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An Assessment of Age Determination Methods, with Age Validation of Greenland Halibut from the Northwest Atlantic

by

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Abstract

Concern with the accuracy and precision of the current Greenland halibut (*Reinhartius hippoglossoides*) age determination method prompted us to examine two age validation methods, chemical marking with oxytetracycline (OTC) and bomb radiocarbon dating (^{14}C released during atmospheric testing of nuclear bombs in the 1960s). In addition we analyzed growth of tag-recaptured fish and the precision of three age determination methods, left whole otoliths, otolith sections and scales. Our growth model for tag-recaptured data suggests a growth rate of approximately 2-3 cm/yr for fish in the size range of 55-70 cm. Age bias plots comparing the age interpretations among structures showed that whole otolith age and otolith section age tended to be similar across all ages. However, both otolith preparations underestimated scale ages in older fish, particularly after age 15. Repeated age readings indicated that ageing precision was somewhat lower for the otolith-based methods (coefficient of variation of 8.4% and 11.1% for whole otoliths and otolith sections, respectively) than for the scale ages (5.2%). Three OTC marked otoliths were examined. One of these, a 66 cm female that had been at-large for 3 years, 10 months had an annual growth rate of approximately 1.5 cm/yr. The OTC mark was visible at the edge on the whole otolith but we could not determine what should have been 3 annuli within the new growth area. We were able to make out what we presumed to be three annuli on the otolith cross-section. However, in some areas of the section it was not as distinct as in others and the subsequent interpretation of annuli prior to the mark was difficult. The ^{14}C based age values of mature otoliths indicate that the ages for all but one of these samples were beyond the age determined by either the whole otolith method or the otolith section (the maximum observed age from whole and section ages was 20 years and from ^{14}C it was 33 years). While the section ages were somewhat closer to the minimum age determined by the ^{14}C we were not always able to match the assumed true age based on the ^{14}C. Comparable scale ages were not available.

Introduction

Age determination for Greenland halibut (*Reinhartius hippoglossoides*) in the Northwest Atlantic has primarily been conducted using whole otolith methods although scales are used by some labs. Igashov (2004) states that the best structure to age Greenland halibut are scales, citing a study of age determination structures done by Milinsky (1944), and that scale ages and whole otolith ages are comparable. Smidt (1969) commented that Milinsky (1944) had been successful at using scales to age Greenland halibut from the Barents Sea but he himself had not had any success using scales and chose to use whole otoliths instead. Lear and Pitt (1975) also chose whole otoliths, commenting on the difficulty in determining age from scales.

Smidt (1969), Lear and Pitt (1975) and Bowering and Nedreas (2001) reported age validation results for Greenland halibut using whole otoliths and the Peterson length frequency method. This method, while useful for the youngest ages, cannot be extended to validate the oldest ages.

Observation of systematic differences between age readings made with different methods prompted the International Council for the Exploration of the Seas (ICES) and the Northwest Atlantic Fisheries Organization (NAFO) to organize a workshop to examine methods, held at Reykjavik, Iceland in 1996. An exchange of otolith samples from five labs, carried out prior to the workshop, showed percent agreement was low varying from 30% to 50%. Pair-wise comparisons revealed biases in many cases over either a portion of or the entire age range (Bech, 1996; ICES, 1997). After reviewing several methods the workshop participants could not recommend one method over another although many felt that baking the otoliths did increase resolution between translucent and opaque zones. They went on to recommend further study of this method as well as validation techniques for older ages (ICES, 1997).

If we are to have confidence in age based assessment models applied to Greenland halibut stocks then it is important to address age method validation and to investigate methods that could help to improve precision within and between labs. In this paper we will examine a number of different methods for ageing Greenland halibut from the Labrador Sea (NAFO Div. 0B, 1C, 1E, 1F, 2G and 2J) and Cumberland Sound (Fig. 1), including scales, whole otoliths and sectioned otoliths. We used a variety of age validation methods for assessing the accuracy of these methods: 1) Bomb radiocarbon (^{14}C released during atmospheric testing of nuclear bombs in the 1960s) was incorporated and retained in the otoliths of fish born during that period, creating a dated chemical tag on the otolith (Kalish 1993; Campana 1997). Radiocarbon assays of otolith cores from fish collected in the 1970s to 1990s could thus be assigned to pre-bomb and post-bomb periods; 2) Otoliths that were marked with oxytetracycline in a tag-recapture program conducted in Cumberland Sound were examined to assess otolith growth and validate annulus formation in mature fish; 3) Finally, the growth of tag-recaptured fish from west Greenland was analyzed using the GROTAG model developed by Francis (1988). Based on these age validation results, we comment on the accuracy of all of the ageing methods examined and make recommendations for future work.

Methods

Annulus Validation in Oxytetracycline Marked Fish

A tagging project was conducted by Fisheries and Oceans Canada in Cumberland Sound from 1997 to 2000. The antibiotic oxytetracycline (OTC) was injected into the fish at the time of tagging in order to introduce a chemical mark. OTC is known to be taken up and bound to calcified structures shortly after injection and is visible under ultraviolet light (MacFarlane and Beamish 1987). The number of growth increments distal to the OTC mark in recaptured fish is then compared with the time at liberty to determine if the growth increments are formed annually, and thus are valid age indicators.

A 200 mg/ml solution of Oxyvet ® 200 LA (commercial OTC solution) was injected into the intraperitoneal cavity immediately after the fish was measured and tagged with a plastic Floy tag. A dosage rate of 50 mg OTC/kg was chosen based on information provided by McFarlane and Beamish (1987) and Babaluk and Craig (1990); fish weight was approximated using a weight-length relationship for Greenland halibut from the Cumberland Sound fishery. Preliminary tests done in 1998 on 6 fish that were tagged, marked with OTC at the above-mentioned dosage and placed in a holding cage for 2 days confirmed that the OTC was incorporated into the otolith (John Babaluk pers. comm.).

To date, 14 fish have been recaptured. Otoliths were recovered from 4 of these fish, three of which had been marked with OTC. The whole otoliths were photographed under reflected light and transmitted light as well as ultraviolet reflected light. A whole age was determined using the method described later.

The left otolith was then embedded in epoxy resin and a series of thin sections (0.35 mm) were cut through the otolith. These sections were placed on microscope slides and viewed under UV light using a compound microscope. Photos were taken under UV light as well as reflected and transmitted light.

Age Structure Comparison

Scales and otoliths were collected from Greenland halibut during a research survey in northern Baffin Bay (NAFO Div. 0A) during September 2004. The scales were taken from the dorsal side in an area just anterior of the mid-line of the body between the dorsal fin and the lateral line (Igashov, 2004). Eighty-one samples selected for comparative analysis were evenly distributed between sexes (40 males, 41 females) and across the available size range (20 cm - 82 cm for females and 24 cm to 66 cm for males) with a target of 2 samples per 3 cm length group.

A single age reader conducted three independent age determinations on each of three different ageing structures: whole left otoliths, scales and left otolith sections. The whole otolith age determination method used was the same as that described under the section on validation. Once the three whole age readings were completed the otoliths were embedded in epoxy resin and sectioned through the core as described earlier. The sections were polished and then viewed in water using a dissecting microscope (30x-40x magnification; reflected light). Annuli were usually read on the left slope of the central "dome" (Fig. 14 and 15).

