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Otolith Analysis of Age and Growth of Larval Redfish (*Sebastes* sp.) on Flemish Cap, 1979 and 1980

by

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ABSTRACT

Sagittae of larval redfish (Sebastes sp.) were used to estimate age and growth of 5-25 mm larvae on Flemish Cap in 1979 and 1980. Results indicate increment formation begins prior to extrusion and has a daily periodicity after extrusion. The number of increments in pre-extruded larvae is highly variable, between 1-6, and this number may be related to the stage of development. Average growth rates of 0.143 mm/d and 0.135 mm/d for 1979 and 1980, respectively, agree well with other estimates. These growth rates appeared to be linear through the size ranges examined. Based on these growth rates peak extrusion of larvae on Flemish Cap occurred during 25-30 April 1980.

INTRODUCTION

Since 1978, a major international research program to investigate biotic and abiotic factors influencing year-class strength of redfish (Sebastes spp.) on Flemish Cap has been in operation. An important part of this study has been directed towards the early larval stages. It is widely recognized that the abundance of fish populations at commercial size may be determined or regulated by influences affecting the growth and survival of larvae (Cushing 1974).

At present, it is known that growth of young fish is limited mostly by temperature and/or food supply (Riley 1966; Brett et al. 1969; O'Connell and Raymond 1970; Houde 1975). Food availability is frequently considered as a major controlling factor on growth and survival (Jones 1973; May 1974; Lasker 1975; Arthur 1976).

Studies to elucidate these problems have been hampered by difficulties in directly measuring growth at sea, largely as a result of inability to accurately age fish larvae. Despite the fact that ageing and calculations of annual growth rates in adult fish has been practiced for decades, only recently have daily increments been discovered in otoliths of larval fish (Pannella, 1971). Since then a number of studies have demonstrated the presence of daily growth increments in larval stages of several fish species: Engraulis mordax, Leuresthes tenuis, Morone saxitilis (Brothers et al. 1976); Stolephorus purpureus (Struhsaker and Uchiyama 1976); Chaetodon miliaris (Ralston 1976); Menidia menidia (Barkman 1978) Lepomis gibbosus, L. cyanellus, L. macrochirus, Tilapia mossambica (Taubert and Coble 1977); Oncorhynchus nerka (Wilson and Larkin 1980) and have utilized this method to determine the growth rate of sea-caught larvae: Engraulis mordax (Methot and Kramer 1979) and Clupea harengus (Lough et al. 1980). The objective of this study was to estimate growth rates of larval Sebastes sp. from Flemish Cap in 1979 and 1980 based on larval otolith techniques.

MATERIALS AND METHODS

Ichthyoplankton samples for otolith studies were collected from Flemish Cap (NAFO Area 3M) during survey cruises 23-27 April 1979, 6-13 April 1980, 20-26 May 1980 and 22-28 July 1980 (see Anderson 1981). Larval redfish were picked from the plankton samples as soon as possible and placed directly in vials of 95% ethanol. After a period of 1-3 weeks, the preserving fluid was drained off and replaced with fresh ethanol. This was done because otoliths in poorly preserved samples may partially dissolve, thus erasing the increments (Methot and Kramer 1979). On three of the grid stations sampled 6-13 April 1980 bottom trawls for ripe, adult female redfish were carried out. Larvae were stripped from adults and preserved as above. It was noted that some females contained larvae in various stages of development, ranging from relatively shorter larvae with large yolk sacs to relatively longer larvae with yolk sacs nearly resorbed. No attempt was made to quantify the amount of yolk sac present but the larvae were grouped subjectively, as either yolk sac nearly complete or yolk sac nearly resorbed. All larvae were measured for standard length (S.L.) to the nearest 0.1 mm. Standard length was defined as tip of snout to tip of notochord in pre-flexion larvae and tip of snout to end of hypural plate in larvae undergoing flexion or post-flexion larvae. As sea-caught larvae were dead for variable periods of time before preservation, the associated shrinkage (see Theilacker 1980) may have contributed to variation in the length at age data, especially for the smaller size ranges. There was minimal shrinkage in the pre-extrusion larvae placed directly into 95% ethanol.

