The Growth of the Fish Otolith.

Takaaki IRIE

Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama

(Plates 1—4; Text-figs. 1—8; Tables 1—5)

(I) INTRODUCTION

The sacculus otolith of the fish, together with its scale, is closely connected with the age and the growth of the fish. Most of the studies on the age and the growth of the fish have been carried out usually by means of observation of the zones in the scale because the observation of them is easier than those in the otolith. But, the zones in the scale are more obscure generally than those in the otolith. It is needless to say, therefore, that the studies can be carried out more reliably by means of the observation of the zones in the otolith than those in the scale. The effective factors of the zone formation of the fish otolith have been examined by some investigators;[1)–6)] yet, no accepted or established view on the factor has been attained until now. Accordingly, concerning the age of the fish and its growth history, it is difficult to expect accurate studies on them, and, therefore the number of the studies is actually few.

This is the reason why the study on the fish otolith had been begun by the present writer to discover a procedure for the estimation of the individual growth history of the fish through its zone. The writer already examined the otolith of the fish caught in various seasons by fishing, and the crystal texture and the forming season of the zones were thus made clear.[7)–8)]

In the present study, the otolith in the marine teleosts reared mostly by the writer, not caught from the sea, were directly examined through the year by means of electron microscope, X-rays and radioactive isotope Ca^{45}. Consequently, the formation of the opaque and translucent zones of the otolith and the various factors concerning its formation are here to be elucidated.

(II) MATERIALS

The materials used in the present study are marine teleosts, Fish-A, B_1, B_2, B_3, C_1 and C_2 as are shown in Table 1. They were originally caught in the Inland Sea of Seto (Seto-naikai), and, except for Fish-A, they were reared in the running sea water, and were given enough fish meat as their food once a day till they were used in the following experiments since they were caught. The fish belonging to Fish-B_2 were at least more than one year old when used in the experiment because they were reared by the present writer for more than 10 months.

Table 1. The various marine teleosts used in this study. The body length and the age indicate those at the period in which the fish were caught.

	Scientific name	Japanese name	Time caugth	Body length	Age	Object & Instrument
Fish-A	*Argyrosomus argentatus* (HOUTTUYN)	SHIROGUCHI	October, 1959	12~22 cm.	2	The crystal texture by using X-rays.
Fish-B_1	*Lateolabrax japonicus* (CUVIER)	SUZUKI	April, 1957	about 3 cm.	0	Effects of the food by using Ca^{45}.
Fish-B_2	same as above	---	April, 1958	about 3 cm.	0	The crystal texture by using electron microscope. Effects of the concentration of water by using Ca^{45}.
Fish-B_3	same as above	---	April, 1959	about 3 cm.	0	Formation of $CaCO_3$ by using Ca^{45}.
Fish-C_1	*Mylio macrocephalus* (BASILEWSKY)	KURODAI	August, 1957	about 7 cm.	0	Effects of the concentration of water by using Ca^{45}.
Fish-C_2	same as above	---	August, 1958	about 7 cm.	0	Effects of the water temperature by using Ca^{45}.

(III) EXPERIMENTAL PROCEDURE

Experiment-1: Observation of the otolith slice by means of X-rays.

This observation was performed to make clear the status of micro-crystal grains of the calcium carbonate ($CaCO_3$) which is the main constituent of the otolith. As its specimens, the otoliths (8~11mm. in length, 6~8mm. in width, 3~4mm. in thickness) obtained from Fish-A (shown in Table 1) were used.

Each otolith was polished into a thin slice (0.1 mm. thick) so as to make the surface plane perpendicular to the lengthwise axis of the otolith and to make it cross the center (shown in Fig. 1 and concerning this procedure confer the previous experiment.[7]). Laue photographs of every portion in this slice were taken by using X-rays through a narrow circular slit (0.09 mm. in diameter) emitted from

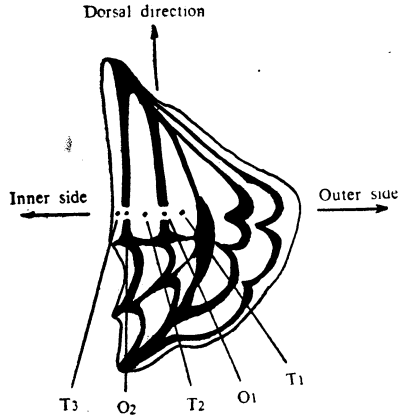


Fig. 1. Cross sectional patterns of the otolith of *Argyrosomus* (Japanese name: SHIROGUCHI, two age, body length: 20.5 cm., Body weight: 200g. Fish-A). The sectional plane is perpendicular to the lengthwise axis of the otolith and crosses its center. T_1, T_2, and T_3 are in the first, second and third translucent zones, and O_1 and O_2 are in the first and second opaque zones respectively.

a micro-focus (0.01×0.02 mm.^{2}) on the Cu-target. In this case, the beam of X-rays strikes a plane of the slice at right angle, and the photographic film was set perpendicularly to the incident beam behind the slice at the distance of 30 mm.

Experiment-2: Electron microscopical observation of the surface of otolith.

The surface of the otoliths which were taken out from Fish-B_2 (shown in Table 1) in February, May, July, August, September and December, 1959, was observed by means of electron microscope in the following way. Each otolith was washed carefully not to injure its surface and then dried in a room. Triafol seat (0.04 mm. in thickness) was adhered closely to its surface using aceton and was stripped off after a few minutes. Then Cr-preshadowed carbon replica was made from this triafol replica. This Cr-preshadowing was done from its lengthwise direction and its inclination to the surface was about 45°. This replica was divided into three divisions, Po., M. and An. as is indicated in Fig. 2-b. These replica was observed by means of AKASHI I. R. S.-50 Electron Microscope.

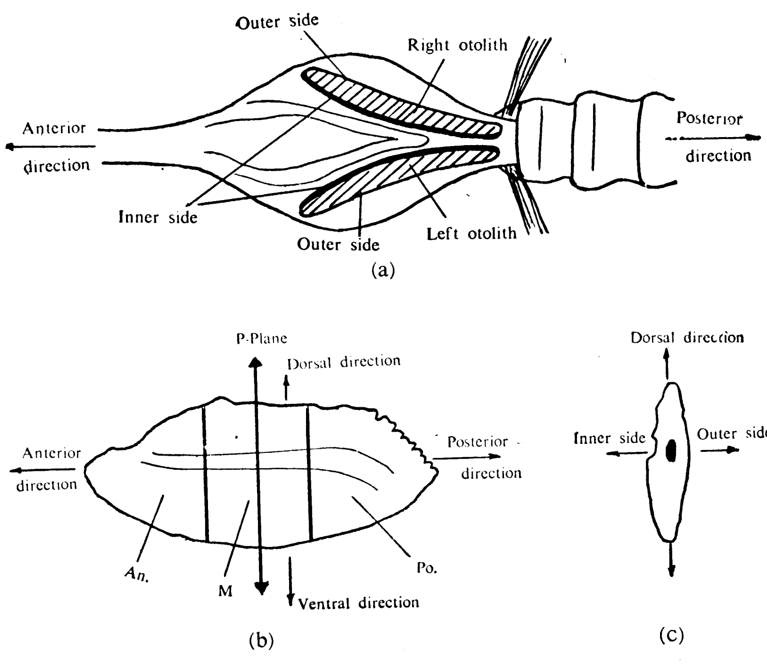


Fig. 2. Otolith of *Lateolabrax* (Japanese name: SUZUKI, Fish-B_1, B_2 & B_3).
(a) View from the ventral direction.
(b) The inner side surface of the otolith. P-Plane is perpendicular to the lengthwise direction of the otolith.
(c) The cross section of the P-Plane perpendicular to lengthwise direction of the otolith.

