An Examination of Criteria for Determining the Age of Pacific Cod *(Gadus macrocephalus)* from Otoliths

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AN EXAMINATION OF CRITERIA FOR DETERMINING THE AGE OF PACIFIC COD (*Gadus macrocephalus*) FROM OTOLITHS

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Biological Station, Nanaimo, B. C.

March 1970

I. INTRODUCTION

At the Nanaimo Station numerous attempts have been made during the past decade to find a satisfactory technique for determining the age of Pacific cod. Various body parts have been examined for consistent patterns (e.g. on scales, vertebrae, otoliths, and opercular bones) which might provide some indication of annual, seasonal (or other) growth zones. None of these hard parts showed any particular promise, though it was recognized at the time that someone with extensive experience with fish scales might have had more success than personnel of the groundfish staff whose experience with age determinations was based largely on the use of otoliths of species other than cod. Such work, as has been published on the growth and mortality rates of cod inhabiting waters off the Canadian coast, has depended partly on the results of tagging and partly on observation of seasonal changes in the size composition of fish of age 2 (completed years) or less (Ketchen, 1961; 1964).

It has been suggested that the lack of distinctive growth zones in the otoliths of Pacific cod is a reflection of the relatively high temperature regime -- since cod inhabiting waters off British Columbia are close to the southern limit of their range (Ketchen, 1961: 534). Results of tagging suggest there is little if any change of growth rate in any particular season of the year. Initially Bering Sea cod otoliths appeared to be easier to "read" than those from regions farther to the south. However, it is now apparent, from comparisons of otoliths from such widely scattered regions as the Sea of Japan, Eastern Bering Sea, and British Columbia, that the otolith of the Pacific cod may be intrinsically divest of clear growth zones, regardless of temperature regime. When otolith specimens from any of the foregoing areas are compared with specimens of Atlantic cod (Gadus callarias), from such areas as the Gulf of St. Lawrence and the North Sea, there is no difficulty whatsoever in distinguishing the former from the latter.

The fact that the Pacific cod otolith, by comparison with otoliths of cod from at least some regions of the Atlantic, is much more difficult to read has been confirmed by cod otolith experts at both the Biological Station, St. Andrews, and the Marine Laboratory, Aberdeen. It is not a complete exaggeration to say that the best (most readable) specimen of Pacific cod otoliths is worse than the worst specimen which the writer has examined from the Atlantic.

Obviously, there does not seem to be any shortage of reasons for abandoning the use of otoliths for ageing of Pacific cod. Results of tagging provide some evidence that there are marked changes in growth rate from year to year (cf. Ketchen, 1964; Thomson, MS) -- to an extent sufficient to argue against the use of an inflexible age-length key developed from tag return information alone. These differences in growth rate may be related to the obviously large fluctuations in abundance and concomitant variations in size composition (see Ketchen, 1967). On the other hand, variations in the amounts and quality of food available for cod may occur from year to year. In any event, much difficulty is experienced in identifying certain sizes of fish with a particular age -- and hence there is great difficulty in estimating strengths of year-classes and other population parameters. The need for more accurate estimates than those now available is therefore a most compelling one which has led to still another attempt to find some objective procedure for estimating the age of cod from otoliths.

This report summarizes work conducted by the writer at various intervals between 1966 and 1968. The study differed from previous efforts in that an attempt was made to relate otolith size (shortest diameter of the transverse section through the centre -- actually the thickness) to length of fish, and from this an attempt was made to quantify the diameters of what seemed to be age zones and to estimate size for age by means of back-calculation.

This treatment is unique, and indeed perhaps foolhardy, since no one to the writer's knowledge has ever attempted to conduct back-calculations from cross-sections of otoliths. On prima facie evidence this procedure seems like a hazardous, error-fraught venture, since much would depend on the consistency of the position of the cross-section and natural variability in the thickness, shape, and peripheral crenulations of the otolith. That such a task was even contemplated, let alone carried out, reflects the desperation of the search for a reliable routine procedure for age determination. The underlying philosophy was that (1) if the otolith diameter bears a reasonable consistent relationship to fish length, and (2) if one were to measure as objectively as possible the regions which appeared to represent annual or seasonal growth checks, then it should be possible to set up some simple criteria for training of otolith readers. For example, it might be possible to define a mean, standard deviation and range of measurements which would provide the otolith reader with clues to the regions on the otoliths where annual or seasonal checks should occur.

II. MATERIALS

Collections of otoliths used in this study were obtained from the following sources:

(a) Juvenile fish collections of the INVESTIGATOR NO. 1 in 1952 (Hecate Strait).

(b) Juvenile fish collections of the G.B. REED in 1965 (Hecate Strait).

(c) Otoliths of cod tagged in 1964 and recaptured during the succeeding two years (Hecate Strait).

(d) Samples from the Sea of Japan and Bering Sea; and Atlantic cod samples from Gulf of St. Lawrence and North Sea.

III. METHODS

1. Grinding and polishing

The otoliths were first broken transversely (Fig. 1a) by hand and then ground and polished with a jeweller's electric grinder with polishing attachments. When the break occurred off-centre, as sometimes was the case, the larger of the two otolith pieces was used for grinding the transverse surface down to the point where the otolith centre was considered to lie.

2. Measurements of the transverse surface

In initial trials, the polished otolith was first mounted on a slab of black plasticine. The beam of a microscope lamp was directed at the exposed side of the otolith and controlled in such a way that no light shone directly on the cut surface of the otolith. The technique of mounting (Fig. 1b) and the special device used for shielding the surface from direct illumination were identical to those described by Bedford (1964: 80). This method permitted control of the amount of light striking the flat (side) surface of the otolith, and hence control of the amount of light refracted upwards through the polished face of the transverse section.

After reading the complete selection of otoliths, it was decided that greater clarity of ring formation could be achieved by imbedding the otolith deep in the block of plasticine (with the transverse surface flush with the surface of the plasticine). In this case direct illumination of the polished surface was from above and slightly to the side (Fig. 1c). The technique is not novel, having been used by various investigators in eastern Canada and Europe.

Under direct lighting, as opposed to refracted lighting as first described, the so-called winter checks being transparent are dark, whereas the opaque summer growth zones appear white. The reverse picture is obtained by the refracted lighting technique. Although the former method is apparently widely accepted and used in studies of Atlantic cod, the latter technique seemed to the author to be somewhat more helpful than the former insofar as Pacific cod are concerned.

Subsequently, during the preparation of specimens for photography, another technique of mounting and lighting was developed which is perhaps superior to either of those mentioned above. The discovery, involving the use of thinly sliced cross-sections of otoliths, came too late for its use in the present analysis. However, a description is given in Appendix C.

We proceed now to the technique of otolith measurement. The polished face of the imbedded otolith was examined through a low power (×12) binocular microscope equipped with a micrometer eyepiece. In an effort to establish a standardized procedure for measurement of otolith and ring diameters, which

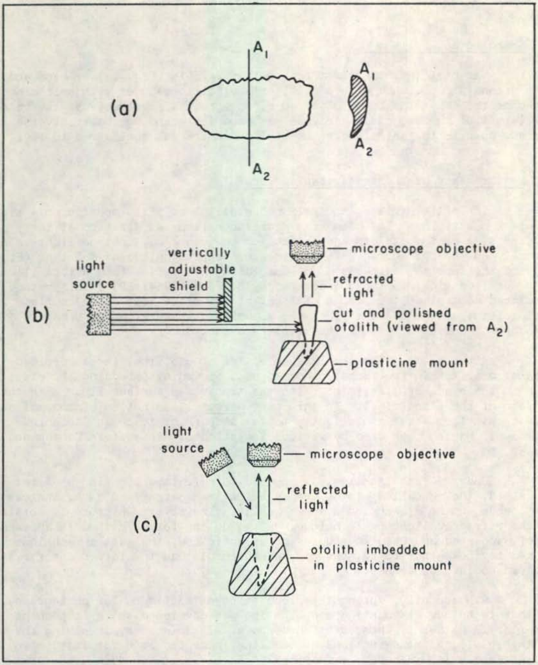

Fig. 1. Methods of (a) cutting and mounting otoliths for (b) refracted illumination, and (c) reflected illumination.

would minimize the effect of natural variations in the shape of the polished face, the otolith was aligned as well as possible in the position illustrated in Fig. 2. The right-hand side of the face lying above the imaginary line X_1X_2 was adjusted so that it was always as close as possible to being parallel to line C_1C_2. In most cases the line PQ joining the points of intersection of line X_1X_2 with B_1B_2 and C_1C_2, represented the position of greatest width (thickness) of the otolith. The shape and thickness varies among fish of a given fish length, being frequently distorted by abnormalities on the face of the otolith (e.g. small globular protuberances from the otolith surface). Thus, at all times a certain amount of judgment was required in order to maintain reasonable consistency in standardizing the axis and the measurements to be made along it.

