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Investigating the timing of annual growth zones in otoliths of southern bluefin tuna (*Thunnus maccoyii*)

Appendix 11 of Final Report for FRDC Project 1999/104

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Introduction

Otoliths are calcified ‘ear-stones’ in teleost fishes used for balance and/or hearing. They are metabolically inert and, unlike other hard parts of fish such as scales and vertebrae, are not subject to resorption (Campana 1999). In addition, otoliths continue to grow throughout the life of a fish and hence contain a permanent chronological record.

Otolith growth is not constant. During periods of fast growth the accreted otolith material is opaque whereas it is translucent during slow growth periods. An opaque zone and subsequent translucent zone is referred to as an ‘increment’ and they appear as a light and a dark band, respectively, when placed on a black background and viewed under a dissecting microscope with reflected light.

Increments in the otoliths of southern bluefin tuna, *Thunnus maccoyii*, (SBT) are formed annually (Clear et al. 2000), with fast growth occurring during the southern summer and slow growth during the southern winter. Thus, the number of increments can be counted to give an age estimate of SBT in years. We refer to this procedure as making a “reading” from an otolith.

There are three pairs of otoliths; in most fishes, including tunas, the sagittae are the largest pair. Because of their size, sagittae are the easiest to handle and prepare so they are most often the otoliths chosen for research, as they were for this study. Previous studies involving direct age estimates of SBT have included two methods of preparing sagittal otoliths for “reading”, one in which the otolith was left whole and the other in which the otolith was sectioned (Gunn et al. In press). The whole otolith method has been used successfully for fish less than about 6 years old (~ 135 cm fork length); after this age subsequent increments are deposited too closely to differentiate them. Sectioned otoliths have been used for all ages, however the first 4 or 5 increments are particularly difficult to decipher. With both methods, if the start of a new translucent zone (or band) is detected on the otolith margin an additional increment (known as the marginal increment) is counted as a year.

Using the number of increments as an estimate of fish’s (integer) age is not straightforward because variation occurs in the time of year when individual fish form their translucent band. Consider two fish from the same cohort. One fish is caught in

April and has not yet started to form the translucent band within the marginal increment. There are *N* increments visible on its otolith, so the fish is assigned age *N*. The other fish is caught two months later. By this time it has started to form a translucent band, so it is estimated to be age $N + 1$, despite the fact that the two fish came from the same cohort.

If we know the date, *d*, when the translucent band becomes detectable, then we can estimate the cohort to which a fish belongs. For example, if we assume that all fish are born on January 1st (the approximate mid-point in the SBT spawning season), then we can correct the ages such that all fish from the same cohort are assigned the same age as follows:

$$\text{age} = \begin{cases} N & \text{if capture date} < d \\ N-1 & \text{if capture date} \geq d \end{cases},$$

where *N* is the number of bands counted, and both *d* and the capture date are expressed in Julian days since January 1 of the year of capture.

As part of this Appendix, we carried out an investigation to determine *d*, the time when the translucent band is formed, using data from a previous age-validation study (Clear et al. 2000). In the Clear et al. (2000) study, a number of SBT that were caught as part of a large-scale tagging program were injected with strontium chloride at the time of tagging. Strontium chloride ($SrCl_2$) is a harmless salt that deposits in the otolith and provides a "time-stamp" of the date of tagging. When the fish are recaptured and the otoliths are removed, the number of increments formed subsequent to marking is determined by counting the number of translucent zones ('bands') deposited after the strontium mark; the translucent zones are narrower and more defined than the opaque zones and hence easier to count. Because the amount of time the fish has been at liberty is known, we can determine whether or not the translucent zone for the recapture year has yet been formed (or is yet detectable).

The results of this investigation did not provide as fine a resolution for determining when during the winter period the translucent band was formed as we had anticipated. This was considered a significant issue for the current growth project because a substantial number of otoliths had been aged from fish captured during these winter months. Results of fitting growth curves to the otolith age and length data were found to be sensitive to assumptions about the time of band formation. As a consequence, additional work was undertaken to see if a more precise and accurate estimate of the time of band formation could be determined.

A pilot study was conducted in which a small number of unread strontium-marked otoliths from fish caught during the winter months were selected from the CSIRO archives. In addition to counting the number of bands formed subsequent to the strontium mark, several additional observations were also made. In particular, the samples were chosen from fish that had had both otoliths extracted so that one otolith could be read using the whole method and the other could be read using the sectioned method. Some preliminary exploration of existing otolith data suggested that the ability to detect a translucent zone forming on the margin may differ between the two methods. The results from the pilot study further indicated that this was a possibility. Consequently, a larger study was conducted in which otolith pairs were read both sectioned and whole and the readings compared.

Methods

Time of band formation study based on strontium chloride marked otoliths

As part of the Clear et al. (2000) age-validation study, 59 otoliths were read from fish that had been strontium injected and recaptured. Details on the strontium chloride tagging experiment can be found in Clear et al. (2000).

