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A Comparative Study of Ageing Methods for Summer Flounder (*Paralichthys dentatus*)

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INTRODUCTION

Summer flounder (Paralichthys dentatus) is an important part of the Middle Atlantic fisheries as reflected by the 1977 recreational catch of 21,300 tons (Resource Assessment 1978). Accurate age information is necessary for any valid assessment of the population. Previous studies using otoliths have presented conflicting results, with the major discrepancy being the size at age one. The inconsistencies of ages warrant further investigation into alternate ageing methods.

This paper presents the results of a study comparing the use of otoliths, scales, and fin rays for ageing summer flounder. Back-calculated lengths at age for the three age structures were compared and then used to determine growth rates.

MATERIALS AND METHODS

Sample collection and preparation

Samples for age determination were collected in Martha's Vineyard Sound during June and July of 1977 by the CAPN' BILL V of Woods Hole, Massachusetts. Additional samples were obtained from Martha's Vineyard and Nantucket Sounds by the Massachusetts Division of Marine Fisheries in May and June of 1976, 1977, and 1978 (Figure 1) (Howe, A.B. 1978). The samples were 12.3% male, all less than 45 cm, so data were combined for both sexes. Length frequencies of the research catches were recorded, and dorsal fin rays, scales and otoliths were removed from the sampled fish. Length frequency data collected on a 1977 ALBATROSS IV August-September Groundfish Survey from Cape Hatteras to Nova Scotia (Grosslein 1969) were summarized and graphed using a three point moving average. Length frequencies were used from survey stations north of Chesapeake Bay because of a difference in spawning time for fish in the north (Smith 1973). Scale samples were collected in 1976 and 1977 from commercial fishing vessels operating in the Long Island Sound and Nantucket Shoal areas. The mean lengths at age derived from commercial scale samples collected during the first half of each year were compared to backcalculated lengths of the 3 age structures. Commercial length frequencies were not weighted by market category.

Otoliths were removed from the specimens, placed in 60% glycerin solution and viewed under a binocular microscope at a magnification of 25X, using reflected light. Thin-sectioning of the otoliths proved unsuccessful.

Scales were taken from the lateral line area a few centimeters anterior to the caudal peduncle. Impressions of the dried scales were made in laminated plastic composed of a thin (0.0051 mm), soft polyethylene layer over a thicker (0.0185 mm) and harder vinyl substrate. The impressions were then viewed on a microprojector at a magnification of 40X.

The dorsal fin rays showing the clearest ring formation, approximately the fortieth from the head, were removed just below the point of articulation. After the membrane was removed, the fin ray was bleached to eliminate any remaining traces of membrane. The fin ray was split longitudinally and each half cross-sectioned at the ridge near the base of the ray, using a low speed macrotome saw (Nichy 1976). This ridge area showed the clearest growth rings and provided a mark for consistent sectioning (Figure 2). The section was covered with oil of clove to enhance the opacity, then aged under a binocular microscope with dark field transmitted light at a magnification of 25X.

Methods for back-calculating length at age

Annular rings on the three age structures were measured to compared back-calculated lengths at age. Scales were measured from the focus to the anterior edge of each annulus. Fin ray sections were measured at the longest radius from the center of the nucleus to the outer hyaline edge of the annuli, with the end of the first annulus taken as the edge of the crystalline zone in the center. Otoliths were also measured from the nucleus to the outer edge of each hyaline zone.

Measurements from the center to the edge of the age structure were used to determine the relationship of fish length to the length of the age structure. Straight lines, exponential curves, logarithmic curves, and power curves were fit to these data and the backcalculated distance to each annulus was adjusted using Ricker's empirical method for a non-linear body:scale relationship (F.W. Resch in Ricker 1968).

RESULTS AND DISCUSSION

Description of annular rings

The annular zones of the scales appear as abrupt changes in the circuli pattern, perhaps due to physical erosion of the scale edge. Monthly samples show the progression of scale growth and annulus formation. The eroded edge starts becoming prominent on scales collected in March-April when the marginal increment is smallest. Poole (1961) concluded the annulus forms on otoliths during February-March. The second annulus is generally the first prominent zone on the scale (Figure 3). The first annulus is barely discernible and is usually estimated by slight changes in the formation of the circuli. Fish in the 10 cm range have scales with faint edge markings believed to be the first annulus. Fin rays from the same fish have an edge which show the beginning of an opaque zone and a clear crystalline zone in the center which is interpreted as the first year of growth. Thereafter the growth patterns on the fin ray sections are clearly visible as alternating opaque zones and hyaline annuli, with an occasional split or check (Figure 3B).

Hyaline zones on the whole otoliths were not clearly defined. The thickness at the center of the otolith made the first few annuli obscure and annuli on the edges of larger otoliths were difficult to distinguish. Evidence for difficulties with otoliths were shown in Smith and Daiber's study (1977) where 20% of their otolith samples were rejected because of a lack of clearly defined annuli. The distorted growth marks probably cause some error in the backcalculation results of the first several years.

Agreement of ages among the three age structures

The closest agreement of ages occurred between the fin rays and otoliths with an average of 95.0% (Table 1). Agreement between scales and otoliths was 90.5% (Table 3) and between fin rays and scales 77.2% (Table 2). No one method gave consistently higher or lower ages. The sample size of otoliths was less than fin rays or scales, possibly influencing the percentage agreement.

Comparison of back-calculated lengths at age

The power curve had the closest relationship with the three age structures as determined by the coefficient of determination. The equations used to convert measurements to backcalculated lengths at age in cm are given below.

Age Structure	Power Curve Equation	Coefficient of Determination
Otoliths	$y = 1.139x^{0.616}$	$r^2 = 0.675$
Scales	$y = 0.299x^{0.947}$	$r^2 = 0.954$
Fin Rays	$y = 0.1961x^{.887}$	$r^2 = 0.928$

The fitted power curves are shown in Figures 4 through 6.

Backcalculated lengths at age were statistically compared using a one way analysis of variance for unequal sizes (Sokal and Rolf 1969) (Table 6). For ages 6 and 7, there were no otolith samples, so a students t-test was used to compare the scales and rays. There was no significant difference in mean length among the three age structures except at the second and third years. The second year had a P value of 0.001 and the third year had a significant difference with P = 0.0387. To determine which age structure accounted for the differences, a t-test for paired samples was used to compare the differences between the adjusted backcalculated lengths at age for each method (Table 7). Fin rays and scales had no difference, with the probability values all less than 0.1. Significant differences were noted with the otoliths at the second and third years. P=0.0013 for otoliths and scales at age 2 and at age 3, P = 0.0117. The fin rays and otoliths comparison yielded P = 0.000003 for age 2 and P = 0.0061 for age 3. Multiple comparison procedures did not detect significant differences because of large within group variances.

Growth equations calculated using the von Bertalanffy equation (Allen 1966), from the mean adjusted backcalculated lengths at age are:

Fin rays: $l_t = 97.11(\text{cm})\ (1-e^{-0.167}(t-o.334))$

Scales: $l_t = 116.32\ (1-e^{-0.127}(t-0.161))$

Otoliths: $l_t = 96.88\ (1-e^{-0.157}(t-0.012))$

Combined Data: $l_t = 101.26\ (1-e^{-0.156}(t-0.247))$

(See Figures 7-10)

The values for L_∞ are all within the same range as the largest reported summer flounder caught, 11,793 g, (Bigelow and Schroeder 1953) which equals 100.95 cm using the length-weight regression equation of Smith and Daiber (1977).

