AUSTRALIAN MUSEUM SCIENTIFIC PUBLICATIONS

Dakin, William John, 1939. The age determination of the Tiger Flathead, *Neoplatycephalus (Colefaxia) macrodon* (Ogilby), by means of otoliths. *Records of the Australian Museum* 20(4): 282–292. [31 March 1939].

doi:10.3853/j.0067-1975.20.1939.578

ISSN 0067-1975

Published by the Australian Museum, Sydney

nature culture discover

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THE AGE DETERMINATION OF THE TIGER FLATHEAD, *NEOPLATYCEPHALUS* (*COLEFAXIA*) *MACRODON* (OGILBY), BY MEANS OF OTOLITHS.

Bу

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(Plates xxvii–xxx.)

Introduction.1

A glance at the fisheries literature which has emanated since 1870 from the countries where fisheries science has chiefly developed, will show that many years had to elapse before the scientific knowledge of even the most common fish species was sufficient for any very definite application.

One of the most essential studies for estimating fish stocks and over-fishing, and for fish conservation generally, has been the collection of data for determining age. The amount of discussion regarding scales and otoliths of the herring, the cod, haddock, and plaice will indicate that the use of such guides is by no means a simple matter. And the proper estimation of the markings on fish scales, otoliths and skeleton has required a thorough study of the life history, migrations, and general ecology of the fish species concerned.

So far as I am aware there seems little or nothing of this kind available for fish of sub-tropical or tropical regions. The most intensely studied marine fish have been the herring, cod, haddock, halibut, plaice and hake, and the northern European countries with Canada and the United States have been mostly concerned. Their chief fisheries have been in cold waters (except for the tuna and pilchard of California). In the southern hemisphere practically no work of the kind has been carried out.

The present work must, therefore, be regarded not only as a pioneer effort in Australian seas, but an incomplete one. It has necessitated more time in the study of one aspect of the problem than the author wished to spend. The results have shown that such a study is unsatisfactory without a thorough scientific knowledge of the life history and habits of the fish.

Unfortunately, to obtain such information it would have required a fisheries investigation vessel so that material could have been obtained at any time and place, and it would have been most desirable to have had fish marked and measured and set free so that some estimates of growth rates in nature could have been obtained. And our knowledge of the Tiger Flathead—the chief trawled fish

¹ The author is indebted very considerably to the technical skill of Miss I. Bennett, who has been responsible for much of the work in preparing otolith sections. The large numbers of Tiger Flathead required were supplied gratuitously and with care by Red Funnel Trawlers, Limited. Thanks are due especially to Captain S. Mills in this connection. The author has been aided in the publication of this work by funds made available from the Commonwealth Government Grant for Scientific Research.

of New South Wales—is still so incomplete that it is unwise to say what are the extreme limits of the spawning season. It was only because of its importance, and because our curiosity regarding age determination in these waters was great, that this research was commenced. The results, whilst interesting and very suggestive, are offered as tentative only. It would, for example, have been easier if some smaller specimens could have been obtained, and if confirmatory growth rates had been determined by other means.

The whole method of age determination by rings of growth in the case of trees, or of fish by markings on scales, otoliths, or other skeletal structures, depends on the fundamental fact that the growth rate is seasonal. Thus where rings of growth are produced as in trees they may be close together in the slow-growing or non-growing winter season, and wider apart in the quick-growing summer season. It is by counting the number of zones of close or wide rings that the age is measured. Where growth is absolutely uniform, such zones would not be recognizable.

In the case of fish otoliths age determination has depended on an alternation of the opaque and translucent areas visible when investigated by transmitted light—the opaque material is regarded as corresponding to a period of rapid growth and the translucent ring as indicating a season of little growth. (By reflected light the opaque rings appear dead white and the translucent rings appear dark. See Pl. xxvii, fig. 2; pl. xxviii, fig. 17; pl. xxix, fig. 24.)

