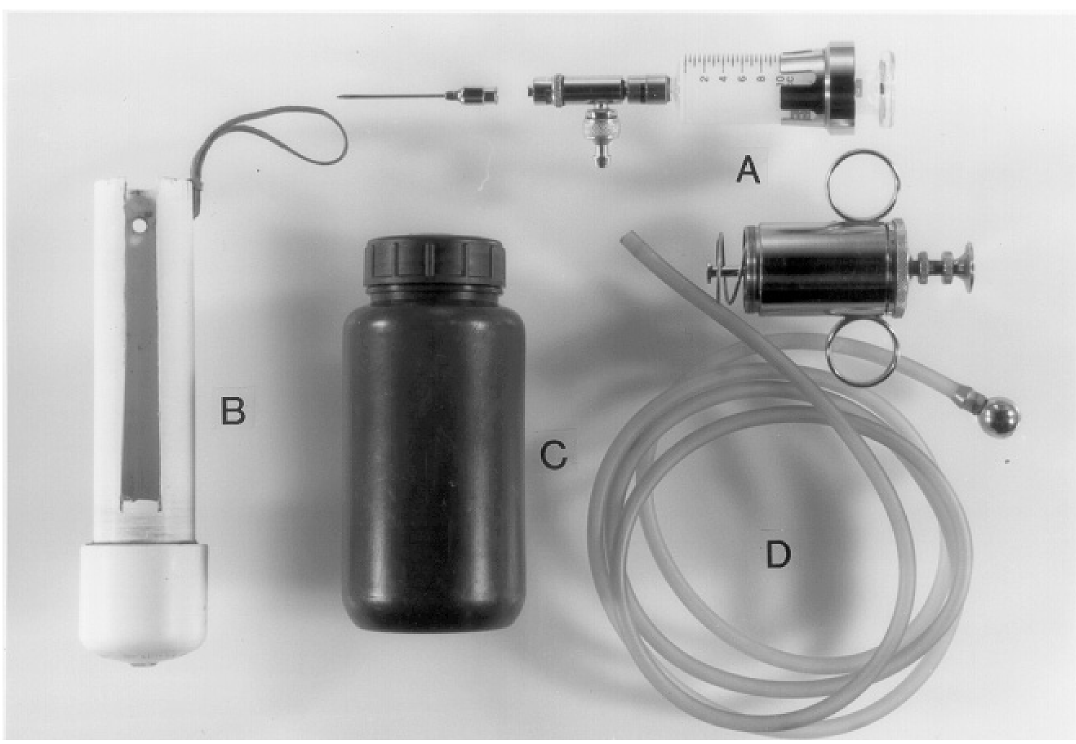

Figure 2. Equipment used in injecting halibut: A. B-D Cornwall 10cc continuous pipetting Syringe outfitted with 18 gauge hypodermic needle. B. PVC holster for field storage of syringe. C. 250ml Nalgene Bottle. D. Quarter-inch surgical tubing with weighted suction ball.

Break and Burn Method

An alternative method of ageing fish is the break and burn method. Otoliths are broken dorso-ventrally through the nucleus and the exposed surface is lightly charred with an alcohol flame (Chilton and Beamish 1982). A thin coating of cooking oil is then applied to enhance the growth- ring patterns. The otolith is placed in plasticene and the exposed surface read under reflected light, through a dissecting microscope.

Only the most experienced age reader estimated ages for the broken and burnt otoliths. Break and burn age reading was not commonly used at IPHC and not all of the age readers were experienced in this method. The criteria used to estimate surface ages were also applied to the burnt sections. An electronic pointer and internal micrometer were used to mark the relationship of the OTC mark on the broken, unburned cross section of the otolith and the position of the otolith in the plasticene filled dish. The otolith was then removed from the plasticene, burnt, and placed back in position in the plasticene for ageing.

Test for Validation

The data from each reader's two surface age readings were pooled to account for within-reader variability and a single reading was analyzed from the break and burn method. We tested for a statistically significant deviation from a 1:1 relationship between the TAL estimated from age readings and the known time at large (known from release and recovery dates). This was accomplished by comparing the mean squared residual from the fitted model ($Y = \alpha + \beta X$) with the mean square from the hypothesized (1:1) model ($Y = 0 + 1X$), where X is the known TAL and Y is the TAL estimated from age readings. An F statistic was calculated from the reduction in the residual sum of squares due to fitting the two-parameter regression:

$$F = \frac{(\text{Sum of squares around 1:1 model} - \text{Sum of squares around fitted model}) / 2}{(\text{Sum of squares around fitted model}) / (n - 2)}$$

where n is the total number of observations. The null hypothesis was that the growth zones formed annually and the 1:1 relationship was correct; a significant F value would indicate the hypothesis was incorrect. Both +/-0 year agreement and +/- 1 year agreement were used to compare differences between the readers and/or ageing methods.

Results

Tag Recoveries

A total of 478 tags from control and injected groups were recovered between 1982-1989. Out of a total of 256 OTC injected recoveries, 139 were obtained with at least one otolith. Recoveries from the control group yielded 222 returns, 106 from which otoliths were obtained. Nearly 99 percent of the fish were recovered near their area of release, but a few had undergone lengthy migrations.

The rates of recovery for 1982 Alaskan Peninsula OTC releases and the 1983 Gulf of Alaska releases were lower than for the respective control groups (Table 1). However, the OTC recovery rate for 1983 releases in the Queen Charlotte Islands was slightly greater than the control group. No significant difference was found between recoveries for the 1982 Alaskan Peninsula (X^2=0.97 with 1 df, P>0.25) and 1983 Queen Charlotte Islands (X^2=0.70 with 1 df, P>0.25) and Gulf of Alaska releases (X^2=2.74 with 1 df, P>0.05). The 1982 Queen Charlotte Islands releases could not be tested due to the lack of a valid control group.