Length frequency data were used as another indication of the similarity between backcalculated lengths and actual lengths at age. The modes from the 1977 ALBATROSS IV length frequency were 16, 27, 37.5, 46, 54, 59.5, and 67 cm for the one through seven year classes (Figure 11). The value of one year olds is larger than the backcalculated lengths at age probably because most young fish inhabit estuarine areas which were not included in the ALBATROSS IV survey. Results of an estuarine survey conducted by the Massachusetts Division of Marine Fisheries in 1976-1977 reflect a closer value of 10 cm for one year olds (Howe 1978). The scales and fin rays had backcalculated lengths for age one at 10.0 cm and 11.3 cm, respectively. The mean lengths of commercial samples were comparable to backcalculated lengths at age, except for the two year olds. The commercial data contain only fish greater than 25 cm, influencing the mean value of the two year olds (Table 8).

The scales and fin rays results confirm Smith and Daiber's conclusion (1977) that the first strong annulus is not apparent until age two. There is no mention in this study of an 0-age group because of the date of January 1 which has been used as the statistical birthdate. Summer flounder spawn in late fall as they are moving offshore (Smith 1973). When the first annular mark is put down in March, the fish have passed the January 1 birthdate. Therefore, the fish are technically one year old fish despite the probability of being only a few calendar months old. The only time 0-age group fish would appear is in November or December following spawning.

Results of previous studies have given conflicting sizes for mean lengths at age. The manipulation of birthdates could be one cause of confusion in the size at age one. Poole (1961) based his ages on growth from the time of annulus formation given as February. Therefore, the fish he called young of the year in July could also be considered one year old fish. As a result his mean lengths at age differed by one year from studies by Eldridge (1962), Richards (1970), and Smith and Daiber (1977). Eldridge (1962) used only length frequency data to estimate a mean value of 17 cm for age one. Using otoliths, the backcalculated size at age one of Smith and Daiber (1977) is comparable to the value determined in this study; 12 cm and 11.4 cm respectively.

The conclusion of this study is that alternative methods using scales and fin rays will give comparable results to those obtained from otoliths. Scales and fin rays are preferred because the annuli are usually more distinct. Fin rays are more time consuming in preparation so may be preferred only for comparison on hard to age samples. Another advantage is scale and fin ray samples can be collected from commercial and recreational catches with little damage to the fish. This could allow routine port sampling to give better age information on a larger percentage of the population. The overall benefit in having alternate methods is being able to verify one set of ages, giving added credibility to age and growth analyses.

ACKNOWLEDGEMENTS

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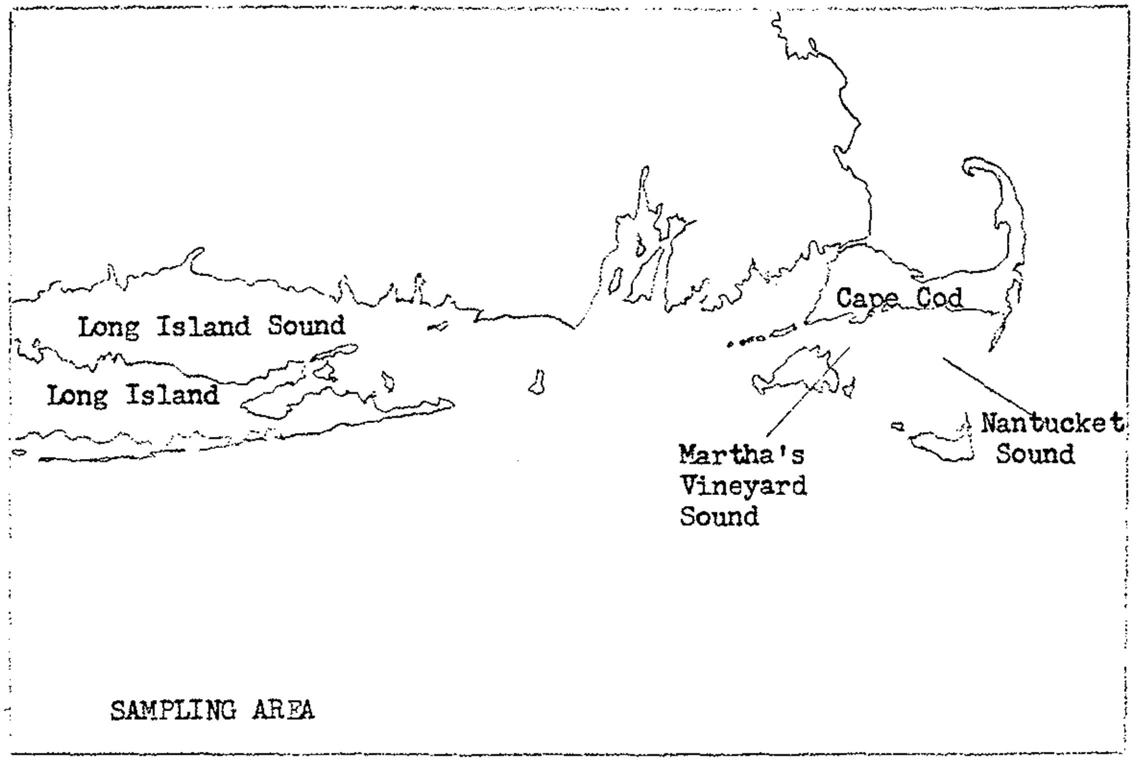


Figure 1.

