

THE SEASONAL PATTERN OF OTOLITH GROWTH AND ITS APPLICATION TO BACK-CALCULATION STUDIES IN *AMMODYTES TOBIANUS* L.

By

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Otolith growth in a population of *Ammodytes tobianus* L. in Langstone Harbour, England, has been investigated during 1969 and 1970, as a basis for the estimation of body growth by back-calculation of mean lengths from otolith zone measurements. Most attention has been paid to I-group spring-spawners.

Linear growth of the otoliths was found to take place mainly from April to August, with the deposition of opaque material occurring in April and May. During the initial period of opaque zone deposition, however, there is little or no otolith growth, and from measurements of the zones in successive samples, it appears that the initial deposition of opaque material is by ingrowth into the hyaline material present at the edge. During the period of opaque zone deposition, body growth is at a maximum, and this is followed by a period of slower growth coinciding with hyaline zone formation in the otolith. As a result, the ratio of otolith length to fish length shows a sharp decrease in spring, followed by an increase during summer to a more stable level.

The application of these data to the back-calculation technique has been examined. Because of ingrowth, values of l_1 derived from measurements of the inner boundary of the opaque zone are estimates of body length before the end of the growing season. Estimates of body length at the time of opaque zone initiation in mid-April can be obtained, however, by using measurements at the split in the opaque zone, but no otolith dimension can be used specifically to estimate body length during the October to March period of no-growth. Assumption of a constant relationship between otolith length and body length was found to result in values of l_1 varying systematically with the time of year at which the samples were obtained. A knowledge of the seasonal variation in otolith-body length ratios and in particular the ratios at the times corresponding to the split and inner boundary of the opaque zone were found to be necessary for accurate back-calculation of mean body lengths.

INTRODUCTION

As part of a study of growth and population structure in the sandeel *Ammodytes tobianus* L., the possibility of back-calculating body lengths from otolith zone measurements has been investigated by examining otolith growth in the main study area, Langstone Harbour, Hampshire. Particular attention has been paid to the relationship between body length and otolith length, and to the seasonal cycle of opaque and hyaline zone deposition.

Work on several species of teleost fish has in general shown that there is a close, but usually curvilinear relationship between otolith size, and body

length and age (HICKLING 1933; TEMPLEMAN and SQUIRES, 1956; LE GALL, 1968). Little attention has been paid, however, to possible seasonal variation in the relationship between the two, although TROUT (1954) for cod, *Gadus morhua* L., and CHRISTENSEN (1964) for sole, *Solea solea* L., have implied that the seasonal growth of both body and otolith proceed in parallel.

Precise estimates of the times of year to which the zonal boundaries refer have not emerged from the many studies involving an examination of otolith edges in samples of fish taken throughout the year, and at least for some species such as the hake, *Merluccius merluccius* (L), (HICKLING, 1933), precision is precluded by the fact that there is only a gradual change from one zone to the other within the population. Although the occurrence of opaque material at the otolith edge is variable both in time and duration, most authors (for example, TROUT (1954) on cod, and HALLIDAY (1969) on the argentine, *Argentina sphyraena* L.) have found that the opaque zone is formed in summer, when body growth is rapid and condition high. Conversely, hyaline zone formation has been associated with the winter months, but periods of growth by hyaline deposition, and periods of quiescence with hyaline material persisting at the edge have not usually been distinguished. In contrast to most other results, IRIE (1957; 1960) found that in Japanese waters, species such as *Lateolabrax japonicus* (Cuvier) and *Mylio macrocephalus* (Basilewsky) deposited opaque zones in their otoliths during the period of slowest body growth in winter and spring.

The otoliths of sandeels have been used by several authors for age-determination (for example, KÄNDLER, 1941; CAMERON, 1958; MACER, 1966), and it was KÄNDLER who first clearly distinguished the autumn- and spring-spawning groups of *A. tobianus* on the basis of otolith structure. CAMERON concluded that in the Isle of Man waters, opaque material was deposited from March to October, and hyaline material from November to February. MACER (1966) and KITAKATA (1957) have both demonstrated, but not quantified, a close relationship between otolith size and body length in *A. marinus* Raitt, and *A. personatus* Girard, respectively.

Most authors have applied the back-calculation technique to scale measurements, but in the Ammodytidae the scales are small and the annual rings not well defined. HICKLING (1933) used otolith measurements for the back-calculation of body lengths at the end of the first three growing seasons in the hake, but found that mean lengths so calculated were greater than observed ones. HALLIDAY (1969) in contrast, found that in the argentine, body-lengths back-calculated from measurements of otolith rings were consistently slightly lower than their empirical equivalents. These have both also involved instances of LEE's phenomenon. In the haddock, *Melanogrammus aeglefnus* (L), SAETERSDAL (1953) encountered difficulties in establishing a satisfactory otolith-body length relationship and concluded that back-calculation from scale measurements would give more reliable estimates of mean body length.

STUDY AREA AND MATERIALS

Langstone Harbour (50°47'N, 01°02'W.) is an extensive, shallow, predominantly muddy inlet receiving a negligible amount of fresh-water inflow, and separated from the Solent by a narrow entrance. Much of the substratum is

exposed at low-tide including two areas of sand-banks at the junctions of the main drainage-channels; it is from these sand-banks that sandeels can be readily caught by beach-seine, and in fact, from May to September, there is a substantial bait fishery for them. Weekly mean water-temperatures range from approximately 3°C to 19°C over the year.

On an annual basis, over 95% of the sandeels caught in the Harbour are *A. tobinus*; the remainder are mostly *Hyperoplus lanceolatus* (LeSauvage), but *A. marianus* Raitt and *H. immaculatus* (Corbin) have also been recorded. Approximately 80% by number of *A. tobinus* caught over the year belong to the spring-spawning group, the remainder to the autumn-spawning group. Free-swimming fish are available between late March and early December, (adults only from June to October) but in addition, small numbers of sandeels can be dug from intertidal sand in the winter months suggesting that some at least over-winter in the Harbour within the sand. The catches are always dominated by either 0- or I-group fish, and, in the case of the spring-spawning group, successive year-classes dominate for a period of about a year, beginning in August when the fish are approximately five months old. Fish greater than three years old are rare and the oldest recorded have been VII-group. Autumn spawned fish first appear in late April when about six months old, and are only regularly caught in their first year of life, between May and October. Spawning probably occurs in February/March and October/November for the spring and autumn spawners respectively, and some fish first spawn when twelve months old.

Thus the material forming the greatest contributions to the present study involves 0- and I-group spring-spawned fish, that is from the age of about five to twenty months; only a limited amount of data is available for older individuals and for the autumn-spawning group.

METHODS

Samples of sandeels were obtained by beach-seining with a net 25 m long attached to 36 m bridles; the mesh size in the bunt was 6 mm bar. The fish were examined either soon after death (1969) or following overnight freezing (1970).