Using the release date of the fish (at which point the strontium mark was deposited in the otolith), the recapture date, and the number of increments subsequent to the strontium mark, we can determine if a translucent band had yet been formed in the year of recapture. For example, if a fish released in January 1990 and recaptured in July 1994 has four translucent bands in its otolith after the strontium mark, we can assume that its translucent band for 1994 has not yet been formed, or at least cannot yet be seen. In doing so, we are assuming that the count of increments subsequent to the strontium mark is accurate. We are also assuming that the only uncertainty in the number of translucent bands that should have formed comes from the year of recapture, and not the year of release. The majority of fish were tagged and released during the summer months of December to March; months during which a translucent band would not be forming. However, two of the 59 fish were released in June and one in May, and we cannot be sure whether the band corresponding to the year of release would have been formed prior or subsequent to the strontium mark. Thus, we excluded these three fish from our analyses.

For each month of recapture, we tabulated how many fish had already formed a band in the year of recapture. We could then estimate the range of months during which band formation can occur, and also an average date by which SBT otoliths exhibit a translucent band.

Pilot study

A pilot study was conducted in which 24 unread strontium-marked otoliths from fish caught during the winter months (May-September) were extracted from the CSIRO archives. The number of bands formed subsequent to the strontium mark were counted and checked against the time at liberty to determine if a band had yet been formed by the recapture date. Several additional observations were also made.

Two methods have been developed for reading otoliths – one in which the otolith is left whole and one in which it is sectioned (Gunn et al. In press). Preliminary exploration of existing otolith data suggested that the ability to detect a translucent zone forming on the margin may differ between the two methods. In particular, a plot of fork length versus estimated age showed obvious disparities in the lengths-at-age of young fish that had been caught in the winter months. Further investigation revealed that these disparities were consistent with fish aged using the sectioned method being one year too young, which would occur if a band forming on the otolith margin was not being detected in the sectioned otoliths. However several confounding factors also existed which could explain the differences, one being that the same fish that were aged using the sectioned method were also caught in a very different area of the ocean than the fish that had been aged using the whole method. We used the pilot study to investigate this issue further. The 24 otoliths that were selected for the pilot study came from fish for which both otoliths had been collected (called sister otoliths). One otolith was read whole and the other otolith was read sectioned. In addition, for both methods the reader recorded whether or not a translucent band was counted on the margin of the otolith.

Whole versus sectioned otoliths study

Exploratory data analyses in conjunction with results from the pilot study suggested that there may be a consistent difference in the time at which the translucent band being formed on the margin of an otolith becomes detectable between otoliths read whole and otoliths read sectioned. For fish caught during the winter months while a marginal band is forming, it is important to know whether in fact a difference does exist. If it does, then the age assigned to these fish could be one year different depending on the method used. A difference in age of one year for small fish can have a significant impact on the estimation of growth rates. Furthermore, it was realized that if a significant difference did exist, it could be exploited to refine the time when bands become detectable with both methods by comparing otolith readings using both methods from the same fish. For example, the first month in which a difference in the number of bands was found between the two methods would indicate the month when bands first became detectable with the method yielding the higher number of bands. This month would mark the beginning of the potential period of band formation. The subsequent month in which both methods yielded the same number of bands would indicate the month when bands were consistently detectable by both methods, and would mark the end limit of the period of band formation.

In order to address the problem of whether a consistent difference did exist and whether it could be used to refine the estimates of the time of detectable band formation, we conducted a study using pairs of sagittal otoliths from the same fish. One otolith was read whole and the other was read sectioned to see if there was a consistent difference in the readings around the time of band formation, and if so, for what months the difference was present.

Pairs of sagittal otoliths (paired otoliths from the same fish are referred to as ‘sister otoliths’) were chosen from the CSIRO Hard Parts Archives. Samples were selected from fish with lengths less than 135 cm since whole otoliths from fish larger than this are usually very difficult to read. Furthermore, otoliths were selected from fish caught during the months of March to November since the translucent zone would almost surely have been deposited sometime within this period. In three of these months (April, August and November), fewer than 20 intact pairs of otoliths were available from the Archives. The small sample sizes could possibly obscure a pattern in the development of the translucent band through the year. However, the numbers of fish in the adjacent months are high, which should still allow for a general pattern to be detected if it exists. Furthermore, the length distribution is fairly similar across months, especially in the potential months of band formation (Figure 1). This would be important if the ability to detect the marginal band using a particular method is related to the size of the otolith, and hence the size of the fish.

Sister sagittal otoliths were prepared using two techniques: one otolith for reading whole and one for reading sectioned. The otolith to be read whole was burned on a 400°C hot plate until it turned golden brown. This accentuates the opaque-translucent banding pattern since the colour change in the translucent zones is greater, making them more visible (Figure 2). The other sagitta of the pair was sectioned along the transverse axis (Figure 3a), producing a 0.35 mm thick cross-section containing the primordium (Figure 3b).

Age estimates were successfully made using both the whole and sectioned method for 227 pairs of sister otoliths. Regardless of the method, two counts were made ‘blindly’ from each otolith (without knowledge of the size of fish or any previous estimates). A third reading was made to determine a final age estimate (FAE); this reading was made with the knowledge of the previous two readings. Two readers, both with extensive experience in age and growth studies of SBT, made all of the readings. The average percentage error (APE) of Beamish and Fournier (1981) was calculated to compare age estimates between the FAEs from each method. This index provides a measure of the precision or reproducibility of age estimates.

