Indirect validation of the age-reading method for Pacific cod (*Gadus macrocephalus*) using otoliths from marked and recaptured fish

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Abstract—Two examples of indirect validation are described for age-reading methods of Pacific cod (*Gadus macrocephalus*). Aging criteria that exclude faint translucent zones (checks) in counts of annuli and criteria that include faint zones were both tested. Otoliths from marked and recaptured fish were used to back-calculate the length of each fish at the time of its release by using measurements of the area of annuli. Estimated fish size at time of release and actual observed fish size were similar, supporting the assumption that translucent zones are laid down on an annual basis. A second method for validating reading criteria used otolith age and von Bertalanffy parameters, estimated from the tagging data, to predict how much each fish grew in length after tagging. We found that otolith aging criteria applied to otoliths from tagged and recovered Pacific cod predicted quite accurately the growth increments that we observed in these specimens. These results provide further evidence that the current aging criteria are not underestimating the age of the fish and support our current interpretation of checks (i.e., as subannual marks). We expect these indirect validations to advance age determination for Pacific cod, which in turn would enhance development of stock assessment methods based on age structure for this species in the eastern Bering Sea.

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Pacific cod (*Gadus macrocephalus* Tilesius, 1810) is an important species in eastern Bering Sea commercial fisheries and is second only to walleye pollock (*Theragra chalcogramma*) in landings (Thompson and Dorn[1]). It is also considered to be one of the most difficult of all commercially important Alaska groundfish species to age. Scientists from both Canada and the United States have experienced similar difficulties in finding an appropriate aging structure, which can be consistently interpreted to track large year classes of cod through time. Historically, scales and otoliths have been the two most common structures used for determining the ages of fish species. Unfortunately, age-readers employing these structures have experienced limited success in the case of Pacific cod (Kimura and Lyons, 1990).

The Pacific Biological Station in Canada stopped aging Pacific cod in 1978, after age estimates derived from scale readings began yielding year classes that were inconsistent with length-frequency time series from field surveys (Westrheim and Shaw, 1982). The Alaska Fisheries Science Center's (AFSC) Resource Ecology and Fisheries Management (REFM) Division is responsible for stock assessment of Pacific cod in the Gulf of Alaska and eastern Bering Sea. The REFM Division's Age and Growth Program used scales for determining the age of Pacific cod from 1976 to the early 1980s. Thereafter, the program used the break-and-burn method with otoliths to age Pacific

[1] Thompson, G. G., and M. W. Dorn. 1999. Pacific cod. *In* Stock assessment and fishery evaluation report for the groundfish resources for the Bering Sea/Aleutian Islands regions (plan team for groundfish fisheries of the Bering Sea/Aleutian Islands), p. 151–205. North Pacific Fishery Management Council, 605 W. 4th Avenue Suite 306, Anchorage, AK 99501.

cod (Thompson and Methot[2]). In the otoliths of young Pacific cod (under 6 years), there is a tendency for subannual marks (also known as "checks") to be very dark and evenly spaced, making them difficult to distinguish from annuli. This confusion makes it difficult to age the species to an exact age.

From 1990 through 1992, the AFSC noticed that the average length at a specific age was smaller than it had been in previous years. The decrease was noticed in ages 1–6 but was especially dramatic in 1-, 2-, and 3-year-olds. It is generally theorized that the shift was the result of one of two scenarios: either the fish population experienced an actual decrease in length-at-age or the age readers were over-aging fish by counting marks other than annuli. Unable to pinpoint the reason for the shift and given the inherent difficulty of aging cod, production (large-scale) aging of Pacific cod was indefinitely suspended at the AFSC.

Pacific cod stock assessments in Alaska have since depended largely on length-frequency data alone to model population age structure because of the difficulties in obtaining age estimates (Thompson and Dorn[1]). However, the use of length-frequency data as proxies for age data can be problematic. If external factors such as ocean conditions affect somatic growth to such a degree that length-at-age within the population is highly variable, such as appears to be the case for Pacific cod, then the population becomes difficult to model. Otoliths, on the other hand, are permanent records of growth that are more independent of external factors.