Experiment-3: Observation of the otolith slice by means of autoradiograph using Ca^{45}.

In order to make clear the growing direction of the otolith, its velocity and the depositing location of calcium, this observation was done by means of autoradiograph using Fish-B_3 (shown in Table 1).

In June, 1959, similar sized fish taken out from the group of Fish-B_3 (grown up to about 6 cm.) were put into the rearing tanks at the rate of 3 fish per 30 *l.* of the sea water, and then the preparatory rearing of these fish was carried on for the following 5 days. When the preparatory rearing was concluded, radioactive isotope Ca^{45} ($CaCl_2$ in HCl solution) was added at the rate of 4 μc. per *l.* of the water. Now, the amount of this Ca^{45} was determined through many preparatory experiments in the previous year. These fish were reared continually in the water containing Ca^{45} for the following 10 days. Half of the water in the tank was exchanged every other day for the fresh sea water which has the concentration of 18‰ in chlorinity, its temperature, 21~25°C and saturating degree of disolved oxygen, above 95%. The amount of $CaCl_2$ added together with $Ca^{45}Cl_2$ was so small that no significant variation was discovered concerning the pH, the concentration and the amount of calcium. Fish meat was given for their food sufficiently once a day and they took it with positive appetite. When this way of rearing using Ca^{45} was concluded, these fish were put into the running sea water, and were reared with the same food.

In the following September, these fish grown up to about 12 cm. were put into the water containing Ca^{45} and were reared again by the procedure similar to that in June. In September, however, water temperature was kept at 20~23°C and 2.7 μc./*l.* of Ca^{45} was used in order to keep the radioactivity at the same intensity as the previous one, because 4 μc./*l.* of radioactivity used in June is to decay naturally to 2.7 μc./*l.* in September.

When the second rearing experiment using Ca^{45} was finished, otoliths were taken out from these fish. Each otolith was polished into a thin slice (about 0.2 mm. thick) so as to make the surface plane (P-Plane indicated in Fig. 2-b) perpendicular to its lengthwise axis and to make it cross the center. One side of this slice was adhered closely to a glass plate with wax, and its micro-autoradiograph was prepared by means of the stripping-emulsion method. Then emulsion membrane was stripped off from its surface and was fixed on another glass plate.

By the same procedure as is described above, micro-autoradiographs of the otolith slice were also made using other fish, which were reared for 10 days twice, September and November, in the rearing tank containing Ca^{45}.

Experiment-4: Effect of the concentration of sea water concerning the growth of otolith.

Amount of calcium taken from the sea water and deposited on the otolith surface was examined by using Ca^{45} as a tracer. Nine fish of similar size were taken out from the group of Fish-C_1 (shown in Table 1), and were divided into three, A-, B- and C-group. Each group which has three fish was put into a rearing tank having 30 *l.* of sea water. In the first 5 days, the rearing water of A-group was diluted gradually to 17‰ in chlorinity, B-group to 14‰ and C-group to 10‰ respectively using fresh water. During the following 5 days, preparatory rearing of these fish was carried on in the tank keeping each concentration unchanged. During this preparatory rearing, the enough fish meat was given as their food once a day.

When this preparatory rearing was concluded, radioactive isotope Ca^{45} ($CaCl_2$ in HCl solution) was added to every group at the rate of 3 μc. per *l.* of the sea water. The amount of $CaCl_2$ added together with $Ca^{45}Cl_2$ was so small that no significant variation was found concerning the pH, the chlorinity and the amount of calcium. Then through the following 10 days, the fish were reared without food in the sea water containing Ca^{45}. In this experiment, half of the water in every tank was exchanged every other day for fresh sea water which maintains each concentration, temperature and amount of Ca^{45} in the same conditions as before. After this rearing was concluded, the otoliths were taken out from each fish.

This kind of rearing experiment was repeated six times during the period from September, 1957 to June, 1958. Various conditions for this experiment are denoted in Table 2. Similar experiment was also carried out concerning Fish-B_2 (shown in Table 1).

Table 2. Conditions for Experiment-4. Each datum indicates the average value. But the item of Water temperature indicates the deviation of temperature through each experiment. And the item of Dissolved oxygen indicates the minimum value and it was 95 ~ 100 % through almost all period. The amount of food taken through the preparatory rearing is denoted as follows.
FF…Taken with appetite F…Taken a little N…Taken nothing

		September 1957	October	December	February 1958	May	June
Fish length (cm.)		8.5	8.8	9.6	9.5	9.8	10.6
Fish weight (g.)		16	22	25	26	26	30
Feeding in preparatory rearing		FF	FF	N	N	F	F
Otolith (length) × (width) (cm.)		0.39×0.26	0.41×0.28	0.46×0.29	0.46×0.30	0.51×0.33	0.53×0.33
Water temperature (°C)		22±1	20±1	10±1	8±1	18±1	23±2
Dissolved oxygen (% in saturating degree)		>90	>90	>92	>96	>90	>91
A-group	Concentration of water (‰ in chlorinity)	17	17	17	17	17	17
	Dissolved Calcium (g./*l.*)	0.37	0.36	0.37	0.36	0.36	0.36
B-group	Concentration of water (‰ in chlorinity)	14	14	14	14	14	14
	Dissolved calcium (g./*l.*)	0.31	0.31	0.30	0.30	0.30	0.29
C-group	Concentration of water (‰ in chlorinity)	10	10	10	10	10	10
	Dissolved calcium (g./*l.*)	0.23	0.22	0.23	0.22	0.23	0.22

Measuring method of radioactivity.

Each otolith removed from the fish was washed carefully not to injure its surface and was dried sufficiently in the room. Then a pair of otolith was placed in the center of a dish (2.8 cm. in diameter, 0.5 cm. in depth) which was made of stainless steel as is shown in Fig. 3-a. Radioactivity of the pair of otolith was counted N_1 counts/min. from one side of its surface by using a radiation counter, TOKYO SHIBAURA Electric Co. R. G. D-100-B. The distance from the mica window of G-M tube to the half height of the otolith was always kept 1.0 cm. as is shown in Fig. 3-b. Then, the pair was turned upside down and their radioactivity was counted N_2 counts/min. in the same way (shown in Fig. 3-c).