Along line PQ measurements were made, first of the otolith diameter and then the diameters of what might be regarded as checks in the annual growth pattern.

IV. RESULTS

1. Relationship of otolith diameter to fish length

A total of 571 otoliths of cod ranging from 17 cm to 88 cm in total length, and collected at various dates (between May 1964 and May 1966) from Hecate Strait, were measured to determine the relationship between otolith diameter and fish length. The regression of fish length on otolith diameter (thickness) is shown in Fig. 3 and the line of best fit (by least squares) is expressed by

$$Y = 2.00096\ X^{1.62841}$$

where $\hat{Y} = \log_{10}$ fish length in mm, and $X = \log_{10}$ otolith diameter in mm. The predictive value of this equation, as indicated by the 68% confidence limits, is clearly subject to increasingly large error for otolith diameters over 2.0 mm. Nevertheless, the equation will form the basis of the computations which follow.

The calculated curve passes almost exactly through the origin -- so close that there is no necessity for a correction factor in back-calculation.

2. The otoliths of 1-year-old cod

There is sufficient evidence at hand to provide assurance that cod of one to two years of age can be identified by length composition alone. From earlier work it was considered that the mean length at age 1.0 years (at the

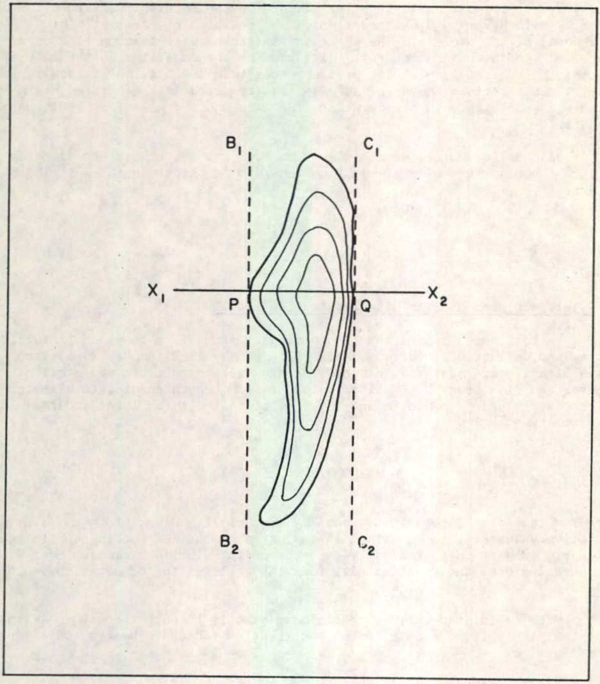

Fig. 2. Method of standardizing the alignment of the cut face of the otolith for measurements along the X_1X_2 axis.

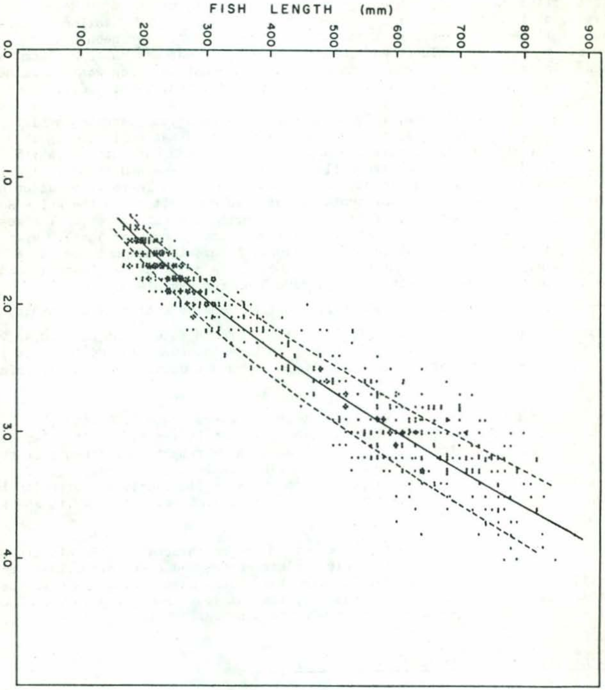

Fig. 3. Regression of fish length on otolith diameter (thickness). Broken lines indicate 95% confidence limits for individual values of Y, fish length.

first birthday, ca. mid-February) was in the vicinity of 26 cm in Hecate Strait, as in the Strait of Georgia (Ketchen, 1964). It was cautioned, however, for the former region, that the mean length might have been biased upwards by mesh selection. Possibly the true value was as low as 22.3 cm, as back-calculated from the von Bertalanffy growth equation which was determined from growth of tagged fish of age 2.0 or older (Ketchen, 1964: 1061).

On March 14, 1964, a large sample of 1-year-olds (assuming a birth date of February 14: 13 months or 1.08 years of age) was collected in the vicinity of the principal spawning ground in northern Hecate Strait (White Rocks ground). These fish were collected with a small-meshed trawl (1 1/4-inch codend) and had an average length of 23.0 cm (Table I). There is no assurance that the sample was fully representative of size composition on the White Rocks ground, or for that matter, of all areas of northern Hecate Strait. The mean length is in close agreement with the aforementioned length at age 1.0 years projected from the von Bertalanffy equation. If growth does not come to a complete halt in winter we might expect these fish of 1.08 years were somewhat smaller on the assumed birthday -- to have been for example possibly $\frac{1.00}{1.08} \times 230$ or 214 mm at age 1.0 years. Neither the selection of a February 14 birth date nor the assumption of linearity in growth rate is on particularly solid ground, thus no real significance can be attached to the differences in the estimated length at age 1.0 years. There must undoubtedly be some differences from year to year.

From the sample of 946 fish caught at assumed age 1.08 years, a stratified subsample of 242 fish was used for calculation of otolith diameters in each centimeter size class. The subsample measurements were then prorated to the full sample and the frequency distribution is shown in Table I. The distribution is expressed as a percentage in Table II (Sample A) for which the corrected* mean otolith diameter was found to be 1.66 mm, with $\underline{s}$ = .15 and range of 1.3 to 2.2 mm.

Referring back now to the question of growth zones on the otoliths, one would expect that if a discernible winter check had been established around the time of the first birthday, its diameter should have parameters corresponding to figures slightly less or possibly the same as those given above. The evidence in support of such a check will be considered later.

3. Otoliths of cod in their second year

On June 16, 1952, samples of 43 cod were collected from the Horseshoe Ground in southern Hecate Strait. These fish were in their second year of

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*To obtain true measurements from the micrometer eyepiece, all measurements of mean diameters of otoliths or circuli were multiplied by a calibration factor of 0.977.

Table I. Frequency distribution of otolith diameters of cod at estimated age 1.08 years (13 months - March 15, 1965).

Fish Length (mm)	Otolith diameter (mm)										Total Number
	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	
170	..	2	..	2	1	..	..	..	..	..	5
180	1	3	5	1	4	..	..	..	..	..	14
190	2	12	16	10	5	2	..	..	..	..	47
200	..	8	36	20	8	8	..	..	..	..	80
210	..	7	20	20	48	27	7	..	..	..	129
220	..	..	35	48	41	14	7	..	..	..	145
230	..	..	17	60	60	9	9	9	..	..	164
240	..	..	..	33	20	39	46	..	..	..	138
250	..	..	5	14	27	32	5	9	..	..	92
260	..	..	..	..	13	25	16	9	..	..	63
270	..	..	..	4	4	11	13	8	..	2	42
280	..	..	..	..	..	2	5	2	4	1	14
290	..	..	..	..	..	2	2	3	1	..	8
300	..	..	..	..	..	..	1	2	..	..	3
310	..	..	..	..	..	1	..	1	..	..	2
Total	3	32	134	212	231	172	111	43	5	3	946
Mean fish length (mm)											230

Table II. Frequency distribution of otolith diameters of cod at 1.08 years of age (Sample A) and at approximately 1.33 years of age (Sample B).