Body length measurements, to the nearest mm, were made from the tip of the lower jaw to the caudal fork using vernier callipers.

The saccular otoliths (sagittae) were examined and measured intact since grinding and splitting of the small, thin structures proved difficult and unnecessary; the zones were adequately clear and conspicuous in surface view, and their measurement, in otoliths before and after splitting, indicated that the dimensions observed in the intact otoliths represent the true zone limits (Figure 1). Thus, overgrowth of opaque material onto the surface plane of the otolith was not interfering with the accuracy of measurements; although in some otoliths, overgrowth could be detected in surface view, it was clearly distinct from the true limits of the opaque zone. The opaque material forming the centre of the otolith and deposited in 0-group fish, was not measured because of the lack of a definite boundary between opaque and hyaline areas in most fish. In general, the outer boundaries of all opaque zones were found to be more diffuse than the inner boundaries.

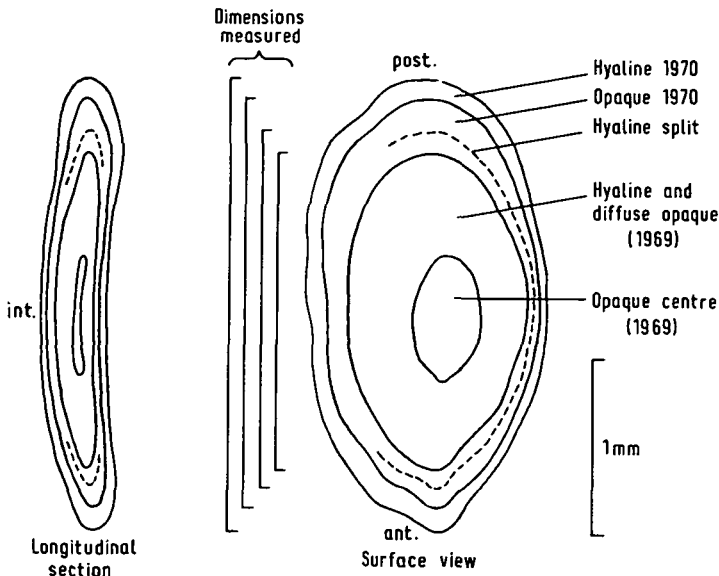


Figure 1. Left saccular otolith of spring-spawning *Ammodytes tobianus*, 1969 year-class, caught on 13. July 1970, showing the dimensions measured and the distribution of hyaline and opaque zones. The fact that the length of the inner boundary of the opaque zone is identical in both surface and sectional views, indicates that overgrowth onto the surface plane is not interfering with the accuracy of the zonal measurements made on the intact otoliths.

The otoliths, immersed in xylol on black plastic cavity slides, were examined using reflected light, a monocular magnification of $30\times$, and an eye-piece graticule. The maximum lengths of the whole otoliths, and the lengths at the boundaries between the opaque and hyaline zones and at the split in the opaque zone (Figure 1) were measured in eye-piece units (where 1 e.p.u. = 0.029 mm) to the nearest whole unit.

The ratio of otolith length to body length is given as

$$\left[\frac{\text{otolith length (e.p.u.)}}{\text{body length (mm)}} \right] \times 10^3$$

Quantitative data given in the text are mean values with 95% confidence limits, unless otherwise stated, and the term 'significance' has been used in the statistical sense referring to significance at the 95% level.

In 1969, otoliths were measured from fish selected from 0.5 cm length-groups, and calculations of mean otolith length of the sample were based on the means for each length-group and the length frequency distribution of the sample. In 1970, otoliths were measured from individual fish of known lengths forming a random sample, thus enabling confidence limits to be calculated. Because of this, and the greater frequency of sampling, most attention has been paid to the 1970 data.

Water temperature data for Langstone Harbour have been abstracted from the records of the Admiralty's Exposure Trials Station at Portsmouth, but, during the period of study, complete records have been unavailable. Thus the curve in Figure 3 has been constructed from the monthly means between 1962

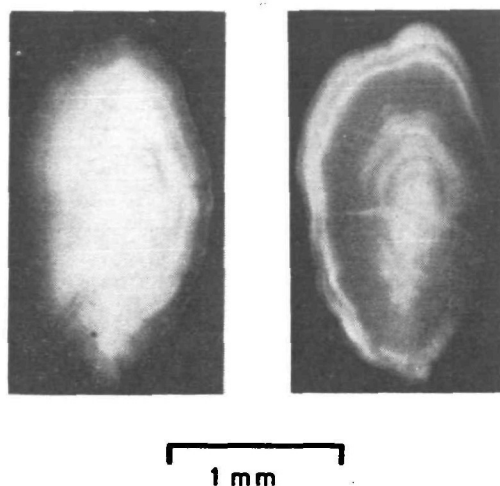


Figure 2. Photographs of left saccular otoliths of *A. tobianus* caught on 11. June 1970. Left: 0-group autumn spawner; right: 1-group spring spawner.

and 1968 in order to give an indication of the annual cycle; the observed data for 1969 closely followed this mean pattern, but in 1970 the rapid increase in temperature in spring was later than usual.

RESULTS

OTOLITH STRUCTURE

The otoliths of *A. tobianus* are elongated antero-posteriorly, flattened, and slightly curved; the posterior end is broader and less pointed than the anterior, and the zones there are at their widest and most conspicuous.

In the otoliths of spring-spawned fish (Figures 1 and 2) a dense opaque centre is surrounded by a variable amount of more diffuse opaque material within a wide hyaline zone. Outside this zone lies a wide dense opaque zone (the first opaque ring) with a sharp inner boundary, but usually with a more diffuse outer boundary. Further opaque zones are much narrower, and are also typically narrower than the hyaline zones which separate them. In most otoliths, a narrow hyaline split appears to be present in the opaque zone, but this rarely forms a continuous ring and is often obscure. In many cases the opaque material inside the split is distinctly brighter than that outside it.

The otoliths of autumn-spawned fish (Figure 2) also have an opaque centre, but usually this is effectively confluent with the wide opaque zone which immediately follows it; thus the otoliths have a larger and more conspicuous central opaque area compared with those of spring-spawned fish.