Now it has been common to speak of the zones on fish scales and otoliths as winter and summer rings, and if such were the case, the time from one winter ring to another would obviously represent a year. The temperature of the sea bottom in the region investigated by us is only a matter of 5° warmer in summer than in winter. The Tiger Flathead is more common in slightly deeper water, but probably the temperatures vary even less in that environment. Of migrations of the flathead we know nothing.

One or two indications have, however, already appeared in the literature, which suggest that differences of temperature are neither the only nor chief causes of differentiated growth rings on the skeletal parts of fishes. In 1910 Schneider held that the so-called winter rings of the herring were due to spawning. Dannevig in 1925 experimented with codling in a rearing pond; the fish were fed in the summer, but had to find their own food from the fauna of the pond in the winter. The highest temperatures were reached between June and August. According to scale readings the greatest growth took place at the time of lowest temperature. Other experiments have shown that feeding produces a more marked effect than temperature change.

It just happens that in the halibut, plaice and cod, three much studied fish from the point of view of age determination, the winter season practically coincides with the season during which the gonads are ripening. There is the probability, therefore, that the so-called winter and summer rings on animal tissues are responses to other rhythms which may happen to coincide with the seasons and not direct effects of the actual temperature of the environment. This will be discussed later.

Now it soon became clear from an examination of the carefully prepared Tiger Flathead otoliths that one could find excellent series of opaque and translucent growth rings exactly as in the otoliths of European fishes so much investigated. In some examples they are beautifully definite and regularly placed in a way suggestive of order. In other cases they are much less regular and, as will be seen later, confusing and difficult of interpretation.

So, growth rings being present, we may proceed to discuss the conditions presented, and to see to what extent there is any periodicity suggestive of a regular seasonal rhythm. We can then glance at the question as to whether, say, an opaque ring means a winter ring or other particular season, or whether the opaque and translucent regions must just be left as the expression of changes in metabolism due to some inherent physiological rhythm or to causes yet unknown.

Technique.

The first attempts to determine the age of New South Wales marine fish species in the Department of Zoology, University of Sydney, were made several years ago under my direction, by the examination of a number of otoliths just as they were taken from the fish. Probably because of the opacity and the rough and irregular form of these structures, and because of the high percentage of "difficult" specimens which we have found in our recent work, it is not surprising that the early studies did not appear hopeful and were given up. Several methods of examination have since been tried. Otoliths have been examined whole, immersed in glycerine, benzol, and Canada balsam respectively. Other otoliths were cracked transversely (across the nucleus) and the sectioned surfaces polished. A third method was to grind sections transversely and longitudinally. But the best method, and the one which we carried out with hundreds of specimens, has been to break off a small piece of the otolith at the anterior end, in order to reduce the total convexity, and then to grind down slightly the concave surface until flat enough to cement with balsam on to a piece of plate glass, using heat. The convex surface of the otolith is next ground slightly until the whole is transparent enough for examination, care being taken not to go far enough to spoil the outer margins. The degree of grinding required is only slight and the process is watched carefully, using a microscope. For the purpose we designed and had constructed a small machine embodying an electric motor (Figure 1). The circles of plate glass with



Figure 1.—Grinding apparatus with small electric motor. Carborundum powder is dusted on the plate of glass, and the small circle of glass on which the otolith has been cemented is placed in the holder above it, then lowered on to the plate, and the motor set in motion. The weight of the holder is such that no pressure is needed when grinding.

the attached otoliths were finally warmed up again until the balsam had softened and the otolith removed to an object glass and permanently mounted in balsam.

The Factors Determining Opaque and Translucent Rings.

As pointed out above, most of the age determinations on marine fish by scales and otoliths—one might almost say all—have been made in the north temperate seas and frequently in waters where the winter and summer temperatures were conspicuously different. It has also been customary to find that the fish spawn at a definite season, the spawning period being a limited season of the year. The Petersen method of age determination by length measurements (applied to such an extent in the case of Northern fish species) has depended upon these facts.

One is faced, then, at the very beginning, with the problem of the breeding season of the Tiger Flathead and, strange as it may seem, this apparently simple problem has not proved by any means easy of solution.