Scales were read in water under a dissecting microscope outfitted with circular polarizing filters (20x-30x magnification; transmitted light). A pattern of alternating dark and light bands was visible under polarized light. The typical compression of *circuli* to form annuli does not appear to be present in this species. A single pair of dark and light bands was considered an annulus.

Age bias and length at age plots were prepared using the first set of age readings for each structure. Bias due to the ageing method was evaluated using age bias plots (Campana, 2001). The precision of the age readings associated with each structure was calculated with the coefficient of variation (Chang *et al*., 1982).

Growth Analysis using Tag-Recapture Length Data

The Greenland Institute of Natural Resources (GINR) conducted a Greenland halibut tagging program from 1986 to 1998 (Boje, 2001). A total of 7,244 Greenland halibut were tagged within the fjords along the west and east Greenland coast and offshore areas of Baffin Bay and Davis Strait. Of the 517 recaptured, 137 had associated length data and date of recapture information suitable for growth analysis. Information on the sex of recaptured fish was not available so a comparison of female and male growth rates was not possible. We also included 6 samples from a tagging study conducted by Fisheries and Oceans Canada in Cumberland Sound (Treble, 2003) for a total of 143.

Francis (1988) developed a model using maximum likelihood estimation which analyzes changes in length over time (growth) collected from tagging data (GROTAG). An Excel-based application of Francis' GROTAG model developed by Simpfendorfer (2000) was used to analyze our data.

The full dataset and a subset of the data (time at large >0.9 years) was also analyzed using a Gulland and Holt (1959) model, with annual growth rates plotted against mean length ((length at tagging-length at recapture)/2).

Carbon-14 Age Validation

Development of the Reference Curve

Although the timing of the appearance of bomb radiocarbon in surface marine waters around the world is well established (Campana, 2001), Greenland halibut may live at depths where the appearance of the bomb signal was delayed. Therefore, a ^{14}C reference chronology unique to Greenland halibut was developed using approximately 36 otolith pairs of young (age 0-3) Greenland halibut born between 1955 and 1997 selected from collections archived at DFO's Northwest Atlantic Fisheries Laboratory in St. John's, Canada (Div. 0A, 0B, 2G and 1C) and the Greenland Institute of Natural Resources in Nuuk, Greenland (1A inshore, 1E, 1F and 2J). These samples were effectively known-age (± 1 yr), since the lengths of such young fish are relatively accurate indicators of age. The left and right otoliths were combined to form a single sample so as to bring total sample weight to at least 3 mg. All otolith material was then decontaminated, stored in acid-washed glass vials, and submitted for ^{14}C assay by accelerator mass spectrometry (AMS) (described in Campana, 2001). AMS assays also provided δ^{13}C values, which were used to correct for isotopic fractionation effects. Radiocarbon values were subsequently reported as Δ^{14}C, which is the per mil ($^{0}/_{00}$) deviation of the sample from the radiocarbon concentration of 19th-century wood, corrected for sample decay prior to 1950 according to methods outlined by Stuiver and Polach (1977).

To extend the reference chronology to the years before 1959, otolith cores from Greenland halibut aged 10 years or older captured in the early 1960's in Div. 1F were also assayed for ^{14}C. Although these samples were not extracted from fish of known age, the fact that pre-bomb (pre-1958) radiocarbon levels are relatively low and stable within a given region indicates that these samples should provide reliable pre-bomb radiocarbon values even if the age assignments of the fish were incorrect. The methods used for core extraction are described below.

^{14}C values for two samples analyzed from Div. 0A, collected in 1978, fell well below the other values. We had only a single survey in this area during the period of interest we chose not to include them in the reference curve.

Radiocarbon Age Validation

Twenty pairs of otoliths, from 5 males, 11 females and 4 sex unknown, were selected for age validation from archived material collected from research surveys carried out in Davis Strait (0B, 1C), Northern Labrador (2G) and West Greenland (1E) between 1967-1989. These were in addition to samples of mature fish that had been analyzed previously as part of our assessment of the suitability of the ^{14}C technique for Greenland halibut and the development of the reference curve (4 from Cumberland Sound, sex unknown; and 4 females from 2G). Fish presumed to be 13-20 yr old, which may have hatched in the 1950s and 1960s, were selected since these are the year classes most suited to bomb radiocarbon dating. Otolith cores with pre-bomb levels of radiocarbon (as indicated by the reference chronology) must have been born before 1958, since post-bomb radiocarbon levels are always higher. Therefore, comparison of the radiocarbon levels of the validation otolith cores with the reference chronology allowed a minimum age for the fish to be determined.

Whole ages were determined for both the left and right otoliths (if both otoliths were in good condition) prior to embedding them in epoxy resin. The whole otolith was viewed in water under reflected light using a dissecting microscope under 10x magnification. The otolith core was identified and subsequent annuli (a pair of opaque and translucent bands) were counted to determine the fishes age. For thicker (older) otoliths reflected light was used initially but transmitted light was used subsequently to help determine outer annuli.

Thin sections (1.0-1.5 mm thick) of each otolith were prepared with a low-speed, diamond-bladed saw by sectioning transversely through the core. After polishing lightly to improve clarity, digital images of each section were taken and enhanced using Adobe Photoshop. No other treatments were applied to the sections. Ages were determined along three transects ("dome", left "arm" and right "arm") (Fig. 2b). The radii of the first three presumed annuli were confirmed through measurements of the dimensions of intact sagittae collected from Age 0-3 individuals.

To isolate otolith material for bomb radiocarbon assay, otolith cores corresponding to the first three years of growth were extracted from each thin section. Cores were isolated with a Merchantek computer-controlled micromilling machine using steel cutting bits and burrs. Since individual core weights were insufficient for assay (3 mg minimum), cores were isolated from each otolith of the pair and pooled. All core material was then decontaminated and submitted for ^{14}C assay by AMS as described earlier.

To assign a minimum fish age to the validation sample, the ^{14}C value for each sample was compared to the reference chronology to determine the most plausible range of year-classes. Confidence limits for each sample were assigned based on year-classes in the reference chronology whose radiocarbon level lay within the polygon describing the inner- and outer-most LOESS curves.

Results

Examination of Growth in Oxytetracycline Marked Fish

Three Greenland halibut marked with OTC in April 1999 were recaptured during the winter fishery in March 2001, April 2002 and March 2003 (Table 1). Two of these fish had grown very little, less than 1 cm in 2 to 3 years. The other had grown 6 cm in almost 4 years for an annual growth rate of approximately 1.5 cm/year.

The OTC was visible on the surface of the first two fish's otoliths, fluorescing green under UV light (Fig. 3). In the third fish, the otolith surface had been covered by new growth but we could see the OTC marked material as a band along the outer edge in certain parts of the left otolith, e.g. the rostrum on the proximal side and the "finger" area on the distal side (Fig. 4). While bands could be seen when the whole left otolith was viewed under reflected light, the material at the edge was translucent and 3 annuli were not visible. Using transmitted light you could clearly see more light and dark banding but again 3 annuli were not visible within the area of new growth at the edge.

The fluorescent OTC mark could be seen on cross-sections taken through the left otolith of all three recaptured Greenland halibut, although in some areas a higher magnification was required (Fig. 5 and 6). The sections show that these otoliths grew slightly more in thickness ("dome" area) than they did at the edges. Using higher magnification and reflected light it was possible to make out presumed annuli in numbers that correspond to the number of years since marking. However, in some areas of the section it was not as distinct as in others, and additional growth bands (that might be interpreted as annuli) could be observed under different focal lengths (Fig. 11).