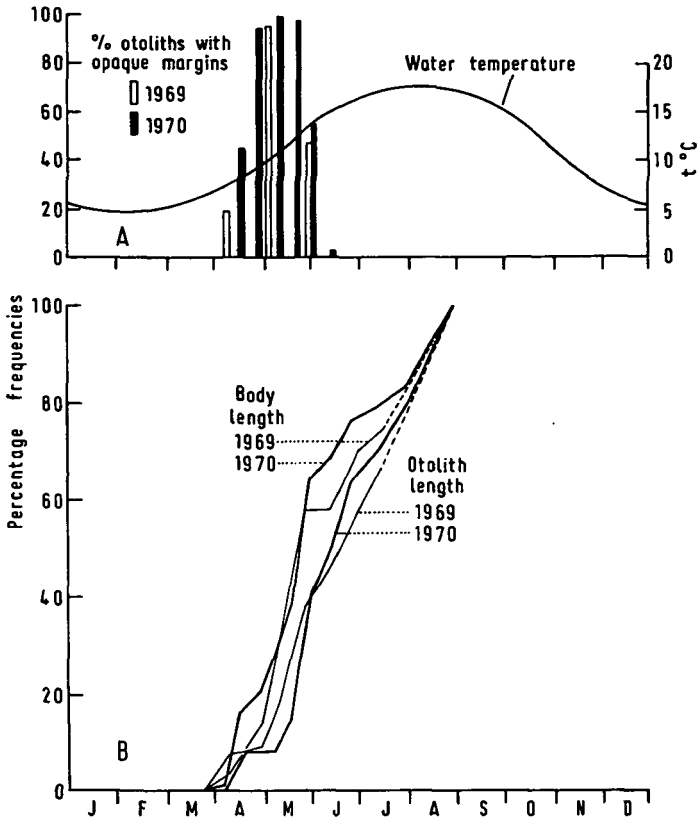


Figure 3. The seasonal pattern of growth and opaque zone deposition for the I-group fish during 1969 and 1970 (1968 and 1969 year-classes respectively). A) % of otoliths with opaque margins, together with water temperature cycle for Langstone Harbour based on monthly means 1962-1968. B) otolith and body growth expressed as cumulative percentage curves of the annual increments.

TABLE 1. The percentage of otoliths of I-group (1969-class) spring-spawning *A. tobianus* with opaque material deposited in the spring of 1970.

	Without opaque material %	Opaque material at posterior end only %	Opaque material at both ends %	No. in sample n
4. April.....	100	0	0	98
15. April.....	56	44	0	92
27. April.....	5	47	48	106
8. May.....	0	8	93	96
18. May.....	1	1	98	124
1. June.....	0	0	100	97

SEASONAL CHANGE AT THE OTOLITH EDGE

The results of examining the otolith edge in successive samples are shown in Figure 3A. It can be seen that one opaque zone is deposited each year,

and that, in the case of I-group spring-spawners, deposition occurs between mid April and early June; the period during which at least 50% of the otoliths had opaque margins was from about the third week in April to the end of May in both 1969 and 1970. It was observed in 1970 that opaque material was deposited at the posterior edge in advance of the anterior edge. This progression of opaque zone formation is shown in Table 1 and it emphasises the fact that the initiation of opaque zone formation occurs rapidly throughout the population.

Otoliths with narrow hyaline edges were frequently encountered in late April and early May at a time when formation of the opaque zone was clearly not complete. It is possible that these edges represent the split in the opaque zone apparent in more advanced otoliths, and the occurrence of hyaline material at the edge at this time was thus ignored in the construction of Figure 3a.

No comparable information is available for older fish, since these are not caught in the area until early June. When they do appear, the opaque zone is usually complete and the otolith edge is hyaline. The otoliths of 0-group autumn-spawners are completely opaque when the fish first appear in late April, and hyaline material is not apparent at the otolith edge until early June.

OTOLITH GROWTH

The growth in length of the otoliths of the 1968 and 1969 year-classes of spring-spawners in 1969 and 1970 is shown in Figure 4A and Appendix Tables I and II.

When 0-group fish appear as juveniles about 6 cm long in late July, the otolith margins are hyaline, although initially some diffuse opaque material is also deposited. Body and otolith growth continue until October, after which there is neither body nor otolith growth until the following spring when the fish are I-group.

The main growth period of the otoliths of I-group fish begins in May, but appears to end earlier than in 0-group fish, that is, about the end of August. Since there was no significant difference between mean otolith length in November 1969 and March 1970, it can be inferred that there was neither resorption of material from the otolith edge, nor growth, during the winter months. Formation of hyaline material takes place mainly from June to August, but also to a slight extent in early April, and, as has already been established, opaque material is formed between mid-April and late May.

Measurements show, however, that during the initial period of opaque zone formation, the otolith is not increasing in length. This suggests that some of the opaque material is being deposited within the existing otolith structure. Since it is considered (in Methods above) that overgrowth is not affecting the validity of the observed zone dimensions, measurements of the inner boundary of the opaque zone lend support to this view; from mid-May onwards they decrease to become significantly smaller than total otolith length measured at the time of the first appearance of opaque material. Thus, in 1969, the inner boundary was of the order of 55 e.p.u. whereas otolith length on 9. April was 59 e.p.u.; the equivalent lengths in 1970 were 58 and 65 e.p.u. (15. April)

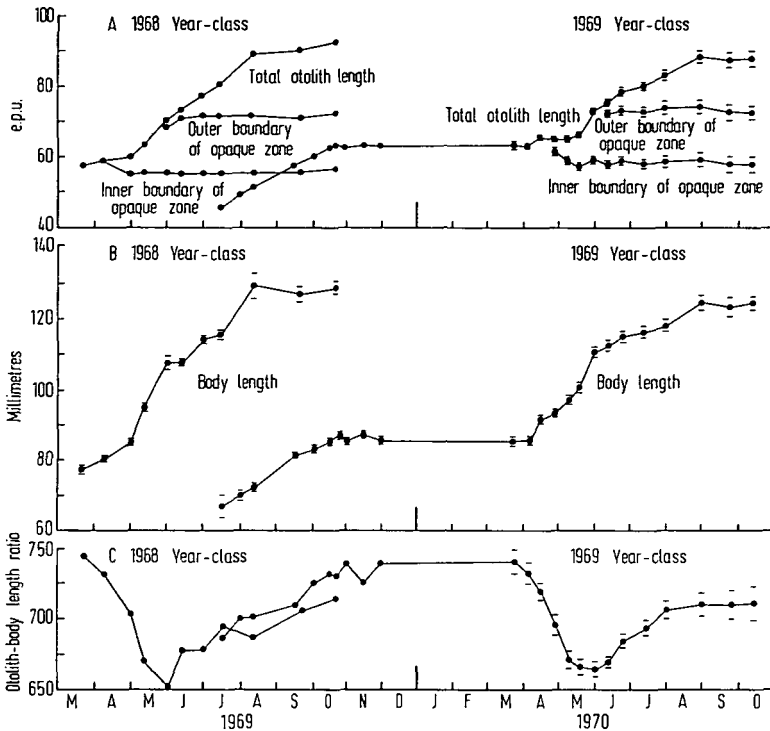


Figure 4. Linear growth of otolith and body for the 1968 and 1969 year-classes from 21. March 1969 to 13. October 1970. A) otolith length in e.p.u. B) body length in mm C) otolith-body length ratio $\times 10^3$. These data are taken from Appendix Tables I and II and Table 2, and are expressed as mean values $\pm 95\%$ confidence limits where available.

respectively. If opaque material was only being deposited outwards from the otolith edge the inner opaque boundary would be expected to be approximately equal to otolith length at the time of the first appearance of the opaque zone, whereas in fact, in 1970 it corresponded to the otolith length of the previous September.