Our samples of flathead of all sizes taken through all the months of the year showed some fish with well developed ovaries or testes in practically every month. But rarely were the ovaries in that absolutely ripe condition in which the eggs easily run out on the application of pressure. A. N. Colefax, when measuring flathead and attempting to obtain fertilized eggs on trawler expeditions, only obtained female fish with eggs capable of fertilization during the summer months, but this period probably lasted six months. On the other hand, the fishery inspectors speak of spawning flathead being found in all months, and the same information has been supplied to us by Mr. G. L. Kesteven, B.Sc., of the N.S.W. Fisheries Department. Obviously, if the Tiger Flathead spawns in all months of the year off the coast of New South Wales, the method of determining age by length measurements would be difficult. At the present moment we cannot believe that the evidence produced does indicate such a condition. It is mentioned here, however, to indicate the difficulties one is up against where even the most fundamental knowledge of our fish is still inadequate. There is little doubt that the spawning period is extensive. We would suggest from data obtained so far that it extends over at least six months, and that the high period comes between the beginning of October and the end of March.

Reference has been made to the use of the terms *winter* and *summer* rings of growth. Now it is by no means certain that Tiger Flathead are subjected to temperature differences which are sufficient to produce winter and summer rings or to exert a major influence on growth. It was, for example, impossible by an examination of otoliths to find any definite correlation between the most external ring and the season of the year when the fish was taken. Otoliths were removed from fish caught in each season. It was impossible to find any evidence that the winter was the period of growth limitation.

It is important in this connection to refer to a paper in which the conditions presented by fish in the tropics are set out. Erna Mohr in 1921 investigated a number of species, examples of which were taken out of the Zoological Museum of Hamburg. Six species were from fresh water lakes. There are rings on the scales as in fish species from temperate latitudes. Mohr points out the need for more research. She seems to suggest, however, that possibly the spawning seasons are responsible for the rhythmic indications in her examples. Her conclusion is that even if we do not know what causes the different rings in these tropical fish, they can be used for age estimations in fishery science. It has been pointed out that Dannevig experimented with codling in a rearing pond and found that rapid growth periods could be produced more easily by modifying the feeding conditions than by the alternating seasonal temperatures, and Gray and Setna (1931) have shown that for the Rainbow Trout feeding is a most important feature in determining growth rate.

Bhatia, too (1932), found that in Rainbow Trout there were no "periodic rings" on the scales when the fish were *fed uniformly*. Under these modified circumstances and notwithstanding the temperature, so-called summer rings could be produced in the winter.

Harold Thompson (1926) also showed that the growth rate of haddock was increased considerably by plentiful supply of food given to fish in tanks, and that abnormal growths on scales could be produced in this way.

M. P. Chevey concludes that the evidences of seasonal rhythms on the scales of fish taken in tropical waters at the mouths of the Mekond and Bassoe Rivers are due to alternations of good seasons with abundance of food, and relatively bad seasons, and that this is due to a congregation of fish, etc., at each season of flooding of these rivers, which bring down enormous quantities of nitrogenous matter. He then refers to similar rhythms seen on scales of fish from the lakes and rivers of Cochin China, and suggests the occurrence of good and bad seasons— "un véritable hiver physiologiquement parlant". No experiments are indicated and curiously enough no reference whatever is made to breeding seasons.

But seasonal food conditions are not the only factors which can affect growth.

A recent paper by Hickling (1936) shows not only how there is a seasonal cycle in the weight and condition of the somatic tissues of mature fish, but affirms that in the hake these seasonal changes are foreshadowed in the immature fish, both in the gonad and somatic tissues. This foreshadowing of a seasonal rhythm had been previously suggested by Graham, Russell and Ramalho for cod, haddock and pilchard.