Age Structure Comparison

Age structures from small (20 cm female), medium (36 cm male) and large (61 cm female) Greenland halibut were selected to illustrate the appearance of annuli on the three structures (Fig. 7 and 8). Age bias plots comparing the age interpretations among structures showed that whole otolith age and otolith section age tended to be similar across all ages. However, both otolith preparations underestimated scale ages in older fish, particularly after age 15 (Fig. 9). Repeated age readings indicated that ageing precision was somewhat lower for the otolith-based methods (coefficient of variation (CV) of 8.4% and 11.1% for whole otoliths and otolith sections, respectively) than for the scale ages (CV of 5.2%).

The maximum age determined for whole otoliths, otolith sections and scales was, 21, 22 and 28 respectively (Fig. 10). Given that the ageing samples included relatively large Greenland halibut, these ages may represent something close to the longevity of the species in this area.

Growth Analysis using Tag-Recapture Length Data

Time at large for tag-recaptured fish varied from 0.08 yrs to 7.17 yrs, with length at recapture ranging from 44 to 87 cm. Average growth rate plotted against average length for both the full data set and the subset for those at large longer than 0.9 years are shown in Fig. 11.

Although the GROTAG model is designed to properly deal with measurement or sampling error, we felt that a decrease in growth beyond the 5 cm level was an unrealistic level of error; therefore 18 samples were removed from the analysis leaving 125 fish. Reference lengths of 55 cm and 70 cm were chosen for estimation as the majority of the data fell between these lengths. The full model (#3, Table 2) was determined to be the best fit for the data since λ decreased by 10.95 with the addition of the two additional parameters (m and p). Growth variability (v) was low (0.46) but not close to 0 and the standard error for measurement error (s) was not too high at 2.77. Therefore, we concluded that the model can differentiate between growth variability and measurement error (Francis, 1988).

The estimated growth rate of 3.01 cm/yr for 70 cm fish was slightly greater than the 2.86 cm/yr estimated for 55 cm fish. Both estimates of growth rate were consistent with the empirical analysis suggested by the regression of growth rate on average length (Fig. 11).

^{14}C Validation

The Δ ^{14}C reference chronology for otoliths of known age 0-3 year old Greenland halibut is shown in Fig. 12. Compared to the reference chronology characteristic of the NW Atlantic off of Nova Scotia (Fig. 12), the Greenland halibut chronology is somewhat depleted in the years after 1970, presumably reflecting greater water mixing. However, the period of bomb radiocarbon increase is similar in both chronologies, albeit with a possible slight delay in the Greenland halibut chronology due to a lag in the penetration of the bomb signal to the 200+ m depths of the halibut. Some of the variability in the Greenland halibut reference chronology was associated with spatial variability, whereby more northerly samples were depleted compared to more southerly samples.

The period of increasing radiocarbon values (1958-1970) in the Greenland halibut reference curve results in a relatively narrow range of $\Delta^{14}C$ values (-10 to -40) that can be used for validation purposes. Within this time period, otolith cores could be assigned to a year-class relatively accurately, since high (> -10) and low (< -40) $\Delta^{14}C$ values could only be found in post-bomb (after 1970) and pre-bomb (before 1958) year-classes, respectively. Based on this range of suitable $\Delta^{14}C$ values, a core year-class could be determined for 11of the mature Greenland halibut samples tested for age validation. A conservative "minimum" core year class (minimum age) for these samples were calculated based on possible year-classes representative of the core radiocarbon assay (Table 3, Figure 13). Of the 11 samples used for validation, 7 had been assigned a whole age (6 for the left otolith and 7 for the right) and a section age while 4 had only a section age.

Table 3 and the age bias plots (Fig. 14) indicate that the minimum core age determined from ^{14}C overestimated the whole otolith age by a range 3 to 17 years. Minimum core ages overestimated the section age by a range of 1 to 15 years. Since minimum core ages represent the minimum possible ages consistent with the radiocarbon data, these results indicate that the age readings of the whole otolith and sections underestimated the actual age of these fish. Age underestimation was most pronounced in the oldest fish. The maximum observed age from whole and section ages of this subset of otoliths was 20 years, while the maximum ^{14}C age was 33 years (Table 3).

A plot of length *versus* minimum core age from ^{14}C is shown in Figure 15. It shows a sharp increase in length in the first three years continuing to an asymptote varying between 70 cm and 80 cm beyond approximately age 10-15 years. Since this figure is based on minimum possible age, it is possible that growth rate is slower than depicted here.

Discussion

The left whole otolith has traditionally been used to age Greenland halibut, a particular region of which is preferred over other areas, primarily because it is readable on a more consistent basis. Other areas can become thick and opaque, particularly in older fish and it is not possible to see the annuli (e.g. Fig. 3b). However, our results suggest that the whole otolith method is likely underaging Greenland halibut, particularly larger mature fish.

The OTC mark was visible in certain fast growing areas of the whole otolith and throughout the cross-section. However, for the whole otolith it was difficult to see the mark in the traditional age reading zone (marked by the grid applied to the first image in Fig. 9a) without increased magnification. As a result we believe that it is very likely that the third re-captured Greenland halibut (66 cm female) would have been under-aged by at least 4 years using the typical whole age determination method.

We found that while we could identify annuli beyond the OTC mark in the section this did not necessarily help us to determine annuli prior to the mark, particularly in the thinner portion of the otolith (i.e. "arms") where there appears to be false annuli or splitting of annuli. The area to the left of the "dome" was better and is the area used for age determination in the comparative analysis portion of our research. Further study into staining or photo enhancement techniques may be helpful in improving the section method.

We found that the pattern of annuli in Greenland halibut scales becomes apparent when viewed under polarized transmitted light. It is not known if this was the method used by Milinsky (1944) but Savvatimsky and Gorchinsky (1998) state that polarized transmitted light was used in the age determination of *Macrourus berglax* using scales so although it may not be a common method it is not a new method for scale age determination.

The tag-recapture analysis indicated the growth rate that should be expected of mature Greenland halibut. The growth rate estimated by the GROTAG model indicates that growth is fairly uniform in mature halibut: in the order of 3 cm/yr. This estimate is similar to, but more robust than the 2 cm/yr estimated by the regression line in the Gulland and Holt analysis. This growth rate is also comparable with that observed in Fig. 10 based on length at age. However, the relationship between annual growth rate and mean length is almost flat, so the Gulland and Holt (1959) model estimates for K and L_∞ could not be calculated.

For the tag-recapture analysis of growth we had expected to see a decline in growth rate with increasing size. However, the similarity in growth predicted by the model for the two sizes we tested may be a result of the relatively narrow length range available to us. The smaller/younger and faster growing portion of the population was not represented. Also, males typically reach a maximum size of 60-70 cm while females grow larger, reaching a size of approx. 90-100 cm (Bowering and Nedreaas, 2001). We might expect the growth rate for a male that has reached maturity and is near the maximum size at 55 cm to be different from that of a female at 55 cm. Bowering and Nedreaas (2001) found that Greenland halibut from NAFO Div. 2J and 3K grew faster than males although the differences were small. Unfortunately, sex information was not available for the tag-recapture data we examined so our analysis could not be done separately by sex.

A growth rate of approximately 2 cm/yr appears to be similar to that observed for 50 cm to 70 cm fish for the whole otolith age method in our methods comparison research (Fig. 10). The rate for the other two methods appear similar but empirical estimates of growth rate using these data has not been calculated.