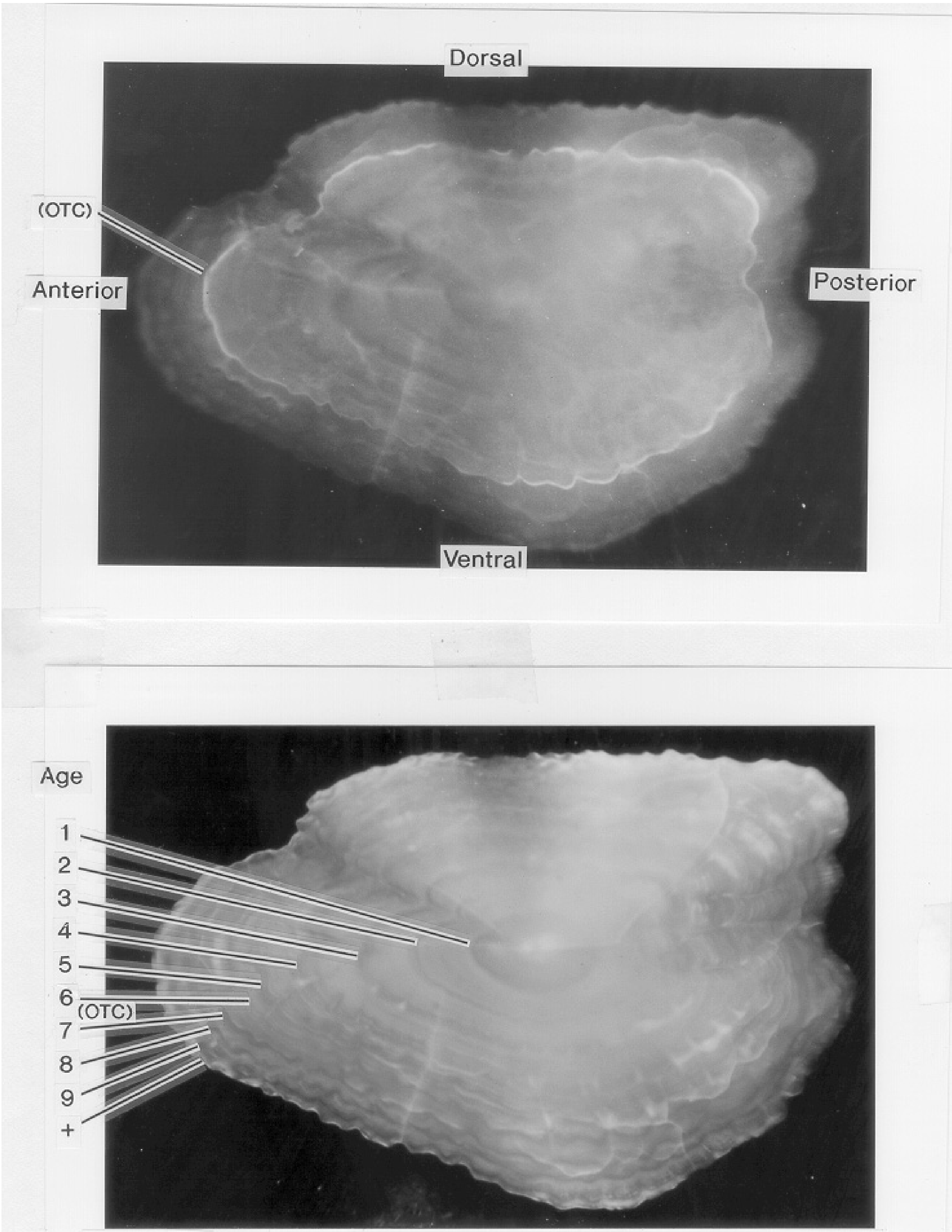

Figure 3. External surface of a 9 year old halibut with current year's growth (+), taken under UV and natural lighting. Tag number 74838 time-at-large was four years.

Age Validation

Surface Ageing

Recoveries of OTC-injected fish confirm the absorption of OTC during formation of new material on the otolith (Figure 3). OTC marks were found in otoliths from injected fish recovered less than a year from tagging. Of the 139 otoliths recovered from OTC-injected halibut, 75 were agreed upon by three age readers as having an identifying OTC mark and used for analysis. Eighteen otoliths were unsuitable for reading due to breakage, crystallization, or lack of a left otolith and the balance of the otoliths were rejected as not having an identifiable OTC mark on each of the two readings.

Estimated ages of recovered OTC-injected fish ranged from 4 to 21 years, with an average age of 11 years (Table 2). Results suggest that the estimated number of annuli during the TAL was slightly higher than known TAL (Table 3 and Figure 4). There was a significant difference in estimated TAL when compared to the known TAL for Readers 1 and 2 ($F_{2,148}$=11.7, P<0.00002 and $F_{2,148}$=10.5, P<0.00005, respectively). No significant difference was found between the estimated and known TAL for Reader 3 ($F_{2,148}$=2.4, P=0.094). As a group, the readers correctly identified the TAL 60% of the time and within ±1 year 94% of the time. The best accuracy occurred at 0,1,2,4 and 5 years-at-large and worst at 3 and 6 years-at-large.

The time-of-release and time-of-recovery were examined to see if they had any effect on the ability of the readers to correctly identify the TAL. Releases and recoveries were divided into two groups: the early group included releases or recoveries in the months from January through June and the late group, July through December (Table 4). We found that age readings from the early releases (late May, early June) recovered from January through June exceeded the actual TAL by one year when compared with the known TAL. A similar difference was apparent in early releases and late recoveries. Late releases (July, September, and November) and early recoveries showed a more evenly distributed error about the known value. Only two otoliths represented late-release, late-recoveries, so no conclusions could be drawn.

Break and Burn Ageing

The break and burn ages were analyzed in a manner similar to the surface ages. The test of the differences between the number of estimated and known from the TAL shows a significant difference ($F_{2,73}$, F=78.8, P<0.001). Break and burn ages underestimated the time at large. A consistent error in underestimating the time at large showed up for early releases and early recoveries. There was less of an underestimating error in early releases and late recoveries. However, underestimating the TAL was pronounced in late releases and early recoveries. As in the surface readings, so few otoliths from late releases and late recoveries precluded any meaningful interpretation.

Discussion

This study differed from most age validation studies on adult fish using OTC as a time-marker in that validation was attempted for ages estimated from both the otolith surface and the cross-section of the otolith (Kobayashi et al. 1964; Holden and Vince 1973; Casselman 1974; Beamish et al. 1983; Smith 1984; Leaman and Nagtegaal 1987). Few studies attempted to validate ages beyond 3 or 4 years TAL, whereas this study examined

Table 2. Year of recovery, tag number, ages and time at large (TAL) estimated from OTC mark for otoliths selected for analysis. Otoliths appear in random order, as they were read.

		READER 1				READER 2				READER 3				BREAK/BURN			
Recovery Year	Tag Number	Age 1	TAL 1	Age 2	TAL 2	Age 1	TAL 1	AGE 2	TAL 2	AGE 1	TAL 1	Age 2	TAL 2	Age 1	TAL 1	Known TAL	Resolved Age
1986	72827	12	4	12	4	12	4	13	4	11	3	11	3	11	3	3	11
1983	73475	9	4	8	0	9	4	8	0	8	0	8	0	9	0	0	8
1986	87519	14	4	15	3	14	3	14	4	16	4	14	3	12	3	3	14
1989	82073	14	6	12	5	13	5	14	5	12	3	13	4	14	5	6	14
1988	74677	14	5	15	5	14	5	14	5	15	5	15	5	15	5	5	15
1984	82155	7	1	7	1	7	0	7	1	6	0	7	1	6	0	1	7
1986	87625	15	4	14	2	15	3	15	3	15	3	15	3	15	3	3	15
1985	87547	16	2	18	3	19	4	19	3	15	2	15	2	20	3	2	17
1989	87006	16	7	15	6	17	7	17	6	16	6	14	5	16	6	6	16
1989	73067	14	6	13	6	13	6	14	4	14	6	14	6	14	4	6	14
1987	87652	16	4	16	4	17	4	17	4	17	4	17	4	17	4	4	17
1986	82178	13	3	13	3	15	3	13	3	14	4	15	4	11	2	3	13
1985	72819	10	3	8	3	10	2	10	3	9	3	10	3	8	2	2	10
1985	79489	13	5	12	4	14	6	12	4	12	3	11	3	20	3	3	12
1983	74725	9	1	8	0	8	0	8	0	7	0	8	0	8	0	0	8
1988	72894	13	5	13	4	14	5	13	4	13	5	13	5	12	3	5	13
1984	73049	10	3	9	2	8	1	7	1	7	1	7	1	8	2	1	7
1987	87132	16	3	13	3	14	5	14	3	13	3	14	4	11	3	4	14
1985	73171	9	3	9	2	9	2	9	2	9	2	9	2	8	1	2	9
1986	87489	21	3	19	3	20	2	20	2	20	2	22	3	21	3	3	21
1984	82162	10	1	10	1	10	0	10	1	10	1	10	1	10	0	1	10
1987	82077	11	5	11	3	12	4	12	5	10	4	11	4	10	4	4	11
1989	74770	14	5	12	5	13	6	14	6	14	6	12	5	13	6	6	14
1986	87515	24	4	20	3	18	2	24	6	21	4	21	4	20	3	3	20
1988	72001	12	6	12	5	13	6	13	6	12	6	12	5	13	6	6	13
1986	87726	12	3	13	4	15	5	13	4	12	3	12	3	11	2	3	12
1986	82126	10	3	14	4	11	2	11	3	9	3	10	2	8	2	3	10
1987	74888	11	4	12	5	11	4	11	4	11	4	11	4	12	4	4	11
1986	73461	8	4	8	4	8	4	8	3	8	3	8	4	8	3	3	8
1985	87455	11	2	11	2	11	2	12	3	12	2	11	2	11	1	2	11
1984	73387	7	1	7	1	8	1	6	1	6	1	6	1	6	0	1	6
1984	82103	6	1	6	1	7	0	7	1	6	0	6	1	6	1	1	6
1988	72385	11	5	12	7	12	6	11	4	11	4	12	6	11	4	6	12