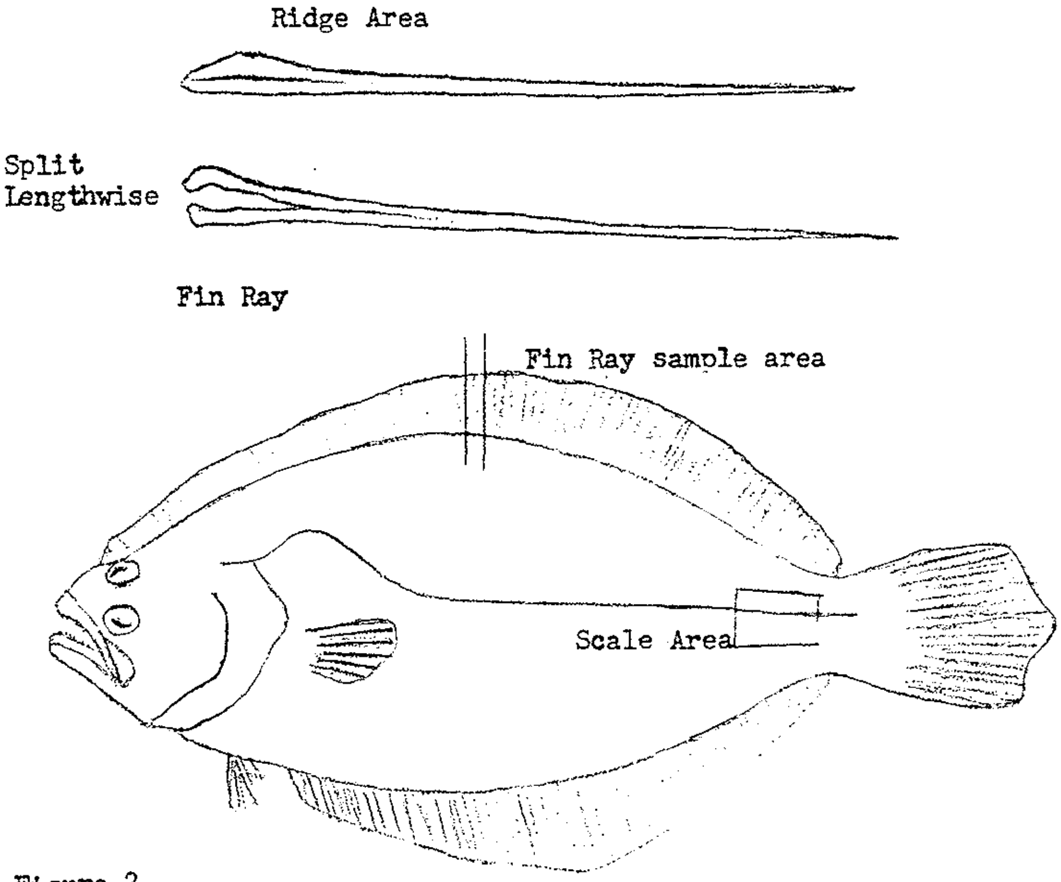
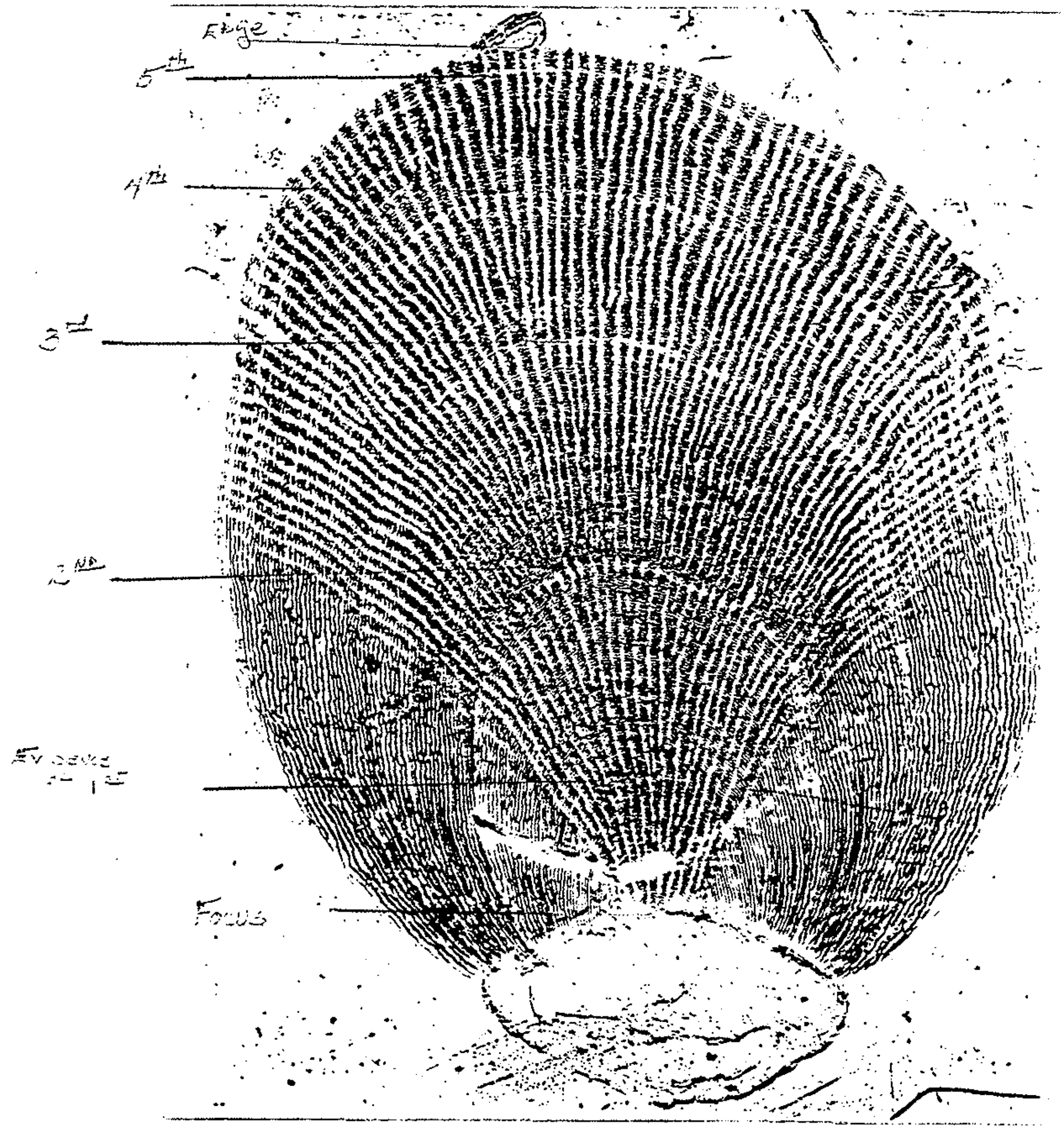
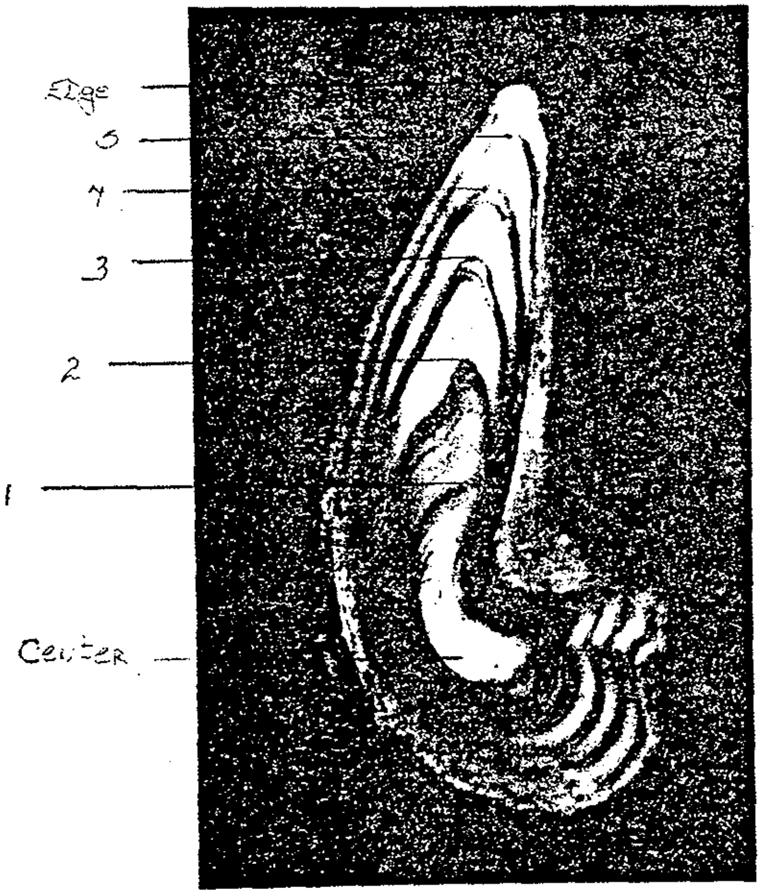


Figure 2.