Consequently, the Age and Growth Program initiated a new study in 1998 to re-examine the otolith aging structure for Pacific cod. This study used otoliths from tagged Alaska Pacific cod to validate aging criteria for otoliths.

Methods

Otoliths and length data were collected during a tagging study conducted by the AFSC. Between 1982 and 1990, 12,396 Pacific cod were tagged and released in the eastern Bering Sea during summer bottom-trawl surveys (See Shimada and Kimura, 1994). Fish were measured to the nearest 0.5 cm fork length, tagged with uniquely marked spaghetti tags, and set free. Over a period of 13 years, commercial fishing vessels recaptured 375 (3%) of the tagged fish and returned otoliths from 112 fish (106 of which were usable) (Table 1). More details on the tagging methods can be found in Shimada and Kimura (1994).

[2] Thompson, G. G., and R. D. Methot. 1993. Pacific cod. *In* Stock assessment and fishery evaluation report for the groundfish resources for the Bering Sea/Aleutian Islands regions as projected for 1994 (plan team for groundfish fisheries of the Bering Sea/Aleutian Islands), p. 2–28. North Pacific Fishery Management Council, 605 W. 4th Avenue Suite 306, Anchorage, AK 99501.

Otolith preparation

One sagittal otolith from each recaptured fish was selected for our study. We did not discriminate between left and right otoliths based on the results of Sakurai and Hukuda (1984) who were unable to detect any consistent differences between the weight and length of right and left Pacific cod otoliths.

Each otolith was cleaned and preserved in 95% ethanol. After having been preserved for approximately one month, a line was penciled across the otolith center from the dorsal apex to the ventral apex to ensure that the otoliths would later be sectioned at the core.

The otolith was then placed in a polyester mold and set in black resin (Technovit 3040, Energy Beam Sciences, Agawam, MA), forming a block of resin. A slow-speed saw was used to cut the blocks in half. This produced two smaller blocks, each with an exposed view of the otolith in the transverse plane and cut through the center. One of the two blocks was selected and glued (otolith side down) to a glass slide. The glass slide was mounted to a Hillquist thin section machine (Hillquist Inc., Fall City, WA) and the section was ground down to a thickness of 0.25 mm. A coverslip was permanently glued on the top of the section with marine-grade epoxy.

Sections were placed on a piece of black velvet (which added contrast) on the stage of a 50× dissecting microscope, and reflected light was used for illumination. The sections were viewed on a computer monitor by using a Cohu 6500 monochrome video camera, Integral Flashpoint 128 frame grabber and Optimas 6.5 imaging software (Media Cybernetics, Silver Spring, MD).

Age-reading criteria

Traditional qualitative aging criteria were used to distinguish annuli from checks. The criterion for identification as an annulus was a continuous translucent band that could be seen along the entire structure or as a ridge or groove on the structure (Secor et al., 1995). Checks (i.e., subannual marks) are translucent zones that appear very similar to annuli. They were determined primarily by the incompleteness of the zone around the entire section, by zone darkness, and by spacing between zones. When translucent zones could be classified as either annuli or additional subannular marks, they were classified as checks. Annuli, checks, and edges (the space between the last annulus and the edge of the otolith) were traced by using the Optimas 6.5 software package and measurements of their areas and major axis lengths were collected (Fig. 1). All otoliths were read blind; that is, information about fish length and date of capture (Table 1) was withheld from the reader to prevent bias.