Table 2. continued

1989	87231	19	4	20	7	20	7	19	5	19	6	19	6	16	4	6	19
1986	82067	12	4	11	3	12	4	12	4	11	4	11	3	11	2	3	11
1986	87317	18	3	19	4	18	4	19	4	19	5	16	3	13	4	3	18
1987	82128	12	5	10	3	12	5	12	5	10	4	10	4	10	4	4	11
1984	87704	13	2	12	1	14	3	12	2	13	2	13	2	11	1	1	12
1985	87776	11	2	11	2	11	3	12	3	11	2	11	2	11	2	2	11
1987	73339	8	4	8	4	8	4	8	4	8	4	8	4	8	4	4	8
1989	73224	12	7	15	8	13	7	12	6	11	6	11	6	11	4	6	11
1985	82169	9	2	9	2	10	3	9	2	10	3	9	2	9	2	2	9
1986	87189	12	4	12	3	15	5	15	4	15	4	16	4	12	2	3	14
1985	87812	14	2	14	2	14	2	15	2	14	2	14	2	14	2	2	14
1989	71870	13	6	16	8	13	7	14	7	12	6	12	6	10	4	6	12
1986	87356	13	3	14	3	14	3	14	3	14	3	14	3	12	2	3	14
1987	79496	16	5	15	5	13	5	13	4	13	5	13	5	12	4	5	13
1986	71899	10	3	9	3	9	3	9	3	9	3	9	3	9	3	3	9
1984	74857	6	1	7	2	6	1	6	1	5	0	5	1	6	1	1	6
1989	71914	13	7	14	8	13	7	13	7	13	7	12	6	11	4	6	12
1984	87170	15	1	15	1	15	1	15	1	17	2	15	2	15	1	1	15
1985	71901	7	2	9	3	9	3	9	3	9	3	8	2	8	2	2	8
1986	74901	10	4	9	4	10	4	10	4	9	3	10	4	10	3	3	9
1987	74869	13	4	12	4	12	4	12	4	12	4	11	3	12	4	4	12
1986	71926	10	3	11	3	10	3	9	3	10	3	10	3	11	3	3	10
1984	79470	12	2	12	1	12	2	13	2	12	2	12	2	14	1	2	12
1986	73116	9	4	10	3	9	3	9	4	9	4	10	3	9	3	3	10
1985	73286	8	2	7	2	8	3	8	3	8	2	8	2	9	1	2	8
1987	74838	9	4	9	4	9	4	9	4	9	4	9	4	9	4	4	9
1987	79494	12	5	11	4	12	5	11	4	12	5	12	5	10	3	5	12
1984	74903	6	1	7	2	5	1	5	1	4	1	4	1	4	0	1	4
1985	74859	9	2	9	3	9	2	9	2	8	2	8	2	6	1	2	8
1987	74645	7	3	10	6	10	4	9	3	10	4	10	4	7	2	4	10
1987	73270	10	4	11	5	10	4	11	5	10	4	10	4	9	3	4	10
1984	82109	9	1	8	1	8	1	9	1	8	1	8	1	8	0	1	8
1987	72864	11	5	11	5	12	6	12	5	12	5	11	5	9	3	4	11
1987	73136	10	5	10	5	11	5	11	5	10	5	11	5	8	3	4	10
1985	82118	9	2	10	2	10	3	9	2	8	1	8	1	8	2	2	9
1985	82136	10	2	12	2	12	2	10	2	12	3	9	2	7	0	2	11
1985	71883	9	2	9	2	10	2	9	2	9	2	9	2	8	1	2	9
1984	73282	6	1	6	1	6	1	6	1	6	1	5	1	7	1	1	6
1984	73158	9	1	9	1	9	1	9	1	9	1	8	1	8	0	1	9
1984	87180	14	1	16	2	14	1	14	1	14	1	14	1	12	1	1	14
1984	74773	6	1	8	2	8	2	8	2	7	1	6	1	7	1	1	7
1984	73445	6	2	7	1	8	3	7	1	7	0	7	0	7	0	1	7

Table 3. The model fit to the pooled estimates of time at large estimated from the OTC mark (Y) and the time at large determined from release and recovery dates (x) takes the form, $Y = \alpha + \beta x$.

Reader	α	β	R^2
1	0.465	0.940	0.81
2	0.528	0.926	0.77
3	0.226	0.938	0.86
break/burn	-0.046	0.829	0.79

halibut at large for 6 years. Our findings show the otolith surface method overestimated the TAL and paradoxically, the break and burn method under-estimated the TAL. Beamish et al. (1983) found that when the reader was unaware of the known TAL, a similar over-ageing tendency occurred for sablefish at liberty for 3 years, but deduced the error was not sufficiently large enough to produce a serious overestimate of age. When the TAL was known, Beamish et al. (1983) found that age readers identified the correct TAL. Leaman and Nagtegaal (1987), however, found that the number of marks designated as annuli beyond the OTC mark was consistent with the number of years at liberty for yellowtail rockfish

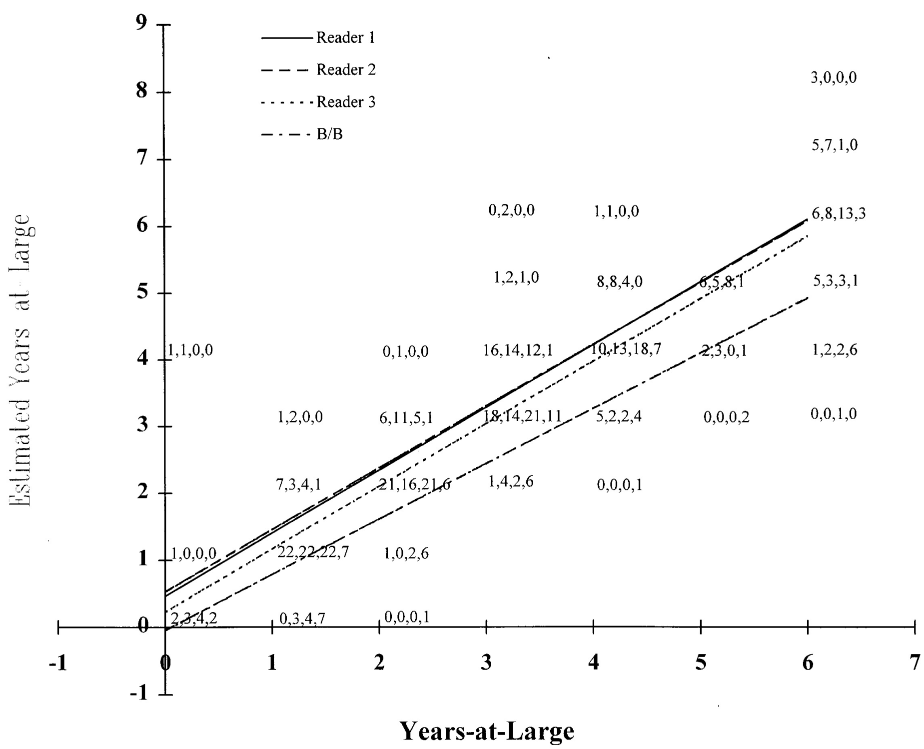

Figure 4. Regressions of estimated annuli vs. actual years-at-large by Readers 1, 2, 3, and Break and Burn (B/B). Numbers separated by commas represent data points for estimated annuli for combined readings.