Dissections of the head region were carried out at 20-40X on microscope slides under glycerin and both sagittae were removed and allowed to stand in glycerin for times ranging from 2-24 hours. The length of time in glycerin was not found to affect the clarity of the otoliths but dissection under glycerin was found to be convenient for otolith manipulation. The glycerin was removed by successive washings in 95% ethanol and the otoliths allowed to dry on the slides. Using fine dissecting needles, they were then transferred to clean slides and placed in a drop of either Spurr or Epon resin without a coverslip. The resins were polymerized at 60°C and 70°C overnight for epon and Spurr, respectively.

Growth increments were counted at 125-800X with transmitted light. Both sagittae of each fish were counted except where ring structure was obscured. An increment is defined as one pair of adjacent light and dark bands. All counts were made at least twice, or until successive readings were within 5% of each other. In older sagittae, some adjacent increments intersect, particularly at the sides of the otolith. In this case the maximum number of bands was counted.

RESULTS

The sagittae of 146 sea-caught Sebastes sp. larvae and 26 pre-extrusion larvae were examined. Of these, 139 of the sea-caught and 25 of the pre-extrusion larvae were readable. The distribution of samples taken on each cruise is shown in Fig. 1. The length at age information with mean growth rate per day is summarized for each length group in Tables 1 and 2 (1979 and 1980 respectively). For 1979, a total of 38 otoliths, from larvae ranging from 5.6-14.6 mm were examined. Mean increment counts ranged from 2 in the 5.0-5.9 mm length group to a high of 58 in the 14.0-14.9 mm length group while the mean growth ranged from a low of 0.116 mm/d for the 7.0-7.9 mm length group to a high of 0.310 mm/d for the 8.0-8.9 mm length group.

For 1980, a total of 101 otoliths were examined from larvae ranging from 5.4 mm to 24.9 mm in length. Mean increment counts ranged from 2.5 in the 5.0-5.9 mm length group to 116 in the 22.0-22.9 mm length group, while the mean growth rate ranged from a low of 0.080 mm/d in the 7.0-7.9 mm length group to a high of 0.241 mm/d for the 7.0-7.9 mm length group .

Analysis of growth rates of larval redfish on Flemish Cap in 1979 and 1980 appeared to be linear over the lengths examined (Fig. 4 and 5). Linear regression by the least squares method yielded:

Year	Regression	n	R^2
1979	$Y = 0.143X + 6.6$	38	0.80
1980	$Y = 0.135X + 6.3$	101	0.91

where Y is the standard length in millimeters and X is increment count in days. The slopes of 0.143 and 0.135 mm/d estimate larval growth rates from the onset of increment formation. There was no significant difference between the slopes for 1979 and 1980 data ($P > 0.05$).

To test for non-linearity, the 1980 data set was repeatedly subdivided into two groups, based on increment counts, and t-tests were run on the pairs of groups. This procedure indicated a division of the data between 45 and 55 increment counts provided a maximum difference. However, subsequent regressions of the two data subsets did not yield significantly different slopes, indicating there was no significant inflection point in growth rate for this data.

Of the pre-extruded larvae examined 11 had yolks sacs nearly complete while in 14 the yolk sac was mostly resorbed. The mean increment count of each group was 2.55 (Range = 1-6) and 3.29 (Range = 2-5) but these means are not significant different at the 0.1 probability level ($t = -1.52$, $P > 0.15$).

Observation of the sagittae of the pre-extrusion larvae indicated that growth increments were already present prior to extrusion (see Fig. 2). These increments may not be "true" increments in the sense that they seldom completely encircled the sagitta. They usually consist of broken lines observable only on part of the sagittal rim, but indications are that these increments persist on older otoliths (see Fig. 3).

In older post-extrusion sagittae, there is typically a wide check-like band after the first few (typically 3) increments followed by a group of adjacent narrow increments usually numbering 10-15 (Fig. 3). Increments after this point are usually more widely spaced but tend to become narrower after 75-85 increments. Increments are believed to be formed daily, at least after extrusion (see Discussion).

DISCUSSION

For the April-July period on Flemish Cap results of larval ageing of Sebastes sp. indicated mean growth rates of 0.143 mm/d and 0.135 mm/d in 1979 and 1980, respectively. These results compare favourably with an estimated growth rate of 0.146 mm/d for May-July, 1980, based on field samples (Anderson MS 1981). In general, these growth rates are lower compared to other species. Larval herring growth rates averaged 0.195 mm/d on Georges Bank (Lough et al. MS 1980) and ranged from 0.21-0.29 mm/d in the Bay of Fundy during September-November (Das 1968). Northern anchovy larvae, Engraulis mordax, grew at rates of 0.34-0.55 mm/d (Methodt and Kramer 1979). An overall lower growth rate in larval redfish might be expected due to the slow growth of redfish throughout its life history, compared to other species.