	Diameter (mm)												Total
	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.5	
Sample A													
No.	3	32	134	212	231	172	111	43	5	3	..	..	946
%	0.3	3.4	14.2	22.4	24.4	18.2	11.7	4.5	0.5	0.3	..	..	99.9
Sample B													
No.	..	..	..	1	1	5	4	10	6	12	2	1	43
%	..	..	..	2.3	2.3	11.6	23.2	14.0	27.9	4.7	2.3	2.3	99.9

	Uncorrected (mm)		Corrected (mm)	
	$\bar{x}$	s	$\bar{x}$	s
Sample A	1.70	0.15	1.66	0.15
Sample B	2.04	0.18	1.99	0.18

growth (4.0 months beyond the February 14 birthday) and hence were, according to the classification used here, approximately 1.33 years of age. As shown in Table II (Sample B), the diameter of the otoliths ranged from 1.6 to 2.5 mm and had an average diameter of 1.99 mm with s = 0.18. Again, assuming that transparent and opaque zones ("winter" and "growing season" zones) are discernible in the otolith, the above values would indicate the approximate position of the uncompleted summer growth.

4. Otoliths of 2-year-old cod

Ketchen (1964) concluded, from results of sampling conducted aboard the research vessel, A.P. KNIGHT, that Hecate Strait cod (in 1960) reached an average length of 43.8 cm at completion of the second year of life, i.e. at age 2.0 years. The range was estimated as 32 to 55 cm with s = 3.5 cm. Assuming that growth is reasonably constant from year to year up to age 2.0 years, we may estimate from the equation on page 6 that the corresponding mean otolith diameter at that time is 2.47 mm (range: 2.0-2.8; s = 0.12). If a growth check or winter zone is laid down at the time of the second completed year (age 2.0), its diameter should correspond approximately to the above parameters.

5. Otoliths from recaptured tagged cod

(a) Cod tagged at age 2.25 years and recaptured at 2.45 years

(i) In mid-May 1964 extensive sampling and tagging, principally of fish considered to be age 2.25 years, was conducted along the northern Hecate Strait "edge". A total of 3674 cod were caught, of which close to 90% could be considered with reasonable certainty to be in their third year of growth. Rough fitting of a normal curve to the size distribution of these fish, provided an estimated mean length of 474 mm; s = 3.4 and a range of 360 to 570 mm.

It should be emphasized that these values apply to all captured fish, not just those which were tagged. If we refer again to the equation on page 6, we may estimate the corresponding figures for otolith diameters (viz. mean = 2.54 mm; s = 0.10; range: 2.20-2.90).

(ii) Tagged cod which are recaptured by fishermen or retrieved during processing ashore are usually turned over to FRB port observers who then take a measurement of length and remove an otolith for subsequent laboratory examination. In July 1964 there were 56 usable tag recoveries, of which 29 were probably of age 29 months or 2.42 years. It was from this group of fish, ranging in length at time of tagging from 360 to 570 mm, that measurements were made, first of the otolith diameters and then the diameters of the first and second so-called winter checks. These checks will be considered later, since at the moment we are concerned only with the probable otolith diameters at known ages. The diameters are shown in the third column of Table III.

Table III. Frequency distribution of diameters of suspected first and second winter checks, as well as marginal diameters of otoliths from cod tagged at assumed age of 2.25 years and recaptured at age 2.45 years.

Otolith diameter (mm)	First check	Second check	Margin
1.0	..	..	..
1.1	..	..	..
1.2	..	..	..
1.3	1	..	..
1.4	5	..	..
1.5	5	..	..
1.6	5	..	..
1.7	9	..	..
1.8	3	..	..
1.9	1	..	..
2.0	..	2	..
2.1	..	3	..
2.2	..	5	1
2.3	..	9	..
2.4	..	3	2
2.5	..	6	3
2.6	..	1	8
2.7	..	..	8
2.8	..	..	1
2.9	..	..	3
3.0	..	..	3
3.1	..	..	..
N	29	29	29
$\bar{x}$*	1.56	2.22	2.61
<u>s</u>	0.27	0.16	0.19

*Corrected for microscope calibration.

(b) Cod tagged at age 2.25 years and recaptured at 3.45 years

In July 1965 there were 62 tagged cod recaptured from the May 1964 tagging, of which there was reasonable assurance that 41 were in their third year at time of tagging and hence in their fourth year of growth when recaptured. By July 1965, then, they should have possessed three winter checks as fish of age 3.45 years (41 months).

The frequency distribution of the diameters of otoliths of these 41 fish is given in the fourth column of Table IV. The suspected distributions of the first, second, and third winter "checks" are shown in the other columns and will be considered below.

6. Comparison of diameter of "winter checks" with diameter of otolith margin

In the foregoing calculations it may be assumed the measurements of otolith diameters were made with reasonable objectivity -- granted, of course, that there may have been errors in technique or abnormalities in the shape of the otolith. Measurements of winter checks, on the other hand, were subjective, being dependent on what the writer judged to be a valid check (i.e. a transparent deposition separating the opaque summer growth zones).

The "objective" measurements of otolith diameters (mean, standard deviation and range) are shown in Fig. 4, part I, as sets A, B, C. D, E, and F, derived from sections 2, 3, 4, 5 (a) (i), 5 (a) (ii), and 5 (b), respectively, above. The solid line was fitted by eye to show the trend of the mean diameters, while a similar fit was applied to the standard deviation (broken line) and range (dotted line).

The "subjective" observations (Fig. 4, part II) are the combined measurements for ages 1 and 2 from Tables III and IV; the combined measurements for these and older ages as obtained from a stratified sample of 171 otoliths collected in May 1966 (Table V). All of these data have been superimposed on the eye-fitted curves of mean diameter, standard deviation, and range reproduced from Fig. 4, part I.

The mean and standard deviation of data for fish of age 1.0 appear to fit closely the expected values. Thus there appears to be reasonable confidence in the interpretation of the first winter annulus. Data for age 2 would have to be shifted appreciably to the left, i.e. to about age 1.5 (18 months) in order to fit the expected distribution. Data for age 3 would require a major shift (to age 2.17 years or 26 months) in order to accord with the expected distribution, while those for age 4 would have to be shifted back to age 3.5 (42 months). Obviously, there is no uniformity in the degree to which the observed diameters depart from the expected and hence it would be difficult to postulate that the so-called winter check is deposited at some consistent time other than the assumed February 14 birth date.

Table IV. Frequency distribution of diameters of suspected first, second, and third winter checks and marginal diameters of otoliths from cod tagged at assumed age of 2.25 years and recaptured at age 3.45 years.

Otolith diameter (mm)	First check	Second check	Third check	Margin
1.2	..	..	..	..
1.3	6	..	..	..
1.4	6	..	..	..
1.5	7	..	..	..
1.6	9	..	..	..
1.7	8	..	..	..
1.8	2	1	..	..
1.9	1	3	..	..
2.0	2	3	..	..
2.1	..	9	..	..
2.2	..	9	..	..
2.3	..	5	1	..
2.4	..	6	6	..
2.5	..	3	10	..
2.6	..	1	2	3
2.7	..	1	6	4
2.8	..	..	10	9
2.9	..	..	2	3
3.0	..	..	2	8
3.1	..	..	1	6
3.2	..	..	..	4
3.3	..	..	1	2
3.4	..	..	..	1
3.5	..	..	..	1
3.6	..	..	..	..
N	41	41	41	41
$\bar{x}$*	1.53	2.17	2.61	2.89
s	0.19	0.20	0.22	0.23

*Corrected for microscope calibration.