Hickling states that immature fish show a loss of condition at the same season that the adults are losing condition owing to a ripening of the gonads. The bearing of this on the rings of growth on otoliths is most interesting. Thus it is pointed out that Graham (1928) (who made an exhaustive study of the literature), concludes that in "no species of fish has a satisfactory relation been shown between conditions in the external environment and the modification in the growth of the skeletal structures which manifests itself as a growth ring". Graham is inclined to think that growth rings in the cod are due to an internal factor. Dahl in 1907 implied the same thing by speaking of a physiological winter.

There can be no doubt about the occurrence of alternations in growth rates in the life history of the Tiger Flathead, and from the otoliths there is pretty good evidence that there is a fundamental regularity in rhythm, although, as in other fish, it can be disturbed. The theory of Graham, supported by Hickling, appeals to the present author as that which best fits the conditions elucidated at present.

It may be suggested that there is just as much likelihood that the translucent ring (the one so often called a *winter ring*) represents the season when the gonads are approaching ripeness as that it indicates anything else. And for the present it will be assumed that a regular alternation of opaque and clear rings represents the alternation of breeding and non-breeding seasons. Then the possibility of spawning in different months or/and under different conditions of food supply and temperature might well account for the variations seen in individual otoliths. In other words, the rings are not due at least directly to the environment, but to physiological stresses of the spawning cycle.

Description of the Otoliths of the Flathead.

After the examination of a very considerable number of otolith preparations, it was possible to set on one side a number in which more regularly spaced zones were found. An examination of these indicated that the centre of the otolith was marked by an oval rather opaque nuclear region, often with an uneven 'tail' projecting anteriorly. (All otoliths presented a more pointed anterior end and almost invariably the rings towards this end were more irregularly drawn out and difficult to interpret. The posterior portion, actually more than two-thirds of the otolith, is the best part for examination.) In a large number of the good examples (both sexes being included and taken from different seasons of the year) the actual length of the nuclear region was pretty consistent and in the neighbourhood of 4.5 to 5 mm. in length. Sometimes it was less and sometimes more. There seemed, however, to be more chance of interpreting the growth rings (i.e., they were more regular) when the length of the nucleus was about 4.5 mm.

The nuclear region is surrounded by the *first* translucent ring (comparable with the first winter ring in the plaice). We have never had a fish small enough to give this amount and no more. Our smallest Tiger Flathead was 15 cm. in length, and so far as we are aware no smaller specimen exists in any collection. Considerable effort was made to obtain smaller specimens, and it was thought that the Danish seiners fishing in shallower coastal waters might obtain some. We received baskets of very young flathead from them as the result of this request for help. But, alas, all were of a different flathead species! We have, then, no flathead of the "0" year group.

By analogy with the results of other workers, we might assume that the beginning of deposition of the next ring, the first opaque ring, marks approximately the first anniversary after hatching. A fish with an otolith presenting a nucleus, a clear ring and the beginning of the first opaque ring would then be about 15 months old. This condition seemed to be reached in fish ranging from 15 to 22 centimetres in length (Pl. xxvii, figs. 2-6). The next opaque ring begins 12 months later in fish ranging between 27 and 29 centimetres, so the length between 15 and 26 centimetres can represent the I group.

The assumption made above depends amongst other things on the reading that one opaque and one translucent ring are laid down in a year, and this leads to the matter of otoliths where the conditions are not clear.

The number of otoliths in which there is less regular alternation of opaque and translucent rings is a large percentage, so much so that it would be very difficult to interpret the meaning of an otolith, as is done in this paper, unless a considerable number of examples were examined. Probably amongst the variability which occurs, the most constant feature is to find an obliteration of one or more of the translucent rings. (The occurrence of 'false' translucent rings is also to be noted as in other well-known cases.) The tendency is thus for the greater part of the more central part of the otolith to be opaque, which means, we take it, that the growth period is more prolonged in these examples. Thus the first translucent ring to be seen may actually represent the second one or even the third (see Plate xxvii, figures 8, 9, 10, 18, 26, 27). It is remarkable, however, how frequently the distance of these later growth rings from the centre of the nucleus agrees in older otoliths, and if the distance in millimetres is measured from the centre of the nucleus a little help can be obtained in the identification of the growth rings. Gradually, and after making a series of observations, a rough scale for the positions of the different rings was acquired.