The ^{14}C based age values of mature otoliths indicate that the ages for all but one of these samples were beyond the age determined by either the whole otolith method or the otolith section. While the section ages were somewhat closer to the minimum age determined by the ^{14}C we were not always able to match the assumed true age based on the ^{14}C. Comparable scale ages were not available. However, the ^{14}C results support the other results presented above and lead us to the following conclusions and recommendations:

1) The whole otolith method underestimates the true age for Greenland halibut.

2) Growth for Greenland halibut in the size range of 55 cm to 70 cm is 2-3 cm/yr. The three OTC marked fish from Cumberland Sound showed even lower growth rates, <1-1.5 cm/yr.

3) The section method did not produce the results we expected. It was difficult to determine the annuli with confidence and ages tended to be lower than the ^{14}C based age and the scale ages (in our comparative study). However, further work with staining or image enhancement techniques may improve this method.

4) Scale ages were comparable with otolith ages for the younger fish, but produced older ages in larger fish. Scale annuli were more distinct and more precise than in either whole otoliths or otolith sections. However, validation of the scale method was not carried out, and it was not possible to determine if scale ages accurately represent age in older fish.

5) Given the cost of preparing otolith sections (both in time and materials) there is merit in considering the use of scales in the routine age determination for Greenland halibut stocks. However, the scale method of ageing needs to be validated before it can be considered for routine ageing. We recommend that scales be included in any future exchange or workshop held to study Greenland halibut age determination. In addition, further research is needed to see if our findings hold for Greenland halibut stocks in other areas.

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Table 1. Data for three fish marked with oxytetracycline during the Cumberland Sound tagging project. All three recaptures were tagged in 1999 at 65.97° N and -66.68° W and were recaptured 2 to 4 years later in the same general area.

No.	Date Tagged	Length at Tagging (mm)	Date Recaptured	Length at Recapture (cm)	Rd. Wgt. (g)	Sex	Time Since Tagging	OTC Dose (cc)
1	April 20, 1999	550	March 15, 2001	55	1550	M	1yr, 10+ months	0.41
2	April 15, 1999	635	April 4-7, 2002	64	2340	F	2 yrs, 11+ months	0.65
3	April 20, 1999	600	March 4, 2003	66	2730	F	3 yrs, 10+ months	0.54

Table 2. GROTAG Model results.

Parameter	Model 1	Model 2	Model 3
λ	-353.2714	-352.7500	-342.3175
g 55	2.927	3.404	2.857
g 70	1.775	2.139	3.013
v	0.778	0.663	0.458
s	3.00	2.989	2.771
m	0	-0.615	-1.10
p	0	0	0.026

Table 3. Results of ^{14}C assays for mature Greenland halibut otoliths selected for validation.

Year Sampled	NAFO	Length	Sex	Whole Age-Left	Whole Age-Right	Section Age-Dome	Core Year Class - ^{14}C	Min. Year Class - ^{14}C	Min. Age - ^{14}C Based	^{13}C	^{14}C
1986	0B	70		16	16	17	1965	1966	21	-3.5	-20.7
1986	0B	74		17	18	15	1965	1966	21	-5.6	-21.9
1986	0B	72		20	20	20	1960	1964	23	-3.4	-37.6
1986	0B	72		14	16	12	1962	1966	21	-3	-29.2
1987	2G c	84	2	-	-	19	1965	1966	22	-2.38	-17.4
1981	2G c	74	2	-	-	18	1957	1958	24	-1.2	-69.1
1981	2G c	75	2	-	-	15	1958	1959	23	-2.13	-51.8
1981	2G c	79	2	-	-	17	1963	1964	18	-1	-28.8
2000	0B	86	2	16	24	20	1967	1968	33	-1.89	-11.2
1986	1C	74	2	16	22	12	1959	1960	27	-2.51	-55.8
1971	1C	85		-	19	-	1958	1960	12	-2.3	-49

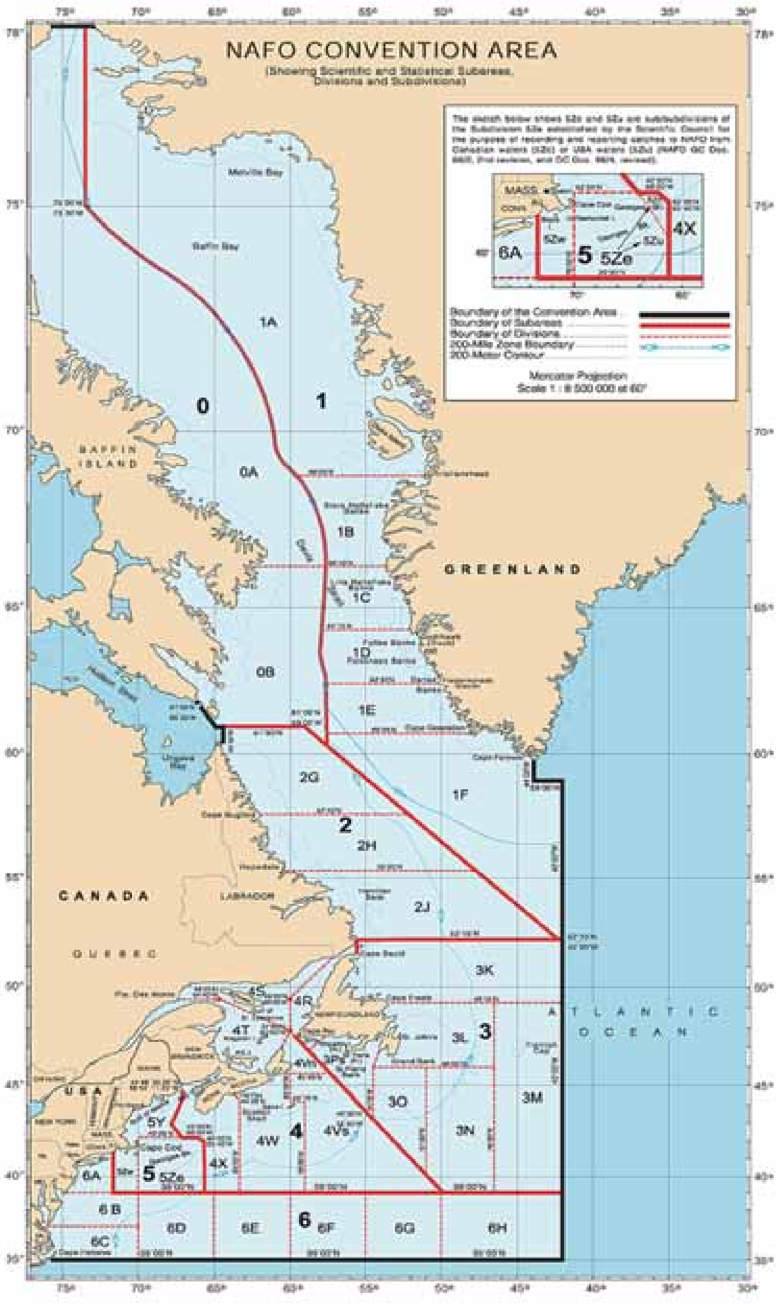


Fig. 1. Map of the Northwest Atlantic Fisheries Organization Divisions. Our samples were collected from surveys conducted in Divisions 0B, 2G, 1C, 1E, and 1F).