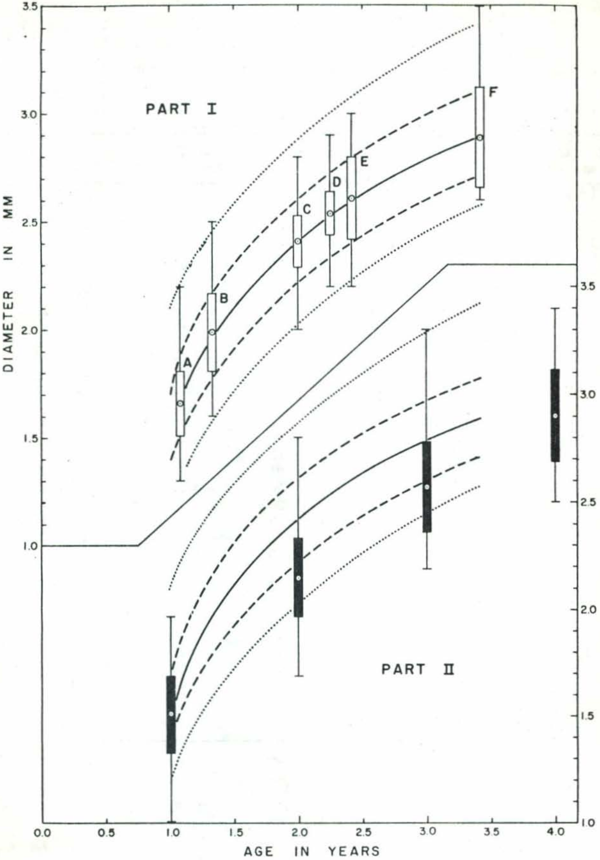

Fig. 4. Part I: "Observed" otolith diameters of cod of known age, with curves of mean size, standard deviation, and approximate range. Part II: Diameters of checks supposedly representing ages 1.0 through 4.0 years, superimposed on the curves derived in Part I. (See text for further details.)

Table V. Frequency distribution of suspected winter checks and marginal diameters of otoliths contained in a special stratified sample of cod from Hecate Strait, in May 1966. (Size range: 36 cm to 88 cm).

Otolith diameter (mm)	First check	Second check	Third check	Fourth check	Margin
1.0	2	..	..	..	..
1.1	2	..	..	..	..
1.2	4	..	..	..	..
1.3	12	..	..	..	..
1.4	25	..	..	..	..
1.5	39	..	..	..	..
1.6	38	..	..	..	..
1.7	27	..	..	..	..
1.8	17	1	..	..	..
1.9	3	5	..	..	..
2.0	2	24	..	..	2
2.1	..	43	..	..	6
2.2	..	27	1	..	9
2.3	..	19	5	..	7
2.4	..	13	13	..	7
2.5	..	10	21	2	16
2.6	..	2	20	..	10
2.7	..	..	20	3	6
2.8	..	1	8	12	10
2.9	..	..	5	8	17
3.0	..	..	3	12	18
3.1	..	..	1	5	8
3.2	..	..	3	6	17
3.3	..	..	..	2	17
3.4	..	..	..	3	8
3.5	..	..	..	..	3
3.6	..	..	..	..	3
3.7	..	..	..	..	2
3.8	..	..	..	..	3
3.9	..	..	..	..	1
4.0	..	..	..	..	1
N	171	145	100	53	171
$\bar{x}$	1.51	2.14	2.56	2.90	
$\underline{s}$	0.18	0.17	0.23	0.20	

The second possibility, namely, that the data for age 2.0 are measurements of a false check has rather little support. Removal of the data for age 2.0 and a shift of the data for age 3.0 to 2.0, and similarly age 4.0 data to age 3.0, would over-correct in the sense that the means and standard deviations would turn out to have higher values than those expected from Fig. 4, part I.

V. DISCUSSION

1. Interpretations

The initial interpretation of Fig. 4, part II is that either (a) the diameter of the so-called winter check was fairly consistently underestimated for ages greater than 1 year, or (b) assuming accuracy of the curve linking means of A through F, the check which was interpreted as a winter zone was in fact a check deposited at some time other than the winter "birthday", or (c) the set of data representing age 2.0 fish reflect a false annulus.

If there are substantial year-to-year changes in growth rate, then it would not be unreasonable to find departures from the expected pattern of winter checks similar to those portrayed in Fig. 4 (part II). On the other hand, the discrepancies may be the result of selection -- namely, at all ages (except age 1.0) the cod which are vulnerable to capture are the larger individuals of those age groups. Thus the otolith diameter of fish captured at any given time of the year is larger than the true population mean, and hence back-calculations will always give smaller measurements of the winter growth check. That some sort of selection (creating a Lea's phenomenon) may exist is not unreasonable, but it does seem unlikely that the discrepancies would be as large as those indicated. As we shall see later, they are not supported by observations in nature nor by information on the growth of tagged fish.

A point which suggests that the discrepancies might be even <u>greater</u> than those discovered concerns the personal bias of the writer. For a reliable test, the otolith reader (measurer) should have no previous knowledge of the expected diameters of the three winters' checks (if such do indeed exist). In actual fact, as is apparent from the procedures which have been described, the writer was well aware of the diameters of otoliths for various ages of fish before the actual calculation of ring diameters was attempted. In theory, such prior knowledge should have forced the data of Fig. 4, part II, to much closer conformity with those of part I than was actually the case.

It is instructive to compare measurements of "winter checks" for a particular year-class and also make comparisons among year-classes, the latter being desirable in order to determine whether deviations of the observed distribution from the expected one may be due to year-to-year changes in growth rate. Table VI compares the observed diameter of the second "annulus" on tagged fish

Table VI. Comparison of the frequency distribution of diameters of the second "winter check" among cod of the 1962 year-class caught at three successive ages.

Month and year of capture	July 1964	July 1965	May 1966
Estimated age	2+	3+	4+
Diameter (mm)			
1.8	..	1	..
1.9	..	3	1
2.0	2	3	12
2.1	3	9	15
2.2	5	9	12
2.3	9	5	5
2.4	3	6	1
2.5	6	3	1
2.6	1	1	1
2.7	..	1	..
2.8	..	..	..
N	29	41	48
$\bar{x}$	2.22	2.17	2.14
s	0.16	0.20	0.14

of the 1962 year-class with the diameter of the same "annulus" as it appeared on tagged fish retrieved 1 year later. The last column of the table provides data on the same second annulus from the stratified sample collected in 1966 (Table V), namely, when the 1962 year-class had reached age 4+. Although there is no statistical difference (as determined by t test) in the mean diameters, there is a suggestion of a downward trend in the diameter of the second "annulus" with increasing age. Here again, we have indications of Lea's phenomenon. If, individually, the three sets of data were superimposed on the curves of Fig. 4 (part II), they would still fall below the expected values at age 2.0. Unless the 1962 year-class contained individuals which were smaller than average at each age, the consistency in the "underestimation" must be regarded as no accidental phenomenon. Although the cod probably were aged correctly, what was taken to be the second annulus (or more precisely a check laid down at the time of the second birthday) was not so.

Table VII compares the second "annulus" of two different year-classes. The data for the 1962 year-class for this purpose have been summed from the first two columns in Table VI. According to a t test, the mean diameters of the second annulus of the 1962 and 1964 year-classes do not differ significantly.

2. Growth determined from tagged fish as compared with that from otolith measurements

For each annulus diameter the equation on page 6 was used to compute the expected fish length. In this case we are determining a single estimate of $\hat{Y}$ from a single measurement of X. There must, of course, be a large error term when we recall the variability of Y as portrayed by Fig. 3. The calculated distributions of fish length at various birthdays are shown in Fig. 5. Superimposed on these distributions is the growth as observed from tagged fish. Shown separately are four growth curves, three of which were determined from three length groups, 44 cm, 47 cm and 50 cm at time of tagging* -- three groups which in all probability were composed almost entirely of age 2+ years (actually 2.25 years). The data on which these observations are based may be found in the table marked Appendix A. This table contains the combined results of taggings conducted in May 1964 and May 1965, and shows the quarterly growth increments of recaptures for all 3-cm length groups tagged.

*Fish at time of tagging which were 43 to 45 cm in length were identified as the 44 cm group; 46 cm to 48 cm, as the 47 cm group, etc.