Summer Flounder 54.5 cm ♀ June 1978 F/V Frances Elizabeth
Scale

Figure 3A

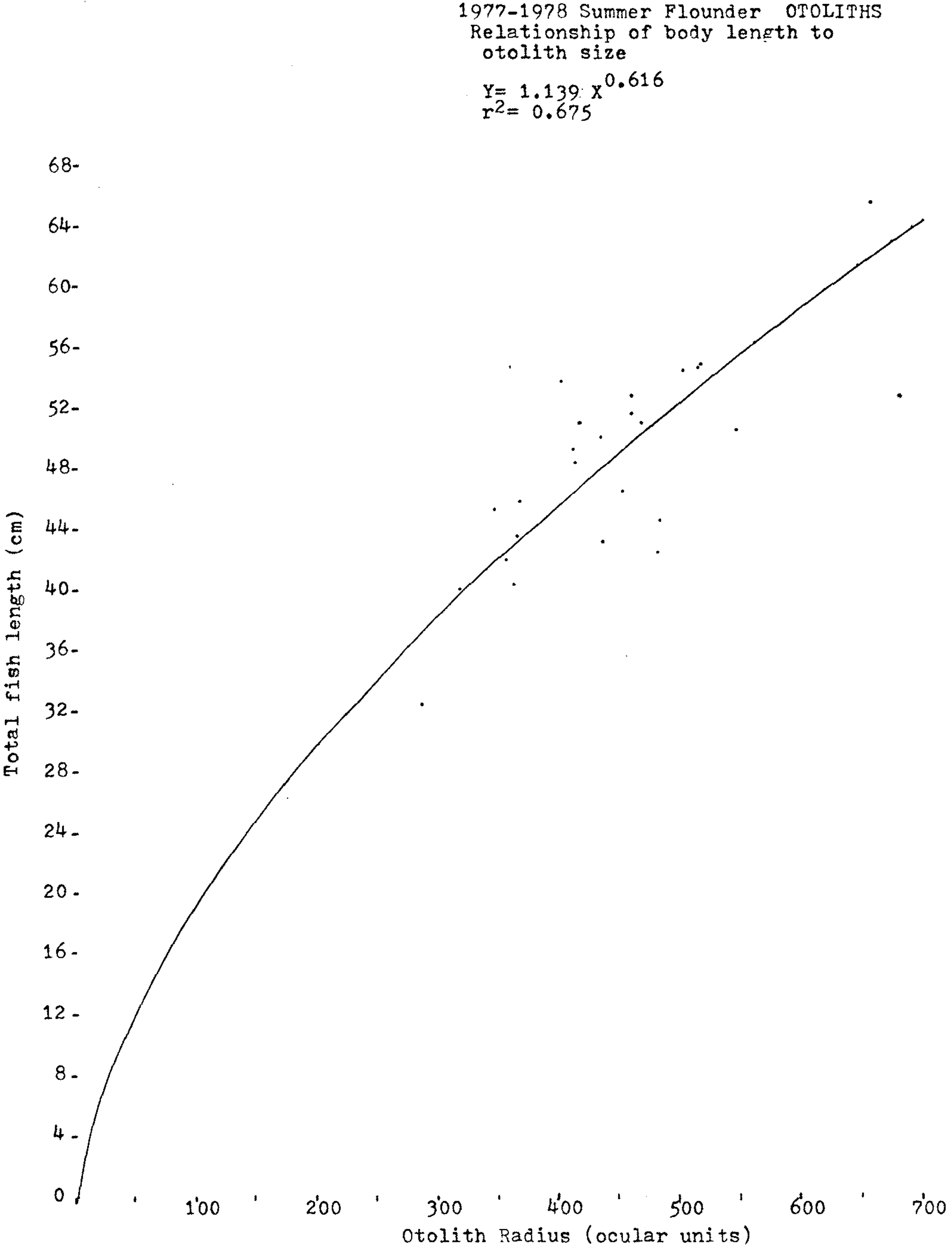


Summer flounder
fin ray section

♀ 54.5 June 1978

Figure 3B

Figure 4.



1977-1978 Summer Flounder OTOLITHS
Relationship of body length to otolith size

$Y = 1.139\ X^{0.616}$
$r^2 = 0.675$

Figure 5.