Results

Time of band formation based on strontium chloride marked otoliths

None of the fish recaptured in the months of January to May (inclusive) had a detectable band on the otolith margin, i.e. the band had not yet begun to form during the year of recapture (Table 1). Of the 11 fish caught in June, two had formed a band in that year, while three of the four fish caught in July had formed a band. All fish recaptured after July had already deposited a band in their otolith by the time of recapture. This data implies that band formation can occur during June and July, with July being most common. Unfortunately, we have no data for August so we cannot say anything about band formation during this month. Likewise, the sample sizes in all months surrounding the identified period of band formation are so small that we cannot be sure about the boundaries of the period, or reliably estimate the probability of a band being formed and becoming detectable during these transition periods. From the available information, we infer that band formation can occur any time during the months of May to September, with July 1 as the approximate average time.

It is important to note that all of the otoliths used in this strontium chloride marking study had been read using the whole otolith method. Thus, the results only apply to the time when bands become detectable with this method, but this also means that there is no potential confounding based on the method of reading. In addition, all of the otoliths came from fish recaptured in years 1992 to 1995 in waters south of Australia between 110°E and 170°E and 32°S and 44°S, with the majority caught in the Great Australian Bight or off Tasmania.

It is conceivable that the year or the spatial environment of the fish may affect the timing of band formation. The formation of otoliths is thought to be determined in part by the environmental conditions experienced by the fish (temperature, salinity, etc.) and in part by other factors such as ontogeny, growth rates and physiological processes, although their relative contributions remain uncertain (Campana 1999). To add to this complex picture, the ability of SBT to maintain body temperature above ambient temperature may possibly obscure the water temperature signal to some unknown degree. However, if fast and slow growth periods are related in part to hot and cold water temperatures respectively, then either a year with an unusually warm autumn, or else a warm place of residence during autumn, might prolong the fast growth period and hence delay the formation of the translucent zone. For this reason we stratified the data in Table 1 by recapture year and by recapture area respectively (Table 2 shows the results stratified by year). We found the pattern in the number of bands formed by each month to be similar across years and across areas.

The recapture ages of the fish (as determined by the number of increments in the otolith) ranged from one to six, with the majority of fish aged three or four. The detection of the marginal band may be affected by the age of the fish. In juvenile SBT the width of the new translucent zone decreases with each subsequent increment formed, so for older fish the marginal band might be more difficult to detect until it has been more fully formed. Thus, we stratified the data in Table 1 by recapture age. Again, we found the pattern in the number of marginal translucent bands formed by each month to be similar across ages.

Caution must be used in interpreting these results because the sample sizes in each month after stratification are so small that the data are not very informative. The results certainly cannot be taken as conclusive, and they cannot be generalized outside the range of the data. The fish included in the study came from a fairly narrow range of the population with respect to years, areas, and ages. It is possible that one or more of these factors play a role in the formation or detection of bands that we were unable to identify.

Pilot study

Of the 24 pairs of sister otoliths selected for the pilot study there were 3 pairs that had to be omitted completely from the study due to both otoliths being unreadable or the release and recapture information being unbelievable. Of the remaining otolith pairs, the whole otolith was unreadable in 3 cases and the sectioned otolith was unreadable in 2 cases. For investigating the time of the band formation, this left 18 whole otoliths and 19 sectioned otoliths; for comparing the two reading methods, this left only 16 pairs with both readings available.

The results from the investigation on the time of band formation (Table 3) were not very informative for clarifying the period of band formation. This was due largely to the small sample sizes. The results from both the sectioned readings and whole readings indicate that bands can form in May, June and July. Only one sample from each of August and September was included in the study. In both cases, the whole otolith was unreadable and the sectioned otolith exhibited a band on the margin, but clearly nothing can be concluded about these months with a sample size of one. The small sample sizes are unfortunate but were due to several reasons: a) this was only intended as pilot study and resources were limited (the process of preparing an otolith and obtaining a final reading is expensive and time-consuming), b) there are limited numbers of strontium-marked otoliths available (only 2 remain in August, which is a critical month for defining the period), and c) strontium-marked otolith samples for which both sister otoliths are available are even more limited.

Recall that for each sectioned otolith, the number of bands deposited after the strontium mark was counted, and it was also recorded whether a band was detected on the margin of the otolith. For 100% of the sectioned otoliths, if a band was detected on the margin of the otolith, then the post-strontium count indicated the presence of a band in the year of recapture; if a band was not detected on the margin, then the post-strontium count indicated a band was not present in the year of recapture. Because both sets of results are equivalent, we will only refer to the detection of a marginal band results for sectioned otoliths.

Despite the small amount of data, some interesting results were obtained. The proportion of fish with a marginal band in May was high, whereas the proportion of fish with a marginal band in July was small, and these observations were consistent whether using the whole otolith or sectioned otolith. These results are somewhat contradictory to those from the previous study (see Table 1), but the difference is not startling since the sample sizes for May and July are so small in both studies. A more interesting result is that the proportion of fish with a marginal band in June was high based on whole otoliths and low based on sectioned otoliths.[1]

[1] In the previous study, which used only whole otoliths, June had a low proportion of otoliths with a band detected in the year of catch. However, the results from the two studies are not completely comparable – (continued on next page)

In comparing the whole and sectioned readings on sister otoliths, the whole reading was generally one greater than the sectioned reading for fish caught in June (Table 4). The two readings tended to agree in the other months, although June is the only month with a reasonable sample size for comparison. This finding supported our hypothesis that the ability to detect a marginal band may differ between the two methods, with a band becoming detectable sooner with whole otoliths than with sectioned otoliths. This led to a more comprehensive study comparing whole and sectioned readings on sister otoliths, the results of which are presented below.