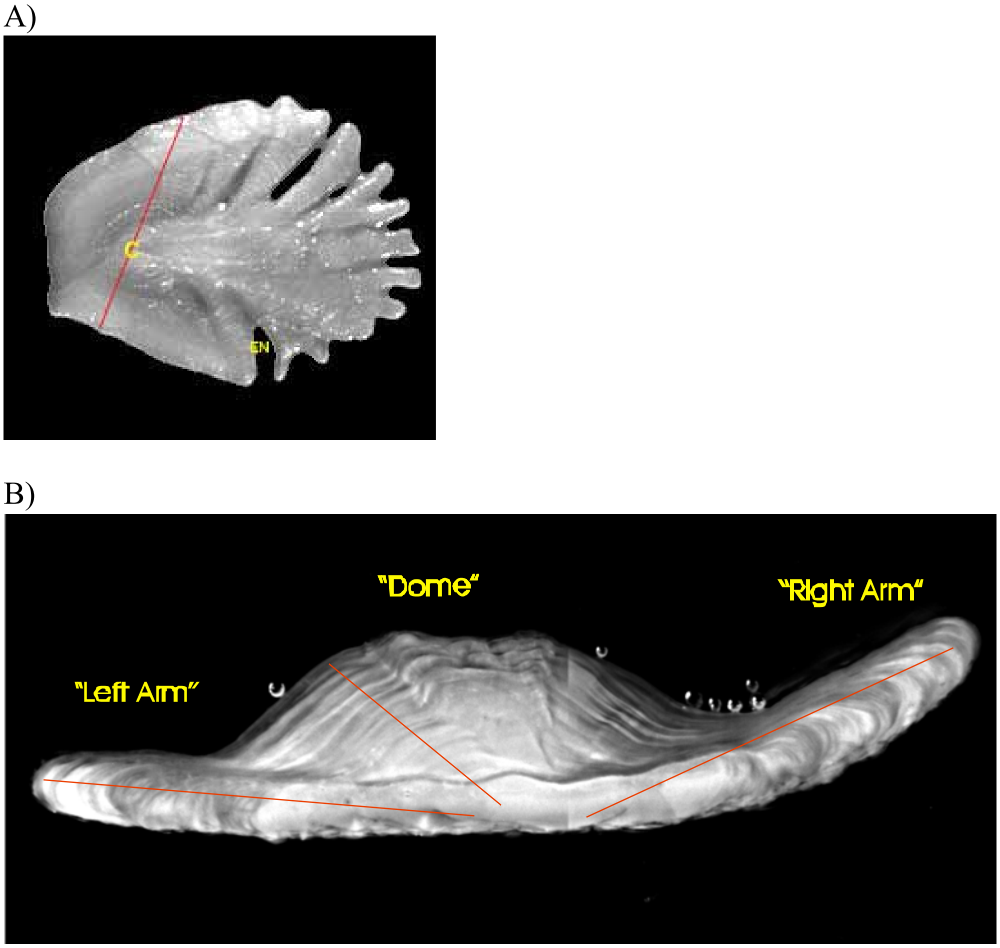


Fig. 2. a) Image of whole otolith showing section plane used in ^{14}C study (C=Core) and b) image of section showing age reading zones (left "arm", "dome" and right "arm") .

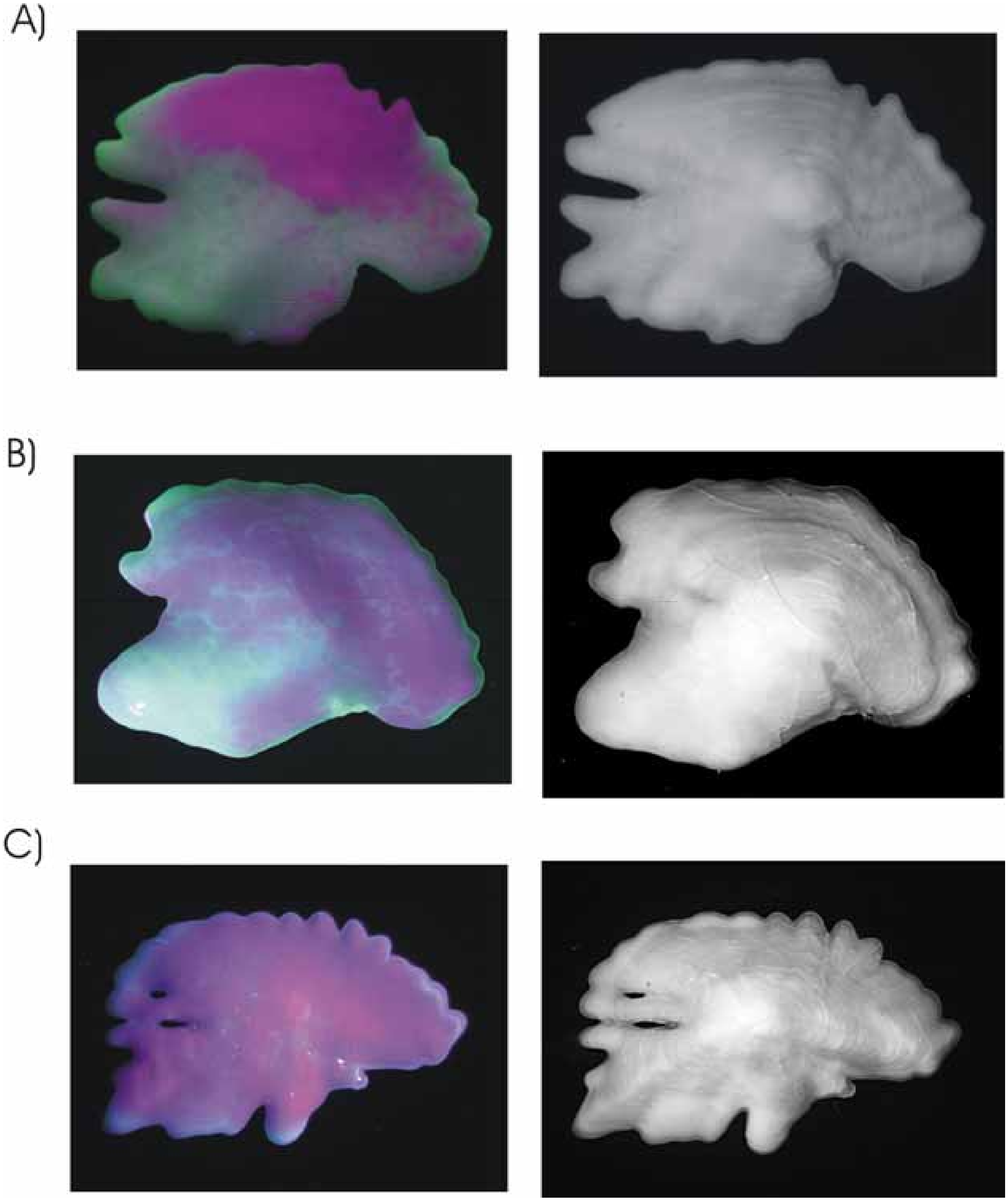


Fig. 3. Otoliths of OTC marked Greenland halibut shown under both ultraviolet and reflected light. The material that has incorporated the OTC shows up as a green to light purple and becomes less visible as time since marking increases: a) 1 year 11 months; b) 2 years 11 months; c) 3 years 10 months.