When all the otoliths had been aged and measured, the age reader returned to each otolith section to estimate the area and length of the otolith when the fish was tagged. This was accomplished by following a two-step process. The first step was to approximate the location of the otolith cross-section that corresponded to

Table 1

Mark and recapture data for spaghetti-tagged Pacific cod (*Gadus macrocephalus*). L_1 = fork length at tagging (mm), L_2 = fork length at recapture (mm), d_1 = time at liberty (months), a_1 = age estimated from L_1 and d_1, a_2 = age at recapture estimated by using the fish's otolith and age-reading criteria, and NR = not reported.

L_1	L_2	d_1	a_1	a_2	a_1–a_2	L_1	L_2	d_1	a_1	a_2	a_1–a_2
430	480	9	4	3	1	625	550	3	6.5	5	1.5
680	875	21	8	7	1	740	850	26	8	8	0
690	830	30	8	8	0	650	670	4	6	7	–1
590	500	4	6	7	–1	525	550	5	5	4	1
530	690	30	7	7	0	645	720	9	7	6	1
430	500	10	4	4	0	835	890	14	7	8	–1
600	620	8	6	7	–1	560	600	5	5	4	1
390	490	11	3.5	3	0.5	645	830	38	9	8	1
630	660	5	6.5	4	2.5	405	412	4	3.5	2	1.5
670	750	9	7	8	–1	460	550	8	4	3	1
630	850	49	9.5	10	–0.5	735	760	1	6	7	–1
590	650	7	6	6	0	620	650	1	5	6	–1
450	570	25	5	6	–1	545	550	4	4	6	–2
630	660	7	6.5	6	0.5	555	560	2	4	5	–1
440	525	14	4.5	5	–0.5	680	730	19	7.5	6	1.5
705	890	35	9	8	1	640	680	4	6.5	4	2.5
556	680	25	6	8	–2	730	730	6	7	7	0
480	540	7	5.5	3	2.5	540	655	12	5	5	0
620	660	2	5	9	–4	600	720	14	6	6	0
630	730	17	7	8	–1	540	700	17	5	6	–1
600	730	44	8.5	7	1.5	530	920	50	8	9	–1
630	623	1	5.5	6	–0.5	610	691	16	6	8	–2
702	760	11	7	8	–1	690	800	10	7	5	2
330	530	22	4	3	1	555	630	7	5	6	–1
540	706	20	6	5	1	790	NR	21	8	7	1
390	578	22	4.5	4	0.5	560	630	8	5	5	0
670	710	4	6	9	–3	520	550	2	4	4	0
820	825	2	6	8	–2	535	NR	18	5.5	6	–0.5
690	710	8	7	8	–1	460	530	7	4	3	1
725	850	17	7.5	6	1.5	590	930	93	12.5	15	–2.5
530	590	9	5	5	0	690	742	5	6.5	6	0.5
660	690	3	6	7	–1	720	770	8	7	9	–2
580	770	57	9.5	10	–0.5	870	924	7	7	8	–1
450	695	25	5	5	0	520	523	7	5	3	2
815	860	10	7	8	–1	470	530	6	4.5	3	1.5
540	670	22	6	7	–1	630	740	13	6.5	7	–0.5
740	670	7	7	6	1	510	630	13	5	6	–1
670	705	2	6	8	–2	650	720	13	7	6	1
650	640	0	6	7	–1	570	675	20	6	8	–2
710	860	19	8	7	1	690	660	20	7.5	8	–0.5
670	810	20	8	7	1	660	760	23	8	8	0
780	770	1	6	8	–2	690	750	7	7	7	0
810	850	7	7	8	–1	560	630	8	5	5	0
485	640	21	5.5	6	–0.5	530	700	7	5	7	–2
305	850	39	4.5	8	–3.5	600	620	10	5.5	4	1.5
695	710	9	7	6	1	595	970	50	8.5	9	–0.5
610	810	30	8	6	2	520	910	32	6.5	7	–0.5
660	820	30	9	7	2	540	800	30	6	7	–1
610	725	26	7	7	0	820	830	2	6	6	0
580	720	30	7.5	7	0.5	585	782	34	7.5	6	1.5
530	830	33	7	6	1	680	790	9	7	6	1
600	760	17	7	6	1	676	1080	70	11.5	11	0.5
515	730	26	6	5	1	585	590	0	4.5	5	–0.5