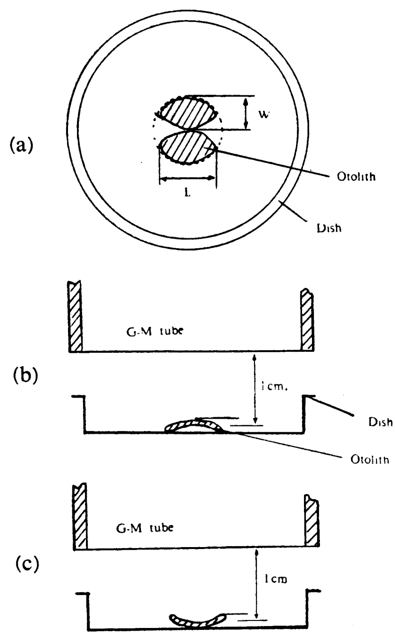


Fig. 3. (a) Top view of a pair of otolith of *Mylio* (Japanese name: KURODAI. Fish-C_1 & C_2) in the dish. (b), (c) Cross view of G-M tube, the dish and the otolith

When the radioactivity is counted from one side of the otolith, it is clear that the amount of this counts contains not only the counts of that side but also those of radioactivity penetrated from the other side. In order to make clear the amount of counts of the latter, the following experiment was carried out using many otoliths of various size. At first, the otolith was coated using dilute solution of $Ca^{45}Cl_2$, and after it was dried, radioactivity of its surface was counted T counts/min. Then, it was turned upside down and was counted t counts/min. The value of the ratio of both counts $R = t/T$ was thus found approximately to be 0.08 concerning the otolith used in the present experiment. It can be estimated therefore that total radioactivity of a pair of otolith is approximately $\frac{N_1 + N_2}{1.08}$ counts/min.

The length and the width of each otolith were measured by using microscope. L in the following researches shows the average length of the pair of otolith and W shows their average width. The pair of otolith of *Mylio* could be arranged almost uniformly in a dotted circle of radius W cm. as is shown in Fig. 3-a. Therefore, the radioactive source may be regarded approximately as a circular plane of radius W cm. Geometrical efficiency G of G-M counter was obtained by means of Cook's method[9]. The radioactivity of the standard source (producted by the Scientific Research Institute, and its disintegration, D_o per minute) was counted n counts/min. by using the same counter. Geometrical efficiency G_o of the standard source was obtained by Cook's method too. If the correction for the absorption of β-rays in the mica window of G-M tube and in the air between G-M tube and the specimens, is denoted by f_w, the following equation will be obtained approximately.

$$D_\circ = \frac{n}{G f_w} \quad \text{(dis./min.)}$$

From this equation, the f_w was calculated. The amount of total disintegration (D dis./min.) of the pair of otolith can be got as follows.

$$D = \frac{N_1 + N_2}{1.08\, G f_w} \quad \text{(dis./min.)}$$

Therefore, the amount of radioactive calcium (R μc.) contained in a whole surface of the pair of otolith can be got as follows.

$$R = \frac{D}{3.7 \times 10^4 \times 60} = \frac{4.2\,(N_1 + N_1)\, D_\circ G_\circ \times 10^{-7}}{Gn} \quad (\mu c.)$$

The amount of radioactivity 3 μc./*l.* of Ca^{45} which was put into the rearing water in the beginning of this experiment, will be decreased to 2.9 μc./*l.* during the following 10 days by its natural decay. When the amount of calcium contained in the water is K g./*l.*, the amount of calcium Z g., taken from the sea water and deposited on the surface of the pair of the otoliths during these 10 days, is obtained from the following formula.

$$Z = \frac{KR}{2.9} = \frac{1.4\,(N_1 + N_2)\, D_\circ G_\circ K \times 10^{-7}}{Gn} \quad (g.)$$

From the amount of Z of each fish, calcification rate Z′ g. per cm^2. of the otolith surface per day was calculated from the following formula.

$$Z' = \frac{Z}{LW \times 0.70 \times 4 \times 10} = \frac{Z}{28\,LW} \quad (g./cm^2.\ day)$$

In this formula, $LW \times 0.70 \times 4$ cm^2. was obtained through many observations of otoliths and may be regarded the approximate total surface area of the pair of otoliths in *Mylio*.

Yet, in the case of the otolith of *Lateolabrax*, the radioactive source may be regarded approximately as a circular plane of radius $1.2 \times W$ cm. (Fig. 4).

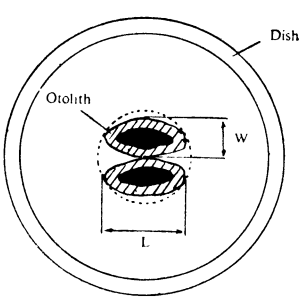


Fig. 4. A pair of otolith of *Lateolabrax* in the dish. Radius of the circle drawn by a dotted line is 1.2 W cm. and most otolith of *Lateolabrax* could be arranged almost uniformly in this circle.

Surface area of the pair of otolith was approximately $LW \times 0.74 \times 4\,cm^2$. Hence, the calcification rate Z' was obtained as follows.

$$Z' = \frac{Z}{LW \times 0.74 \times 4 \times 10} = \frac{Z}{30LW} \quad (g./cm^2.\ day)$$

The correction for the self-absorption loss of the radioactivity was made by using Comar's data[11]. In the present study, the amount of calcium deposited upon the otolith during these 10 days was mostly less than $0.5\,mg./cm^2$. In this range, no correction was made. But, according to Comar's data, the counts were corrected 3 % when the amount of calcium was $0.5 \sim 1.0\,mg./cm^2$., and were corrected 6 % when it was $1.0 \sim 1.5\,mg./cm^2$.

The central part of one side surface of various sized otolith (dark part shown in Fig. 4) was coated by using the solution of $Ca^{45}Cl_2$. But the radioactivity of this surface could not be counted from the other side. Therefore, back-scattering can be neglected concerning these otoliths used in the present experiment. And it was found that the value of R is due to the thin hem of the otolith (the part denoted by oblique line in Fig. 4), because, in the central part of the otolith, the value counted from one side did not contain the counts penetrated from the central part of the other side.

Experiment-5: Effect of the water temperature concerning the growth of otolith.

The following experiment was also carried out using Ca^{45} as a tracer. In November, 1958, nine fish of similar size were taken out from the group of Fish-C_2 (shown in Table 1), and divided into three, S-, H- and M-group. Each group which has three fish was put into a rearing tank having 30 *l.* of sea water of 15°C. In 5 days, the water temperature of S-group was raised gradually to 22°C, and H-group to 20°C, while M-group was kept at 15°C constantly. The concentration of the water in every tank was kept at 17 ‰ in chlorinity. For the following 5 days, preparatory rearing of these fish was carried on in the tank keeping each temperature unchanged. During this period, enough food was given once a day.

When this preparatory rearing was concluded, Ca^{45} ($CaCl_2$ in HCl solution) was added to every group at the rate of $3\,\mu c.$ per *l.* of the sea water. Then, through the following 10 days, these fish were reared without food in the tank containing Ca^{45}. Through these procedures, half of the water in every tank was exchanged in the similar way mentioned before. When this rearing was concluded, the otoliths were taken out from each fish.