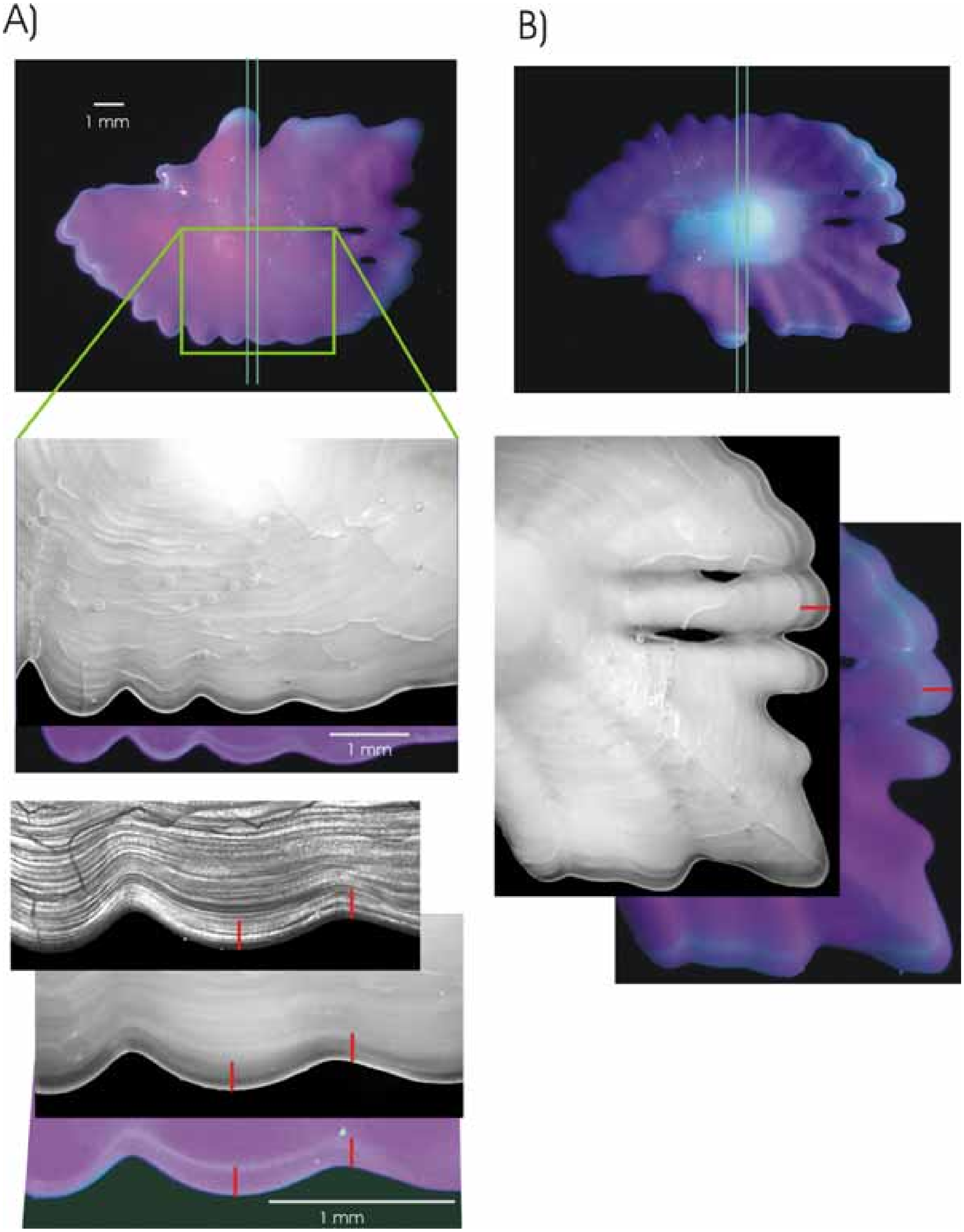


Fig. 4. The OTC mark is visible at the edge in certain areas of the proximal (A) and distal (B) sides of the otolith sample taken from a Greenland halibut we recovered 3 years 10 months after tagging. Reflected light views of the same area are shown as well as a transmitted light view of the edge in A). The sectioning plane is also indicated.

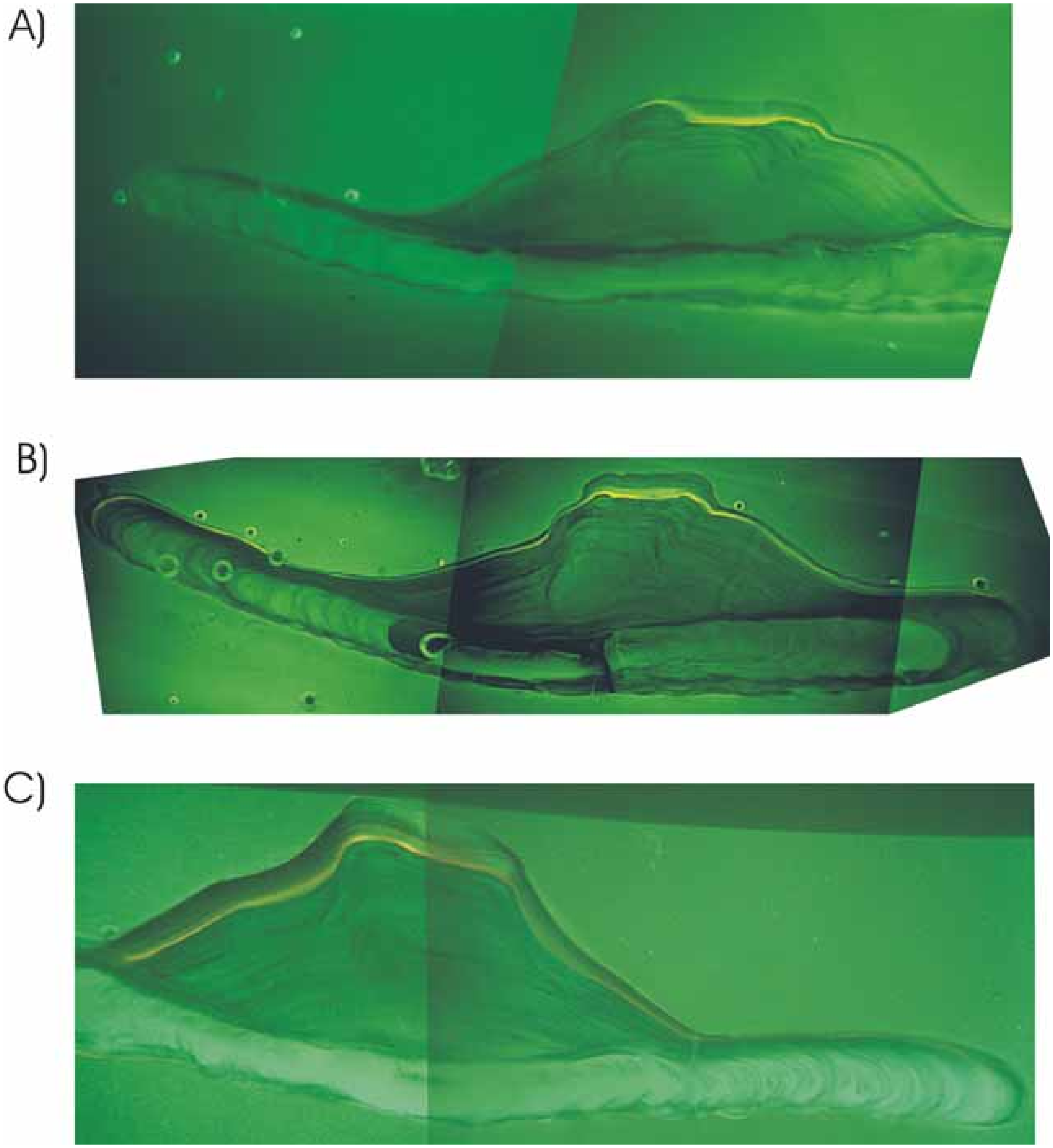


Fig. 5. Photos showing the entire otolith section under UV light for three Greenland halibut marked with OTC and recovered after: A) one year and 11 months; B) 2 years 11 months; and C) 3 years 10 months. The OTC mark shows up as a yellow band near the edge of the section.