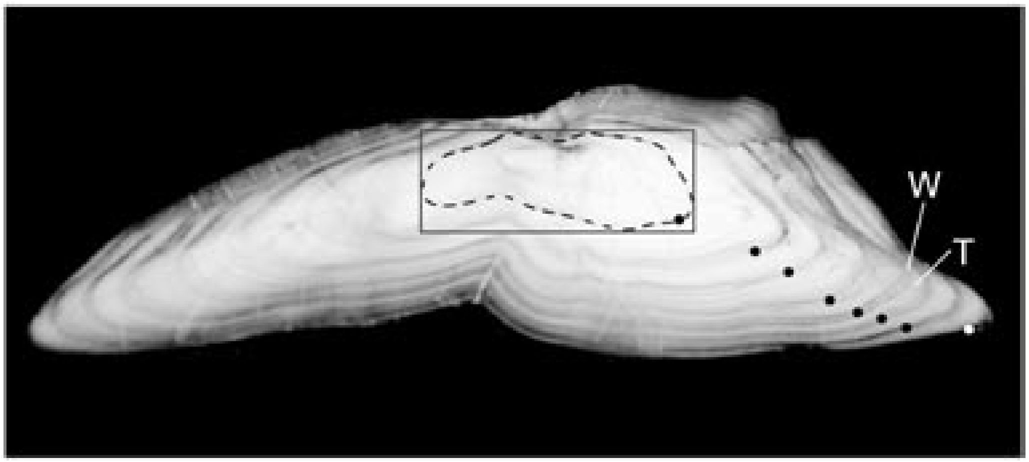

Figure 1

Transverse cross-section of a Pacific cod (*Gadus macrocephalus*) otolith with an unusually clear annulus pattern. The otolith was taken from a 830-mm, 8-year-old fish that was recaptured 30 months after tagging. Annuli (black dots), checks, and edges (the space between the last annulus and edge [white dot]) were traced and measurements of their areas (from the center of the otolith to the outer margin of the translucent zone [dotted line]) and major axis lengths (from the smallest rectangular box that will hold the translucent zone) were collected. *T* is the estimated otolith size (length and area) at the time of tagging and was used to back-calculate fish length at tagging. *W* corresponds to the annulus preceding *T*.

the time of tagging. The second step was calculating the area and length of that region, producing an estimated otolith size at the time of tagging

First, to approximate the location of the otolith that corresponded to the time of tagging required the reader to know how long (years) the fish had been at liberty, after tagging. Using this knowledge and starting from the last annulus before the edge, the reader counted towards the center of the otolith, the number of years (as represented by annuli) that the fish had been at liberty. (In cases where the fish had been at liberty for less than one year before being caught again, the reader began at the edge rather than at the last annulus before the edge). Assuming that all annuli are laid down by late winter, the reader would end up on the annulus that preceded the summer of tagging. This annulus represents the size of the otolith just prior to tagging and for sake of further explanation, its area and length will be identified as *W* (Fig. 1). To complete the procedure, the reader needed only to measure the summer increment which followed *W*, divide it in half and add it to *W*. These calculations were assumed to reflect the size of the fish's otolith at time of tagging and were used as O_i values (the size of the otolith at tagging) to back-calculate fish size at initial capture.

Estimating fish length by using tagged fish and back-calculations

Annuli on tagged fish otoliths can be used to estimate the length of each fish at an earlier age. Smedstad and Holm (1996) compared six different back-calculation formulae on tagged Atlantic cod (*Gadus morhua*) and found that the age-independent, nonlinear, body proportional (nbp) hypothesis worked the best. Pacific cod is a gadid closely related to the Atlantic cod; therefore we also used the nonlinear, body proportional formula

$$L_i = (O_i / O_c)^v L_c,$$

where L_i = the predicted fish length at tagging or desired age;

O_i = the size (either radius or area) of otolith at tagging;

O_c = the size of otolith at time of recapture;

v = the slope from the regression of Ln(L) on Ln(O); and

L_c = the fish length at recapture.