Table 4. Distribution of time-at-large error for combined surface readings by readers 1,2,3 and break/burn.

Error in Years	Early release Early recovery	Early release Late recovery	Late release Early recovery	Late release Late recovery
+4		1,1,0,0		
+3	0,1,0,0		0,1,0,0	
+2	5,5,1,0	0,1,0,0	1,0,0,0	
+1	26,25,17,2	11,10,3,1	5,7,5,0	1,1,0,0
0	41,40,54,15	20,21,29,15	22,16,20,6	2,3,4,1
-1	5,7,6,12	2,0,2,4	6,9,6,7	1,0,0,2
-2	1,0,0,5	0,1,0,2	0,1,2,3	
-3			0,0,1,0	

and had no knowledge of the TAL (Leaman, pers. comm.)[2]. We chose not to provide readers with any information beyond the month of recapture. A review of the literature is unclear as to how much ancillary information researchers give age readers when assessing TAL. This author suggests that age-readers given too much information will fit the data to provide the "correct" answer. The less an age reader knows about a fish's bio-characteristics, the better age criteria can be evaluated.

Beamish and McFarlane (1987) point out misclassification of age would be a significant error in short-lived species such as Pacific herring *(Clupea harengus pallasi)* or Pacific salmon *(Onchorynchus* spp.*)* and would have major implications for harvest and management strategies. Ageing errors as small as ±1 can smooth differences in year-class strength and make strong year classes look weaker and weak year classes look stronger (Richards et al., 1992). This may also confound attempts to estimate stock recruitment relationships and to relate year-class strength to environmental factors (Heifetz et al., 1999). Pacific halibut is a relatively long-lived species with the oldest male and female each aged at 55 years (Forsberg, 2001). Most of the ageing errors in this study fall within ±1 year of the actual TAL. Although our total agreement was not particularly strong, our agreement to ±1 year suggests our discrepancies are minor and correctable.

The greatest source of ageing error in our study was the inability of the readers to consistently apply the criteria for defining an annulus. A major shortcoming of this study was the lack of systemized documentation of the edge type around the otolith. We cannot conclusively state, therefore, that the over ageing is caused by the misinterpretation of the edge growth. Examination of results by time of year of the OTC injection also suggested that a check or mark was produced when the fish was actively depositing opaque material on the otolith. Time of year of injection, particularly May, is believed to have caused our readers to misinterpret the relationship of the annulus to the OTC mark. Fargo and Chilton (1987), however, found no trouble in correctly estimating the TAL for rock sole

[2]Leaman, B. M. International Pacific Halibut Commission. P.O. Box 95009, Seattle, Washington, 98145-2009.

(Lepidopsetta bilineata) injected with OTC during the same month of the year. In their study, they used the break and burn technique but did not experience any difficulties discerning the position of the OTC mark or assigning the edge growth.

Dunlop (1935, unpublished) set forth the basic criteria used by IPHC for determining halibut annuli. He contended that only distinct zones should be counted. Obscure opaque and transparent zones were to be treated as secondary in nature (i.e., "checks"). He acknowledged the possibility that opaque zones may be completely hidden from view and not counted. For these reasons he regarded his age determinations as minimum values. Dunlop also postulated that, in spite of discrepancies, the ages obtained from surface readings for young of the year through age 2 were irrefutably in agreement with the examination of the length-frequencies. Although we clearly had problems distinguishing the relationship of the OTC mark to the annulus and assigning the edge growth, further research suggests the error is most likely associated with assigning edge growth (Blood 2003). We acknowledge that the inability to obtain consistent results using criteria developed to describe an annulus is disturbing and needs further examination. We have taken steps to document our ageing criteria and edge typing through the use of an age manual (Forsberg 2001). Regrettably, we cannot conclusively state we have validated the surface or break and burn age technique for halibut at this time.

The process of ageing fish is fraught with difficulties (Kimura and Lyons 1991), so care must be taken to minimize design-induced error. Future mark-recapture validation experiments should pay close attention to the time of year the translucent zone is formed. Injection of fish should take place so that the absorption of the marking-agent is likely to take place within the time period the fish is actively growing. We believe the best time for marking fish is well beyond the time of translucent zone formation. We recommend that a marginal increment analysis study be undertaken to fully understand the scope and range of months when the translucent zone is being formed. For Pacific halibut, further examination of the criteria describing the annulus needs to be done. Using three readers to validate ageing techniques increased the probability for interpretive error, but also proved invaluable in quantifying between reader discrepancies. Additional testing should be conducted between readers to compare annuli designation and, where necessary, develop new criteria for annular designation. Finally, a comparison of the ages derived from surface and break and burn techniques should also be conducted; initially between otoliths, and then, between readers.

Acknowledgments

I would like to express my thanks to Dr. Robert J. Trumble and Dr. William G. Clark for their advice and guidance and to Shayne MacLellan and Doris Chilton for their assistance with photography and counsel, and to Dr. Bruce M. Leaman and Gregg Williams for their encouragement in completing this work. I am also thankful to all of those who provided critical comments in review of this manuscript.

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Abstract

A comparison of the surface and break and burn age reading methods for 1,324 Pacific halibut otoliths produced results which are very close for ages 6-15 years. Break and burn ages were slightly, but consistently older than surface ages beginning at age 7 and resuming from age 9 and older. At ages 6 and 8, there was no difference between surface and break and burn ages. The edge growth may have been incorrectly interpreted resulting in a higher age than by the surface method. We recommend re-examining otoliths which are 7 years to 10 years old when aged by the break and burn method to clear up this discrepancy between surface and break and burn ages.

II. Comparison of surface and break-and-burn otolith methods of ageing Pacific halibut

Calvin L. Blood

Introduction

By the early 1980s, age researchers on the Pacific Coast were using an ageing method known as otolith breaking and burning and estimating higher ages when compared to ages obtained by the otolith surface method (Chilton and Beamish 1982; Beamish and McFarlane 1987). Rockfish (*Sebastes* spp.) and sablefish (*Anoplopoma fimbria*) were among the first species studied which yielded ages far greater than previously thought. Age validation studies confirmed the higher ages (Bennett et al. 1982; Leaman and Nagtegaal 1987). The method gained wider acceptance as a result of these studies and is considered a standard procedure for long-lived species. At least one study, however, involving arctic char (*Salvelinus alpinus*) (Baker and Timmons 1991), ran contrary to the new findings. They found surface ageing still produced older ages than break and burn.

The International Pacific Halibut Commission (IPHC) has long used the surface reading technique (Dunlop, unpub.)[1] for ageing Pacific halibut (*Hippoglossus stenolepis*). Dunlop cautioned that while evidence strongly suggested annular formation in halibut otoliths, this was by no means conclusive. He stated that some otoliths were occasionally found with indistinct transluscent zones and were regarded as accessory marks and were not counted. He also maintained that until the nature of those marks could be proven, surface ages should be regarded as minimum values. Age validation of Pacific halibut by both the surface and break and burn ageing methods was attempted in the early 1980s when halibut were injected with oxytetracycline and externally tagged. When the tagged and injected halibut were recaptured, the otoliths were extracted and viewed under ultraviolet light (UV). The UV light revealed a mark on the otolith laid down at time of injection. A comparison of the UV mark and subsequent annular rings deposited during the time at large was analyzed. While the results did not conclusively establish validation, the findings strongly suggest the age rings are deposited annually (Blood 2002).