At what point differences in larval growth rates become significant is not known. Comparison of larval Sebastes sp. growth rates of 0.143 and 0.135 in 1979 and 1980, respectively, based on otoliths indicated the slopes were not significantly different ($P < 0.05$). This is not surprising given the high degree of scatter about the lines (Fig. 4 and 5). In addition, analyses of both sets of data indicated growth was linear and also that no inflection point between higher and lower growth rates could be demonstrated. Again, the high degree of scatter and corresponding small number of observations would tend to obscure any non-linear relationship. Our expectations were that growth rate would be a decreasing exponent with time (Lough et al. MS 1980). Comparisons of growth rates versus length (Table 1 and 2) indicated this may in fact be the case for larvae <12-14 mm standard length, after which growth is approximately linear.

During development in the body cavity, the larvae are presumably growing in an optimal environment with large reserves of yolk and even the possibility of some maternal nutritional contribution (Moser and Butler 1981). Therefore, if there is an exponential growth phase during the larval period, it is most likely to be at this time. If there is no exponential phase in the pre-extrusion period, we expect that pre- and post-extursion growth will possibly be best described by a two-stage linear growth equation of the style proposed by Zweifel and Lasker (1976) for larval anchovy. Further work will be necessary to clarify growth characteristics in the pre-extrusion period.

Based on the study of 25 pre-extrusion larvae it appears that increments began to form in the post-hatch period prior to extrusion. Comparison of increment number to the amount of yolk sac remaining, while highly variable, indicated larvae with greater yolk sac absorption had more increments. Thus, sagittal growth appears to be related to the stage of development in the body cavity. The periodicity of this increment formation is not known. In otoliths from pre-extrusion larvae both means and ranges compare closely with observations of 5-7 mm sea-caught Sebastes sp. (Tables 1 and 2), suggesting larvae are extruded with a mean number of 3 increments. This is in contrast to observations by Radtke (MS 1980) who concluded increment formation began at the time of extrusion. Our data indicates redfish larvae are extruded at widely differing stages of development relative to otolith increment formation and yolk sac absorption. Consequently, variable extrusion of larvae at different stages of development may have a pronounced effect on larval mortality and subsequent survival.

In sagittae of pre-extruded larvae the increments are characterized as discontinuous bands observable only on part of the sagittal rim. They can be counted, however, and there is no indication these pre-extrusion increments become obscured by later growth, as reported for sockeye salmon (Wilson and Larkin 1980).

Exactly what causes the onset of increment formation is not known (Simkiss 1974). Larvae of the centrarchid, Tilapia mossambica, which are mouth-brooded for 10-12 days after hatching, do not begin increment formation until leaving the female's mouth (Taubert and Coble 1977). Anchovy form daily increments beginning with yolk sac absorption, while increments are laid down at the time of hatching with even, pre-hatch increments also apparent (Brothers et al. 1976). In contrast, herring larvae do not form daily increments until approximately three weeks after hatching (Lough et al. MS 1980). Hence, the onset of formation and early characteristics of larval otolith increments appears to be highly variable from species to species.

Periodicity in otoliths of larval redfish is assumed to be daily, at least for the post-extrusion period. Back-calculations of increment counts, corrected for pre-extrusion increments, from the date of capture during May and July surveys on Flemish Cap and compared with length frequency data (Anderson MS 1981) indicated peak extrusion in 1980 occurred on April 28 (±3-4 days). This corresponds very closely with the estimates by Anderson (MS 1981) and general published accounts of time of peak extrusion for redfish on Flemish

Cap (Templeman 1976). This evidence, the close agreement in growth rates compared to changes in length-frequencies (Anderson MS 1981) and the seemingly universal occurrence of daily growth increments in other species (e.g. Ralston 1976; Brothers et al. 1976; Pannella 1971, 1974; Struhsaker and Uchiyama 1976; Barkman 1987; Taubert and Coble 1977; Wilson and Larkin 1980), indicates the increments in larval Sebastes sp. are formed with daily periodicity.