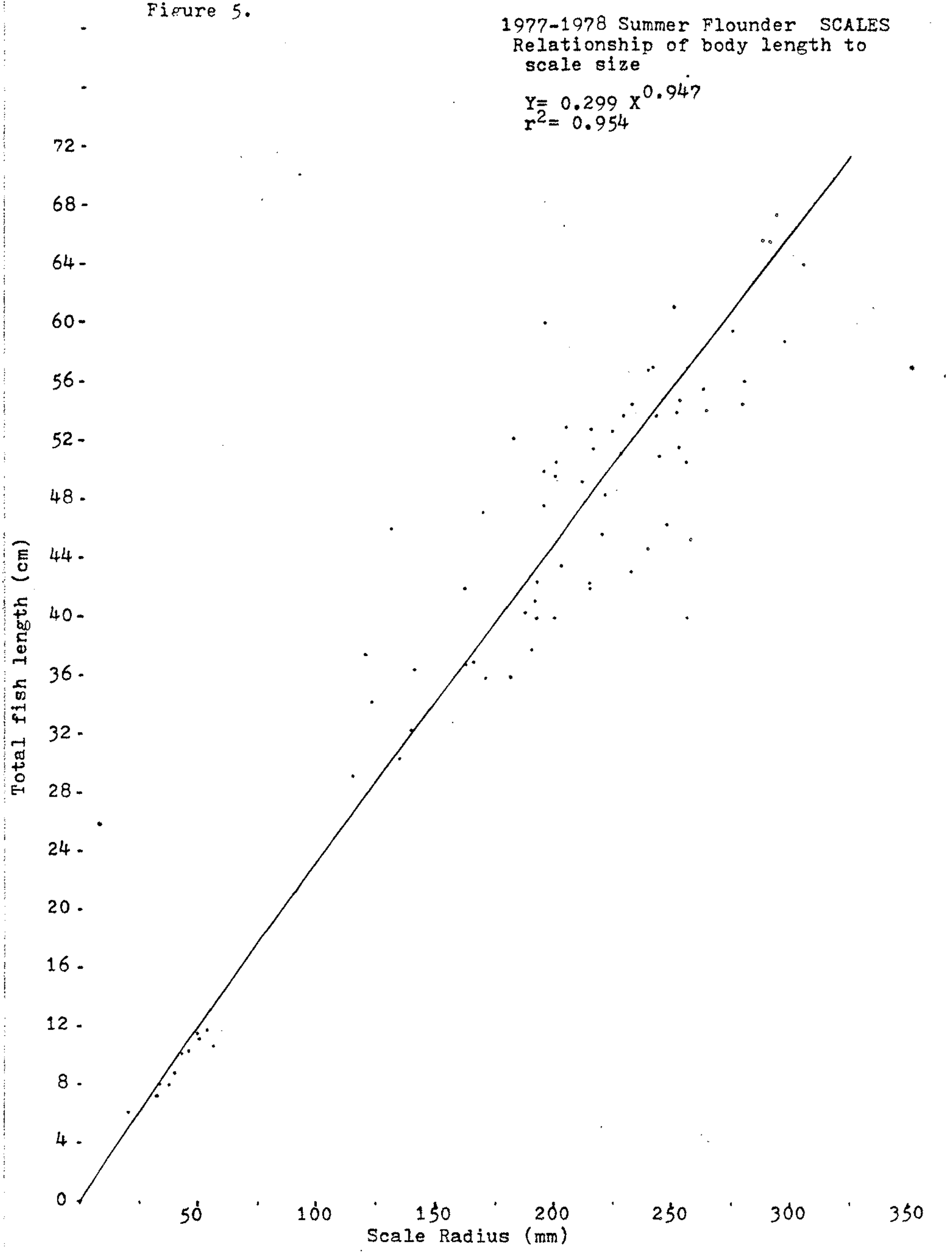
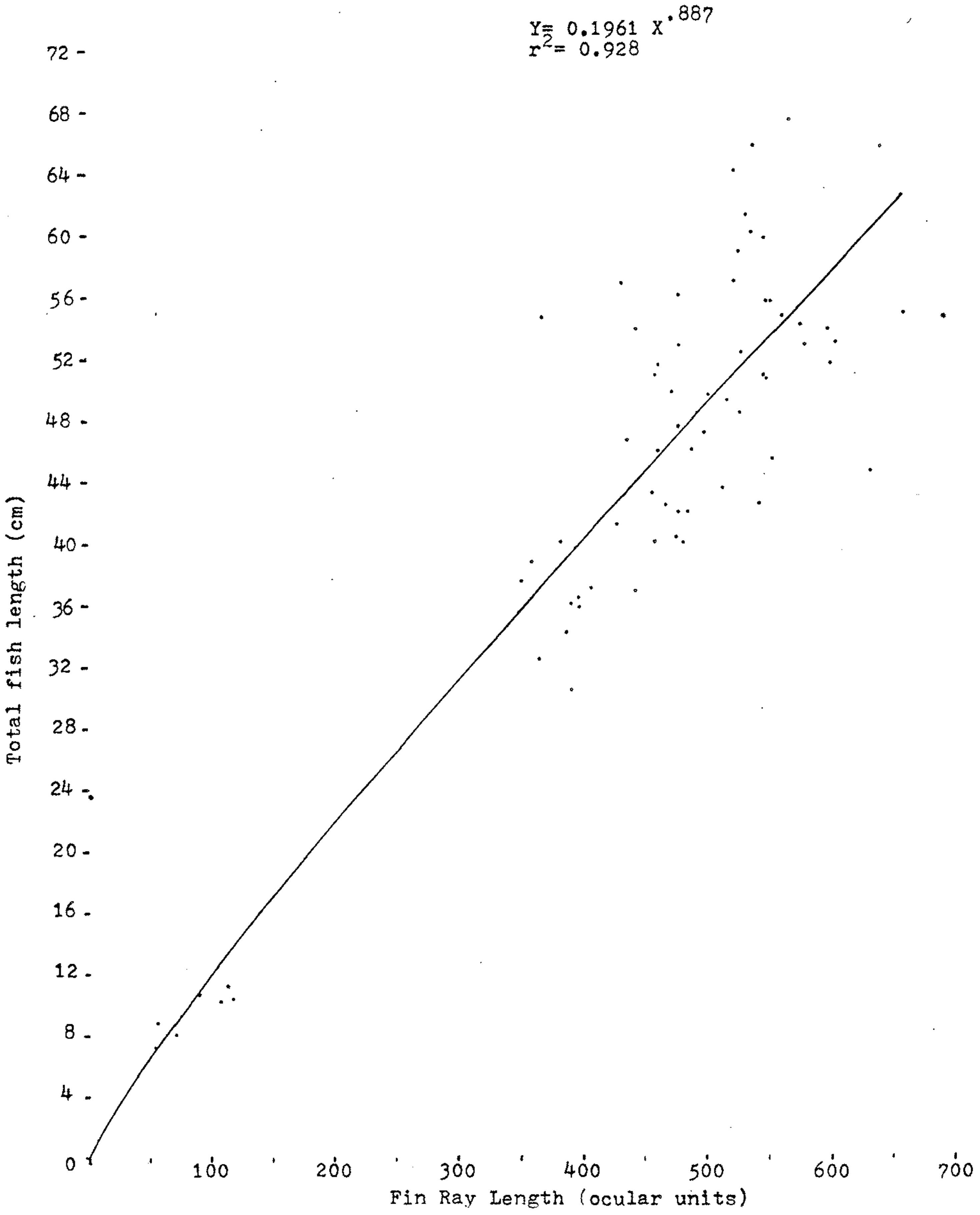

1977-1978 Summer Flounder SCALES
Relationship of body length to scale size
$Y = 0.299\ X^{0.947}$
$r^2 = 0.954$
Total fish length (cm)
72
68
64
60
56
52
48
44
40
36
32
28
24
20
16
12
8
4
0
50
100
150
200
250
300
350
Scale Radius (mm)

Figure 6.



1977-1978 Summer Flounder FIN RAYS
Relationship of body length
to fin ray size

$Y = 0.1961\ X^{.887}$
$r^2 = 0.928$

Figure 7.

1977-1978 Summer Flounder Fin Rays
Adjusted Backcalculated Lengths fitted to
von Bertallanfy Growth Curve at points •.