Some halibut otoliths collected in 1992 from samples of the commercial harvest in the Bering Sea and Aleutian Islands yielded much older ages by the break and burn method than the surface method. Structurally, these otoliths are thick in relation to their length and width. None of this type of otolith was present in the oxytetracycline age validation samples.

A test group of five particularly thick otoliths from halibut collected on a research survey in the Bering Sea and Aleutian Islands indicated the break and burn method could

[1] Dunlop, H. A. Unpublished report. Age Studies on the Pacific halibut *(Hippoglossus stenolepis* Schmitt). Int. Pac. Halibut Comm. P.O. Box 95009, Seattle, WA 98145.

yield much higher ages than surface readings (Table 1). In this group, a break and burn aged otolith produced an estimated age of 55 years for a male halibut, whereas the surface age indicated an age of 27 years. This doubled the previous record for male halibut and was 30 percent higher than the record age (42) for all halibut.

To determine if these results applied to only thick otoliths, and to compare surface with break and burn ages, a larger sample of otoliths from the same survey were examined. This report presents the results from this examination.

Table 1. Comparison of surface and burnt-section ages from otoliths collected from the Bering Sea and Aleutian Islands in 1992.

Fork length (cm)	Sex	Surface age	Burnt-section age
108	unsexed	19	42
120	unsexed	17	34
126	unsexed	21	37
118	male	27	55
138	female	17	19

Materials and Methods

A sample of 1,324 otoliths collected in 1992 during an IPHC research cruise in the Bering Sea and Aleutian Islands was read by one reader using both the surface and break and burn methods. The surface and break and burn readings for each otolith occurred on separate occasions, without reference to the previous reading.

The surface method is simply viewing the whole otolith under reflected light against a black background with 5-10X magnification and counting the opaque and translucent zones outward from the center (Figure 1). One year of otolith growth is composed of one opaque and one translucent zone. These zones correspond to the summer and winter growth periods. The winter zone is often referred to as the annulus. If a complete annulus is not apparent on the edge of the otolith, a judgement is made based on the time of year when the otolith was collected whether to include the incomplete annulus at the edge in the age estimate. IPHC age readers count this edge if the current growth is more than half of the previous year's growth for age 10 and younger when the otolith is collected between January and June. For fish aged 11 and older the annuli are spaced closely together and for this reason the edge growth is generally counted if the otolith is collected in that same time period. At least two transects are read to confirm the age. The otolith is rolled and tilted to view the edge and expose the maximum amount of zones.

With the break and burn method the otolith is broken through the nucleus (Chilton and Beamish 1982) (Figure 2). Each broken cross-section is heated over an alcohol flame which increases the contrast of the wide and narrow growth zones. The burned surface is lightly coated with cooking or mineral oil and examined under 25-50X magnification. The preferred transect is close to the sulcus on either the dorsal or ventral edge, but others may be chosen if the pattern at the original break is not clear (Figure 3). The posterior-half of halibut otoliths appears to be the preferred section but, depending on the clarity of the burn, the anterior end is also acceptable.

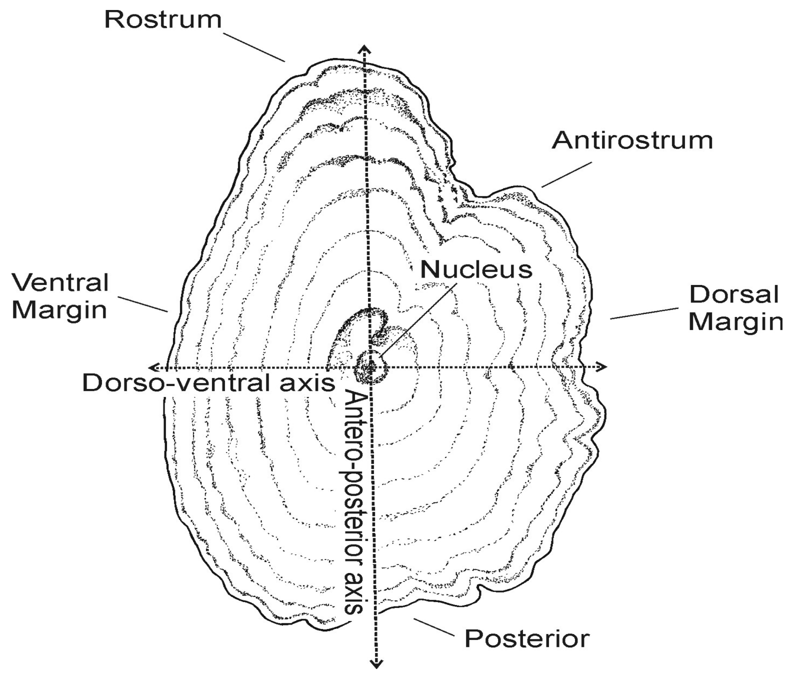

Figure 1a. Distal surface of left sagittal Pacific halibut otolith (Terminology from Harkonen 1986).

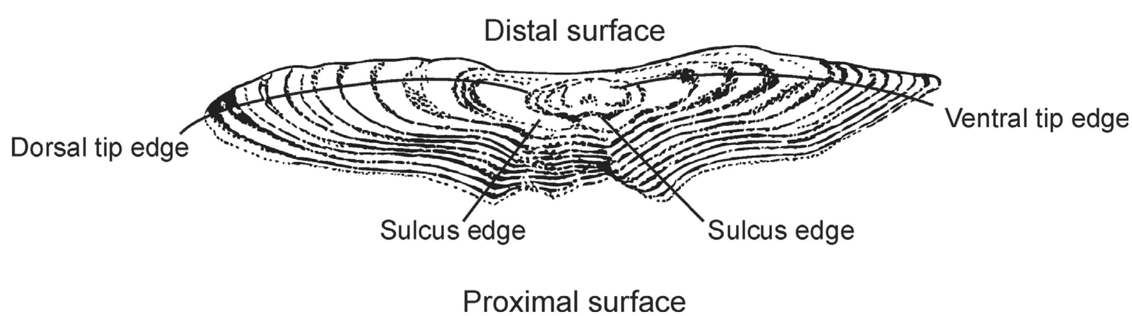

Figure 1b. Cross-section of Pacific halibut otolith.

We wanted to determine if surface ages differed significantly from break and burn section ages, e.g., are break and burn ages older in halibut as they are in other groundfish species? Clark et al. (1986), found that by testing systematic differences between the two methods on the otoliths of yellowtail rockfish (*Sebastes flavidus*), a simple t-test could detect whether the differences between the paired readings came from a distribution with mean zero. If halibut ages were similar to other long-lived groundfish species, we would expect break and burn ages to yield higher age estimates than surface readings. Data were grouped into 5 classes: 0-5 years old, 6-10 years old, 11-15 years old, 16-20 years old, and >20 year olds, and tested for a significant difference within each group.

Otoliths were also classified by clarity and thickness to see if older-aged halibut could be quickly identified. We wanted to know how the clarity and thickness affected differences between surface and break and burn readings.