This kind of rearing experiments were also repeated in January, March and May, 1959. Various conditions for this experiment are shown in Table 3.

Experiment-6: Effect of the amount of food concerning the growth of otolith.

The following experiment was also carried out using Ca^{45} as a tracer. Similar sized 10 fish were taken out from the group of Fish-B_1 (shown in Table 1) and were divided into two, F-and N-group. Each group which has 5 fish was put into

Table 3. Conditions for Experiment-5. FF, F and N are similar to Table 2. Activity of the fish is denoted as follows.
VS……Moved very actively, S……Moved sensitively,
D……Moved dully.

		November 1958	January, 1959	March	May
Fish length (cm.)		9.5	9.5	9.2	9.7
Fish weight (g.)		24	20	20	28
Otolith (length) × (width) (cm.)		0.44×0.28	0.44×0.29	0.42×0.28	0.46×0.30
Water temperature (°C) / Food : Activity	S-group	22 ± 1 / FF : VS	---	---	22 ± 1 / FF : VS
	H-group	20 ± 1 / FF : VS	20 ± 1 / FF : VS	20 ± 1 / FF : VS	20 ± 1 / FF : VS
	M-group	15 ± 1 / F : S	15 ± 1 / F : S	15 ± 1 / F : S	15 ± 1 / F : S
	L-group	---	10 ± 1 / N : D	10 ± 1 / N : D	---
Concentration of water (‰ in chlorinity)		17	17	17	17
Dissolved calcium (g./*l*.)		0.36	0.37	0.37	0.35
Dissolved oxygen (% in saturating degree)		>96	>96	>99	>93

a rearing tank having 30 *l*. of the sea water, and then preparatory rearing was carried on for the following 10 days giving plentiful fish meat once a day.

When the preparatory rearing was finished, Ca^{45} ($CaCl_2$ in HCl solution) was added to every group at the rate of 3 μc. per *l*. of the sea water. Through the following 10 days, the fish of F-group were given food continuously, but N-group was reared without food. Every remains of food in the former case was taken out instantly. Half of the water in every tank was exchanged in the similar way as was mentioned before.

This method of rearing was repeated eight times through the period from June, 1957 to June of the next year. However, during the period from December to June, 1958, 60 *l*. of water instead of 30 *l*. was put into each tank, because the fish had grown more than 15 cm. in length. Various conditions for this experiment are shown in Table 4.

When the rearing continued for 10 days in the sea water containing Ca^{45} was concluded, the otoliths were taken out from each fish. Their radioactivity was counted in the same way as was explained before. In this case, it may be inferred that Ca^{45} could not be contained in the calcium compounds of given food (for instance, bone, fin etc.). Therefore, the amount of calcium Z g. taken from the sea water and deposited to the surface of the otoliths can be obtained by the above-mentioned method.

Mylio and *Lateolabrax* used in Experiment-2 ~ Experiment-6 were not adult but young fish reared artificially. The difference, however, could not be found

Table 4. Conditions for Experiment-6. FF, F and N denote the amount of food taken by F-group similarly to Table 2.

	June 1957	July	August	September	December	February 1958	May	June
Fish length (cm.)	6.5	7.2	9.1	10.9	15.3	16.4	16.3	16.5
Fish weight (g.)	5	6	12	21	56	68	60	70
Amount of food of F-group	FF	FF	FF	FF	N	N	F	F
Otolith (length) × (width) (cm.)	0.39× 0.21	0.43× 0.23	0.51× 0.27	0.62× 0.31	0.81× 0.41	0.84× 0.42	0.83× 0.40	0.89× 0.42
Water temperature (°C)	23 ± 2	26 ± 1	26 ± 1	22 ± 1	10 ± 1	8 ± 1	18 ± 1	23 ± 2
Concentration of water (‰ in chlorinity)	17	17	17	17	17	17	17	17
Dissolved calcium (g./l.)	0.35	0.36	0.35	0.37	0.36	0.36	0.36	0.35
Dissolved oxygen (% in saturating degree)	>94	>94	>93	>96	>94	>96	>93	>94

between the zones of the adult fish and those of reared ones. Moreover, the rearing conditions were regulated as nearly as possible to natural conditions. And, it is probable therefore that no essential difference may be seen between these two kinds of fish.

(IV) RESULTS

From the above-mentioned experiments, the following results were obtained.

(1) Laue photographs of every portion of the otolith slice were taken through the Experiment-1 and the typical examples of them are shown in Plate 1. In these Laue photographs, Fig. A, B, C, D and E are the radiographs of the portions denoted by T_1, O_1, T_2, O_2 and T_3 in Fig. 1 respectively. Therefore, Fig. A, C and E are radiographs of the portions in the translucent zone, and Fig. B and D are those of portions in the opaque zone. From these radiographs, the followings were obtained. The micro-crystals of $CaCO_3$ in any part of the otolith of *Argyrosomus* are in the form of aragonite and are fibrously arranged similarly to *Pseudosciaena*[7]. The size of the micro-crystal grains of $CaCO_3$ in the translucent zone is larger than that of the opaque one generally.

(2) Electron micrographs of the otolith surface were taken through the Experiment-2 and representative ones are indicated in Plate 2 and 3. Magnifications of these micrographs are all 5,000 ×. The observations obtained from these micrographs are shown in Table 5. From this table, the followings were induced.

(a) In summer and autumn, the size of crystal grains in the otolith surface is generally larger than in winter and spring.

(b) In summer and autumn, the groove between the boundaries of the grains are deeper than in winter and spring. In winter and spring, much amount of something which may be a kind of organic matter can be observed in the groove, and the surface is smooth comparatively. While it can be observed little in summer

Table 5. The surface texture of the otolith observed using the electron micrographs. Division An., M. and Po. are denoted in Fig. 2-b.

• ......the small sized grain
● ......the medium sized grain
⊙ ......the large siged grain
Or......much organic matter in the groove.
× ......little organic matter in the groove.
v ......the shallow groove between grains.
V ......the ordinary groove between grains.
V ......the deep groove between grains.

	Division An.	Division M.	Division Po.
February	• v Or	• v Or	• v Or
May	• v ×	• V Or	• v Or
July	● V ×	● V ×	● V ×
August	● **V** ×	● **V** ×	● **V** ×
September	⊙ **V** ×	⊙ **V** ×	⊙ **V** ×
December	● V ×	• V ×	⊙ V ×

and autumn.

(c) No significant difference is found between the divisions, An., M. and Po.