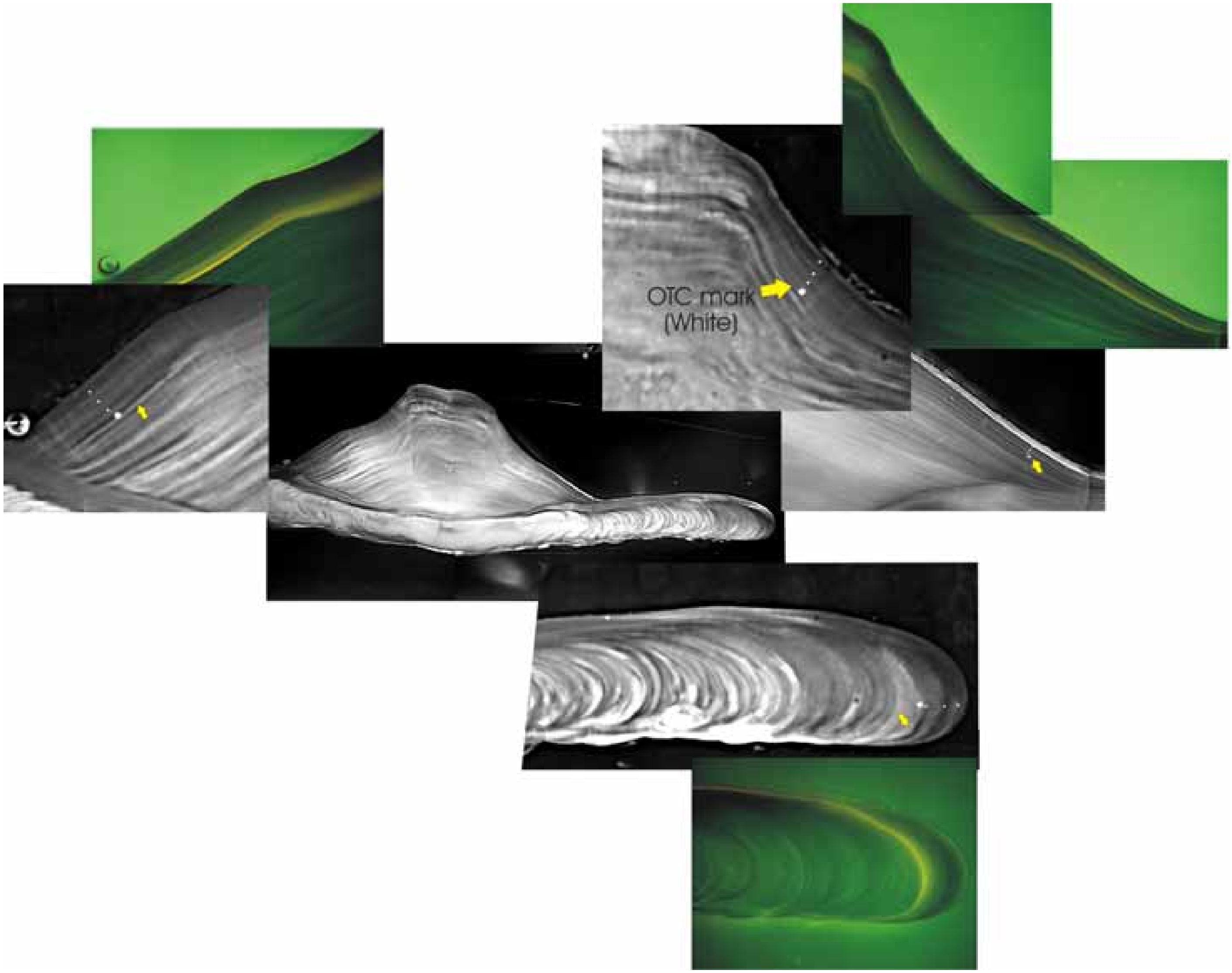


Fig. 6. Section C from Figure 10 is shown under reflected light with images taken using both reflected and ultraviolet light under increased magnification to highlight the location of the OTC mark in relation to annuli.