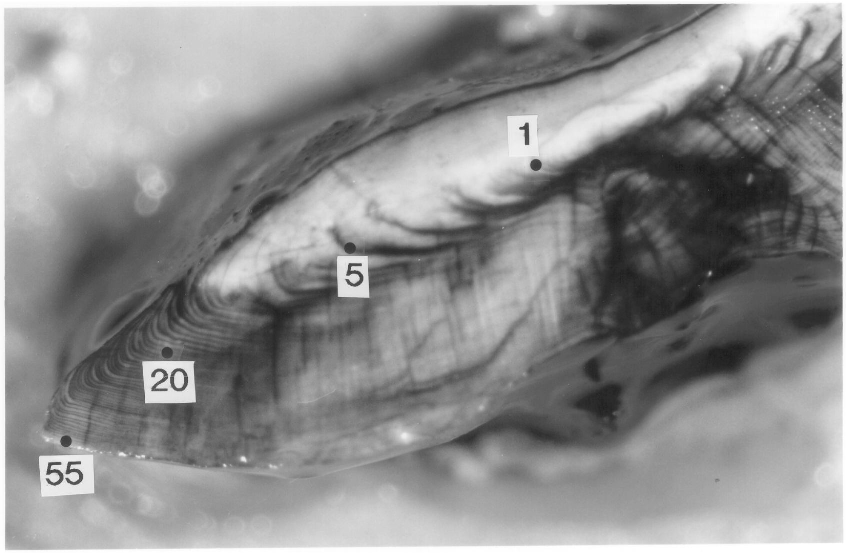

Figure 2. Cross-section of the otolith after breaking and burning of a 55 year-old Pacific halibut.

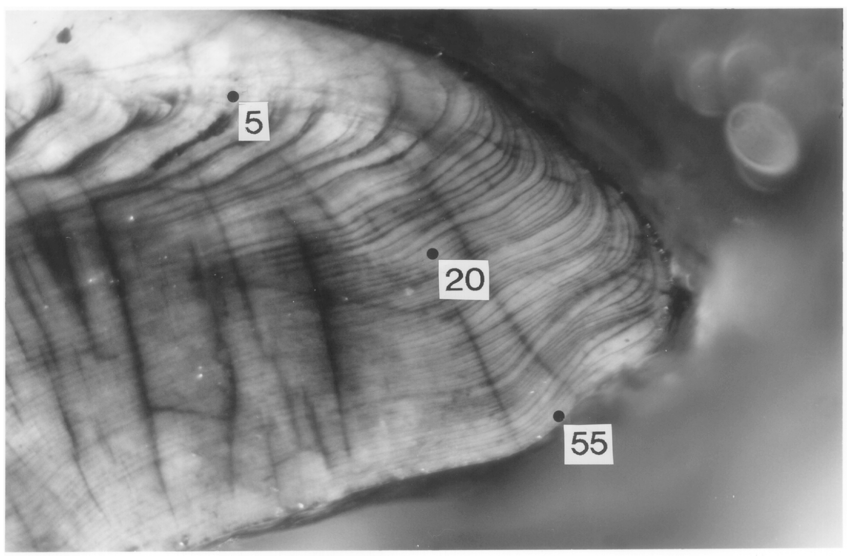

Figure 3. Enlargement of ventral-half of burnt-section otolith from a 55 year-old Pacific halibut.

Results

The break and burn method produced ages greater than surface readings (Table 2). The observed differences detected were highly statistically significant for all groups. Age group 0-5 contained only six comparisons and was eliminated from the results. Age differences were mainly confined to ± 1 to 2 years and never exceeded 5 years, below the age of 20. For halibut aged 6-15 the absolute difference was frequently only one or two years, and clearly the break and burn age method yields slightly higher ages.

There generally appears to be good agreement between the surface and break and burn ages. Over 96 percent of the ages between 6-15 years were within ±1 year (Table 3).

Table 2. Difference between surface and burnt section age readings.

Age group (Section)	Mean difference	Standard deviation	Sample size	P-value
6-10	0.05	0.41	804	0.000**
11-15	0.26	0.70	426	0.000**
16-20	0.61	0.97	66	0.000**
21+	1.59	2.44	22	0.003**

** Significant at P=0.05

Table 3. Joint distribution of surface and burnt section readings of all otoliths included in the study.

		Surface																											
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Sums
break	4	1																											1
and	5	1	1	3																									5
burn	6		5	177	5																								187
	7			7	65	2																							74
	8				15	74	15																						104
	9					25	232	16																					273
	10					5	23	133	5																				166
	11					1	4	29	51	15																			100
	12						1	6	20	111	6																		144
	13								4	32	30	6	1																73
	14									1	9	59	3																72
	15										4	14	15	4															37
	16										1	1	3	6	3	1													15
	17												2	9	9														20
	18												1	1	5	7													14
	19													1		2													3
	20														1	1	4												6
	21																4	4											8
	22																1	2											3
	23																1		2	4	1								8
	24															1				1									2
	25																				2								2
	26																												0
	27																							1					1
	28																				2			1		1			4
	29																												0
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	38																												0
	39																												0
	40																												0
	Sums	2	6	187	85	107	275	184	80	159	50	80	25	21	18	12	10	6	2	5	5	0	0	3	0	2	0	0	1324

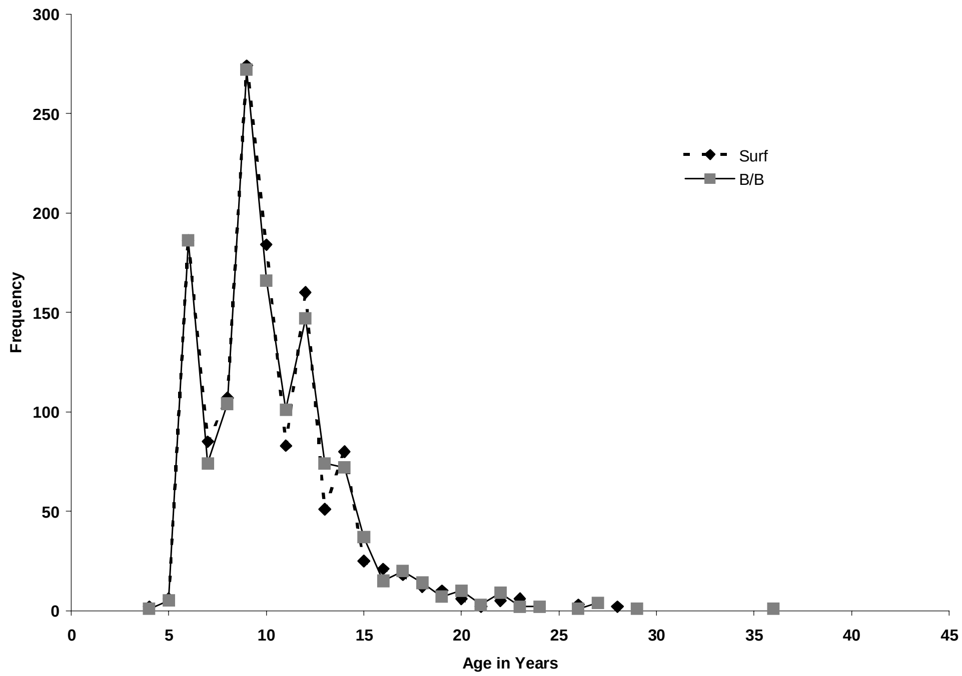

Figure 4. Age composition generated by surface and burnt section age readings.