Accepting the above, the following scheme is suggested as an interpretation of the otoliths:

15-22	23-26	27-29	30-33	34-35	36-38	39-40	41-44	45	51
1st opaque ring in varying stages of thickness.	1st opaque ring complete.	2nd opaque forming.	2nd opaque complete.	3rd opaque ring forming.	3rd opaque ring complete.	4th opaque forming.	4th opaque complete.	5th opaque forming.	Difficult to decide after 6 years.
I		 II				IV		v	-
Group		Group		Group		Gr	oup	Group	

Length of Fish in Centimetres.

The age of a large Tiger Flathead is thus 5 to 6 years or more. The largest fish that we have had since otoliths were being collected was 55 centimetres in length. At this size the otolith shows rings beyond an age of six years, but it would be unsafe to say definitely whether such a fish was more than eight years of age, if that. In other words, the maximum age is not definitely to be stated. A fish of 67 centimetres was measured by Colefax. It was regarded as exceptional by the trawlermen. Several fish at 65 centimetres were measured, this size also being regarded as exceptional. Whilst, as indicated later, the large females above 50 centimetres are not common, it must be distinctly remembered that the Tiger Flathead is a fish of the deeper coastal waters, and that since catches are practically limited to the grounds at present fished by trawlers, it does not follow that an accurate estimate of the number of large fish can be made. The trawlers are practically always inside the 100 fathom line.

Our figures indicate that the annual increment in growth of the fish is as follows. The length range between the second and third years of age is about 7 centimetres, and for fish of approximately 37 centimetres in length the next year's growth would add about 5 centimetres. Once again it may be stressed that these figures are all regarded as tentative. They are the first suggestions put forward for an Australian marine fish species, and are based on one type of age reckoning alone. It now remains for a fuller biological study of the Tiger Flathead to determine by other methods whether the system adopted here is applicable.

It may be argued, however, that the figures obtained do not seem unreasonable. Incidentally they are valuable as indicating the extra viability of the female (see later), and they show that the fish are on the trawling grounds and are caught first at approximately 12 to 15 months old, the size then being 15 to 22 centimetres.

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AGE DETERMINATION OF TIGER FLATHEAD—DAKIN.

At this age and size it is not possible to distinguish the sex by the reproductive organs; they are quite immature. The legal minimum size is at present 13 inches (33 centimetres), at which length the fish are, according to our estimates, about 2 to $2\frac{1}{2}$ years of age. The most usual size in the markets is probably round 38 to 40 centimetres.

A glance at some of the illustrations might make it appear that the estimating of age by the otoliths is not so difficult. It must be stressed that the more regular ones chosen for illustrating the different years are not always in the majority. Amongst the facts which appear with some certainty is one which helps to show that the alternating opaque and translucent bands are not mere accidents, but are due to very definite factors. This is the surprising resemblance which may sometimes be found between fish of the same size from the same catch. Thus the otoliths from three different fish taken from one catch and nearly resembling one another in size are illustrated (Plate xxvii, figs. 2, 9, and 10). None of these shows a clear first translucent ring. They present very similar characteristics. It would be possible to deduce that all three fish had experienced similar environmental conditions.

Age and Sex.

In view of the fact that the largest Tiger Flathead obtained by us—size 50 to 58 centimetres—were all females, and in view of the rarity of male flathead of a length greater than 48 centimetres, a distinct possibility of a differential growth rate follows. It is not uncommon amongst fish for the female to grow faster than the male. On the other hand it is possible that the differences in the sex ratio of the largest sized flathead might be due to a differential death rate. As there was some indication at first that smaller flathead were usually males (information received), we commenced our work with some expectation of a difference in the growth rate in the two sexes.

It was interesting, therefore, to find that the otolith readings gave no indication of any considerable differences between the two sexes in regard to speed of growth. So far as we can judge at present from our material, there is little difference between the growth rings of otoliths from male and female flathead up to 45 centimetres in length.