(3) The micro-autoradiographs of the otolith slice were taken through the Experiment-3 and typical one of them is shown in Fig. A of Plate 4. It is clear that the darkness of these zones depends on the amount of Ca^{45} deposited to the otolith. Fig. B of Plate 4 is a photomicrograph of the same slice, and it can be seen that the whole area of this slice is translucent except its central area. Fig. 5

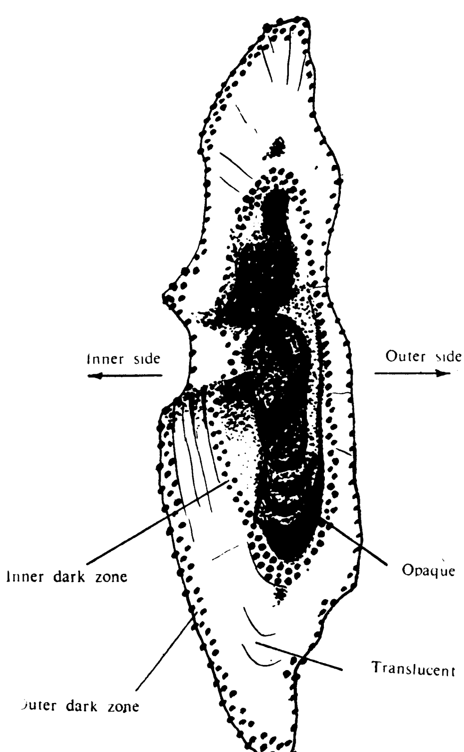


Fig. 5. The otolith slice of *Lateolabrax* (Fish-B_3) and its autoradiograph. Dark central part written with small dotts is a opaque part of the slice and the other part is translucent. Two zones written with large dotts are the dark zones of the autoradiograph.

is a figure traced by putting Fig. A upon Fig. B. As is shown in this figure, the shape of the outer hem of the outer dark zone is nearly the same with that of the edge of the slice, and that of inner hem of the inner dark zone is nearly the same with that of the outer edge of the opaque zone. Between those two dark zones, a light zone whose breadth is more than two times of these dark zones can be seen. Accordingly, it can be inferred that the inner and outer dark zones were produced by Ca^{45} taken from the rearing water and deposited on the surface of the otolith in June and September, and the opaque zone in the central part of this slice was already formed before the experiment in June was begun.

Moreover, those two zones which can be seen in the upper, lower and left part (inner side of the actual otolith as is shown in Fig. 2-a) are darker and wider than those in the right part (the outer side of the actual otolith). And concerning the distance between these two zones it was observed that the largest is the upper and lower, the smallest is the right and the medium is the left part. Similar results were found also in the radiographs of the otolith in the fish reared twice in the rearing tank containing Ca^{45} in September and November. Accordingly, it may be concluded that the otolith grows mainly by depositing upon its surface calcium contained in the sea water, and that, concerning the rapidity of its growth, the largest is the two extreme ends of the otolith, the smallest is its outer side, and the medium is its inner side.

In Fig. 5, it is seen that the outer dark zone of radiograph protrudes more or less beyond the outer edge of the slice. Such a little disagreement may be due to the fog of the radiograph. And the same fog can be imagined to exist on all hem of these two zones. Therefore, though the hem line is not sharp but rather dim, it is imagined that the two zones exist in a definite and clear shape. This fact shows that otolith grows piling up the layer gradually for outward direction. The boundary between the inner hem of the inner zone and the outer edge of the opaque zone is to show the turning point in its growth from the piling of the opaque layer to that of the translucent layer, but it is hard to examine its fine structure minutely owing to the inaccuracy of this radiograph. It was confirmed originally and systematically for the first time through these experiments, however, that the opaque zone was already formed before June every year, and the translucent zone was begun to be formed from June and was continued to September of the same year.

(4) Concerning the concentration of the sea water and the calcification rate Z' of the otolith, the results shown in Fig. 6-a were obtained through Experiment-4. Water temperature in this experiment was shown in Fig. 6-b. From those figures, the followings were found.

(a) In any season, no significant difference of the calcification rate was discovered between A-, B- and C-group. In other words, the calcification rate of the otolith of *Mylio* and *Lateolabrax* is independent of the concentration of the sea water surrounding the fish.

(b) From June to October, the calcification rate of the otolith is larger than

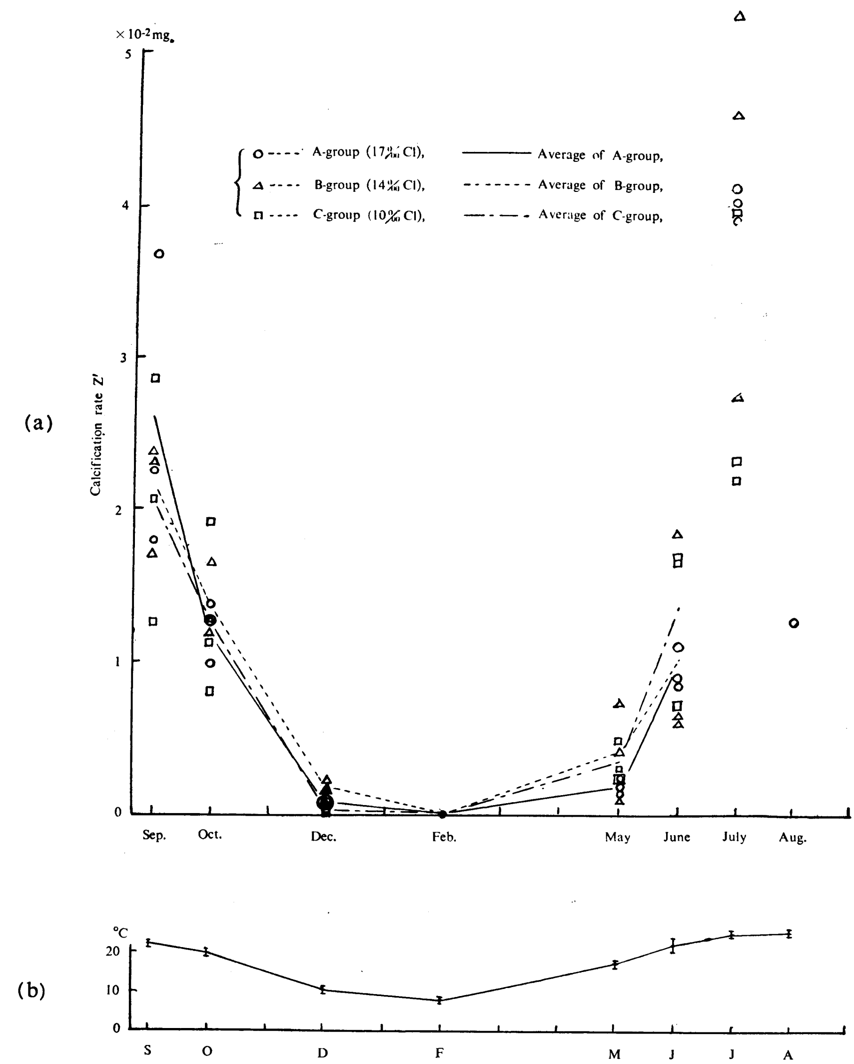


Fig. 6. (a) Seasonal changes of calcification rate Z′ mg./cm2. day of the otolith of Fish reared in various concentrations of the sea water (Exp.-4). Values in July were obtained by using *Lateolabrax* (Fish-B_2), and the others were *Mylio* (Fish-C_1). (b) Temperature of the rearing water in each experiment.

in other months. Especially, in February and March, it becomes very small.