This analysis was performed by using two different measures of otolith size, the cross-sectional area and major axis (i.e., length) of otolith increments (Fig. 1).

Estimating growth increments in fish length from tagged fish

We can use tagged fish otolith ages to estimate how much each fish grew in length after tagging, in a manner similar to Fabens' equation (Ricker, 1975), using the von

Table 2

Results of regressing Ln *fish length* on Ln *otolith area* and Ln *fish length* on Ln *otolith major axis* by using tagged fish data. Fish length was measured in mm, otolith area in mm^2, and otolith major axis in mm, n=96.

	Coefficients	Standard error	t stat	P-value
Regression of Ln *Fish length* on Ln *Otolith area*				
Intercept	4.637299	0.117551	39.44929	2.75E-60
Slope (v)	0.65732	0.040471	16.24168	4.96E-29
Regression of Ln *Fish length* on Ln *Otolith major axis*				
Intercept	4.27009	0.229693	18.5904	3.01E-33
Slope (v)	1.012865	0.102305	9.900426	2.99E-16

Bertalanffy equation (Ricker, 1975). However, because we could estimate the age of fish at time of recapture, we were able to manipulate the von Bertalanffy equation to obtain the following equation:

$$L_2 - L_1 \approx L_{inf}\left[\left(e^{-K(a_2-d-t_0)}\right)-\left(e^{-K(a_2-t_0)}\right)\right],$$

where L_1 = length at tagging;
L_2 = length at recapture;
L_{inf} = maximum size;
K = growth rate;
a_2 = estimated age at recovery determined from our otolith ages;
d = time at liberty; and
t_0 = age at length 0 mm.

Given von Bertalanffy parameters and age at recovery, a (fish) length increment for time after tagging can be predicted. Using published L_{inf} and K estimates from tagging data, L_{inf} = 1043 mm, K = 0.222 (Kimura et al., 1993), we estimated L_2–L_1. One weakness in these estimates is that the growth parameters estimated by Kimura et al. (1993) were based on only positive growth increments (there were some instances where recaptured fish were smaller than they were at tagging, demonstrating negative growth increments).

A value for t_0 was estimated iteratively in the von Bertalanffy equation by using the subroutine Solver (Frontline Systems Inc., Incline Village, NE) from the Excel software package with the following parameter values: K = 0.222, t = 1 year old, L_{inf} = 1043 mm (Kimura et al., 1993), l_t = length at age one = 180 mm (from the 1977 year class [Foucher et al., 1984]). Because these von Bertalanffy parameters are not based on age determination, they provide an indirect method for validating aging criteria. In addition, ages determined by readers were scaled smaller (by 0.75) and larger (by 1.25) in order to simulate the results of younger and older aging criteria. Plots of observed and predicted growth increments should agree if the aging criteria for a_2 reflect the true age of fish.

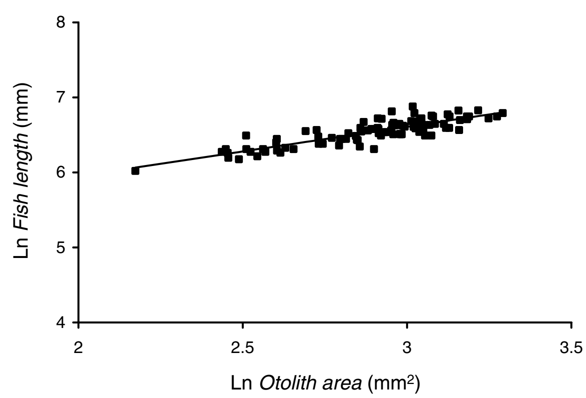

Figure 2

Relationship between Ln *fish length* and Ln *otolith area* based on tagged and recaptured Pacific cod (r^2=0.735, Y=4.6+0.66X, n=96).