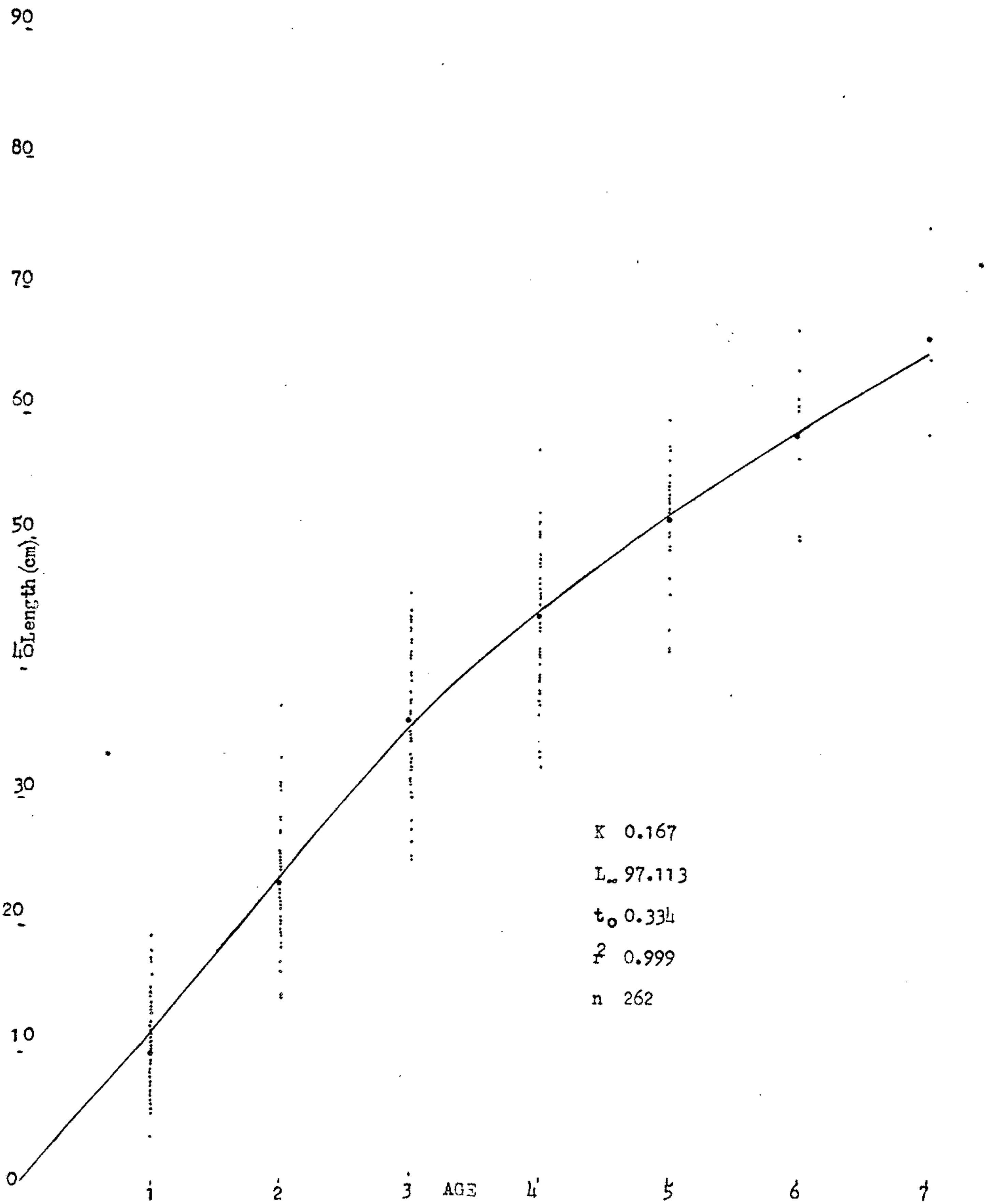


Figure 8.

1977-1978 Summer Flounder Otoliths
Adjusted Backcalculated Lengths fitted to
von Bertallanfy Growth Curve at point • .

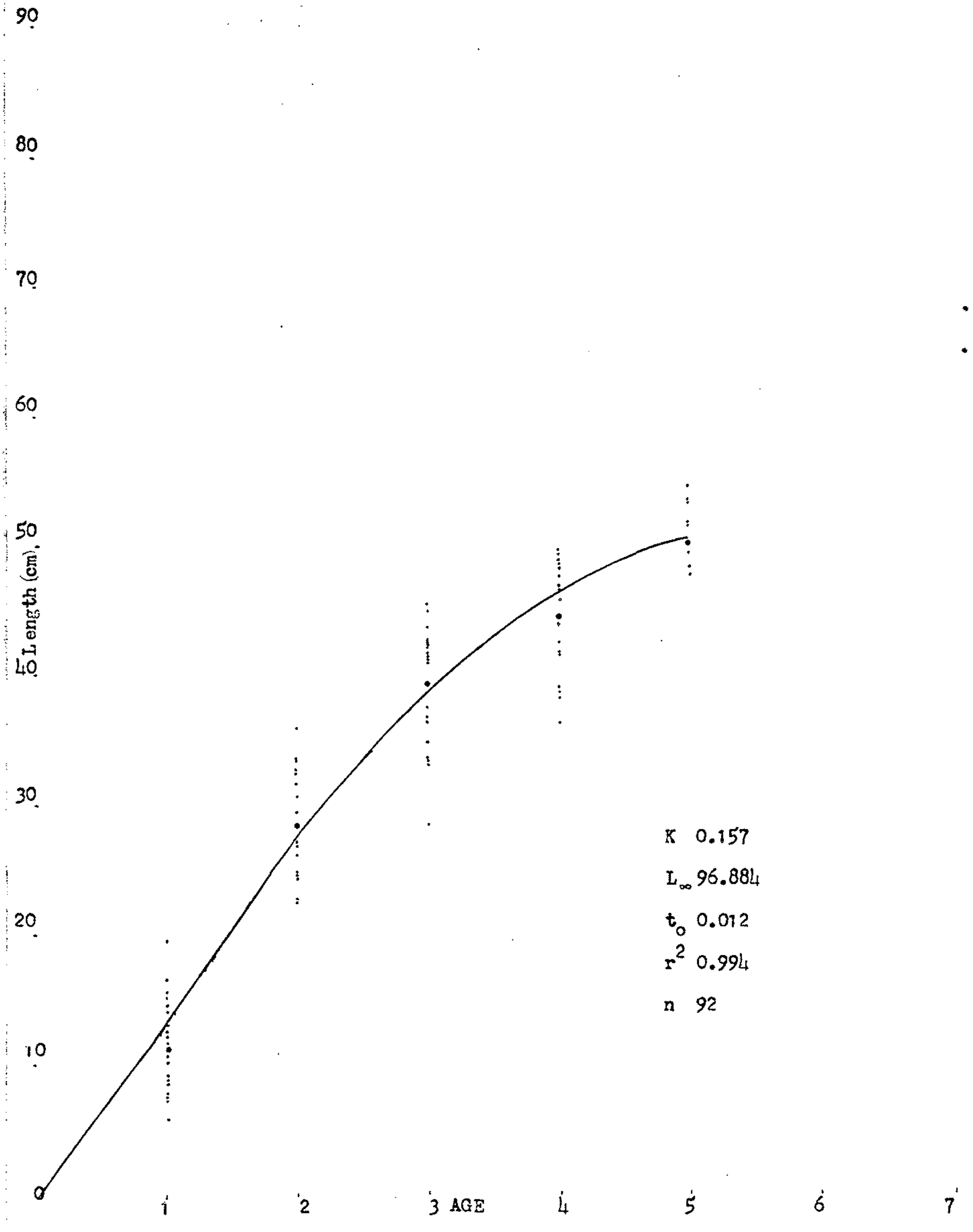


Figure 9.

1977-1978 Summer Flounder Scales
Adjusted Backcalculated Lengths fitted to
von Bertallanfy Growth Curve at points •.