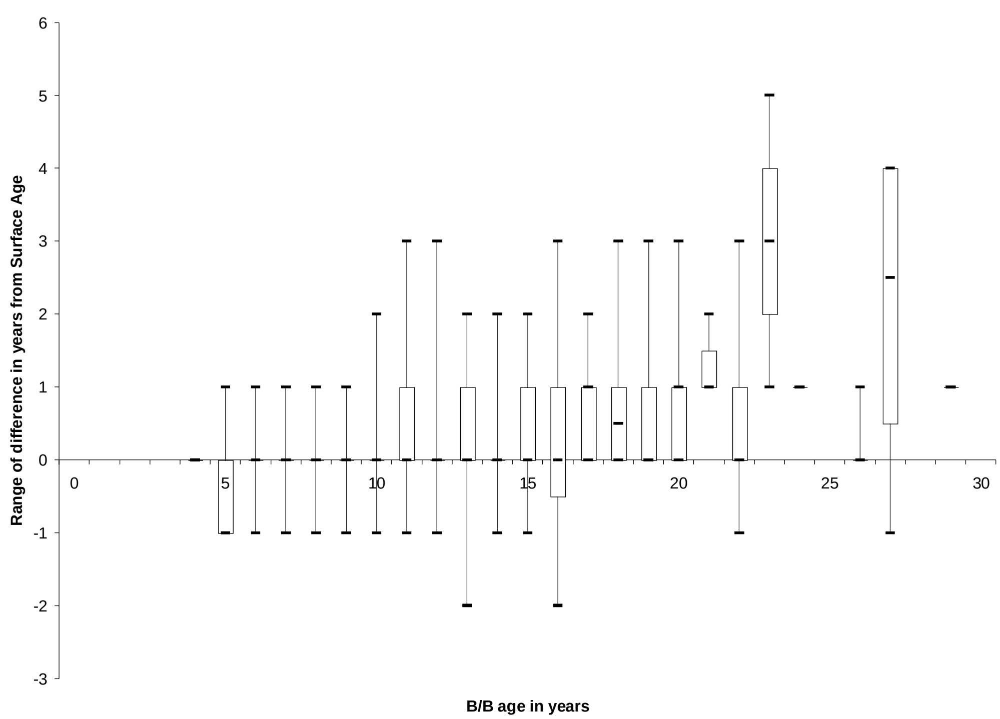

Figure 5. Box plot comparisons of break and burn age with differences between surface and break and burn ages through age 29.

A frequency distribution of the ages shows that the surface and burnt section methods produce nearly the same age composition (Figure 4). However, a comparison of the differences between the break and burn and surface ages reveals that the surface ageing method consistently underestimates ages produced by the break and burn method (Figure 5). Further examination of the p-values for ages 6-15 reveals that there is a significant difference in ages 7 and 9-15 (Figure 6). Ages 6 and 8 showed relatively little difference and the ageing error was distributed equally. The reason age 7 did not show the same error distribution is not clear, though it may be related to misclassification of the edge assignment. The two ageing methods also digress at different points for mean length at age for males and females. Males differ between surface and break and burn at 14 years old (Figure 7) and females at 16 (Figure 8). A von Bertalanffy growth curve (Figures 7 and 8) was fit to each of the break and burn and surface ages for both male and female mean length at age. The growth curves show little change in the growth schedules for each ageing method.

Otoliths were also classified by four types to determine if clarity and thickness could predict whether an otolith should be broken and burnt (Table 4). The four categories were; 1) clear, 2) opaque, 3) clear and thick, 4) opaque and thick. Clear otoliths typically had a high degree of total agreement between the two methods. The total agreement dropped considerably for opaque and thick otoliths. Within ±1 year there was excellent agreement. Clearly the differences in the two methods are influenced by the opacity and thickness of the otoliths. Additional annuli are obscured and can only be observed in cross-section.

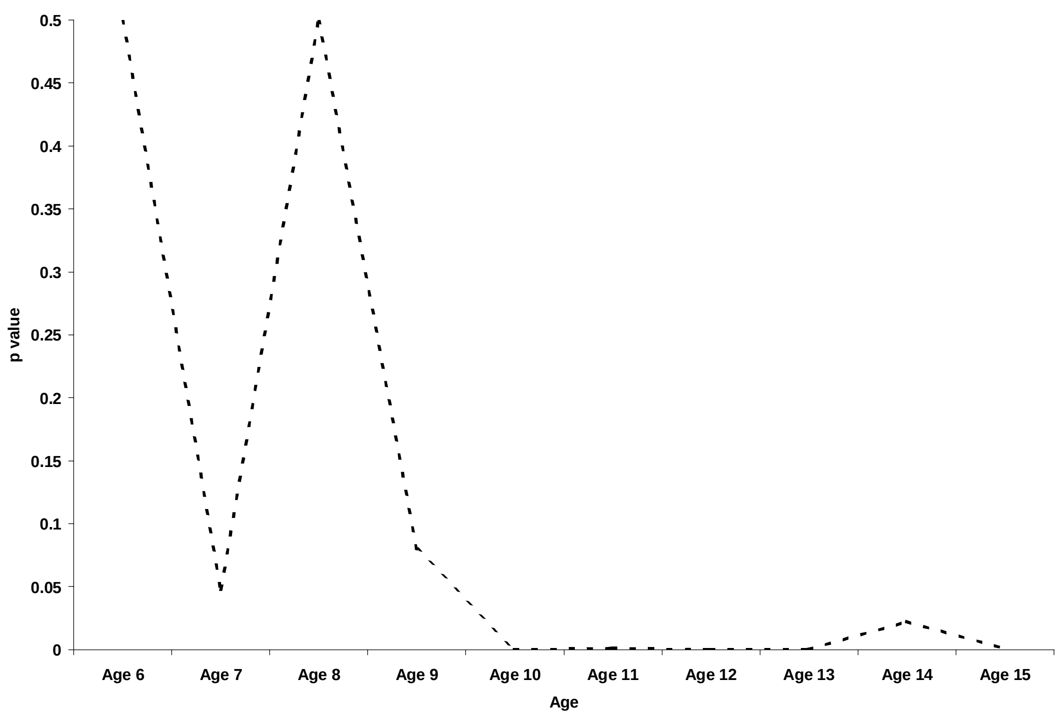

Figure 6. P-values for individual comparisons of ages.

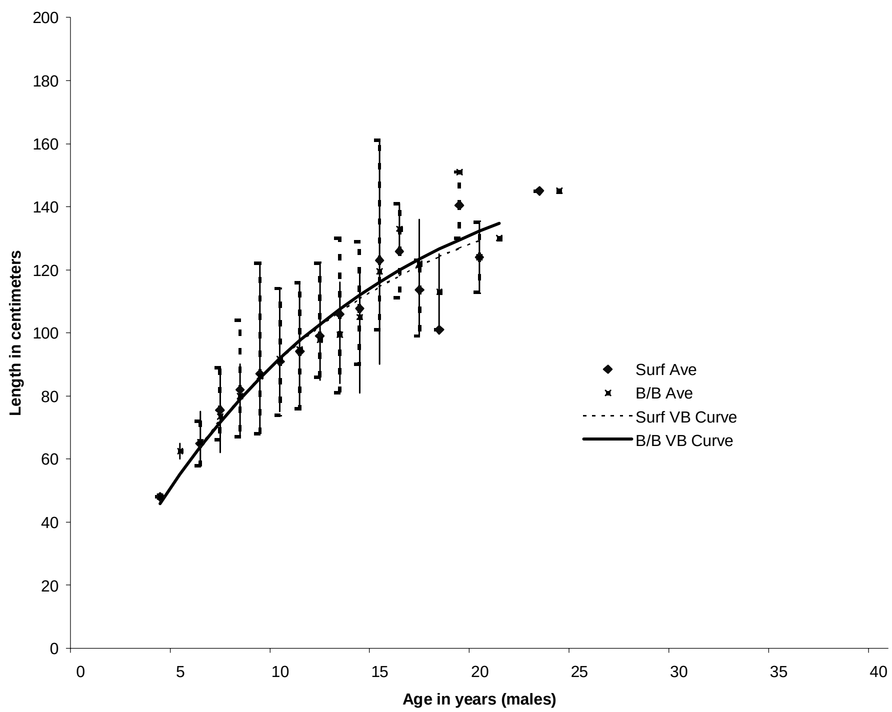

Figure 7. Mean length at age of Pacific halibut males by surface and break and burn readings, with von Bertalanffy growth curves.