After the length of the inner opaque boundary has reached its lowest level in early May, a two to three week period of rapid otolith growth begins during which opaque material is deposited at the edge of the otolith as it grows (outgrowth); this is then followed by a prolonged period of growth involving hyaline material deposition from June until August. A precise end to the growing season is, however, difficult to determine; in 1970 no significant increases were apparent after August in either otolith or body length. In 1969 growth appeared to continue until October but there is an indication from the larger size of the zone dimensions in the October sample that it may have included a greater proportion of larger individuals compared with other samples, thus giving rise to an apparent increase in mean length.

In contrast to the inner boundary, measurements of the outer boundary of the opaque zone show that this dimension closely corresponds to the observed length of the otolith at the time when opaque zone formation is complete, that is approximately 71 e.p.u. in 1969 and 73 e.p.u. in 1970.

A further dimension measured, but only in the 1. June and 13. July 1970 samples, was the length at the hyaline split in the opaque zone which can be seen in most otoliths; the values obtained were 64.87 ± 0.95 and 63.85 ± 1.03 e.p.u. respectively. These are similar to the observed otolith lengths during the period of no-growth in late April and early May, and it is thus possible that the split represents otolith length at the time of opaque zone initiation, and the changeover point from ingrowth to outgrowth. It is, however, a dimension which is much less conspicuous than the zonal boundaries, and less easy to measure; in the 1. June and 13. July samples, about 10% of the otoliths failed to reveal a measurable split.

Measurements of the inner boundary of the opaque zones of II-group fish in 1970, (1968 year-class), were smaller (87.49 ± 1.69 e.p.u.) than the total otolith lengths of the same year class at the end of the previous growth season (91.66 ± 1.43 e.p.u.). Similarly, for the 1967 year class, the equivalent measurements in 1970 and 1969 were 97.40 ± 3.71 and 100.60 ± 2.08 e.p.u.; although the latter are not significantly different, there is clearly reason to suppose that the phenomenon encountered with the I-group fish is operating in other age-groups.

It appears therefore, that the pattern of growth of the otoliths in I-group fish takes the following form. The growth period extends from April to August, but in late April and early May little or no increase in length takes place. At this time, opaque material is being deposited by ingrowth within hyaline material already present at the otolith edge and probably in part laid down the previous autumn. After this, increase in length is rapid and involves first, a brief period of opaque deposition at the otolith edge, and then a more prolonged period of hyaline deposition.

THE RELATIONSHIP BETWEEN OTOLITH GROWTH AND BODY GROWTH

The growth in body length of the 1968 and 1969 year-classes in 1969 and 1970 is shown in Figure 4B and Appendix Tables I and II. It is apparent that, although body growth takes place from April until August, there is a conspicuous period of very rapid growth in late April and May coinciding with the formation of the opaque zone in the otoliths, and thus in part with their period of little or no growth. Body growth thus precedes otolith growth and this is shown again in Figure 3B where the curves are superimposed and reduced to the same scale. In both years, 50% of the annual body growth increment was reached three to four weeks earlier than that of the otoliths. This lag is reflected in the ratio of otolith length to body length (Figure 4C, Table 2 and Appendix Table I) which decreases sharply over the April–May period, and then increases throughout the remainder of the growing season as the relative rate of body growth slows down.

Comparable data are not available for older fish because of their absence from the catches before early June, and because of their general scarcity even

TABLE 2. Otolith-body length ratios ($\times 10^3$) in 1970, expressed as mean

	<i>n</i>	0 (spring) (1970 year-class)	<i>n</i>	I (spring) (1969)
23. March.....	-	-	59	741 \pm 8 (29)
4. April.....	-	-	98	732 \pm 6 (27)
15. April.....	-	-	93	719 \pm 6 (28)
27. April.....	-	-	108	696 \pm 6 (29)
1. May.....	-	-	-	-
8. May.....	-	-	100	670 \pm 6 (24)
18. May.....	-	-	129	661 \pm 6 (29)
1. June.....	-	-	107	664 \pm 6 (30)
11. June.....	-	-	97	668 \pm 4 (32)
25. June.....	-	-	89	684 \pm 6 (22)
13. July.....	-	-	98	693 \pm 5 (27)
30. July.....	19	703 \pm 16 (34)	75	707 \pm 6 (27)
28. August.....	49	715 \pm 8 (28)	58	709 \pm 9 (32)
25. September.....	51	734 \pm 6 (22)	32	709 \pm 9 (26)
13. October.....	54	728 \pm 8 (28)	34	711 \pm 12 (33)

when they do appear. Small, but adequate, samples of II- and III-group fish were, however, obtained from June onwards in 1970, and the otolith-body length ratios are given in Table 2; they show no significant seasonal variation, but samples are not available for the period of greatest change found in I-group fish. Similarly, samples of 0-group fish (as larvae) are not available for the spring period, and by the time they appear as juveniles in late summer, the opaque centre is already formed. There is an indication, however, that, in the autumn, the ratio for 0-group fish has increased from a summer level lower than that of I-group fish, and that their late autumn to winter level is higher than that of the latter. Further, in 0-group autumn-spawners, which are present from April onwards in their first year of life, the ratio in 1970 was initially very low at 593 ± 13 (1. May) (Table 2) when they were about six months old, but had increased rapidly to 692 ± 11 by 13. July, thereafter continuing to be similar to the ratios found in samples of I-group spring-spawners. Thus, although complete data are unavailable for fish other than I-group, there is evidence to suggest that the amplitude of seasonal variation in the otolith-body length ratio decreases with age.

BACK-CALCULATION OF MEAN BODY LENGTHS

The technique of back-calculating body lengths has been developed and used in conjunction with scales rather than other skeletal structures. As originally used by LEA (1910) for herring, the total body length at the time of formation of the first ring is represented by L_1 , and the estimate of that length derived from measurements of the first ring on the scales of older fish is represented by l_1 .