In older, post-extrusion sagittae, there was typically a wide check-like band of 10-15 increments after the pre-extrusion bands. These were then followed by more widely spaced increments to the edge of the otolith (see Fig. 3). This check-like band may represent the period of growth immediately following extrusion. If this is true, then increment counts in sea-caught larvae may be corrected to give age from time of extrusion, instead of from the onset of increment formation. Work on this aspect is continuing.

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Table 1. Number of otolith growth increments and mean growth rate per day per unit standard length for larval Sebastes spp. from Flemish Cap, 1979.

Length group (mm. S.L.)	Otoliths examined	Increments ($\bar{x}$)	S.D.	Range	Growth rate (x mm.d.$^{-1}$)	S.D.
5.0-5.9	1	2.00	-	-	-	
6.0-6.9	6	5.00	3.03	1-10	-	
7.0-7.9	9	8.11	2.93	5-13	.116	.050
8.0-8.9	7	7.86	4.02	4-14	.310	.134
9.0-9.9	7	16.71	6.10	9-24	.190	.080
10.0-10.9	4	26.25	3.77	22-31	.154	.032
11.0-11.9	3	33.00	3.46	29-35	.155	.018
12.0-12.9	-	-	-	-		
13.0-13.9	-	-	-	-		
14.0-14.9	1	58.00	-	-	.138	-

Table 2. Number of otolith growth increments and mean growth rate per day per unit standard length for larval <u>Sebastes</u> spp. from Flemish Cap, 1980.

Length group (mm; S.L.)	Otoliths examined	Increments ($\bar{x}$)	S.D.	Range	Growth rate ($\bar{x}$ mm.d.$^{-1}$)	S.D.
5.0-5.9	4	2.50	1.91	1-5	-	-
6.0-6.9	21	3.19	2.04	0-9	-	-
7.0-7.9	14	11.50	8.04	1-24	.241	.352
8.0-8.9	8	13.88	5.46	8-23	.171	.055
9.0-9.9	13	21.23	5.78	13-33	.161	.038
10.0-10.9	11	25.18	7.61	12-36	.174	.062
11.0-11.9	5	36.40	6.07	29-43	.142	.032
12.0-12.9	5	52.40	16.09	43-81	.126	.027
13.0-13.9	1	85.00	-	-	.080	-
14.0-14.9	1	89.00	-	-	.091	-
15.0-15.9	2	73.50	14.85	53-84	.124	.028
16.0-16.9	-	-	-	-	-	-
17.0-17.9	3	86.00	13.23	71-96	.133	.022
18.0-18.9	3	87.00	3.46	83-89	.141	.010
19.0-19.9	2	104.50	10.61	97-112	.128	.010
20.0-20.9	4	88.75	13.10	72-104	.161	.026
21.0-21.9	2	99.50	7.78	94-105	.155	.011
22.0-22.9	1	116.00	-	-	.138	-
23.0-23.9	-	-	-	-	-	-
24.0-24.9	1	104.00	-	-	.179	-