Results

Predicting fish length from tagged fish and back-calculations

The parameter v used in all back-calculations in our study was estimated by using otolith area and again by using the major axis of the otolith (Fig. 1). Based on the slopes from the regression of Ln *fish length* on Ln *otolith size* (Table 2, [Fig. 2]), the coefficients should be v=1.01 for otolith length and v=0.66 for otolith areas.

Back-calculations were performed by using the otolith area and were repeated by using the major axis. Scatter plots of estimated and observed fish lengths were used to visually inspect how well back-calculation determines fish length (Fig. 3). Assuming that the residuals of the back-calculated length at tagging have independent chi-square distributions, an F-test indicates that back-calculations derived from otolith areas are significantly more accurate than back-calculations derived from the major axis (P<0.05). However, because we used the two different methods on the same otoliths, the residuals were correlated and thus this result can be considered only approximate.

Predicting growth increments of fish length from tagged fish

Using the von Bertalanffy curve fitted with the data from the tagged fish sample, we estimated the value of t_0 to be 0.147.

The amount that each tagged fish grew after tagging was calculated three times by using fish age at recovery and the von Bertalanffy equation (Fig. 4). The calculated sums of squared deviations for the three sets of predicted values are as follows: 433,955 when fish age is scaled by 0.75, 419,477 when fish age is scaled by 1.0, and 761,545 when fish age is scaled by 1.25. The lowest sum of squared deviations accompanied ages that were scaled by 0.86. Assuming that the residuals of the estimated growth increments have independent chi-square distributions, an F-test indicates that residuals were significantly larger ($P<0.05$) when ages were scaled 25% older and there was no significant difference ($P<0.05$) between reader-determined ages and ages scaled 25% younger. The three sets of residuals came from the same otoliths and would be correlated; therefore, this result can be considered only approximate.

Another test of our reading criteria was performed through a more direct comparison: simply "aging" the tagged fish from estimated age at tagging (based on length), plus the time after tagging (Table 1). Out of 106 samples, 75% of these fish were within one year of the age that we had determined from otolith readings, and 94% were within two years. The average percent error (Beamish and Fournier, 1981) was 8.70, and the average deviation from tagged-based age was −0.075. Results of a Z-test indicated that the average deviation was not significantly different from zero ($P=0.724$) and indicated no bias in the age estimates.