The structure and mode of growth of otoliths are, however, very different from those of scales, and it should not be assumed that the two structures can be used in the same way. The present material provides an opportunity

values with 95 % confidence limits and, in parentheses, one standard deviation

<i>n</i>	II (spring) (1968)	<i>n</i>	III (spring) (1967)	<i>n</i>	0 (autumn) (1969)
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	36	593 ± 13 (39)
-	-	-	-	-	-
-	-	-	-	48	621 ± 11 (37)
22	695 ± 13 (29)	5	701 ± 26 (23)	59	642 ± 9 (33)
-	-	-	-	61	663 ± 10 (39)
15	695 ± 17 (32)	5	697 ± 29 (26)	61	686 ± 9 (34)
17	699 ± 16 (31)	5	693 ± 39 (34)	53	692 ± 11 (38)
15	706 ± 13 (24)	3	695 ± 75 (41)	30	704 ± 9 (24)
6	701 ± 15 (15)	-	-	15	705 ± 15 (27)
-	-	-	-	-	-
9	695 ± 13 (17)	-	-	16	712 ± 15 (29)

for investigating the validity of applying the back-calculation technique to the otoliths of *A. tobianus*. In order to do this, back-calculations have been carried out from samples of I-group fish collected between 1. June and 13. October 1970, and the results compared with observed values of body length, the object being to back-calculate mean body length at the end of the 1969 growing season. There are three basic steps to back-calculation and these will be considered separately.

Location of a measurable dimension

Three dimensions associated with the first opaque ring have been measured; these are the inner and outer boundaries of the opaque zone and the split within the opaque zone. The inner boundary was found to be the most distinct and the easiest to measure, and the split, which was not apparent in all otoliths, was the most difficult.

Relation of the dimension to a point on a time scale

Observations on the nature of the otolith edge in successive samples have shown that opaque zone formation takes place between mid-April and the end of May. Thus it could be inferred from such data that the inner boundary of the opaque zone would reflect otolith length in mid-April, and the outer boundary the otolith length at the end of May. In fact, measurements of otolith length in successive samples has confirmed that the latter is so, but, in the case of the inner boundary, otolith length in mid-September, and not in mid-April, is reflected; this can be ascribed to ingrowth of opaque material during the initial stage of opaque zone formation. It has been tentatively suggested, that the length at the split in the opaque zone reflects the mid-April length (which also persists into early May) and thus the time at which opaque zone formation begins.

It would seem to be possible, therefore, to back-calculate body lengths in mid-September, mid-April and at the end of May. Since back-calculated lengths should ideally refer to the end of the growing season it is then necessary to assess the value of such back-calculated lengths as estimates of body length during the period of no-growth in winter. Data on body growth show that well over 50% of the annual increment is attained by the end of May, so that measurements of the outer boundary of the opaque zone can be discounted. Neither mid-September nor mid-April, however, coincide exactly with the end of the first growing season; the former precedes the cessation of growth by about a month and the latter is preceded by the resumption of body growth in early April. Therefore since no otolith dimension has been found to reflect precisely the winter period of no-growth, body lengths calculated from the present material will either be under-estimates or over-estimates of body length during this period. It is suggested that the following terms be used for actual or observed body lengths; $L_{1(A)}$, the body length in mid-September (autumn) and $L_{1(S)}$, the body length in mid-April (spring). The estimates of these lengths (l_1) will be calculated from measurements at the inner opaque boundary, ($h_{1(A)}$), and at the split, ($h_{1(S)}$), respectively. In addition, the estimates can be compared with $L_{1(W)}$, observed body length during the winter period of no growth. Observed values for the 1969 year-class were, $L_{1(A)} = 81$ mm, $L_{1(W)} = 85$ mm, and $L_{1(S)} = 91$ mm, to the nearest mm. Values of $h_{1(A)}$ varied slightly from sample to sample, and, in order to remove this source of variation, the combined mean of all the samples was used so that $h_{1(A)} = 57.82$ e.p.u. Similarly, the combined mean for $h_{1(S)}$ obtained from the 1. June and 13. July samples was used so that $h_{1(S)} = 64.25$ e.p.u.

Relationship between otolith length and body length

In order to convert an otolith measurement into a body length value, it is necessary to know the relationship between the two; more specifically, in the present context, it is necessary to know the relationship in mid-September if $h_{1(A)}$ is being used, and in mid-April if $h_{1(S)}$ is being used. If it were to be assumed that the otolith-body length relationship was constant over the study period, that is at least from September 1969 to October 1970, then the following would be appropriate:

$$l_1 = \frac{h_1}{H} \cdot L \quad (1)$$

where H and L are the otolith and body length of the sample from which back-calculation is to be made.

If, however, the relationship is not constant, a straight line joining either the mid-September or mid-April point to one of the 1. June–13. October points, on a graph plotting mean otolith length against mean body length, will intercept the body length axis, and formula (2) will apply:

$$l_1 = \frac{h_1}{H} \cdot (L - c) + c \quad (2)$$

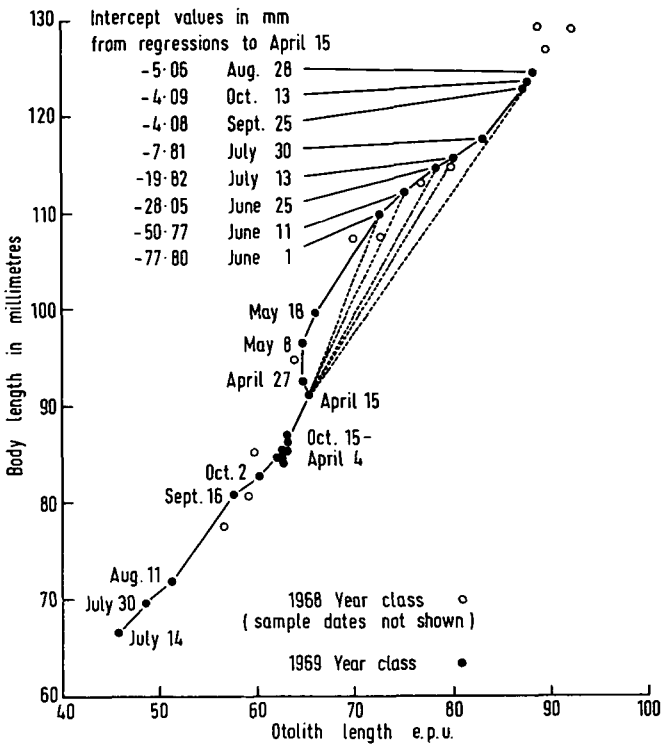


Figure 5. Plots of mean body length against mean otolith length for the 1968 and 1969 year-classes during 1969 and 1970, together with intercept values on the body length axis calculated from regression to the 15. April point from each of the 1. June–13. October points. These, and values for regression to the 16. September point, are also shown in Table 3. For further explanation, see text.

where c is the intercept on the body length axis. The seasonal variation in otolith-body length ratios already described on p. 494, in fact suggest that formula (2) will be the most successful in the present study. In Figure 5, body length has been plotted against otolith length and the rapid change in the otolith-body length ratio during April, May and June is reflected in this curve. The otolith-body length relationships of the 16. September and 15. April samples have been taken to correspond to $h_{1(A)}$ and $h_{1(S)}$ respectively, and on this basis two intercept values have been obtained for each of the 1. June–13. October samples. These were calculated, but could equally well have been derived from an extension of the axes of the graph in Figure 5 and extrapolation of the lines linking each of the 1. June–13. October points to either the 16. September or the 15. April point. The resulting intercept values have been termed either $c_{(A)}$ or $c_{(S)}$ and are given in Table 3.