(c) In any case of concentration of the rearing sea water, calcification of the otolith is seen scarcely when the water temperature is below 10°C, a little when it is about 15°C and conspicuously when it is above 20°C.

(5) Concerning the water temperature and the calcification rate Z′ of the otolith, the results shown in Fig. 7 were obtained through Experiment-5. Values of Z′ in September, October, December and February were reproduced from the value of A-group (17 ‰) in Fig. 6-a. The activities of the fish in the tank were

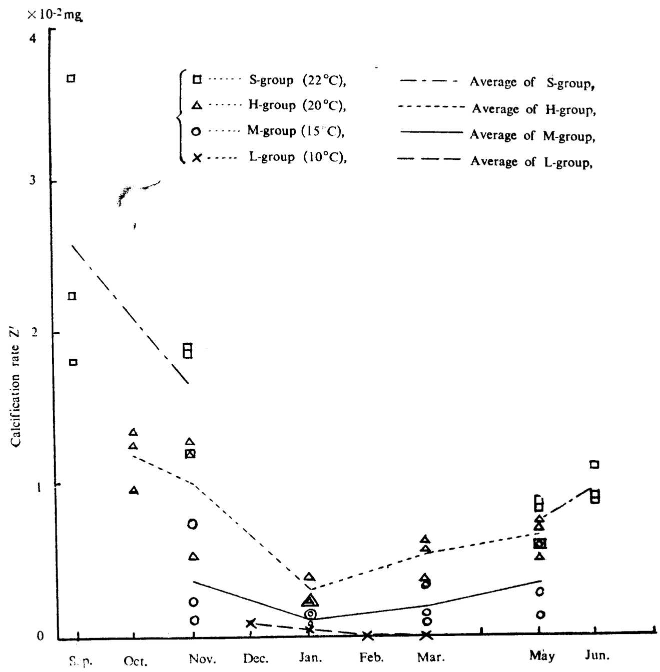


Fig. 7. Seasonal changes of calcification rate Z′ mg./cm². day of the otolith of *Mylio* (Fish-C_2) reared in various water temperature (Exp.-5).

shown in Table 3 (item: Water temperature/Food: Activity). From both this figure and the table, the followings were found.

(a) The calcification rate of the otolith of *Mylio* is to be found, leastly in the water of 10°C, a little at 15°C and high at above 20°C. But in each case of the temperature, it is also affected by the season, and it is natural that the smallest is January and February, and the largest is September and October.

(b) The calcification rate of the otolith is small when the behavior of fish is dull, but is large when its activity is sensitive.

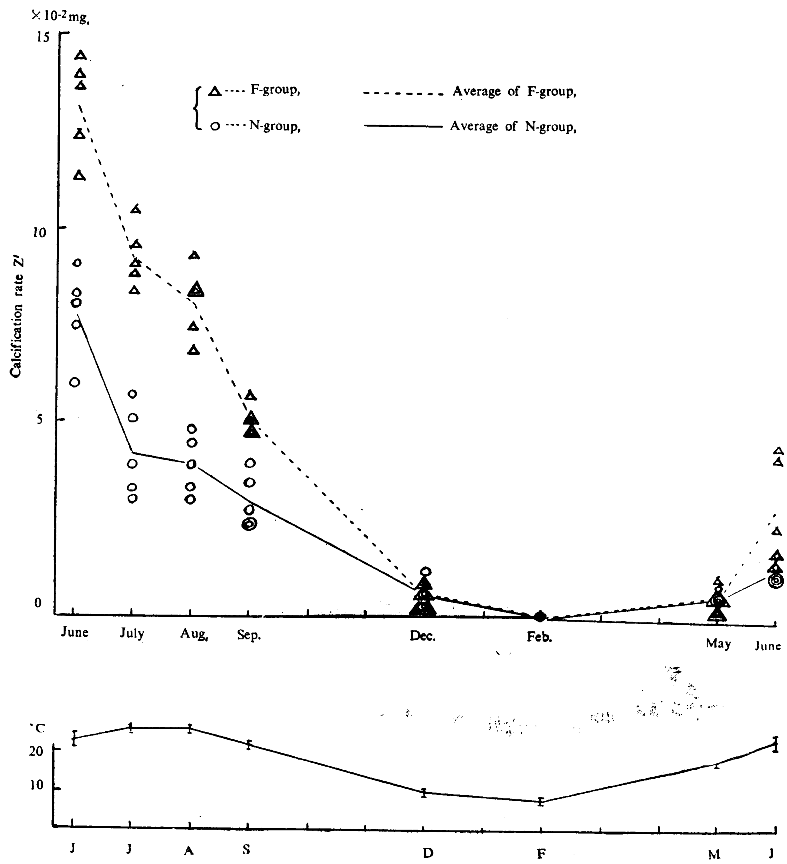
(6) Concerning the amount of food and the calcification rate Z′ of the otolith, the results shown in Fig. 8-a were obtained through Experiment-6. From this figure the followings were gained.

(a) The calcification rate of the otoliths of *Lateolabrax* in summer and autumn becomes low in winter and spring.

(b) The calcification rate of the otolith in the fish which took food plentifully is higher than those given food scantly.

(c) The calcification rate is low when the water temperature is below 10°C, and is very high when it is above 20°C.

(a)



(b)

Fig. 8. (a)Seasonal changes of calcification rate Z′ of the otolith of *Lateolabrax* (Fish-B_I) reared with various amount of food (Exp.-6). The fish of F-group were given food sufficiently, while those of N-group were given nothing for 10 days. (b) Temperature of the rearing water in each experiment.

(V) DISCUSSIONS

(1) The Crystal Texture and the Growth of the Otolith.

Previously, the crystal texture and the arrangement of $CaCO_3$ which is the main constituent of the otolith were examined by means of X-rays and others. And it has already been elucidated by the writer[7)] that the opaque zone which forms a part of the "annual ring of the otolith" together with the translucent zone, is formed in winter and spring, the latter is formed in summer and autumn, and a kind of organic matter (Protein[10)]) is contained more abundantly in the opaque zone than in the translucent one.