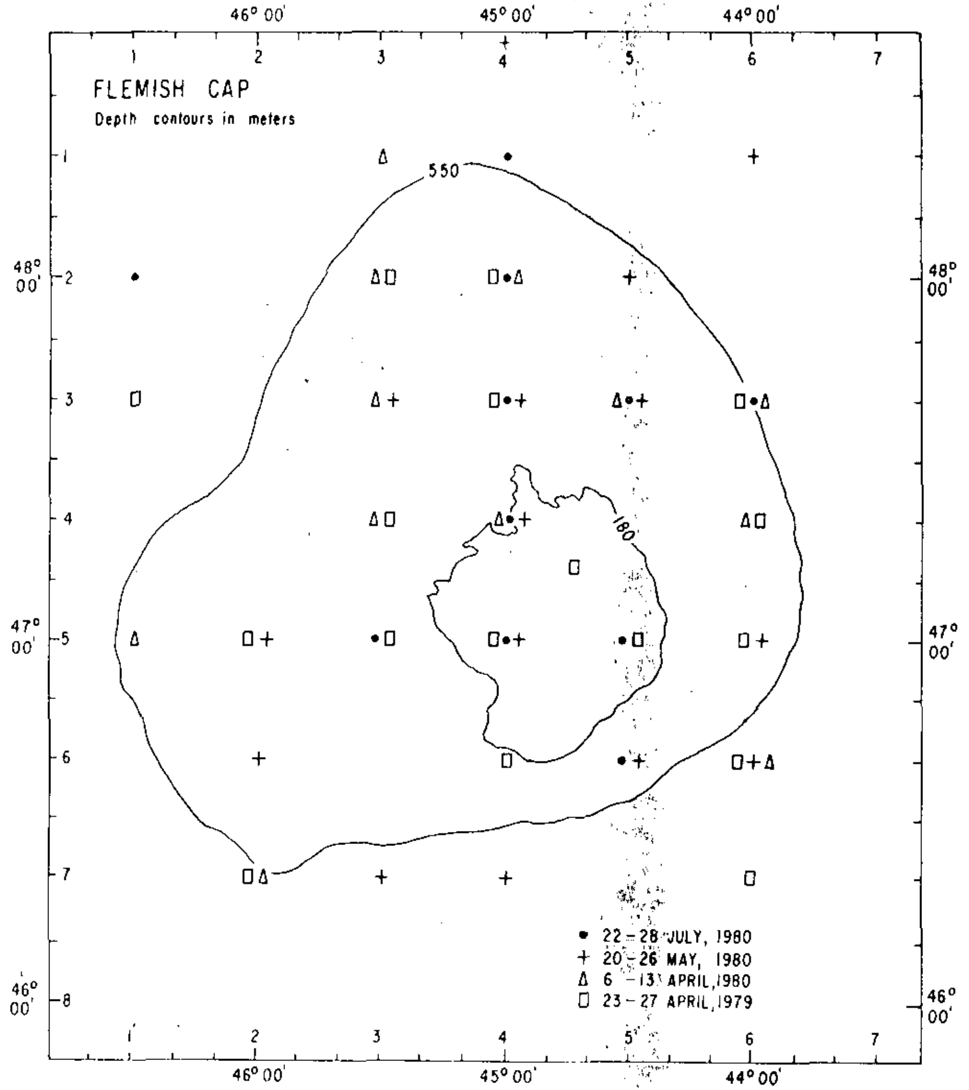

Figure 1: Redfish sample locations in 1979 and 1980 used for otolith examinations.