Whole versus sectioned otoliths study

Using the final age estimates (FAEs) from 227 pairs of sister otoliths, where one sister was read using the whole otolith method and the other using the sectioned otolith method, we calculated the average percent error (APE) between the methods to be 5.8%. This is well within the acceptable level of 10%. For each pair we also calculated the difference between the whole and the sectioned read. Of the 227 pairs included in the study, the whole and sectioned readings agreed in 146 cases (64.3%). The whole reading was one greater than the sectioned reading in 17 cases (7.5%) and two greater in 4 cases (1.8%); it was one less in 55 cases (24.2%) and two less in 5 cases (2.2%). This trend is consistent across months (Figure 4). This would suggest that there is a tendency to count an extra band using the sectioned method versus the whole method. If the difference was due to new bands being detectable earlier in the year in sectioned otoliths than whole otoliths, then we would expect the sectioned readings to be one greater during the early months of band formation and equal later in the year. Instead, all months show the same pattern, which implies that the difference is not due to the timing of band formation.

The main purpose of this study was to see if the method used to read the otolith affects the perceived time of band formation. However, out of interest, we plotted the

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the results from the previous study are based on counts of the number of bands after the strontium mark in whole otoliths, whereas the results obtained from whole otoliths in the pilot study are based on whether or not a band was detected on the margin of the otolith. Post-strontium counts were only made on sectioned otoliths in the pilot study.

fork length of the fish versus the whole and sectioned readings (Figure 5). The sectioned readings show greater variability in length at age than the whole readings, especially at younger ages. From our knowledge about the length-at-age distribution of young SBT, the sectioned readings show a greater range than expected. For example, it is unlikely that a fish of age two is over 100 cm in length. This suggests that the whole readings may be more precise, at least at young ages. This result may not be overly surprising since it has been documented that the first four or five increments are difficult to interpret in sectioned otoliths (Gunn et al. In press).

Discussion

From the data available, we found that the time of year at which SBT deposit a translucent zone in their otoliths can vary from May to September, with July 1 being an approximate average date. Although some of the variability may be due to errors in the increment counts, most of the variability is likely due to the fact that the time of translucent band formation differs considerably from fish to fish. Growth in SBT, and hence the formation of otolith increments, is believed to be related in part (and indirectly) to water temperature, with slow growth occurring when the water is cooler. SBT are found throughout a large area of the ocean with very different environmental conditions. As such, it is not surprising that the time at which the band forms can vary greatly between fish. The annual formation of increments in otoliths may also be, at least in part, physiologically controlled; this too could cause the timing of band formation to differ between fish.

In our comparison of sister otoliths read whole and sectioned, we found no relationship between the difference in the readings and the time of year. This means that comparison of number of bands from the same fish using the same method cannot be used to provide further information on the timing of band formation. However, it also means that the fact that all the otoliths read in the Clear et al. (2000) strontium-chloride experiment were read whole should not affect our findings about the period of band formation, and that the conclusions from this study can be applied to otoliths read with either method.

Although the main purpose of comparing whole and sectioned otolith readings was to see if the methods differed in their detection of the marginal increment, some other interesting results came from the study. For example, although the methods show quite good agreement (64%), there appears to be a tendency for the sectioned count to have one more band than the whole count. The discrepancy between the whole reading and the sectioned reading does not appear to be related to the size of the fish (Figure 6), although the sample sizes in the smaller length-classes are too small to be certain. Based on Figure 5 and our prior knowledge of the length-at-age distribution of young fish, we believe the whole count is more reliable for young (small) fish. However, the whole method cannot be used for larger fish (fork length >135 cm). Thus, with respect to using direct aging data to model growth, it might be preferable to use only data from otoliths read whole up to a given size, and then use data from the sectioned method subsequently. Of course, one would need to decide (probably somewhat arbitrarily) an exact figure to use for the transition size.

There are still some unread otoliths in the CSIRO Hard Parts Archives that were collected from strontium-injected SBT. These otoliths could perhaps be used to better define the period of band formation, but preparation and reading of them was beyond the scope of this study. There are at least 20 unread strontium-marked otoliths collected in each month from January to June (with the exception of 16 in May) that could help to define the start of the period. Unfortunately, there are only 7 unread otoliths from fish caught in July, 2 from August, and 5 from both September and October. This means that there are insufficient samples to reliably estimate the end of this period with any confidence.

All of the strontium-mark otoliths included in our investigation of the time of band formation came from fish aged six or less that were caught in southern Australian waters in years 1992 to 1995. The time of band formation may differ for fish outside these ranges since the physical environment in other regions may differ and there may be differences in habitat use with age. By proposing a fairly broad range of months in which band formation can occur (May to September), we hope to have enveloped the time of band formation for the vast majority of fish.

Without more information, uncertainty of one year exists about the age of a fish caught during the period of band formation. In turn, this uncertainty could induce substantial uncertainty into the estimation of growth curves if fish caught during this transition period are included. While statistical approaches can be developed to deal with this problem (see Appendix 12), their application is complex, requires substantial amounts of data and was outside the scope of the current project. It is important to note that the exclusion of direct aging data from fish captured during the months when bands are being formed would not bias the estimation of growth, but their inclusion with some assumption about the time of band formation could. Thus, only direct aging data from fish caught outside the proposed months of band formation (i.e. from October through April) were included in growth analyses for the current project. In doing this, we believe that we should have excluded almost all, if not all, fish for which the time of band formation could potentially confound the estimation of its age.