Table VII. Comparison of the frequency distribution of diameters of the second "winter check" between two year-classes of cod.

Month and year of capture	July 1964 July 1965	May 1966
Year-class	1962	1964
Diameter (mm)		
1.8	1	1
1.9	3	5
2.0	5	24
2.1	12	43
2.2	14	27
2.3	14	19
2.4	9	13
2.5	9	10
2.6	2	2
2.7	1	..
2.8	..	1
N	70	145
$\bar{x}$	2.25	2.19
s	0.19	0.15

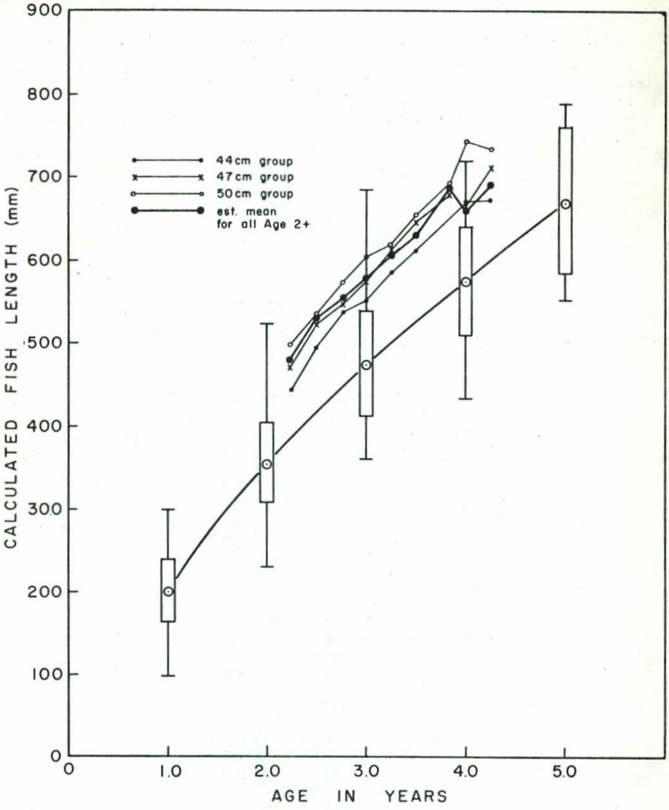

Fig. 5. Length distributions of cod, calculated from otolith measurements, at completed ages 1.0 through 5.0 years, compared with results of tagging (see text for further details).

The fourth and heavier growth curve in Fig. 5 is more subjective for it represents what is possibly the mean growth rate of all age 2+ fish, as provided in Appendix B.*

Although the slopes of the tagged fish curves are similar to that of the curve calculated from otolith measurements, the absolute values, age for age, are markedly higher than the predicted values. There are two possible explanations one of which has already been mentioned, namely, that there was consistent underestimation of the true diameters of winter check at each age (other than age 1). The other is that the tagged population was unrepresentative of the age 2+ fish, being comprised of the larger, faster growing individuals of that age group. Doubtless there would be some tendency in this direction but, again, not to the extent observed. In light of all observations made in Hecate Strait since 1960 the conclusion that cod of age 2.0 have a mean length of 35.5 cm is completely untenable. The true mean is closer to 43-45 cm or not unlike the distribution shown in Fig. 5 for age 3.0 fish. If we were to assume that all estimated lengths of fish at age 2.0 years were based on false checks on the otoliths, then the tagging results would conform very well with the growth curve by shifting ages 3.0, 4.0, and 5.0 to 2.0, 3.0, and 4.0, respectively.

Yet on checking back over those otoliths which might have been mismeasured in respect to the winter check, it is impossible to dismiss the second check as an accessory and hence assign an age of 1+ years instead of age 2+, for this would then make the mean size of age 1+ fish far larger than that observed in nature. It has to be concluded that such annuli were misidentified or otherwise mismeasured (because the winter check may actually be laid down before the winter and growth resumes during winter) but not necessarily erroneously aged.

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*To make this calculation it was assumed, partly from observations of the apparent split between age 2+ and age 3+ fish, that age 2+ fish comprised 100% of the size groups up to and including 47 cm; 89.6% of the 50 cm group at time of tagging; 57.5% of the 53 cm group; 5.4% of the 53 cm group; and 0% of the 56 cm group. In addition, correction factors were introduced to account for the obviously higher tagging mortality among the smaller size groups. Note in the last column of Appendix A that the percentage recapture from the 35, 38, 41, and 44 cm groups was markedly lower than those of groups 47 to 65 (the mean recapture rate of which was 30.14%). Thus the number of recaptures in the poorly represented groups was multiplied by factors of 1.99, 2.71, 1.86, and 1.41, respectively, in order to give them weight equal to that of the well represented groups. Such calculations are incorporated in Appendix B.

VI. CONCLUDING REMARKS

There can be no doubt about the difficulty of reading otoliths of the Pacific cod. The attempt to quantify the growth zones seems to have created more problems than it has solved. The hazardous effort to relate otolith diameter (thickness) to fish length may have been one of the reasons for failure.

Perhaps without support from his more skeptical associates, the writer remains convinced that with sufficient experience and improved techniques (such as that described in Appendix C, for example), the otolith can provide an indication of age to the well-trained eye. In this preliminary study, it cannot be denied that much guesswork was involved. Relatively few otoliths show as distinct annuli as do those of Atlantic cod, and there is some basis for suspecting that an independent, unbiased observer would experience much difficulty in reproducing the results obtained by the writer.

It is obvious that the assumption of a winter check being laid down at or near the birth date (mid-February) is open to question. It seems to apply to the first year but not to older ages. Checks must be occurring at times of year other than late winter. Perhaps seasonal variations in the kinds and amounts of avialble food play a greater role in annulus formation than do such seasonal phenomena as changes in water temperature or spawning condition.

At the moment it appears that further attempts to use otoliths for age determination are unwarranted. It is recommended that before work of this kind is resumed, consideration be given to the use of tetracycline, a drug which produces marks on scales, otoliths and other bony parts, as a means of checking the patterns of scale and otolith growth. Heavy tagging (ca. 3,000-4,000 fish) would be needed to accompany this treatment. Even if such an experiment could not be performed soon, a critical study should be made of scales by persons thoroughly experienced in such studies.

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APPENDIX A

CONSOLIDATION OF SIZE AT RECAPTURE DATA ON PACIFIC COD, BY SIZE GROUP AT TIME OF TAGGING AND BY THREE-MONTH PERIODS, FROM THE COMBINED MAY, 1964 AND MAY, 1965 TAGGINGS CONDUCTED IN NORTHERN HECATE STRAIT.

(Figures in parenthesis are the average lengths in cm per size group per recapture period.)

CM SIZE GROUP	0 AT TAGGING Sum of lengths	N	1 JUNE Sum of lengths	N	2-3-4 JUL-SEP Sum of lengths	N
35	1168 (35.4)	33	--	--	450 (45.0)	1
38	2398 (38.1)	63	--	--	450 (45.0)	1
41	10921 (41.2)	265	2949 (42.1)	7	3694 (46.2)	8
44	36139 (44.2)	817	14926 (45.2)	33	17368 (49.6)	35
47	56087 (47.1)	1192	36827 (48.5	76	36957 (52.1)	71
50	57406 (49.9)	1150	39793 (49.7)	80	46120 (53.6)	86
53	47145 (53.0)	890	20613 (52.9)	39	52251 (56.8)	92
56	50913 (55.9)	910	11990 (57.1)	21	63532 (61.7)	103
59	45962 (59.0)	779	10241 (60.2)	17	61258 (62.5)	98
62	27467 (61.9)	444	6427 (64.3)	10	36043 (65.5)	55
65	15531 (64.7)	240	4626 (66.1)	7	17717 (68.1)	26
68	5699 (67.8)	84	2069 (69.0)	3	7777 (70.7)	11
71	4539 (70.9)	64	4335 (72.2)	6	5900 (73.8)	8
74	2734 (73.9)	37	1522 (76.1)	2	4572 (76.2)	6
77	1913 (76.5)	25	--	--	2345 (78.2)	3
80	636 (79.5)	8	--	--	800 (80.0)	1
83	249 (83.0)	3	--	--	--	--
86	--	--	--	--	--	--