With the basis for a back-calculation study established, values of l_1 have been calculated using a combination of $h_{1(A)}$, $h_{1(S)}$, formula (1), and formula (2). The results are shown in Table 3, and in Figure 6 together with the observed values for $L_{1(A)}$, $L_{1(W)}$ and $L_{1(S)}$.

TABLE 3. Values of l_1 calculated for the 1969 year-class from samples obtained 1. June-13. October 1971. Four separate values have been obtained from each sample using different formulae, otolith zone measurements and intercept values.

L - Body length of sample (mm). H - Otolith length of sample (e.p.u.). $h_{1(A)}$ - Length at inner boundary of opaque zone = 57.82 e.p.u. $h_{1(S)}$ - Length at split in opaque zone = 64.26 e.p.u. $c_{(A)}$ - Intercept value (mm) referring to measurements at the inner boundary of opaque zone. $c_{(S)}$ - Intercept value (mm) referring to measurements at the split in the opaque zone. The calculation of $c_{(A)}$ and $c_{(S)}$ is described in the text.

Date	Intercept value (mm)	Values of l_1 (mm) calculated from the appropriate formula, otolith measurement and intercept value.			
		$l_1 = h/H \cdot L$ $h = h_{1(A)}$ = 57.82 A.	$h = h_{1(S)}$ = 64.26 B.	$l_1 = h/H \cdot (L - c) + c$ $h = h_{1(A)}$ = 57.82 C.	$h = h_{1(S)}$ = 64.26 D.
1. June.....	-	87.52	98.20	81.71	
	$c_{(A)} = -28.71$ $c_{(S)} = -77.80$				89.91
11. June.....	-	86.41	96.96	81.51	
	$c_{(A)} = -20.86$ $c_{(S)} = -50.77$				90.11
25. June.....	-	84.65	94.97	81.59	
	$c_{(A)} = -12.00$ $c_{(S)} = -28.05$				90.27
13. July.....	-	83.85	94.07	81.56	
	$c_{(A)} = -8.14$ $c_{(S)} = -19.82$				90.32
30. July.....	-	82.08	92.09	81.51	
	$c_{(A)} = -1.90$ $c_{(S)} = -7.81$				90.40
28. August.....	-	81.80	91.77	81.50	
	$c_{(A)} = -0.80$ $c_{(S)} = -5.06$				90.42
25. September...	-	81.51	91.45	81.50	
	$c_{(A)} = -0.09$ $c_{(S)} = -4.08$				90.37
13. October.....	-	81.51	91.44	81.50	
	$c_{(A)} = -0.11$ $c_{(S)} = -4.09$				90.43

The use of formula (1) with $h_{1(A)}$ only provides good estimates of $L_{1(A)}$ from samples taken after mid-July (Curve A in Figure 6) and the same applies (Curve B) to estimates of $L_{1(S)}$ derived from $h_{1(S)}$ and formula (1). This seasonal variation in the calculated values of l_1 can be corrected, however, if formula (2), incorporating the appropriate intercept values, is used (Curves C and D).

It is thus possible to obtain accurate estimates of body length at the time of opaque zone initiation and at an apparently arbitrary time during the previous autumn, providing that some information is available on the otolith-body length relationships at these times. There appears to be no way of estimating $L_{1(W)}$ accurately from the present data, but if estimates of both $L_{1(A)}$ and $L_{1(S)}$ are available, it can be concluded that $L_{1(W)}$ will lie somewhere between them. Neither $L_{1(A)}$ and $L_{1(S)}$ are ideal end-points, but the latter can at least be related to a definite point in the life of the fish, that is the initiation of opaque zone formation, and for this reason is to be preferred. On the other hand its associated otolith dimension $h_{1(S)}$ is much less easy to obtain than $h_{1(A)}$, and there are clearly advantages in calculating both $l_{1(A)}$ and $l_{1(S)}$.

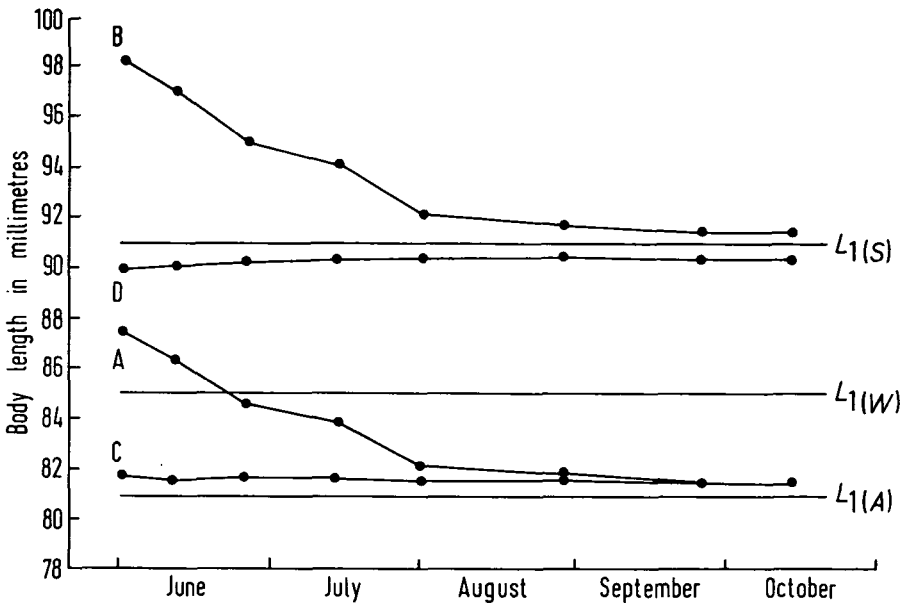


Figure 6. Values of l_1 (mm) calculated for the 1969 year-class from samples obtained between 1. June and 13. October 1970. Curves A–D correspond to columns A–D in Table 3. $L_{1(S)}$ = observed body length at the time of opaque zone initiation (mid-April 1970) $L_{1(W)}$ = observed body length during the period of no-growth (Nov. 1969–March 1970) $L_{1(A)}$ = observed body length at the apparent time of opaque zone initiation (mid-Sept. 1969)