These findings receive further support from observations made by Mr. G. L. Kesteven and kindly communicated by him. He has found not only that equal numbers of males and females are found in the smallest size groups at which the sexes can be distinguished by the gonads, but that in the intermediate sizes the numbers of males and females are also approximately the same.

It seems necessary, therefore, for us to conclude that the length of life is greater for females, or that growth continues to a greater extent in that sex after the length of 45 to 50 centimetres is reached.

As we have previously noted, it is not easy to determine the age by growth rings after a size of 52 centimetres is reached. Beyond indicating that the female flathead probably attains a greater age than six or seven years, we should not like to go. In short, the maximum cannot be definitely stated. It is, however, not of so much consequence in practice, for it would apparently be rare for flathead on the present fishing grounds to reach the maximum possible. It is, however, always conceivable that larger and older Tiger Flathead may occur in greater numbers in deeper water.

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The suggestion that the female flathead is more viable than the male, and that it attains a greater age and greater length, is not something unusual in fishes. It is not claimed, however, that this has been definitely proved. Once again it is necessary to point out that without a *complete* knowledge of the life history of the Tiger Flathead, many sides of the story must be left for future workers.

For example, it is not fair to judge the conditions of fish over 50 centimetres in length whilst the fishing area remains restricted. Maybe it is improbable that the present picture of sizes will be changed, but the writer would very much like to know something of the oceanic limits of range of this fish.

Again, there are species of fish in which the sexes grow at practically the same rate for the first two years and then a greater and greater discrepancy occurs. At present, however, we could not accept this as applying to the Tiger Flathead unless something of this sort occurs after the debatable regions of the otolith beyond the fifth opaque ring had been reached, i.e., in fish over 50 centimetres in length.

Conclusions.

1. An examination of a large number of otoliths of the Tiger Flathead, a commercial fish taken by trawlers off the coast of New South Wales, shows that the otolith presents periodic rings of growth.

2. Despite a large percentage of variable specimens and others difficult of interpretation, there is evidence to indicate that a regular seasonal rhythm is present.

3. This seasonal rhythm can be used for an estimation of age.

4. The Tiger Flathead would appear to fall into the following age groups at approximately the sizes indicated:

Age Groups	••	0	I	11	111	IV	\mathbf{v}
Length	Cms.	0–15	15-26	27-33	34–38	39–44	45

(*Note*: The above is a tentative scheme, see discussion. The range of variation in length is greater than indicated, but a complete statistical study to determine this has not been made.)

5. The so-called "winter rings" are not to be classed as indicating the winter season. There is insufficient evidence to show what is the chief factor determining the seasons of limited growth, but for the present it has been taken that the season when the clear ring is normally laid down represents the season when the gonads are approaching maturity or, in the immature fish, a foreshadowing of this season.

6. There appear to be no sexual differences in growth rate or age length until the size of 50 centimetres is being reached. Over the 55 centimetres there appears to be a far greater frequency of females. It is suggested that this is due to a greater viability of the females, although reasons for further investigation are indicated.

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EXPLANATION OF PLATES.

Otoliths from the Tiger Flathead, Neoplatycephalus (Colefaxia) macrodon (Ogilby).

PLATE XXVII.

Fig. 1.—An unground otolith from a female fish 55 centimetres in length.

Fig. 2.—Ground otolith from fish of 19 cm. length. (Interpreted as indicating nucleus and part of 1st opaque ring.)

Fig. 3.—Ground otolith from fish 20.5 cm. in length. Female. Nucleus + almost complete 1st ring.

Fig. 4.—Ground otolith from fish 23.0 cm. in length. Female. Nucleus + 1st opaque ring.

Fig. 5.—Ground otolith from fish 26.1 cm. in length. Male. Nucleus + 1st opaque ring.

Fig. 6.—Ground otolith from fish 24.0 cm. in length. Female. Nucleus + 1st opaque ring.

Fig. 7.—Ground otolith from fish 24.1 cm. in length. Male. Same age as Fig. 6, but with secondary rings.