APPENDIX A CONTINUED

CM SIZE GROUP	5-6-7 OCT-DEC Sum of lengths	N	8-9-10 JAN-MAR Sum of lengths	N	11-12-13 APR-JUN Sum of lengths	N
35	--	--	--	--	527 (52.7)	1
38	--	--	1050 (52.5)	2	--	--
41	520 (52.0)	1	3732 (53.3)	7	1176 (58.8)	2
44	5192 (51.9)	10	20479 (55.3)	37	9926 (58.4)	17
47	7684 (54.9)	14	26582 (57.8)	46	18510 (61.7)	30
50	13767 (57.4)	24	36251 (60.4)	60	8032 (61.8)	13
53	11948 (59.7)	20	27767 (61.7)	45	7678 (64.0)	12
56	26593 (63.3)	42	17706 (63.2)	28	14273 (64.9)	22
59	20945 (65.5)	32	17953 (66.5)	27	7979 (66.5)	12
62	12205 (67.8)	18	11085 (69.3)	16	7097 (71.0)	10
65	7597 (69.1)	11	8004 (72.8)	11	2104 (70.1)	3
68	1401 (70.1)	2	--	--	736 (73.6)	1
71	2979 (74.5)	4	2311 (77.0)	3	2371 (79.0)	3
74	--	--	1601 (80.0)	2	--	--
77	--	--	--	--	800 (80.0)	1
80	--	--	--	--	--	--
83	--	--	--	--	--	--
86	--	--	--	--	--	--

APPENDIX A CONTINUED

CM SIZE GROUP	14-15-16 JUL-SEP Sum of lengths	N	17-18-19 OCT-DEC Sum of lengths	N	20-21-22 JAN-MAR Sum of lengths	N
35	550 (55.0)	1	--	--	--	--
38	518 (51.8)	1	--	--	--	--
41	3505 (58.4)	6	--	--	1134 (56.7)	2
44	8583 (61.3)	14	--	--	2020 (67.3)	3
47	25141 (64.5)	39	4786 (68.4)	7	4657 (66.5)	7
50	19718 (65.7)	30	2763 (69.1)	4	762 (76.2)	1
53	16763 (67.1)	25	1998 (66.6)	3	2929 (73.2)	4
56	7363 (73.6)	10	4847 (69.2)	7	2085 (69.5)	3
59	5546 (69.3)	8	2180 (72.7)	3	2984 (74.6)	4
62	2872 (71.8)	4	701 (70.1)	1	775 (77.5)	1
65	1376 (68.8)	2	--	--	--	--
68	743 (74.3)	1	--	--	--	--
71	748 (74.8)	1	--	--	--	--
74	--	--	--	--	--	--
77	--	--	--	--	--	--
80	--	--	--	--	840 (84.0)	1
83	--	--	--	--	--	--
86	--	--	--	--	--	--

APPENDIX A CONTINUED

CM SIZE GROUP	23-24-25 APR-JUN Sum of lengths	N	26-27-28 JUL-SEP Sum of lengths	N	29-30-31 OCT-DEC Sum of lengths	N
35	670 (67.0)	1	--	--	--	--
38	1252 (62.6)	2	--	--	--	--
41	2020 (67.3)	3	--	--	--	--
44	4710 (67.3)	7	--	--	--	--
47	10710 (71.4)	15	--	--	--	--
50	3669 (73.4)	5	--	--	--	--
53	3021 (75.5)	4	782 (78.2)	1	--	--
56	720 (72.0)	1	--	--	1561 (78.1)	2
59	1573 (78.7)	2	--	--	--	--
62	1539 (77.0)	2	--	--	--	--
65	--	--	--	--	--	--
68	773 (77.3)	1	--	--	--	--
71	--	--	--	--	--	--
74	--	--	--	--	--	--
77	--	--	--	--	--	--
80	--	--	--	--	--	--
83	--	--	--	--	--	--
86	--	--	--	--	--	--

APPENDIX A CONTINUED

CM SIZE GROUP	TOTAL TAG RETURN*	% RECAP
35	5	15.15%
38	7	11.11%
41	43	16.23%
44	175	21.42%
47	349	29.28%
50	348	30.26%
53	281	31.57%
56	278	30.55%
59	231	29.65%
62	131	29.50%
65	72	30.00%
68	23	27.38%
71	27	42.19%
74	12	32.43%
77	5	20.00%
80	4	50.00%
83	1	33.33%
86	--	--

*Includes data on broken tags, and tags for which no information was provided on size and/or date of recapture.

APPENDIX B

ESTIMATED SIZE COMPOSITION OF PACIFIC COD OF AGE 2+ YEARS AT TIME OF TAGGING, BY SIZE GROUPS AND BY THREE-MONTH PERIODS OF RECAPTURE--AS COMPUTED FROM THE BASIC DATA CONTAINED IN APPENDIX A

CM SIZE GROUP	AT TAGGING (MAY)		JUNE		JUL-SEP	
*35	1168	33	--	--	896	2.0
*38	2398	63	--	--	1220	2.7
*41	10921	265	5485	13.0	6871	14.9
*44	36139	817	21046	46.5	24489	49.4
47	56087	1192	36827	76.0	36957	71.0
**50	51436	1030	35655	72.0	41324	77.0
**53	24280	458	10616	20.0	26909	47.0
**56	2749	49	648	1.1	3407	5.6
TOTAL	185178	3907	11027	228.6	142073	269.6
AVERAGE LENGTH	47.4		48.2		52.7	

*Corrected for effects of differential tagging mortality (see text).

**Corrected for possible presence of age 3+ fish in these size groups (see text).

APPENDIX B CONTINUED

CM SIZE GROUP	OCT-DEC		JAN-MAR		APR-JUN	
*35	--	--	--	--	1049	2.0
*38	--	--	2846	5.4	--	--
*41	967	1.9	6942	13.0	2187	3.7
*44	7318	14.1	28875	52.2	13996	24.0
47	7684	14.0	26582	46.0	18510	30.0
**50	12335	22.0	32481	54.0	7197	11.6
**53	6153	10.0	14300	23.0	3954	6.2
**56	1436	2.3	956	1.5	771	1.2
TOTAL	35893	64.3	112982	195.1	47664	78.7
AVERAGE LENGTH	55.8		57.9		60.6	

APPENDIX B CONTINUED

CM SIZE GROUP	JUL-SEP		OCT-DEC		JAN-MAR	
*35	1095	2.0	--	--	--	--
*38	1404	2.7	--	--	--	--
*41	6519	11.2	--	--	2109	3.7
*44	12102	19.7	--	--	2848	4.2
47	25141	39.0	4786	7.0	4657	7.0
**50	17667	27.0	2476	3.6	683	0.9
**53	8633	13.0	1029	1.5	1508	2.1
**56	398	0.5	262	0.4	113	0.2
TOTAL	72959	115.1	8553	12.5	11918	18.1
AVERAGE LENGTH	63.4		68.4		65.8	

APPENDIX B CONTINUED

CM SIZE GROUP	APR-JUN		JUL-SEP		OCT-DEC	
*35	1330	2.0	--	--	--	--
*38	3393	5.4	--	--	--	--
*41	3757	5.6	--	--	--	--
*44	6641	9.9	--	--	--	--
47	10710	15.0	--	--	--	--
**50	3287	4.5	--	--	--	--
**53	1556	2.1	403	0.5	--	--
**56	39	0.1	--	--	84	0.1
TOTAL	30715	44.6	403	0.5	84	0.1
AVERAGE LENGTH	68.9		80.6		84.0	

APPENDIX C

OTOLITHS OF THE PACIFIC COD FROM HECATE STRAIT COMPARED WITH THOSE FROM OTHER AREAS OF THE PACIFIC AND WITH THOSE OF ATLANTIC COD

1. Introduction

Although the expression "one picture is worth a thousand words" has long been a tired cliché, it is nevertheless most appropriate in any document which endeavours to set down criteria for age determination of fish. Accordingly, this appendix has been prepared as a visual aid to interpretations and conclusions provided in the foregoing text. It deals with typical photographic examples of the kinds of problems encountered in the struggle to establish criteria for the ageing of Pacific cod.