References

Beamish, R.J. and Fournier, D.A. 1981. A method for comparing the precision of a set of age determinations . Can. J. Fish. Aquat. Sci. **38**: 982-983.

Campana, S.E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. **188**: 263-297.

Clear, N.P., Gunn, J.S., and Rees, A.J. 2000. Direct validation of annual increments in the otoliths of juvenile southern bluefin tuna, *Thunnus maccoyii*, by means of a large-scale mark-recapture experiment with strontium chloride. Fish. Bull. **98**: 25-40.

Gunn, J.S., Clear, N.P., Carter, T.I., Rees, A.J., Stanley, C.J., and Farley, J.H. In press. The direct estimation of age and growth in southern bluefin tuna, *Thunnus maccoyii* (Castelnau), using otoliths, scales and vertebrae. Fish. Bull.

Table 1. Direct aging data from 56 southern bluefin tuna that were part of a tag-recapture experiment with strontium chloride. The table shows how many of the fish recaptured in each month had yet deposited a detectable band in their otolith for that year.

Recapture Month	Band deposited yet? No	Band deposited yet? Yes	Total
January	3		3
February	9		9
March	14		14
April	3		3
May	2		2
June	9	2	11
July	1	3	4
August			
September		2	2
October		4	4
November		1	1
December		3	3
Total	41	15	56

Table 2. The data from Table 1 stratified by year of recapture. The time when bands are formed does not appear to be affected by year; however, the sample sizes in each month are much too small to be conclusive.

Recapture Year	Recapture Month	Band deposited yet?		Total
		No	Yes	
1992	3	1		1
	4	1		1
	6		1	1
	7		2	2
	10		1	1
1992 Total		2	4	6
1993	1	1		1
	2	4		4
	3	8		8
	4	1		1
	5	2		2
	6	1		1
	7		1	1
	10		2	2
	11		1	1
	12		3	3
1993 Total		17	7	24
1994	1	1		1
	2	1		1
	3	3		3
	6	4		4
	7	1		1
	9		2	2
	10		1	1
1994 Total		10	3	13
1995	1	1		1
	2	4		4
	3	2		2
	4	1		1
	6	4	1	5
1995 Total		12	1	13
Grand Total		41	15	56

Table 3. Summary results from the pilot study. The table shows how many of the fish captured in each month had a detectable band forming on the margin of their otolith according to a) the whole method and b) the sectioned method.

Recapture Month	Whole Method		Sectioned Method	
	No	Yes	No	Yes
May	1	3	2	3
June	2	8	7	1
July	3	1	3	1
August	0	0	0	1
September	0	0	0	1

Table 4. Difference between whole reading and sectioned reading for otoliths read as part of the pilot study. Only 16 of the otolith pairs could be included in the comparison due to one or both of the otoliths being unreadable using the method for which it was prepared.

Recapture Month	Whole – Sectioned			Total
	-1	0	+1	
May	0	4	0	4
June	0	2	6	8
July	1	2	1	4
August	0	0	0	0
September	0	0	0	0

Figure 1. Fork length versus month caught for the 227 fish selected for the study comparing whole and sectioned readings from sister otoliths. The length distributions are reasonably similar across months, especially in the most probable months of band formation, so length should not be a confounding factor in whether or not a method can detect a band in a particular month.