In the present study, discriminating between translucent zone and opaque one, size and texture of micro-crystal grains were examined by using micro-beam X-rays (0.09 mm. in diameter) emitted from a micro-focus and by using electron microscope. As the result, it was found that micro-crystal grains in any zone are crystalline $CaCO_3$ having the form of aragonite and are fibrously arranged as is mentioned in the first part of the results. It was also found firstly that, in the translucent zone, the size of micro-crystal grains is larger than that in the opaque zone; secondly, that only small amount of protein sticks to the groove between each grains of the translucent zone and therefore groove is deep, while, on the other hand, in the opaque zone, much protein fills the groove and covers the surface of the grain and therefore the groove is shallow and the surface of the grain becomes rather smoother than that of the translucent zone. Moreover, through the measurement of calcification rate of the otolith surface, it was found that the calcification rate becomes high in summer and autumn through which seasons the translucent zone is thus formed, while it becomes low in winter and spring through which the opaque zone is formed consequently. The present writer could only infer in the previous research[7] that the translucent zone may due to the vigorous calcium deposition and the opaque zone may due to its slow deposition, but through the present experiments it was first ascertained to be a definite true. At the same time, it was examined by using X-rays that crystalline $CaCO_3$ in the form of aragonite is formed by combining calcium with organic matter surrounding the otolith. The autoradiograph ~~obtained~~ originally in this experiment shows that most part of the calcium is taken from the sea water and is deposited on the whole surface of the otolith, and that the amount of its deposition in this case differs by the place remarkably. In other words, calcium is deposited, abundantly on the two extreme ends, leastly on the outer side, and mediumly on the inner side. Therefore it may be concluded that the growth of the otolith differs locally, that is to say, the rapid growth can be found towards both ends, slow growth towards the outer side, and the medium growth towards the inner side. Moreover, it was elucidated that the zone mark formed in the otolith once is preserved ever after as it is, and the form of the zone indicates what the shape of the otolith was when the zone was formed.

Lastly, the followings are inferred concerning the formation of the translucent and opaque zone in the otolith. The number of the micro-crystal grains of $CaCO_3$ increases a little in winter and spring and their size is small because the amount of protein surrounding the otolith is much in comparison with that of calcium deposited on the surface in these seasons. Therefore, the groove between these crystal grains is filled with much protein, and the visible rays can hardly penetrate. On the other hand, in summer and autumn, the number of the crystal grains increases rapidly and their size is large because the otolith is surrounded with a less amount of protein in comparison with calcium. In this case, the groove between crystal grains are filled with less protein, and therefore, the zone formed in these seasons becomes translucent to visible rays. It is thus clear that the otolith grows by piling up the translucent layer and opaque layer alternately.

In winter, the calcification rate of *Mylio* is exceedingly low than that of *Lateolabrax*. It can be inferred therefore that the formation of the micro-crystal grains in this season is almost stagnated, and the surface of the otolith remains almost unchanged. It may be due to this reason perhaps that in the margin of the otolith of *Mylio* caught in winter the opaque zone could not be found in the previous research[8] by the present writer. It must be avoided to estimate the season for the formation of these zones only through the zone in the margin of the otolith.

(2) Growth of the Otolith and the Living Condition of the Fish.

As is mentioned above, the growth of the otolith is due to the formation of crystal grains of $CaCO_3$ caused by the deposited calcium upon the surface of the otolith. It was once said that this calcium might be taken from the food[3]. But, it was for the first time found through the present experiments that a large amount of calcium contained in the sea water is also deposited clearly upon the surface of the otolith. It was elucidated moreover that the amount of calcium taken from the sea water and deposited upon the otolith is quite independent of the concentration of the sea water, and that it becomes small below 15°C of water temperature and becomes remarkably large above 20°C. The deposition of calcium, that is to say, the formation of crystal grain of $CaCO_3$, was found to be affected greatly by season even when the rearing water is kept its temperature unchanged or when its concentration unchanged. In any case, it may be concluded that calcium contained in the sea water is deposited abundantly in summer and autumn and a little in winter and spring.

One of the conspicuous difference between the fish in the period from summer to autumn and the one from winter to spring is that of their activity. In winter and spring, the behavior of the fish is dull, but it becomes sensitive and active in summer and autumn. It can be said therefore that the activity of the fish is closely connected with the amount of calcium taken from the sea water and deposited upon the surface of the otolith. On the other hand, in the present experiment, no significant difference in their activity was found between the fish which took food plentifully and that took insufficiently. Nevertheless, the amount of calcium deposited on the otolith of the former was exceedingly larger than that of the latter. Accordingly, it is considered that the speed for the increase of crystal grains of $CaCO_3$ in the otolith, in other words, the growing velocity of the otolith, is not due to their external activity but to their physiological condition. Here it may be concluded that when the environment of the fish is favorable to their living and their physiological condition is good, the otolith grows rapidly by taking up a large amount of calcium from the sea water. On the contrary, when their environment is unfavorable and their physiological condition is not good, the otolith grows slowly. Both physiological conditions, good and not good, can be realized either in summer and autumn or in winter and spring; moreover, it is clear that calcium is taken and deposited to the otolith surface not only from the sea water but also from their food. Therefore, when the calcium contained in the food was measured together with the calcium in the sea water, more conspicuous effects might be expected to be observed.

(VI) SUMMARY

In order to elucidate how the crystal texture of the fish otolith and its growth are influenced by water temperature, its concentration, the amount of food and the season, the present writer chose marine teleosts, *Lateolabrax* and *Mylio*. They were reared through all seasons in tanks and the temperature of the sea water, its concentration and the amount of food were variously changed and experimented. For the observation of the crystal texture of the otolith surface, the electron microscope was used and details concerning the formation of the otolith zone were studied through the autoradiographs. Radioactive isotope Ca^{45} was used as a tracer in order to measure the amount of calcium deposited upon the otolith surface, and, concerning the crystal texture of the annual rings, *Argyrosomus* was used instead of *Lateolabrax* and *Mylio* for convenience' sake, and their Laue photographs were taken by the micro-beam X-rays. As the results, the followings were obtained.

(1) Calcium dissolved in the sea water is deposited on the surface of the otolith, and micro-crystal grains of $CaCO_3$ which are the main constituent of the otolith are thus formed. It goes without saying that the growth of the otolith is mainly due to the formation of micro-crystal grains of $CaCO_3$.

(2) In summer and autumn, in which the fish grows rapidly, a large amount of calcium is deposited on the otolith surface, and the micro-crystal grains of $CaCO_3$ are formed abundantly. A very little protein fills the groove between crystal grains. In this way, the wide translucent zone which contains a small amount of protein is formed.

(3) In winter and spring, in which the fish grows slowly, a small amount of calcium is deposited and the micro-crystal grains are formed very slowly. Much protein fills the groove between crystal grains. This is the way in which the opaque zone containing much protein is formed.

(4) Concerning *Lateolabrax* and *Mylio*, the concentration of the sea water or the amount of the dissolved calcium in this water cannot have influence upon the formation of micro-crystal grain of $CaCO_3$. In other words, the growing velocity of the otolith is always unchanged. But, on the other hand, the formation of the micro-crystal grains is influenced much by the water temperature and the amount of food taken by the fish. The micro-crystal grains of $CaCO_3$ are formed abundantly and the otolith grows rapidly when the environment of the fish is favorable and its physiological condition is good independently of the concentration of the water. On the contrary, when their environment is unfavorable, the micro-crystal grains are formed only a little and the otolith grows slowly. The good environment for the fish in this case means that they are living in the warm water and taking plentiful food, and the contrary means that they are living in the cold water and taking only insufficient food, and both environments can be realized either in summer and autumn or in winter and spring.