2. Acknowledgement

The technique of preparing the cod otoliths for photography is the unique brainchild of Mr. Pat Fraser of the FRB's Nanaimo Station. He was also directly responsible for the photography. Needless to say, the author is profoundly indebted to Mr. Fraser for his ingenuity, perseverance and invaluable assistance.

3. Technique of preparation and mounting

In reports dealing with the photography of Atlantic cod otoliths, (e.g. Messtorff, 1964) the technique of mounting does not differ from the technique of mounting simply for age determination. Namely, the otolith is photographed either by refracted light or when completely imbedded in plasticene, by reflected light. (These mounting techniques were described in the main body of the text and illustrated diagrammatically in Figure 1.) The unique technique employed in the present situation involved the use of reflected light -- from a thinly ground section of otolith placed against a black background.

The procedure was as follows: (1) the otolith was first cracked, ground and polished in the manner described in the text; (2) the half otolith was then reversed and ground down from the other direction. For lack of suitable equipment, it was necessary to hold the otolith between thumb and forefinger while grinding -- hence it was difficult to reduce the section to less than 2 or 3 mm in thickness; (3) this thin section was then glued to a microscope slide with a colorless adhesive (Tensol Cement No. 7) and subjected to further grinding and polishing to a thickness of about 1 mm. The slide together with the affixed otolith section was then placed in a petri dish and immersed in water, in preparation for photography.

4. Photographic technique

The petri dish was placed on the stage of a microscope equipped with a Leitz Aristophot camera system. The objective was a Summar 35 mm U42 lens and the film was Kodak X Pan. A Bausch and Lomb microscope lamp provided reflected illumination from one side. In most cases shutter speeds were set at 1/125th at f.8, but the speeds and stops had to be varied depending on the size of the otolith and hence the amount of reflected light.

In retrospect, there appear to be two improvements which could be made to the procedures. Immersion of the slide should be in alcohol rather than water because of the tendency for air bubbles to form as the liquid becomes heated by the lighting system. Secondly, there should be double side lighting to avoid uneven illumination of the otolith surface.

5. Otolith specimens

The accompanying table provides all pertinent information about the 22 specimens which were used for photography. Nine of the specimens are from Pacific cod of various sizes and ages from the Canadian west coast, 2 from the Gulf of Alaska, 4 from eastern Bering Sea, and 2 from the Japan Sea. For contrast several specimens from Atlantic cod were also examined -- 2 from the Gulf of St. Lawrence and 2 from the east coast of Scotland (northeastern North Sea).

6. Results

(a) Plate I provides photographs of 8 otolith specimens from Hecate Strait. Aside from no.'s 1 and 2, all are from cod of approximately the same size (69 to 71 cm). Specimen no. 1 was selected to show the size and appearance of otoliths of one-year-old cod -- this one from a fish taken in mid-March, measuring 23 cm in length, the mean size and modal length of the sample shown in text Table I. A distinct mark near the margin on the "bulge" in the upper left portion of the otolith appears to denote the first winter check.

Specimen no. 2 is from a 36 cm female fish in its second year, having been captured in the month of September. The first winter check is clearly discernible. When mounted for age determination, but not for photography this otolith was repeatedly read as a 1+. No dark band was present near the margin suggesting the second winter check. When photographed, however, a dark area is quite obvious. This has to be interpreted as an artifact of lighting.

The difficulties which have been encountered in the use of otoliths for ageing of cod from British Columbia waters are apparent in specimens 3 to 8. All were collected during the late spring or summer months, so theoretically there should be no problem with the presence or absence of checks on the otolith margin. All specimens are from cod which probably were of age 3+, but to

DATA ON THE OTOLITHS USED FOR PHOTOGRAPHY

SERIAL NUMBER	THICKNESS OF SECTION	FISH LENGTH	SEX	DATE OF CAPTURE	PLACE OF CAPTURE	ESTIMATED AGE (YRS)	AGENCY*
	mm	mm					
1	1.6	230	⊖	15/3/65	Hecate Strait - White Rocks	1+	FRB
2	1.5	360	♀	12/9/66	Hecate Strait - Two Peaks (H075)	2+	FRB
3	1.7	700	♀	9/7/65	Hecate Strait - Two Peaks	3+	FRB
4	1.6	690	♂	3/5/65	Hecate Strait - Horseshoe (H160)	3+ ?	FRB
5	1.7	700	♀	3/5/66	Hecate Strait - Horseshoe (H162)	3+ ?	FRB
6	1.2	700	♀	3/5/66	Hecate Strait - Horseshoe	3+ ?	FRB
7	1.7	700	♀	5/5/66	Hecate Strait - Two Peaks (H049)	3+	FRB
8	1.6	710	♀	3/5/66	Hecate Strait - Horseshoe (H163)	3+	FRB
9	1.4	710	♀	21/6/61	Shelikof Strait	6+	IPHC
10	1.5	730	♀	27/5/61	Shelikof Strait - West End	6+	IPHC
11	1.6	690	♀	14/6/61	Kodiak Island - SW Side	6+	IPHC
12	1.4	700	♀	22/7/59	Eastern Bering Sea - Polaris Ground	5+	IPHC
13	1.1	700	♀	27/7/59	Eastern Bering Sea - Polaris Ground	7+	IPHC
14	1.3	700	♀	27/7/59	Eastern Bering Sea - Polaris Ground	6+	IPHC
15	1.3	700	♀	?	Southern Gulf of St. Lawrence	8+	FRB St. A
16	1.4	800	♀	?	Southern Gulf of St. Lawrence	8+	FRB St. A
17	1.7	820	♀?	22/7/65	East Coast of Scotland	4+	MLA
18	1.7	870	♀?	22/7/65	East Coast of Scotland	5+	MLA
19	1.6	860	♀	18/7/59	Eastern Bering Sea - Corridor	7+	IPHC
20	1.6	700	♀	11/1/65	Japan Sea	5+	FAJ

continued...

SERIAL NUMBER	THICKNESS OF SECTION	FISH LENGTH	SEX	DATE OF CAPTURE	PLACE OF CAPTURE	ESTIMATED AGE (YRS)	AGENCY*
	mm	mm					
21	1.5	850	♀	1/12/64	Japan Sea	9+ or 8+	FAJ
22	1.7	850	♀	17/9/66	Dixon Entrance - Tow Hill (H070)	6+	FRB

*Otoliths were obtained from a number of areas during Fisheries Research Board (FRB) investigations and through the cooperation of staff at the St. Andrews Station (FRB, St. A), the International Pacific Halibut Commission (IPHC), the Marine Laboratory at Aberdeen, Scotland (MLA) and the Fisheries Agency of Japan (FAJ).

conform with this hypothesis it is necessary to assume that, at least in the case of specimens no. 4 and 5, one or two of the annuli are accessory checks.

(b) Plate II illustrates otolith specimens from the Gulf of Alaska and eastern Bering Sea; again from fish of about 70 cm in length. No's 9, 10 and 11 (Gulf of Alaska) have a decidedly clearer pattern of rings than those from British Columbia waters (Plate I). Possibly only by chance selection they also appear to be clearer than those from Bering Sea (no's 12, 13 and 14).

All six of the specimens from the colder waters north of British Columbia provided estimated ages considerably older than those from fish of comparable size in Hecate Strait (ca. age 5+ versus 3+, respectively).

(c) Plate III shows specimens from the Atlantic Ocean. None shows up quite as clearly as it did in direct observation, presumably because of the imperfect lighting technique used in photographing. Specimens no's 15 and 16 were estimated as age 8+ years (corroborated by specialists at St. Andrews). Those from the North Sea (no's 17 and 18) were estimated as age 4+ and 5+ respectively. Except for specimen no. 15, these otoliths were from relatively large fish -- 80 cm or over.

(d) Plate IV shows a mixed bag of specimens. Specimen no. 19 was from an 86 cm cod taken in the Bering Sea corridor (estimated age 7+ years). No's 20 and 21 were from the Sea of Japan (70 cm, age 5+; 85 cm age 9+ or 8+ years). No. 22 was from a large cod (85 cm) taken in Dixon Entrance and age was estimated as 6+ years. The Sea of Japan specimens are the poorest of the lot and suggest that otoliths of cod from widespread areas of the North Pacific present serious problems of age determination.