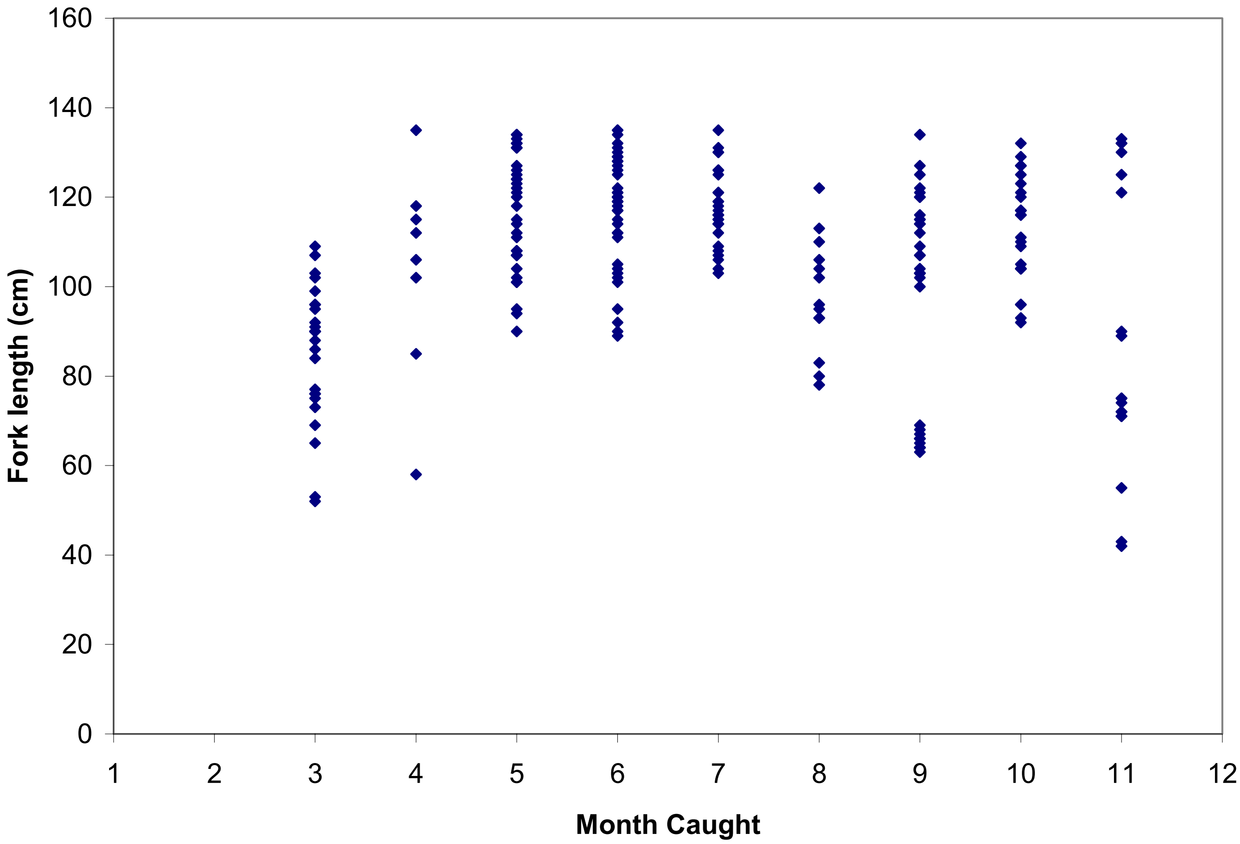


Figure 2. In preparation for being read whole, the sagittal otoliths were burnt on a hot plate accentuating the translucent zones (bands). Scale bar: 1 mm.

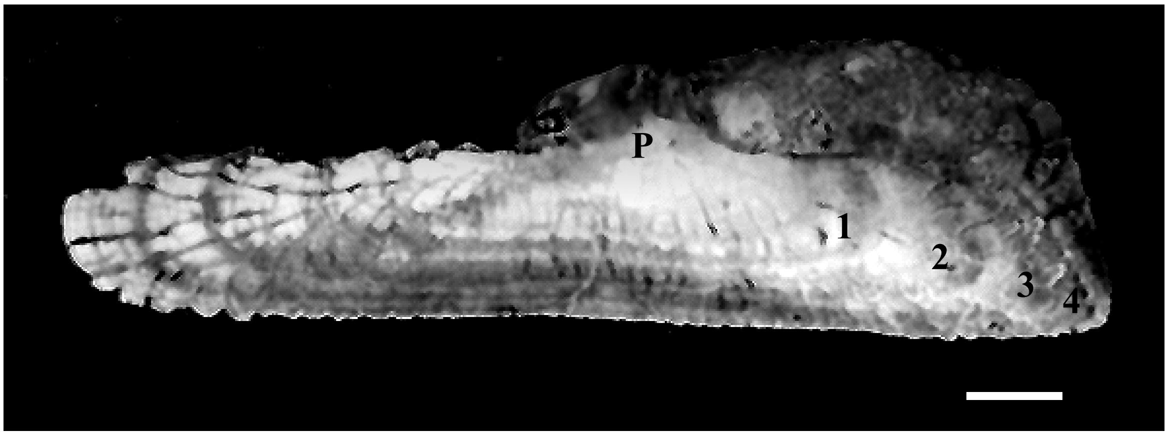
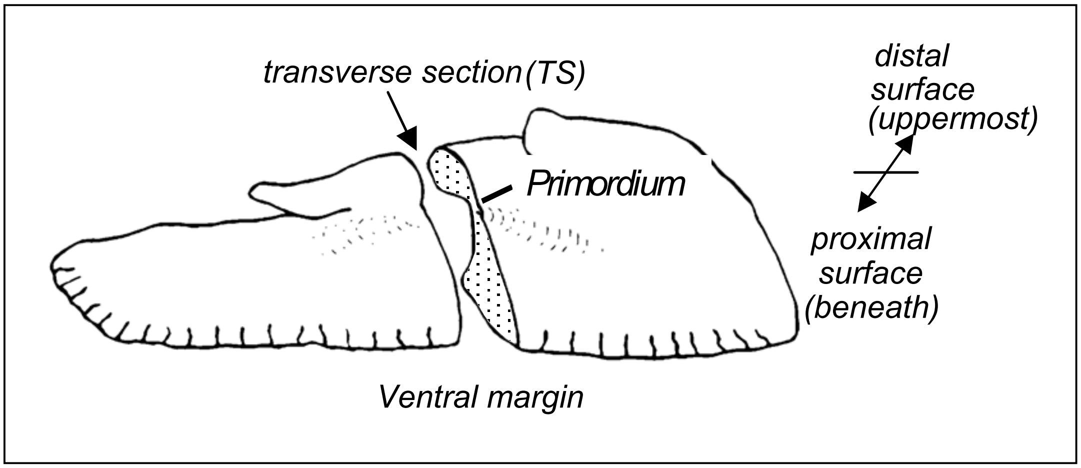