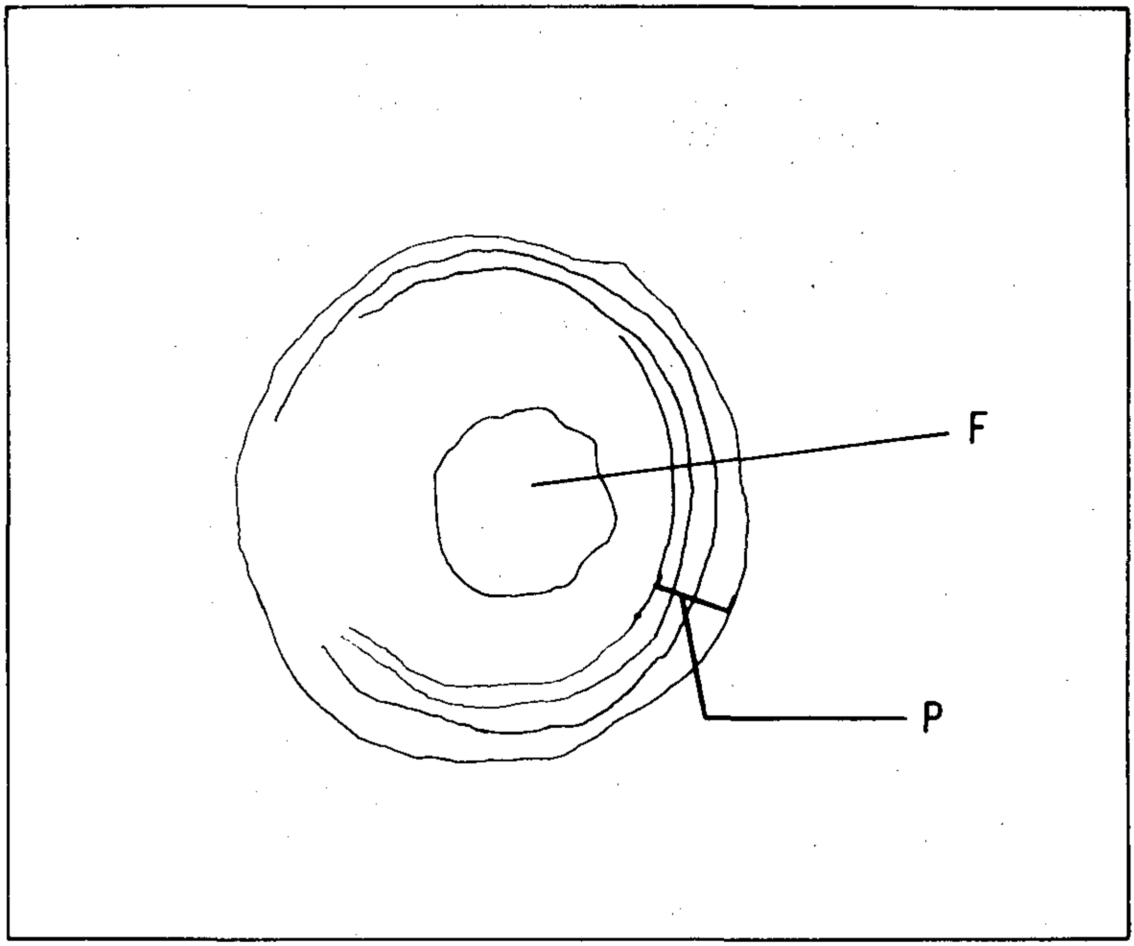

Fig. 2. Larval otolith taken from a 6 mm redfish (<u>Sebastes</u> spp.) pre-extrusion larva stripped from a ripe female. F is the focus; P is a zone of three pre-extrusion increments. (Drawing is done by camera lucida at 800 X).