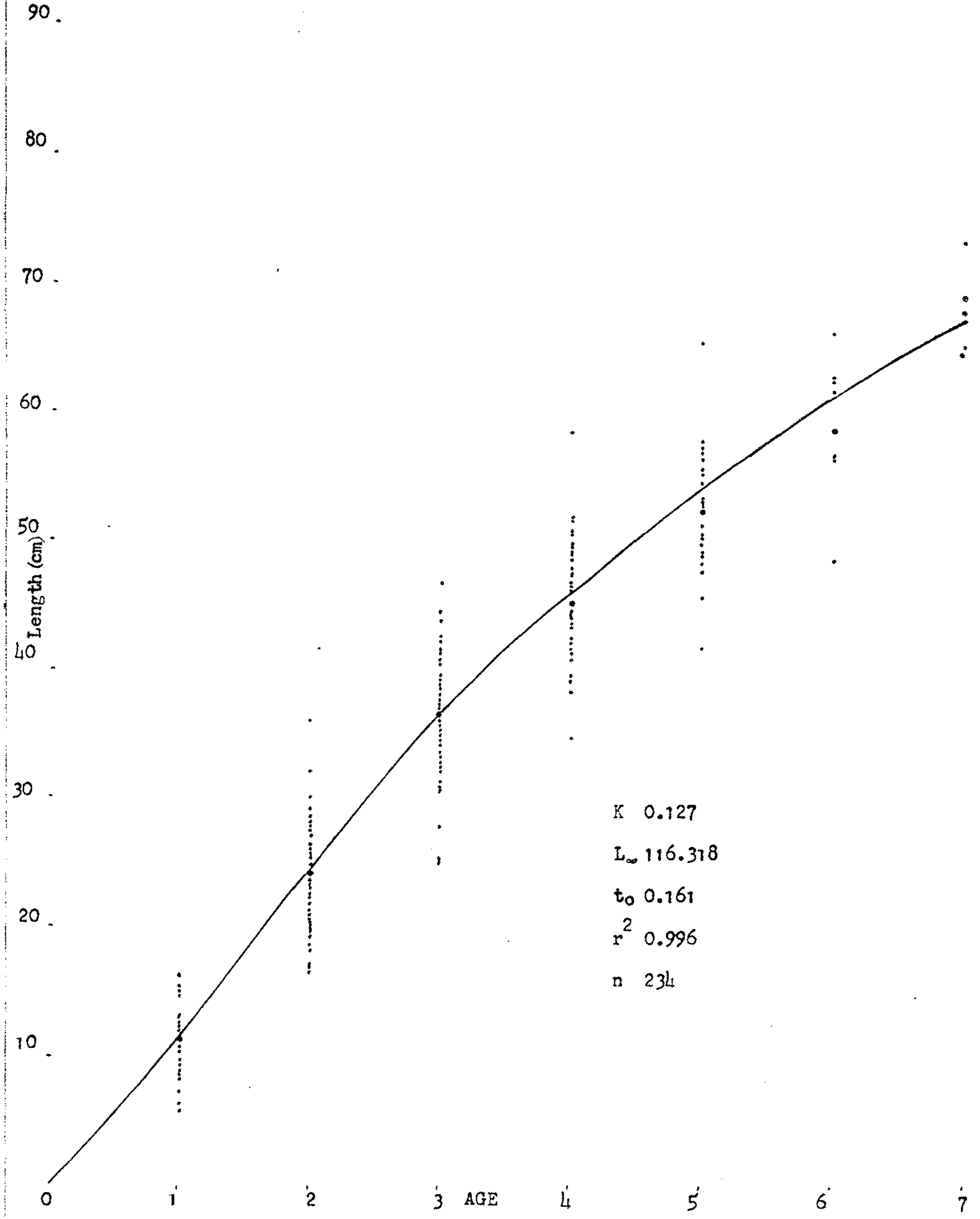


Figure 10.

1977-1978 Summer Flounder Average for Scales, Fin rays, and Otoliths fitted to von Bertallanfy Growth Curve

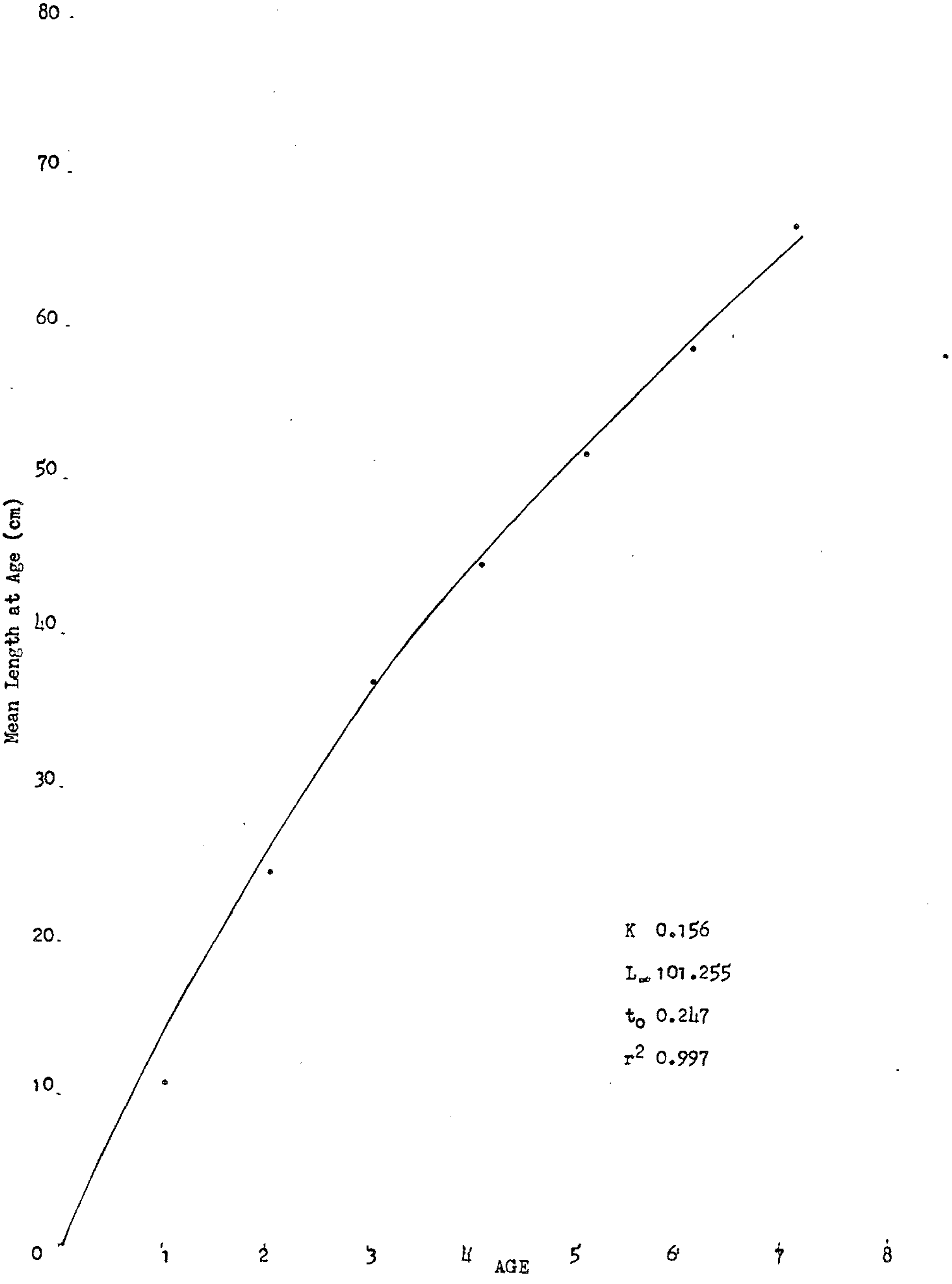


Figure 11.