Accurate estimates of $L_{1(A)}$ and $L_{1(S)}$ have been obtained, but this has required information on the specific otolith-body length relationships corresponding to $h_{1(A)}$ and $h_{1(S)}$. Whether the technique can now be applied to other year-classes or even to other populations where this information is lacking, will depend upon the extent to which the pattern of otolith and body growth established for the 1969 year-class in 1970, can be regarded as an intrinsic property of the population or even the species. More work is clearly needed on this point but the fact that the 1969 data, although not strictly comparable to those obtained in 1970, were similar, suggests that the pattern may be reasonably constant. If so, use could be made of the present results in one of three ways:

1) Samples obtained before the end of July could be excluded from back-calculation studies; 2) a fixed relationship between intercept value and time of year could be used so that, for example, if one was back-calculating to $L_{1(S)}$ from an early June sample one would use formula (2) and an intercept value of about -70 mm; 3) specific otolith-body length relationships relating to $h_{1(A)}$ and $h_{1(S)}$ could be used. The latter would seem to be a particularly promising approach since it would obviate the need for measurements of H and L in the sample from which back-calculations were being made. Thus if an otolith-body length ratio of 720×10^{-3} at the time of opaque zone initiation

could be assumed, then calculations of $l_{1(S)}$ would be given by $l_{1(S)} = h_{1(S)} / 720 \times 10^{-3}$. Alternatively, use could be made of a regression formula, $l_{1(S)} = (h_{1(S)} \cdot b) + a$ with values for b (slope) and a (intercept derived from individual plots of otolith and body length at the time of opaque zone initiation) In fact this was done for the 15. April 1970 sample and a slope value of $b = 1.05 \pm 0.15$, and an intercept value of $a = 22.50 \pm 0.69$ mm were obtained.

It must be emphasised that this study has only explored back-calculation of l_1 from I-group fish. Although data on older fish are not available between October and June, it is considered that the problems encountered in back-calculating l_1 from I-group fish will be less important in situations either where l_1 is being calculated from older fish, or where l_2, l_3 etc. are being calculated. This is because the differences between $L_{(A)}, L_{(W)}$ and $L_{(S)}$ and between $h_{(A)}$ and $h_{(S)}$, together with the amplitude of seasonal variation in the otolith-body length ratio, almost certainly decrease with the age of the fish.

DISCUSSION

In this study of *A. tobianus* it has been found that the formation of opaque material in the otolith occurs during April and May at a time when the rate of body growth is at a maximum, and when sea-water temperatures are increasing rapidly. The coincidence of opaque zone formation with the period of rapid body growth is in agreement with the conclusions of most other workers. The period of opaque zone formation recorded for *A. tobianus* is earlier and of shorter duration than that usually found in other species, however, and is also considerably shorter than the March to October period recorded for *A. tobianus* and other sandeels around the Isle of Man by CAMERON (1958). Although LE GALL (1969) suggested that in the pogge, *Agonus cataphractus* L., opaque formation from April to June actually preceded the main period of body growth, his published data are inadequate and open to alternative interpretation.

HICKLING (1933) associated opaque zone formation with rapid body growth and a high condition factor, but the period of opaque zone formation was from December through to July. For *Gobius paganelus* L., however, MILLER (1961) found that throughout the winter the otoliths had opaque edges, and that the summer growth period was associated with the presence of hyaline edges to the otoliths.

IRIE (1957, 1960) has concluded that in *Lateolabrax japonicus* (Cuvier) and *Mylio macrocephalus* (Basilewsky) from Japanese waters, opaque zone formation takes place in the winter-spring period when body growth is at its slowest. However, he gives no quantitative data for either otolith or body increments, and, in fact, in the text of his papers refers only to activity, rather than growth-rate, being low during the period of opaque zone formation. Thus objective comparison with much of the work carried out in the North Atlantic is not possible, and MINA (1968), for example, may have attached too much importance to the apparent differences between IRIE's work and that of TROUT (1954). Indeed, in view of the known variation in the season and duration of opaque zone formation in North Atlantic species, a close correspondence between unrelated species from different environments would not necessarily be expected.

It is, however, interesting to note that in the present study on *A. tobianus*, some opaque material could be said to be deposited, by ingrowth, into a hyaline structure which was present at the otolith edge during the period of no-growth in body length, thus perhaps offering one possible interpretation of IRIE's work. There is some evidence also, that in *A. tobianus* the formation of opaque zones in the otoliths and the period of rapid body growth are independent. A small number were kept without food in aquaria during April and May, and over this period showed no increase in body length and their condition deteriorated. Nevertheless they did deposit, by ingrowth, a narrow opaque zone in the otolith at the same time as the wild fish which of course were growing rapidly at this time. This would suggest that nutrition is not so important for opaque zone formation as it is for body growth; indeed opaque zone formation may be more directly dependent on water temperature (relating to calcification rate) as MOLANDER's (1947) experiments on plaice, *Pleuronectes platessa* L., have indicated. In this way, a universal correspondence between body growth and opaque zone formation would not be expected.

In his study of the chemical and crystallographic structure of the otoliths, IRIE showed that opaque material, identified as protein, filled the grooves between the aragonite microcrystals. This clearly presents a mechanism for the ingrowth of new opaque material into an existing hyaline layer which appears to occur in early spring in *A. tobianus*. The situation is, however, complicated by BLACKER's (1969) recent conclusion from burning cod otoliths that it is the hyaline rather than the opaque zones which contain most of the protein constituent, a view which is certainly difficult to reconcile with other work, in particular the electron microscopy and isotopic studies of IRIE. Clearly the application of these, and the burning technique, to a wide variety of fish species from different environments is desirable, in addition to studies relating otolith growth and zone deposition to physiological and environmental cycles.

Three aspects of otolith growth in *A. tobianus* have been found to be of particular importance in connection with the back-calculation of body lengths from otolith measurements: the occurrence of some body growth (18% of the annual length increment in 1970) prior to opaque zone initiation in spring; ingrowth of opaque material into an established hyaline layer during the initial stage of opaque zone formation, and seasonal variation in the otolith-body length ratio.

It appears that accurate estimates of body length during mid-April ($L_{1(S)}$, the time of opaque zone initiation) and mid-September ($L_{1(A)}$, because of ingrowth, the apparent time of opaque zone initiation) can be derived from otolith measurements. These estimates require measurements at the split in the opaque zone and at the inner opaque boundary respectively, together with data on the otolith-body length relationships during mid-April and mid-September; neither, however, provides an accurate estimate of body length at the end of the growing season ($L_{1(W)}$). Thus no dimension of the otolith in *A. tobianus* corresponds precisely to the annual rings in the scales.