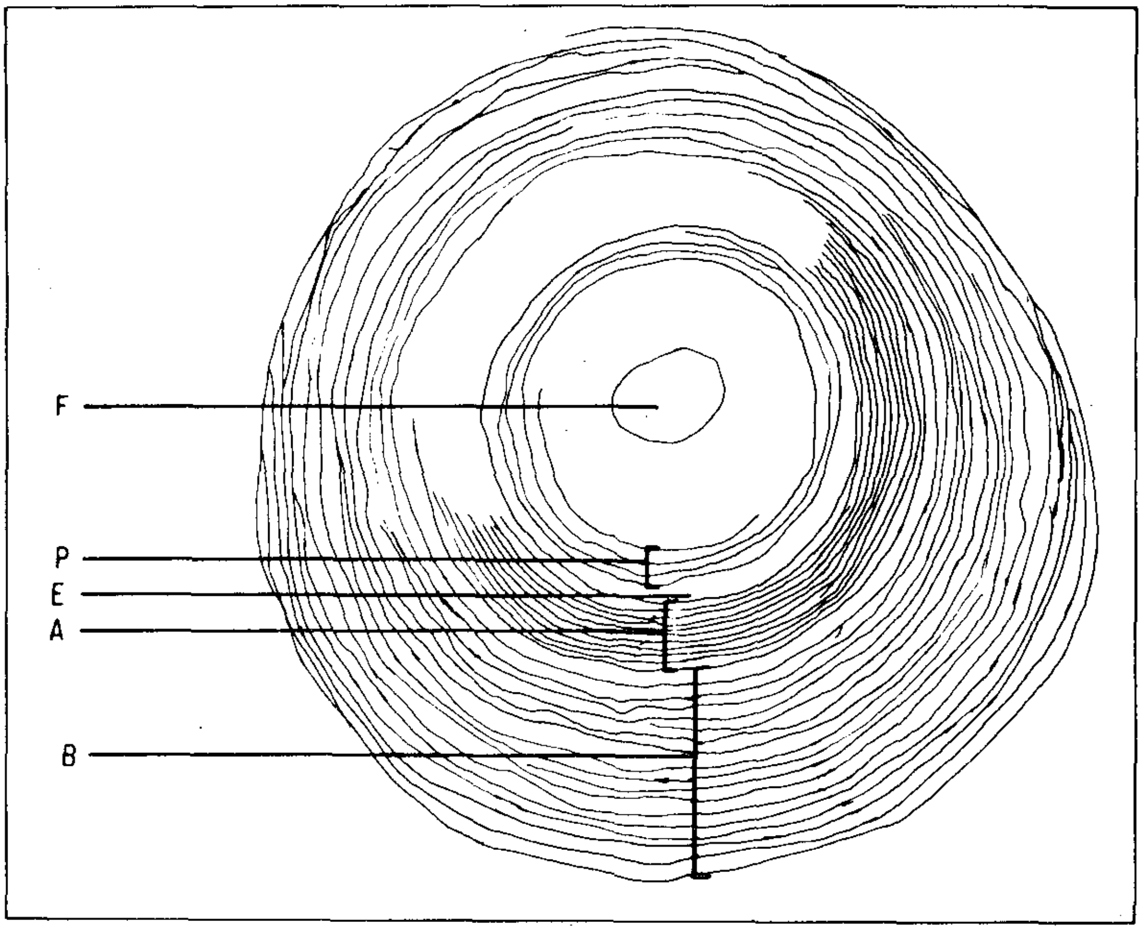

Fig. 3. Larval otolith taken from a 9 mm redfish (Sebastes spp.) larva. The total increment count is 31. F is the focus; P is believed to be three pre-extrusion increments; E is a wide band formed at extrusion; A is a zone of typically narrow early post-estrusion increments; B is a zone of later increments with wider inter-ring distances. (Drawing is done by camera lucida at 800 X).

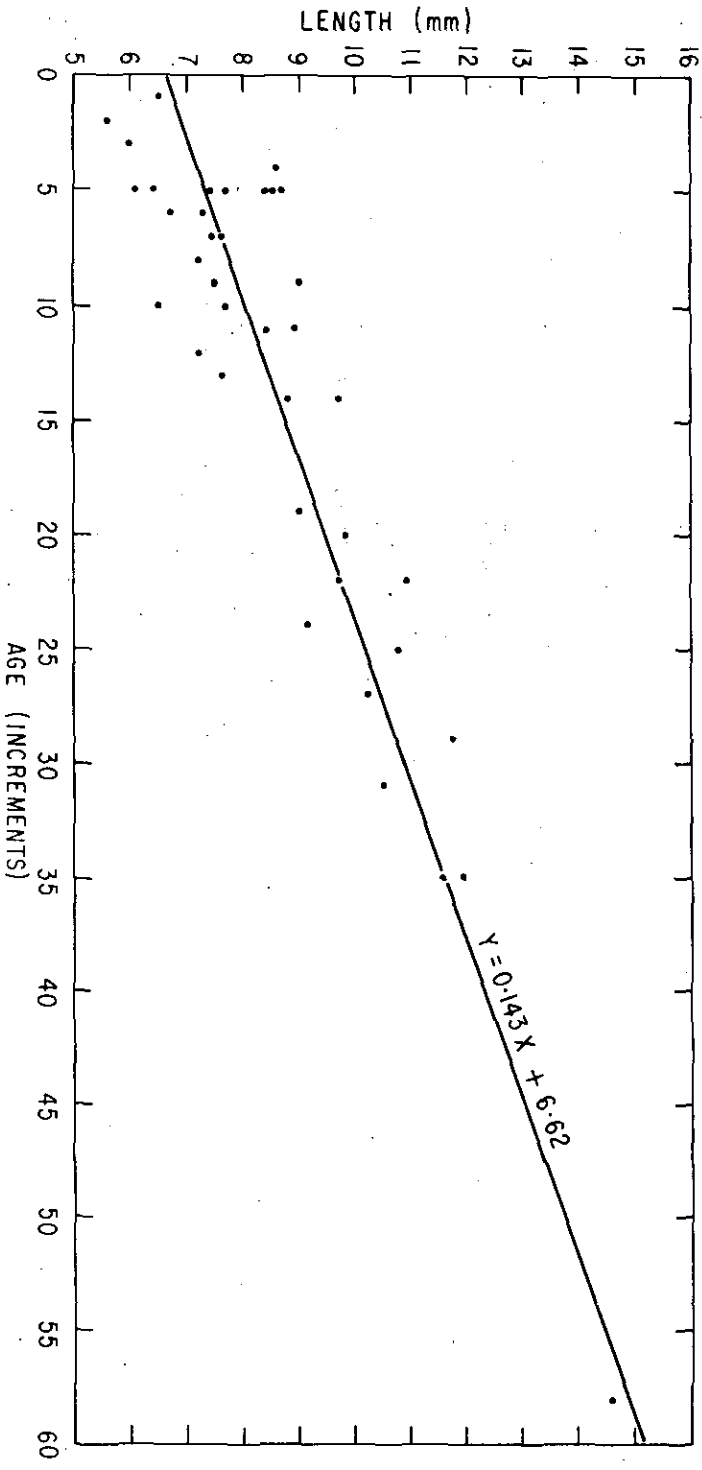

Figure 4: Age from onset of increment formation versus standard length for larval redfish (Sebastes spp.) in 1979 ($R^2 = 0.80$).