(5) As is mentioned above, the formation of the zone in the otolith is closely

connected with the history of the fish and the zone mark once formed in the otolith is preserved ever after as it is. Accordingly it can be used as an effective key to investigate the history of the individual fish.

ACKNOWLEDGEMENT

The writer wishes to express his sincere thanks to Professor Takeo Fujiwara, under whose kind guidance and encouragement this study was carried out. The writer is also grateful to Professor Mutsuaki Shinagawa for his kind guidance concerning the measuring of radioactivity. The present study was supported by the Scientific Research Expenditure of the Ministry of Education.

REFERENCES

1) DANNEVIG, A. : Rep. Norw. Fish. Mar. Inv., **4**, 1, 1—86 (1933).
2) _______ : Ibid., **11**, 7, 1—16 (1956).
3) MOLANDER, A. R. : Sv. Hydrogr.-Biol. Komm. Skr., N. S., Biol., **2**, 8, 1—11 (1947).
4) HATANAKA, M., SEKINO, K. and OTSUKA, A. : Tohoku Journ. Agr. Res., **2**, 2, 25—32 (1952).
5) SAETERSDAL, G. S. : Rep. Norw. Fish. Mar. Inv., **10**, 9, 1—46 (1953).
6) TROUT, G. C. : Rapp. et Proc. Verb. Cons. Intern. Expl. Mer, **136**, 89—102 (1954).
7) IRIE, T. : This Journal, **1**, 1, 1—8 (1955).
8) _______ : Ibid., **1**, 3, 311—317 (1957).
9) COOK, G. B., DUNCAN, J. F. and HEWITT, M. A. : Nucleonics, **8**, 1, 24—27 (1951).
10) DANNEVIG, E. H. : Journ. du Cons., **21**, 2, 156—159 (1956).
11) COMAR, C. L., HANSARD, S. L., HOOD, S. L., PLUMLEE, M. P. and BARRENTINE, B. F. : Nucleonics, **8**, 3, 19—31 (1951).

EXPLANATION OF PLATES

Plate 1 X-ray Laue photographs of the otolith thin slice of *Argyrosomus*. Fig. A, C and E are radiographs of the portions denoted respectively by T_1, T_2 and T_3 (translucent zone) in the Fig. 1. Fig. B and D are the ones denoted by O_1 and O_2 (opaque zone) similarly.

Plate 2 and 3 Electron micrographs of the inner side surface of the otolith. The fish used here are *Lateolabrax* (Fish-B_2). The name of the month when the otolith was taken out, and the division of the otolith of which the micrographs were taken are shown in the following table (5000×).

Month	Plate	Division An.	Division M.	Division Po.
February	Plate 2	Fig. A.	Fig. B.	Fig. C.
May	Plate 2	Fig. D.	Fig. E.	Fig. F.
July	Plate 2	Fig. G.	Fig. H.	Fig. I.
August	Plate 3	Fig. J.	Fig. K.	Fig. L.
September	Plate 3	Fig. M.	Fig. N.	Fig. O.
December	Plate 3.	Fig. P.	Fig. Q.	Fig. R.

Plate 4 Fig. A Micro-autoradiograph of the otolith slice of *Lateolabrax* (Fish-B_3). The age of this fish was O and was reared in the water containing radioisotope Ca^{45} twice for 10 days in June and September respectively. (22×).

Fig. B Photo-micrograph of the otolith slice used in Fig. A. It was taken through penetrated visible rays. (22×).

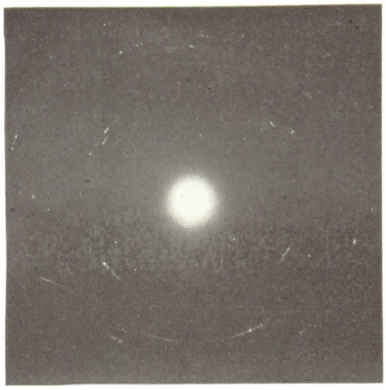


Fig. A

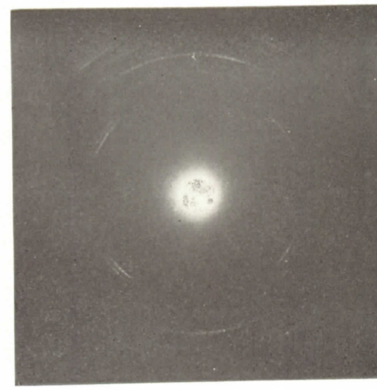


Fig. B

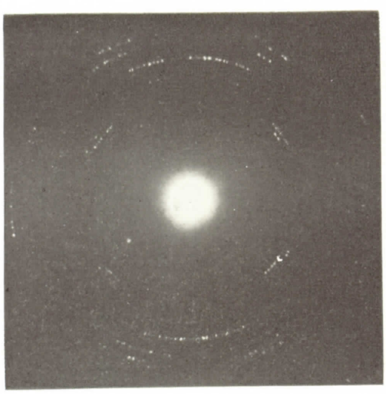


Fig. C

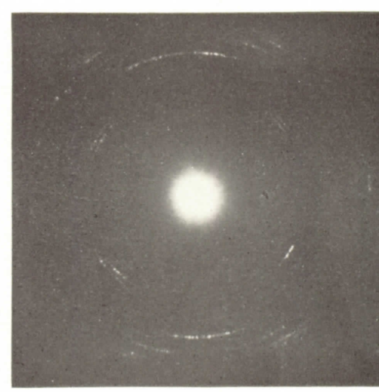


Fig. D

Fig. E

IRIE: The Crowth of the Fish Otolith

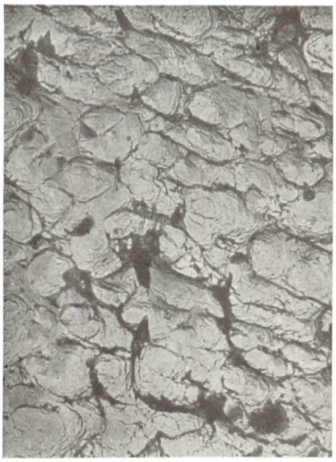


Fig. A

Fig. B

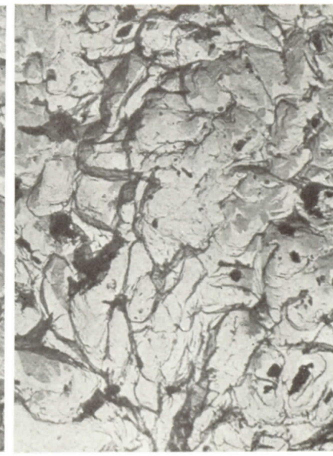


Fig. C

Fig. D

Fig. E

Fig. F

Fig. G

Fig. H

Fig. I

IRIE: The Growth of the Fish Otolith

Fig. J

Fig. K

Fig. L

Fig. M

Fig. N

Fig. O

Fig. P

Fig. Q

Fig. R

IRIE: The Growth of the Fish Otolith

Fig. A

Fig. B

IRIE: The Growth of the Fish Otolith