If data over several years were available it may be possible to estimate $L_{1(W)}$ from either $L_{1(S)}$ or $L_{1(A)}$ with reasonable precision, but at the moment one can only determine $L_{1(W)}$ as lying somewhere between $L_{1(S)}$ and $L_{1(A)}$, that is within a range of about 10 mm for the 1969 year-class. Although $L_{1(S)}$ will be determined by $L_{1(W)}$ it will also presumably, be determined by environ-

mental conditions prevailing in early spring: similarly $L_{1(A)}$ will determine $L_{1(W)}$ but so will conditions in late autumn. There are clearly advantages in estimating both $L_{1(A)}$ and $L_{1(S)}$.

Although the problems raised by this study are important if accurate back-calculations are required, the use of an unmodified back-calculation technique is not invalidated if only approximate values are needed, as in the determination of the overall growth parameters of a species. For example, on the assumption that back-calculation from measurements of the inner boundary of the opaque zone would give estimates of $L_{1(W)}$ the values represented by curve A in Figure 6 could in fact be interpreted as adequate approximations. As a corollary, however, it can be suggested that if data on the otolith growth of young fish is lacking, only approximate estimates of back-calculated body lengths can generally be expected.

Although it would be unwise at this stage to extrapolate too far from the results obtained from this specific study of one age-group of a single population, it is hoped that some of the difficulties likely to be encountered in back-calculation studies have been brought to light. In particular, if initial use of otoliths for back-calculation is found to be unsatisfactory, then it will clearly be worth investigating the occurrence of ingrowth, of seasonal variation in otolith-body length ratios, and of the temporal separation, of growth resumption and opaque zone initiation, as possible sources of error. As noted in the Introduction, relatively little use has been made of otoliths for back-calculating body lengths; as well as emphasising the difficulties and limitations involved, however, this study suggests that at least in situations where some data on otolith growth are available, these structures may be used with confidence.

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APPENDIX TABLE I. Otolith and body lengths, and the otolith-body length ratio, for the 1968 and 1969 year-classes, in 1969. Data given as means, and for body length, as $\pm 95\%$ confidence limits, with one standard deviation in parentheses.

Date	n	Total otolith length (e.p.u.)	Length at inner boundary of opaque zone (e.p.u.)	Length at outer boundary of opaque zone (e.p.u.)	Body length (mm.)	Otolith-body length ratio $\times 10^4$
1968 year class						
(I-group)						
21. March	40	56.5	-	-	77.75 \pm 0.55 (3.95)	726
9. April	19	58.9	58.4	-	80.40 \pm 1.17 (5.31)	733
30. April	44	59.8	54.9	-	84.99 \pm 1.18 (5.36)	704
12. May	31	63.6	55.3	-	94.73 \pm 1.02 (7.04)	671
29. May	34	69.9	55.8	68.5	107.22 \pm 1.71 (5.68)	651
13. June	28	72.7	55.1	70.4	107.30 \pm 1.19 (5.49)	678
30. June	48	76.7	55.5	71.2	113.29 \pm 0.89 (5.95)	677
14. July	51	79.9	55.6	71.1	114.85 \pm 0.99 (6.20)	695
11. August	9	88.6	54.9	71.3	129.22 \pm 4.02 (5.33)	686
22. September	11	89.3	55.4	70.6	126.50 \pm 2.47 (3.50)	706
22. October	72	92.1	56.6	72.0	128.85 \pm 1.27 (6.00)	714
1969 year class						
(0-group)						
14. July	14	45.8	-	-	66.71 \pm 2.98 (5.81)	686
30. July	50	48.6	-	-	69.46 \pm 1.19 (6.71)	700
11. August	30	51.2	-	-	71.89 \pm 0.72 (5.12)	701
16. September	38	57.4	-	-	80.91 \pm 0.84 (4.65)	709
2. October	28	60.1	-	-	82.86 \pm 0.81 (5.98)	725
15. October	60	62.3	-	-	84.97 \pm 0.66 (6.00)	733
22. October	48	63.1	-	-	86.49 \pm 0.72 (5.40)	730
30. October	47	62.6	-	-	84.66 \pm 0.65 (4.69)	739
16. November	100	63.4	-	-	86.78 \pm 0.92 (4.98)	724
28. November	32	63.1	-	-	85.27 \pm 1.14 (4.19)	740

APPENDIX TABLE II. Otolith and body lengths, for the 1969 year-class in 1970. Data given as means \pm 95% confidence limits, with one standard deviation in parentheses.

Date	n	Total otolith length (c.p.u.)	Length at inner boundary of opaque zone (c.p.u.)	Length at outer boundary of opaque zone (c.p.u.)	Body length (mm)
23. March ...	59	62.85 \pm 1.11 (4.28)	-	-	84.54 \pm 1.69 (6.49)
4. April	98	62.18 \pm 0.88 (4.34)	-	-	84.86 \pm 1.09 (5.44)
15. April.....	93	65.29 \pm 0.90 (4.33)	-	-	91.03 \pm 1.16 (5.60)
27. April.....	108	64.59 \pm 0.78 (4.05)	61.09 \pm 0.83 (4.31)	-	92.46 \pm 1.02 (5.31)
8. May.....	100	64.64 \pm 0.79 (3.86)	58.35 \pm 0.87 (4.26)	-	96.35 \pm 0.98 (4.93)
18. May.....	129	65.80 \pm 0.99 (5.63)	56.77 \pm 1.03 (5.90)	-	99.64 \pm 1.38 (7.90)
1. June.....	107	72.60 \pm 0.99 (4.96)	58.60 \pm 1.05 (5.33)	-	109.94 \pm 1.23 (6.34)
11. June.....	97	74.99 \pm 0.95 (4.75)	57.45 \pm 1.03 (5.19)	72.11 \pm 0.95 (4.76)	112.10 \pm 1.37 (6.81)
25. June.....	89	78.17 \pm 1.17 (5.42)	58.24 \pm 1.31 (6.17)	72.83 \pm 1.13 (5.41)	114.53 \pm 1.37 (6.50)
13. July.....	98	79.74 \pm 1.16 (5.77)	57.33 \pm 1.20 (5.95)	72.39 \pm 1.21 (6.00)	115.57 \pm 1.55 (7.76)
30. July.....	75	82.88 \pm 1.15 (4.97)	57.80 \pm 1.33 (5.74)	73.40 \pm 1.26 (5.46)	117.67 \pm 1.48 (6.41)
28. August ...	58	88.02 \pm 1.36 (5.06)	58.93 \pm 1.59 (5.90)	73.98 \pm 1.52 (5.67)	124.50 \pm 1.73 (6.45)
25. September	32	87.09 \pm 1.76 (4.89)	57.34 \pm 1.93 (5.35)	72.21 \pm 1.95 (5.42)	122.81 \pm 2.71 (7.53)
13. October ..	34	87.55 \pm 1.88 (5.29)	57.36 \pm 2.02 (5.67)	71.68 \pm 1.82 (5.11)	123.47 \pm 2.26 (6.37)