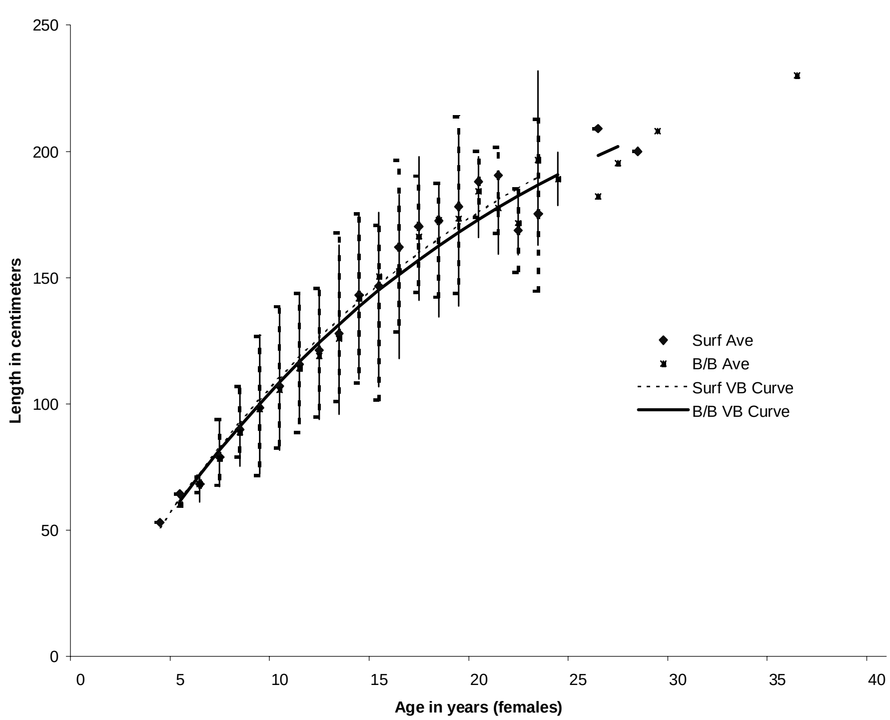

Figure 8. Mean length at age of Pacific halibut females by surface and break and burn readings, with von Bertalanffy growth curves.

Table 4. Percent agreement and otoliths read for four degrees of difficulty in defining otolith clarity.

	Percent agreement		
Degree of difficulty	Total agreement	Within ±1 year	Number of otoliths read
Clear	78 %	98%	1026
Opaque	63%	93%	246
Clear and thick	65%	96%	26
Opaque and thick	31%	77%	26

Discussion

Pacific halibut otoliths from the Bering Sea and Aleutian Islands are among the easiest to read. The annuli are distinct and well defined giving the reader a sense of confidence in obtaining an accurate age. The area from which the large sample was chosen is a relatively lightly exploited fishing ground and we expected to find thicker and potentially older otoliths. If no problems were found with these otoliths, then areas which are more heavily exploited would contain fewer old halibut and, presumably, fewer gross ageing errors.

There is a divergence of the two ageing methods beginning at age 7, with no difference at age 8 but diverging again at age 9. Chilton and Beamish (1982) reported that surface ages may underestimate the break and burn ages for older halibut in a previous cooperative study between IPHC and Canada Department of Fisheries and Oceans. In our study, male ages determined by the two age methods produced growth schedules which were similar to age 14 and female ages to 16 years of age and older. Fundamentally, the reason behind these differences is the shape of the otolith. Male otoliths tend to become more pill-shaped as they grow and are thicker in comparison to their length and width. Their edges are steep or rounded off. Annuli are often obscured beyond the edge of the otolith. Female otoliths, on the other hand, are more convex and knife-edged.

Otoliths are collected in two major stock assessment related activities. One program obtains otoliths from unsexed fish through market sampling of commercial catches. The other is through sexed fish from research surveys on chartered commercial longline and trawl fishing vessels. We recommend testing the two methods, once more, beginning at 7 years and ending at 10 years of age. The question really comes down to which age is the artifact, age 7 or age 8? Clearly as halibut grow older the break and burn method yields an older age than the surface method. One could make a case to break and burn otoliths from age 9 and older, but we prefer to examine the age differences once again before making a recommendation. Although few halibut of age 30 and greater are collected each year, the doubling of previous record-aged fish suggests the growth rate schedules and natural mortality of older fish may be worth investigating. We also recommend testing the two age methods in heavily exploited areas, such as the Gulf of Alaska where thick otoliths are rarely found and annuli are not as easily identified. Finally, we recommend that timing of annulus formation from previous findings be re-examined within a calendar year and throughout the geographical range of halibut. Careful consideration should be given to collect sufficient samples within each region to distinguish annulus formation over the entire year.

The persistent difference by one year between the ages of 7-15 is troublesome and we must be cautious in interpreting these results. One reason for this error may be the obscuring of the edge in the burning process. This author was still relatively inexperienced in the breaking and burning process at the time of this study and conceivably may have incorrectly assigned the edge growth. The generally universal finding in many species of fish that concludes the break and burn method yields older ages than the surface method also may have induced the reader to err on the side of more annuli. Fournier and Archibald (1982) warn that even small ageing errors can cause serious problems in estimating fishing mortality and age-classes by smearing weak and strong year classes. This error is particularly troublesome when strong cohorts are next to weak ones and one assumes a constant ageing error. Shifts from strong to weak cohorts have much greater impact than from weak to strong. Detecting this error at an earlier age further supports our decision to break and burn at age 7, rather than age 9. More rigorous examination of edge formation would improve ageing criteria. This would also likely reduce smearing of age groups and better differentiate between dominant and weak year classes.

Acknowledgments

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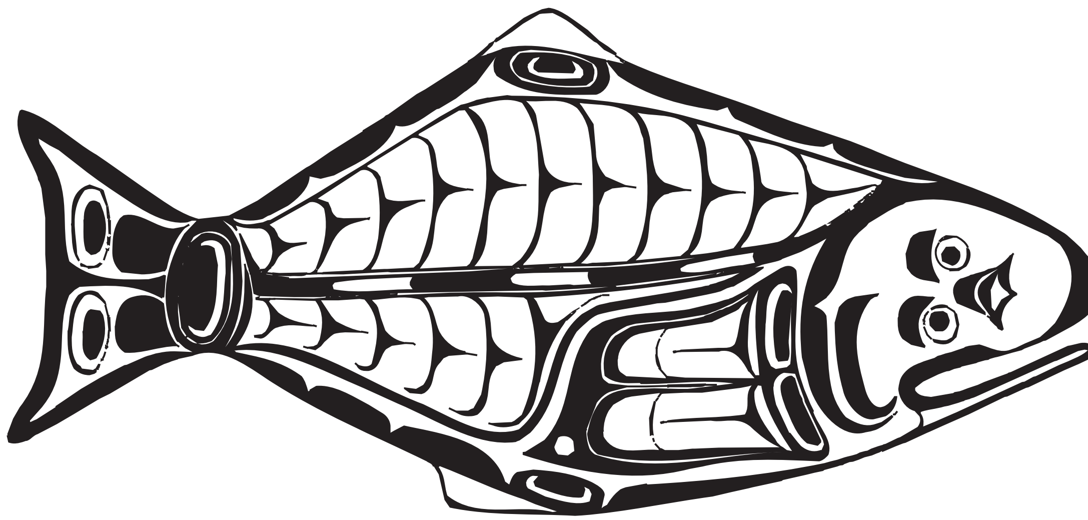

HALIBUT CREST - adapted from designs used by Tlingit, Tsimshian and Haida Indians