Length Frequency of Summer Flounder
Albatross IV Summer 1977
Stations north of Chesapeake Bay
n=227

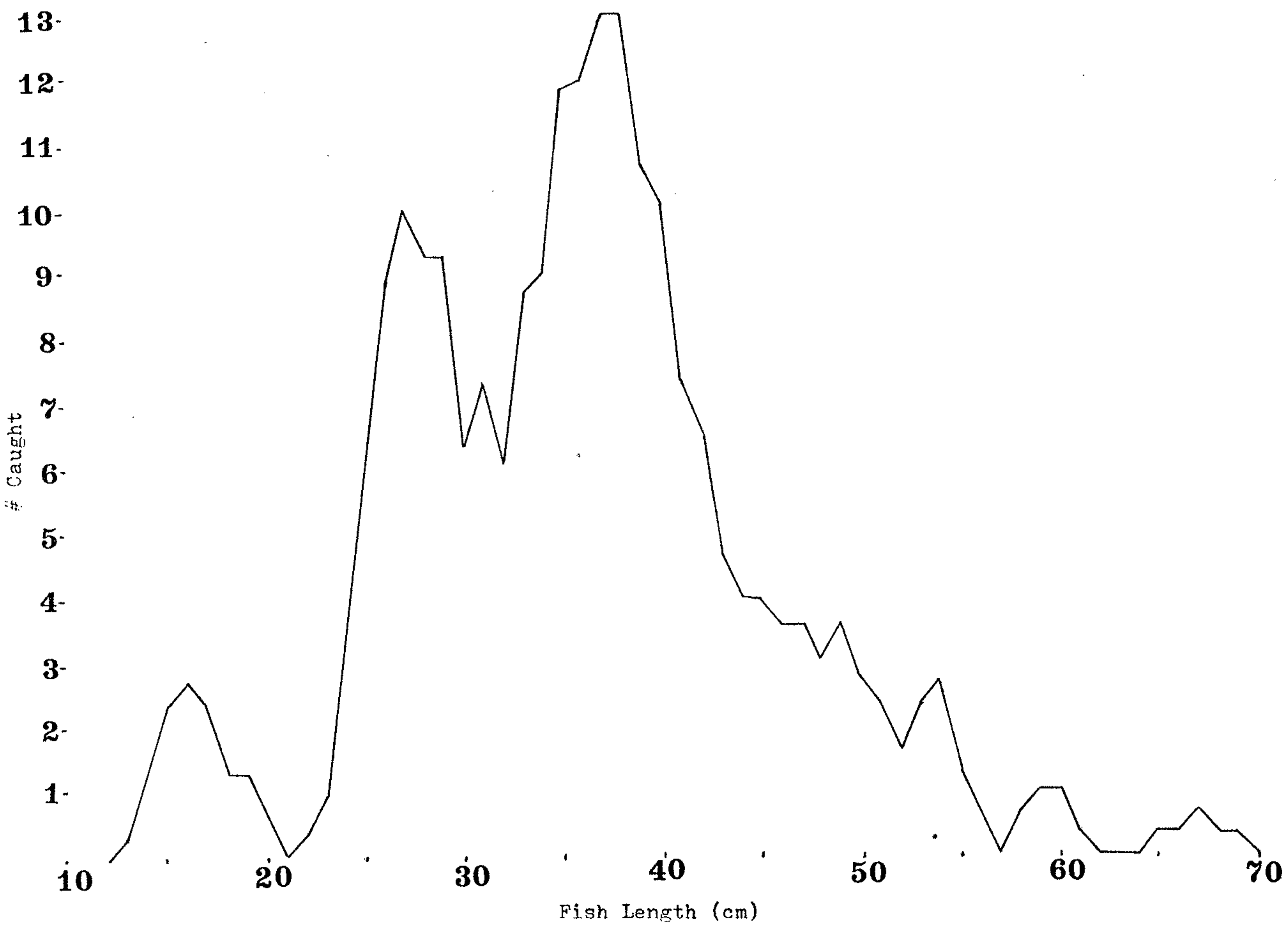


Table 1

Comparison of Age Results

OTOLITHS

FIN RAYS	0	1	2	3	4	5	6	7	8	9	10	11
0												
1												
2												
3												
4					10							
5					1	8						
6							1					
7												
8												
9												
10												
11												

20 Total Number

19 Number of Agreements

1 Number of Disagreements

95.0 % Agreement

Table 2

Comparison of Age Results

SCALES

FIN RAYS	0	1	2	3	4	5	6	7	8	9	10	11
0												
1												
2												
3				9	2							
4				3	16							
5					3	14	2					
6						1	4					
7							2	1				
8												
9												
10												
11												

57 Total Number

44 Number of Agreements

13 Number of Disagreements

77.19 % Agreement

Table 3

Comparison of Age Results

	OTOLITHS											
SCALE	0	1	2	3	4	5	6	7	8	9	10	11
0												
1												
2												
3												
4					11	1						
5					1	7						
6							1					
7												
8												
9												
10												
11												

21 Total Number

19 Number of Agreements

2 Number of Disagreements

90.48 % Agreement

Table 4. Adjusted back-calculated mean length at age. (cm)

				AGE			
	1	2	3	4	5	6	7
Scales							
N	41	57	57	45	24	8	2
$\bar{X}$	11.32	24.17	36.51	45.30	52.43	58.95	69.10
s	3.595	4.039	4.217	4.350	4.839	5.487	5.738
Otoliths							
N	20	21	21	21	9		
$\bar{X}$	11.40	28.45	39.16	44.72	50.09		
s	3.675	4.410	4.462	4.080	3.944		
Rays							
N	64	57	58	47	25	8	3
$\bar{X}$	10.04	23.42	36.11	44.34	51.96	58.77	66.06
s	3.391	5.044	5.267	5.553	4.793	5.898	8.241

Table 5. Comparisons of previous age studies[1]

				AGE					
♂ (MM)	1	2	3	4	5	6	7	8	9
Smith (1977)		260	345	397	448	493	517		
Poole (1961)	251	326	387	427					
Eldridge[2] (1962)	170	240	319	357	381	399	414	426	
♀									
Smith (1977)		280	380	453	511	565	618	661	
Poole (1961)	271	377	465	531	644				
Eldridge[2] (1962)	170	240	377	424	471	518	566	613	657

[1] Smith, W.E., "Fishery Bulletin", Vol. 75, #4

[2] Lengths given for Eldridge at the end of year 1 and 2 are estimates of the average observed length frequency.

Table 6. Anova results for fin rays, scales, and otolith comparison.

	AGE				
	1	2	3	4	5
F	2.157	9.666	3.331	0.457	0.820
d.f.	124.0	134.0	135.0	112.0	57.0
P	0.1200	0.0001***	0.0387*	0.6341	0.4457

Students t-test comparison of scales and fin rays.

	AGE	
	6	7
t	0.062	0.445
d.f.	14.0	3.0
P	0.9514	0.6863

Table 7. Paired T-test comparison of fin rays, scales, and otoliths.

	AGE	
	2	3
Fin Ray Average	23.35	36.19
Scales Average	24.16	36.51
d.f.	55.0	56.0
t	1.079	0.489
P	0.2852	0.6268
Fin Ray Average	22.57	36.90
Otolith Average	28.72	39.19
d.f.	17.0	18.0
t	6.798	3.104
P	0.000003****	0.0061**
Scale Average	23.86	36.75
Otolith Average	28.39	39.19
d.f.	18.0	18.0
t	3.798	2.804
P	0.0013**	0.0117*

* Significant difference

** Highly significant difference

*** Very highly significant difference

**** Very,very,highly significant difference

Table 8. 1976-1977 Commercial Summer Flounder Mean Lengths at Age (cm).

	AGE							
	1	2	3	4	5	6	7	8
1976								
$\bar{X}$		30.7	37.2	45.2	53.1	60.4	67.1	75.5
N		44	562	286	295	53	9	2
1977								
$\bar{X}$		31.9	38.1	45.0	56.2	61.5	69.8	73.8
N		15	380	454	141	129	31	4