On the basis of available material, one might conclude tentatively, that if otoliths of Pacific cod must be used for age determination, the prospect of obtaining reliable results lies more in the Gulf of Alaska than anywhere else.

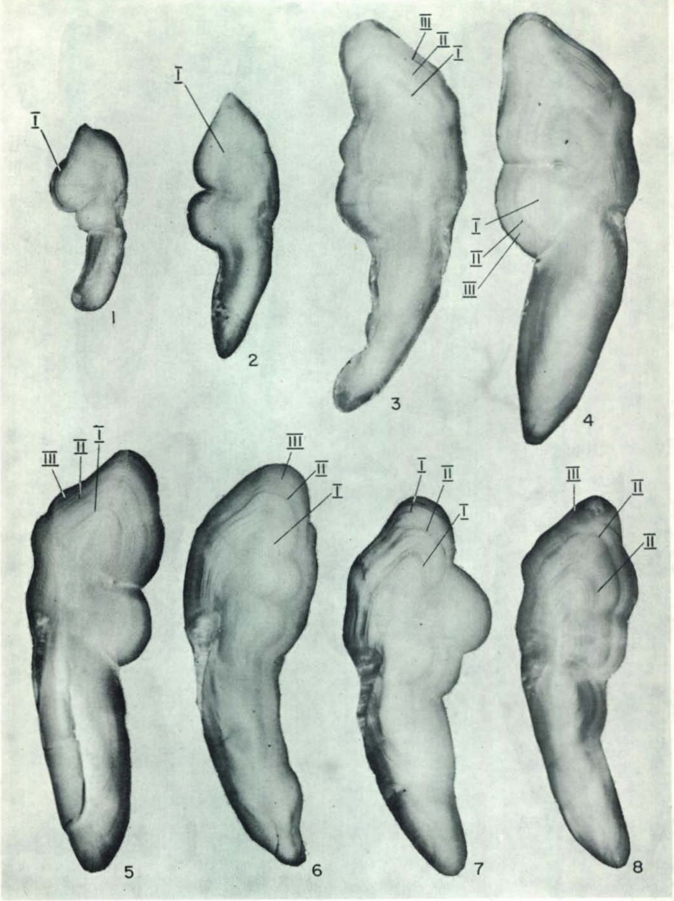

Plate I

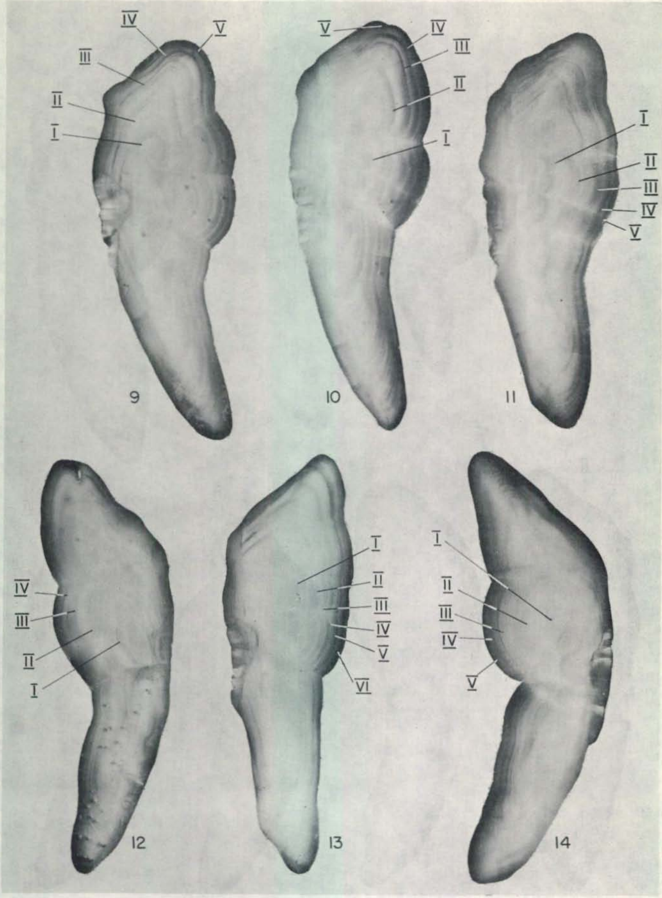
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Plate II

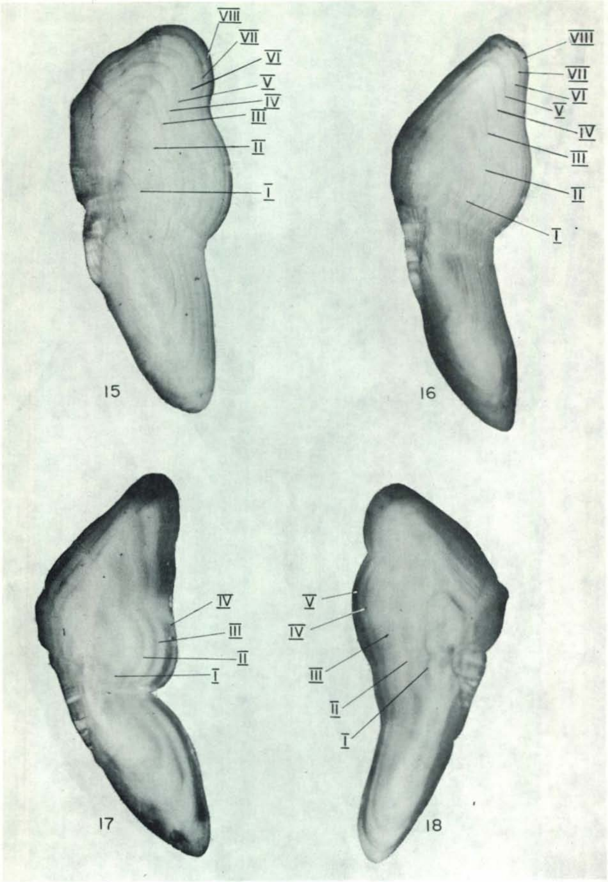
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Plate III

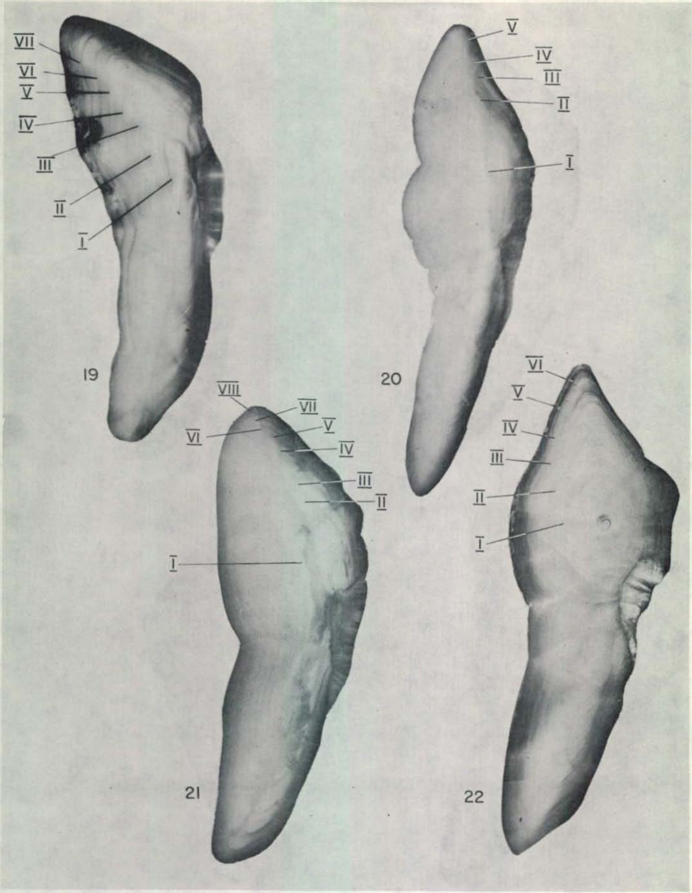
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Plate IV