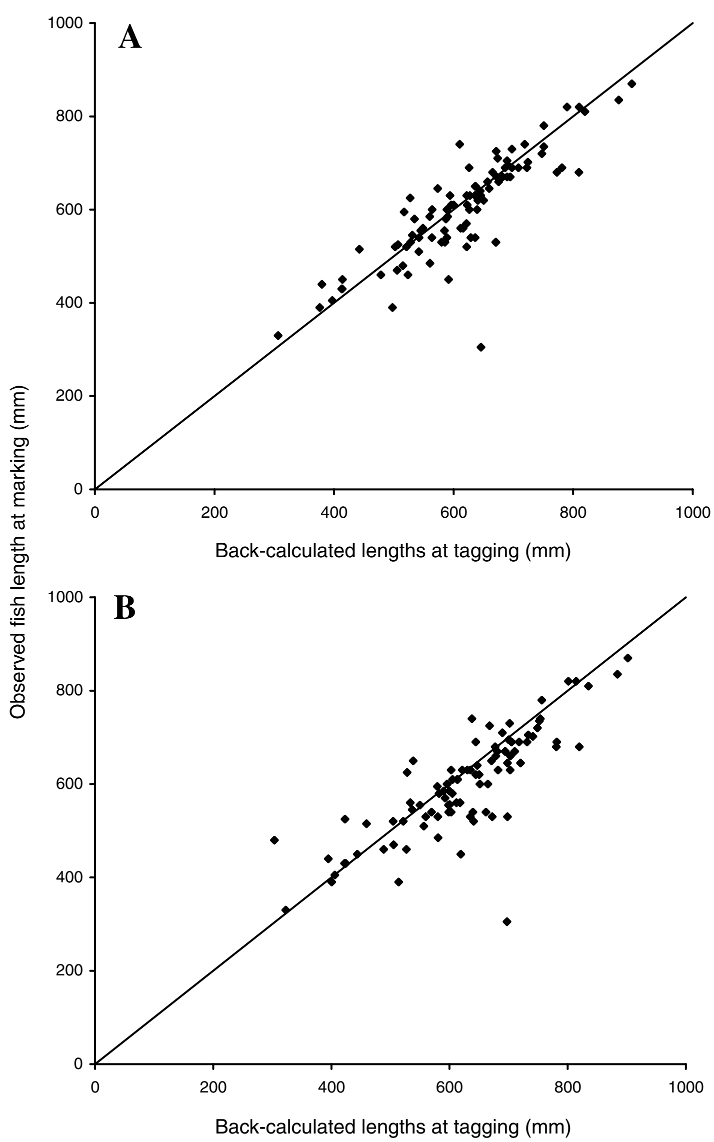

Figure 3

Fish length at tagging was back-calculated from estimated otolith size at tagging and time at liberty. Two separate back-calculations were performed, each with a different measure of the otolith size at tagging: one using (**A**) the area; the other using (**B**) the major axis.

Discussion

Beamish and McFarlane (1983) noted that "validating a method of age determination is as important in fishery biology as standardizing solutions or calibrating instruments are in other sciences." Age determination must reflect the actual age of each fish in order to be a useful tool for use in stock assessments. Although much effort has been devoted in the past to finding an appropriate aging structure for Pacific cod, particularly with dorsal fin rays (Beamish, 1981; Lai et al., 1987; Kimura and Lyons, 1990), scales and otoliths (Lai et al., 1987; Kimura and Lyons, 1990), a directly validated method of age determination has yet to be found (Westrheim, 1996). The otolith seems to be the most promising structure for production (large-scale) age reading of Pacific cod (Kimura and Lyons, 1990); however it is not without weaknesses (i.e., the faint patterns of some translucent zones can lead to low precision between readers and are a constant concern in regard to under- or overestimated ages). The key difficulty of the cod otolith patterns is differentiating the translucent zones that are annual from the translucent zones that are checks, particularly in young fish. It is necessary to have validated criteria in order to confidently eliminate checks without under-aging the fish. In our study, we have given two examples of indirect validation for Pacific cod age determination by using otoliths from marked and recaptured fish.

In the first example, we used back-calculations to test our reading criteria, which exclude counting lighter translucent zones. Early in the study, we found a strong relationship between otolith size and fish length, which supported using back-calculations as a vehicle to test accuracy. Overall, using strong translucent zones to back-calculate fish length at tagging gave fairly accurate results. This finding supports the assumption that translucent zones are laid down on an annual basis.

An ancillary finding was that otolith area measurements provided more accurate estimates of fish length than otolith lengths. Although back-calculations are typically performed by using radial or diametral measurement, the more accurate estimates of fish length from otolith area measurements are not surprising in that otolith area is a more comprehensive measure of otolith three-dimensional growth.

A second indirect validation of reading criteria was possible by estimating how much each tagged fish grew (millimeters) between tagging and recapture by its estimated age at recovery and von Bertalanffy growth parameters (derived only from tagging data). When compared to the observed growth increments, we found that the results support our proposed aging criteria (which exclude lighter translucent zones) because these criteria give the best fit to growth increments based on the mark-recapture growth increments. Aging the fish older (by counting light translucent zones) or younger (counting less annuli by banding translucent zones together) increases the residual fit to the mark-recapture growth increments. Large growth increments of fish length were difficult to estimate (Fig. 4). A possible explanation is that the longer a fish remains at liberty, the more likely that the growth becomes asymptotic, making the relationship between the growth increment and time at liberty less exact.