Figure 3. a) Sagittal otoliths were sectioned along the transverse axis, producing a 3.5-mm thick cross-section that contained the primordium. b) Age estimates were made by counting increments along the longer arm of the cross-section, from primordium to margin. Scale bar: 1mm.

a)



b)

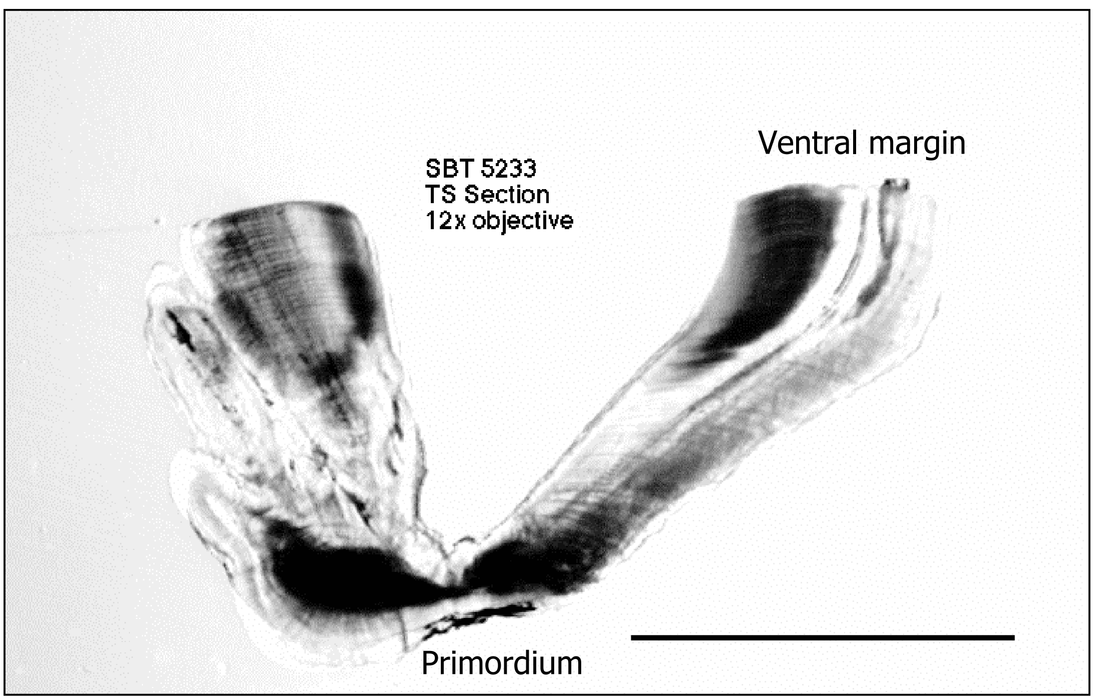
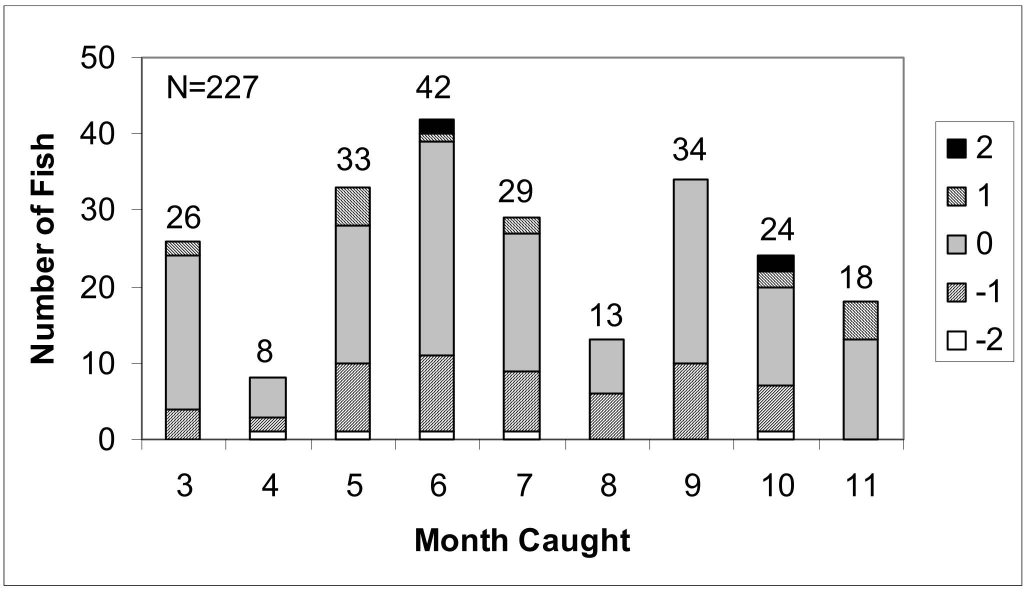


Figure 4. Barplots of the difference in whole versus sectioned readings made from sister otoliths broken down by month. a) Each bar shows the number of otolith pairs from that month for which the whole reading minus the sectioned reading was 2, 1, 0, –1, and –2 respectively. b) Same as a) but showing percents instead of absolute numbers.

a)



b)