Fig.	8.—Ground	otolith	from	fish	$25 \cdot 0$	cm.	in	length.	Female.
Fig.	9.—Ground	otolith	from	fish	$26 \cdot 3$	cm.	in	length.	Female.
Fig.	10.—Ground	otolith	from	fish	26.5	cm.	in	length.	Female.

Similar otoliths from fish of same size and catch, but nucleus larger than usual in all.

Fig. 11.—Ground otolith from fish 27.0 cm. in length. Female. Nucleus + 1st opaque and nearly complete 2nd ring; fish taken in month of June.

PLATE XXVIII.

Fig. 12.—Ground otolith from fish 30.6 cm, in length. Male. Nucleus + 2 opaque rings and with translucent margin.

Fig. 13.—Ground otolith from fish 30.5 cm. in length. Male. Nucleus + 2nd opaque just complete.

Fig. 14.—Ground otolith from fish 33.0 cm. in length. Male. Nucleus + 2nd opaque ring and clear marginal ring.

Fig. 15.—Ground otolith from fish 34.6 cm. in length. Female. Nucleus + 2nd opaque ring and clear marginal ring.

Fig. 16.—Ground otolith from fish 36.7 cm. in length. Female. Nucleus + 2nd opaque ring and trace of 3rd opaque commencing.

Fig. 17.—Ground otolith from fish 37.0 cm. in length. Female. Nucleus + 3 opaque rings, 3rd not yet complete.

Fig. 18.—Ground otolith from fish of 36.0 cm. in length. Female. Nucleus + 3 opaque rings by comparison, but example of difficulty in that 2nd translucent ring is apparently missing.

PLATE XXIX.

Fig. 19.—Ground otolith from fish 36.5 cm. in length. Female. Nucleus + 3 rings, but nucleus unusually small.

Fig. 20.—Ground otolith from fish 36.2 cm. in length. Female. Fig. 21.—Ground otolith from fish 36.3 cm. in length. Male. Fig. 22.—Ground otolith from fish 38.0 cm. in length. Female. Fig. 23.—Ground otolith from fish 39.3 cm. in length. Female. Probably all equivalent to otoliths with nucleus + 3 opaque, but obscure types through secondary rings and irregularities.

Fig. 24.—Ground otolith from fish $38\cdot 8$ cm. in length. Female. Probably nucleus +3 opaque, but 2nd and 3rd opaque rings not clearly defined.

Fig. 25.—Ground otolith from fish 38.7 cm. in length. Female. Probably nucleus + 3 opaque rings, but very small nucleus and secondary translucent rings in nuclear region.

PLATE XXX.

Fig. 26.—Ground otolith from fish 39.0 cm. in length. Female. Probably nucleus + 3 opaque rings, but 1st translucent ring not well defined.

Fig. 27.—Ground otolith from fish 41.1 cm. in length. Male. Nucleus + 4 opaque rings, 1st and 2nd translucent rings not well defined.

Fig. 28.—Ground otolith from fish 45.0 cm. in length. Female. Nucleus + 5 opaque rings. (Indicated by five short black strokes on left side of photo.)

Fig. 29.—Ground otolith from fish 51.0 cm. in length. Female. Nucleus + 6 opaque rings.

Fig. 30.—Ground otolith from fish 54.0 cm. in length. Female. Nucleus + 5 distinct opaque rings + undeterminable margin.

Fig. 31.—Ground otolith from fish 55.5 cm. in length. Female. Probably nucleus + at least 6 opaque rings. First 4 rings distinct and then large undeterminable margin.

(*Note*: In most cases the photography is by transmitted light, and the photograph shows the nucleus and rings representing active growth periods as dark regions; in a few cases, increased surface light has produced the opposite effect, that of a photo by reflected light, and the nucleus and opaque rings show white. This is the case in Pl. xxvii, figs. 2-3; Pl. xxviii, fig. 17; Pl. xxix, fig. 24, and Pl. xxx, fig. 26.)

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PLATE XXVII.



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PLATE XXVIII.



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PLATE XXX.



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