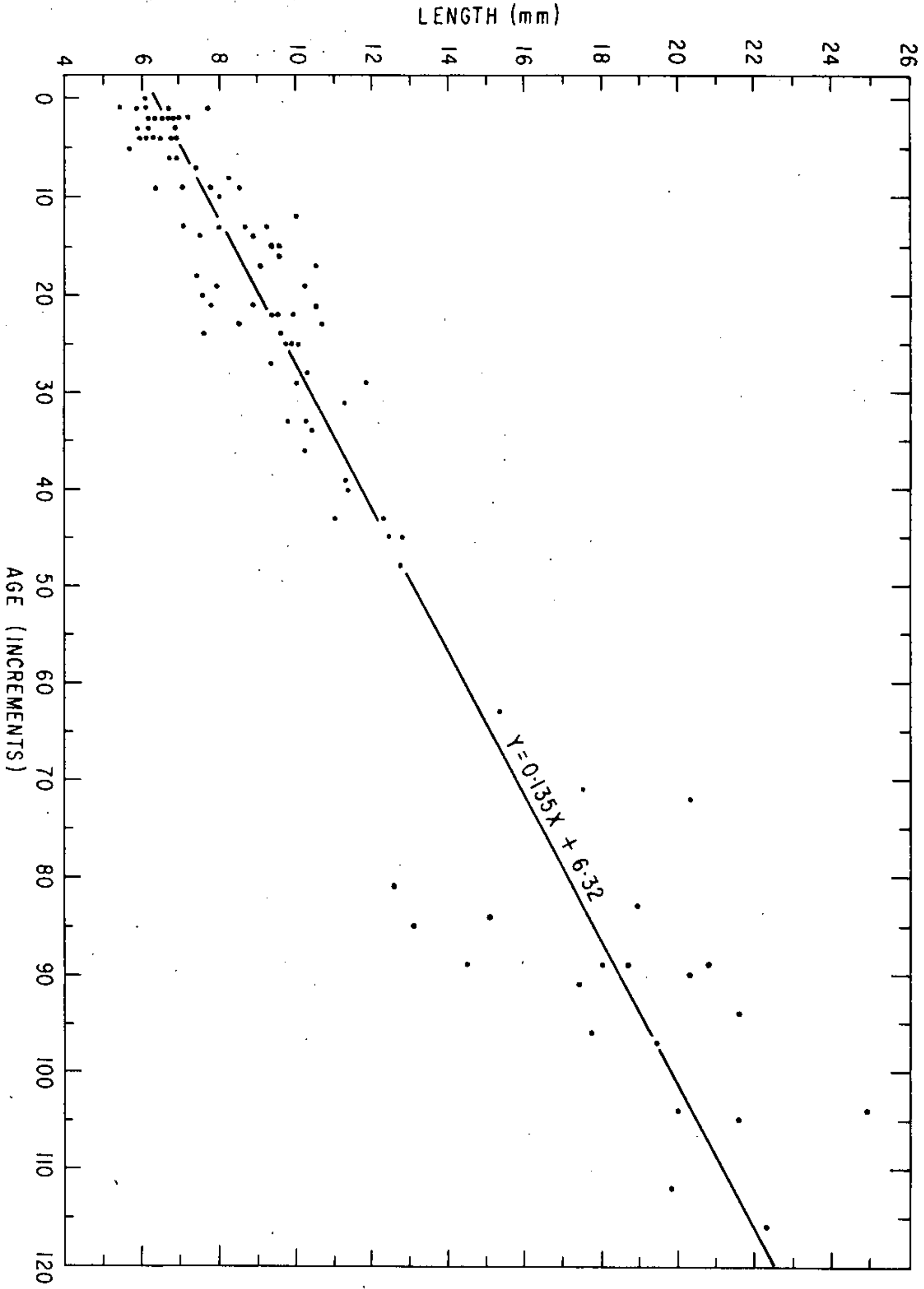

Figure 5: Age from onset of increment formation versus standard length for larval redfish (Sebastes spp.) in 1980 (R^2 = 0.91).