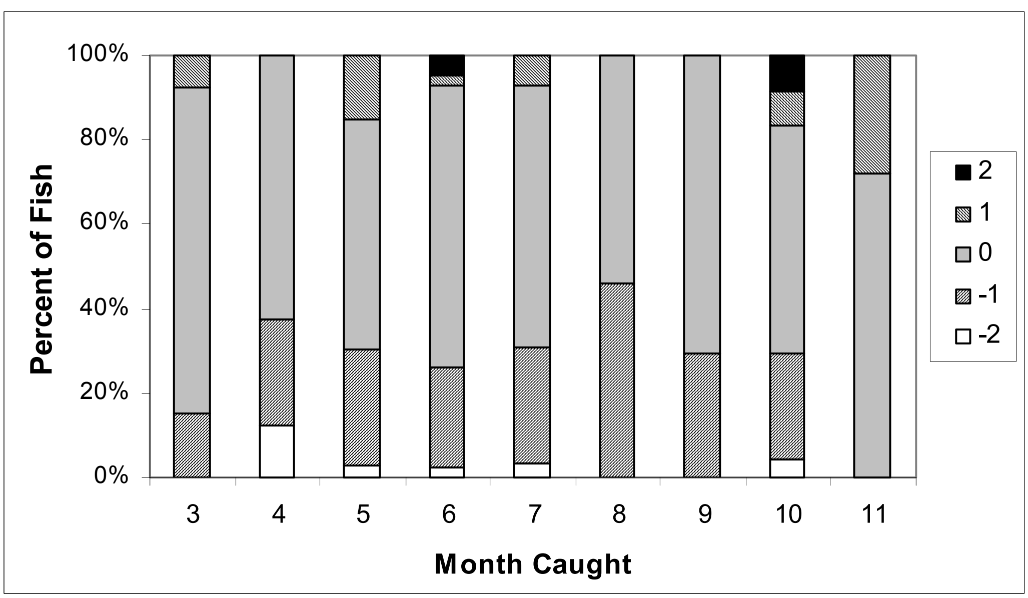


Figure 5. Fork length of fish versus final otolith reading, for both sectioned and whole otoliths.

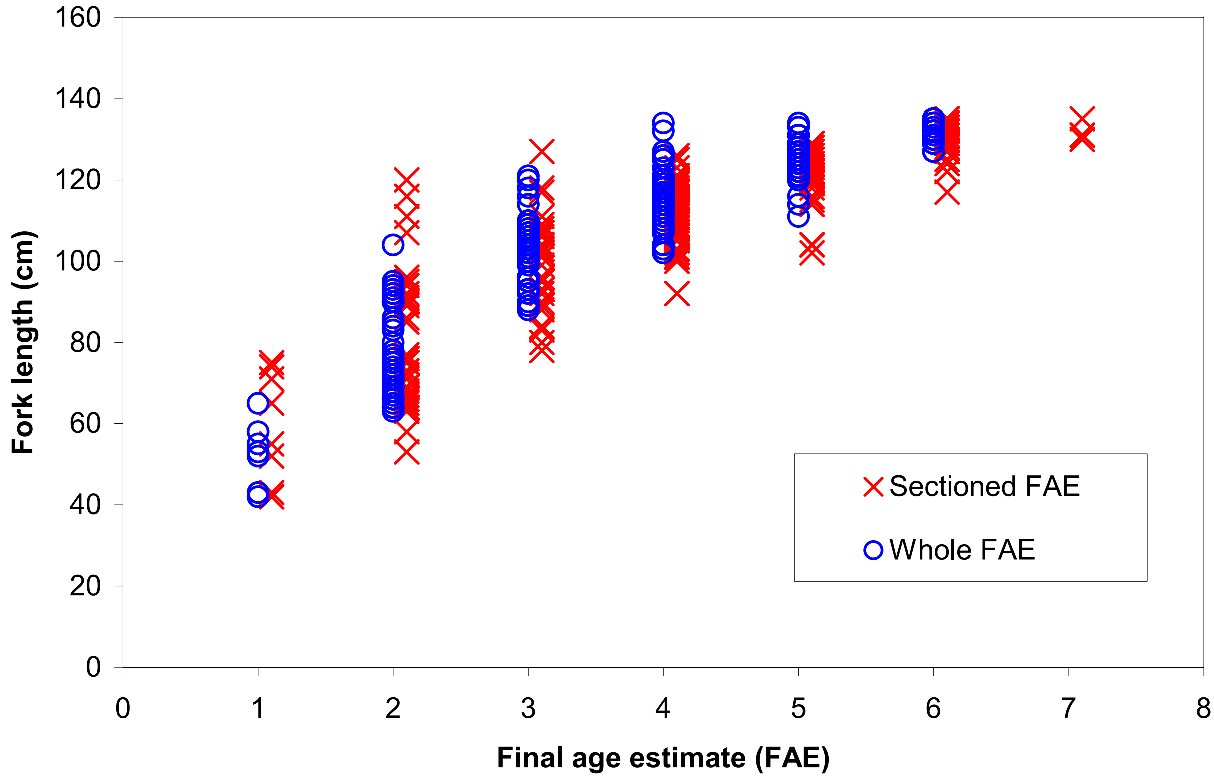


Figure 6. Barplot of the difference in whole versus sectioned readings made from sister otoliths broken down by 10-cm length classes. The x-axis label gives the start of the length class. Each bar shows the number of otolith pairs coming from fish in that length-class for which the whole reading minus the sectioned reading was 2, 1, 0, –1, and –2 respectively.