The final test for reading criteria was performed through a more direct comparison: simply "aging" the tagged fish by its length-at-release plus its time at liberty after tagging and comparing that age to the otolith-based age at recovery. Dwyer et al. (2003) also used this method in their study of yellowtail flounder (*Limanda ferruginea*). Average deviation from tag-based age was −0.075; 75% of these fish were found to be within one year of our age according to otolith readings, and 94% were within two years. These results provide further evidence that the current criteria do not result in the underestimation of the age of the fish and support the practice of not counting checks.

We found that growth information residing in otoliths from tagged and recovered Pacific cod provided significant information applicable to indirectly validating otolith aging criteria. Therefore, it seems that otoliths from other species that were tagged and recovered might be useful for indirect age validation as well. The

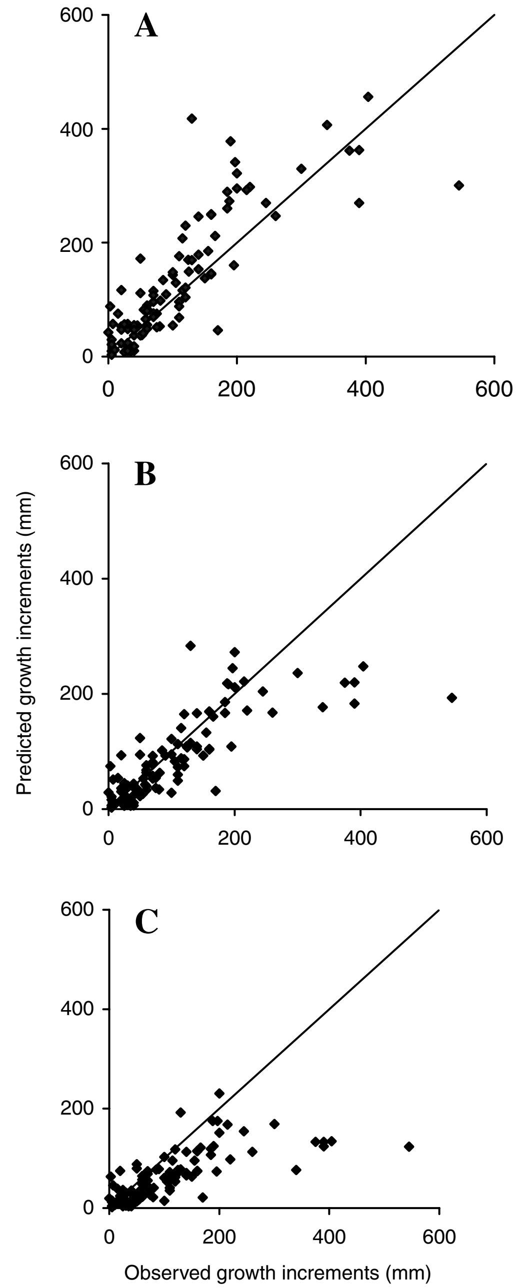

Figure 4

Three plots comparing predicted and observed fish-length growth increments by using recaptured fish from tagging experiments (n=97). Estimated ages at recovery (**B**) were scaled 25% smaller (**A**) and 25% larger (**C**). The lines indicate theoretical 1:1 line of perfect agreement.

information provided in our study indicates that our aging criteria for Pacific cod are roughly correct and that errors are probably within plus or minus 1 or 2 years. However, the problem of the shift in length at age alluded to in the introduction is more difficult to elucidate. Analysis made during this study seems to indicate that both environmental growth factors and changes in otolith aging criteria could have played a role in this apparent shift.

Acknowledgments

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