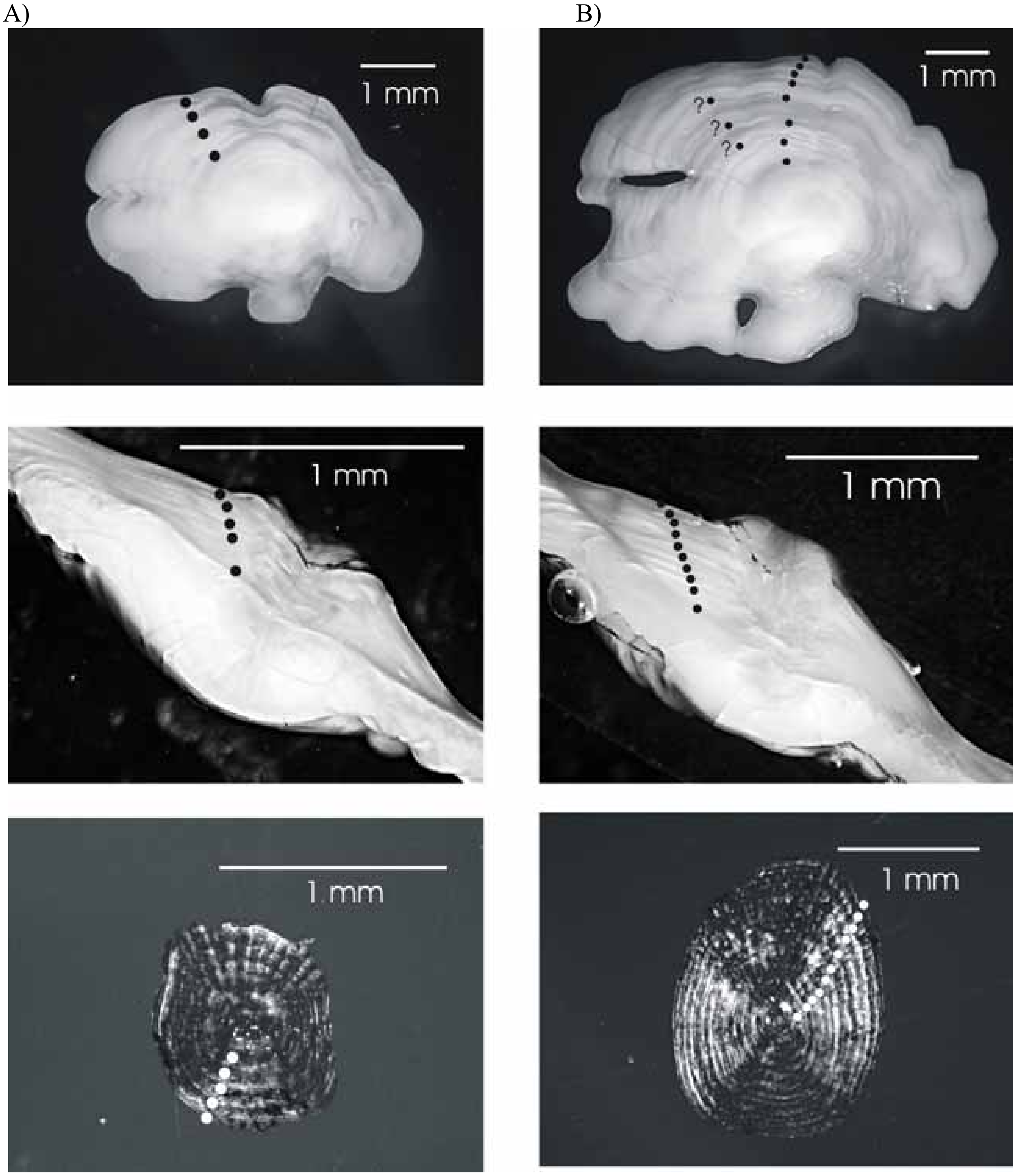


Fig. 7. Age determination structures for; a) a 20cm female (whole ages=4, 4; scale ages=5, 5 and section age=5) and b) a 36 cm male (whole ages=8, 6; scale ages=10, 11 and section age=10).

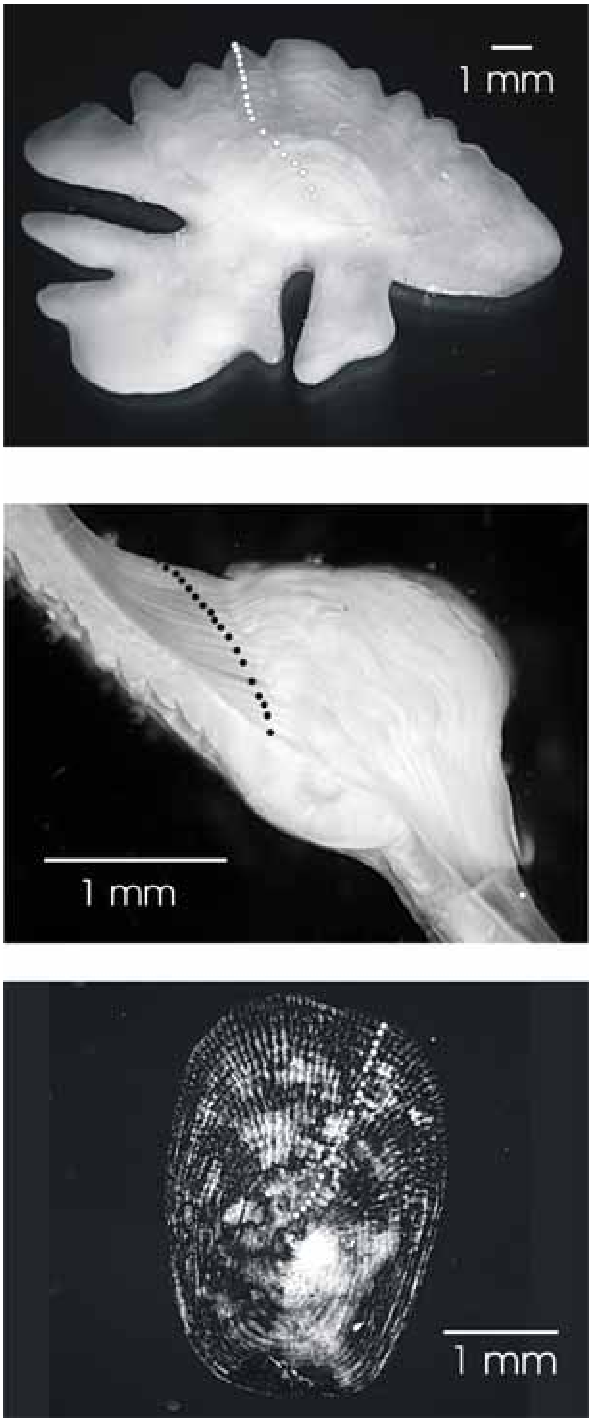


Fig. 8. Age determination structures for a 61cm female (whole ages=17, 18); scale ages=20, 21 and section age=18). The annuli are not marked on the image of the whole otolith because they were not marked at the time the age trials were conducted and it was too difficult to place them without having the original structure available for comparison.

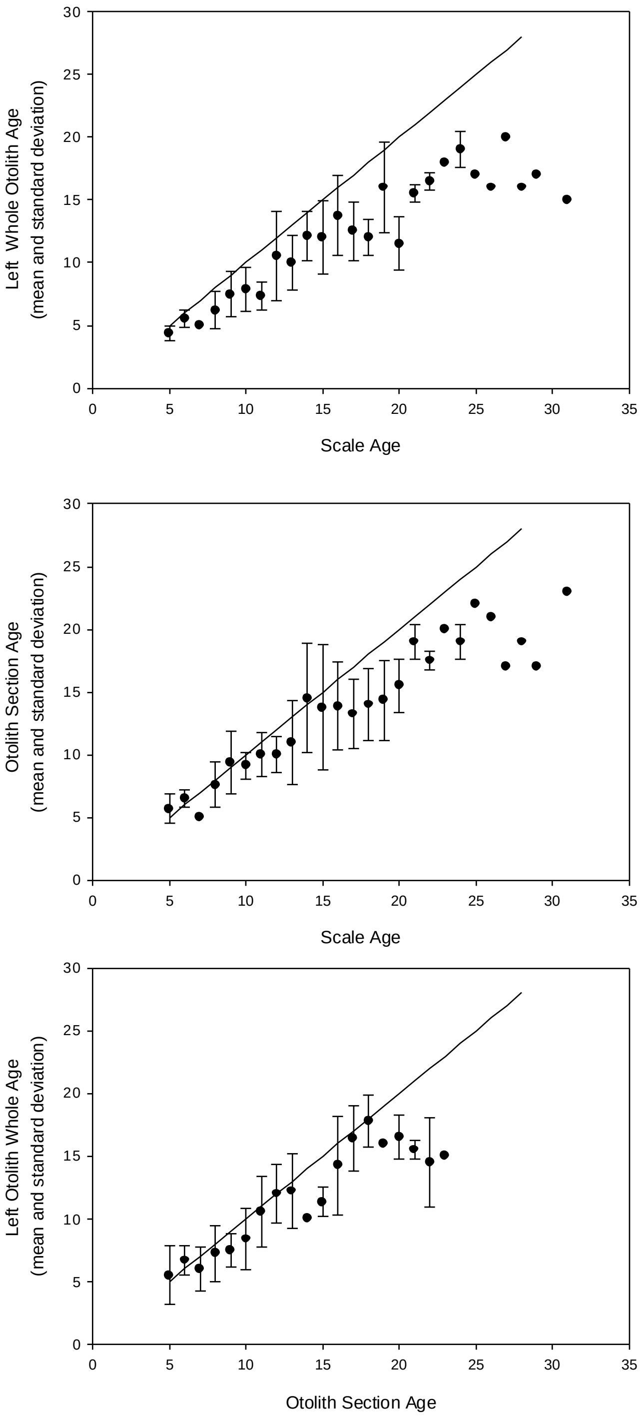
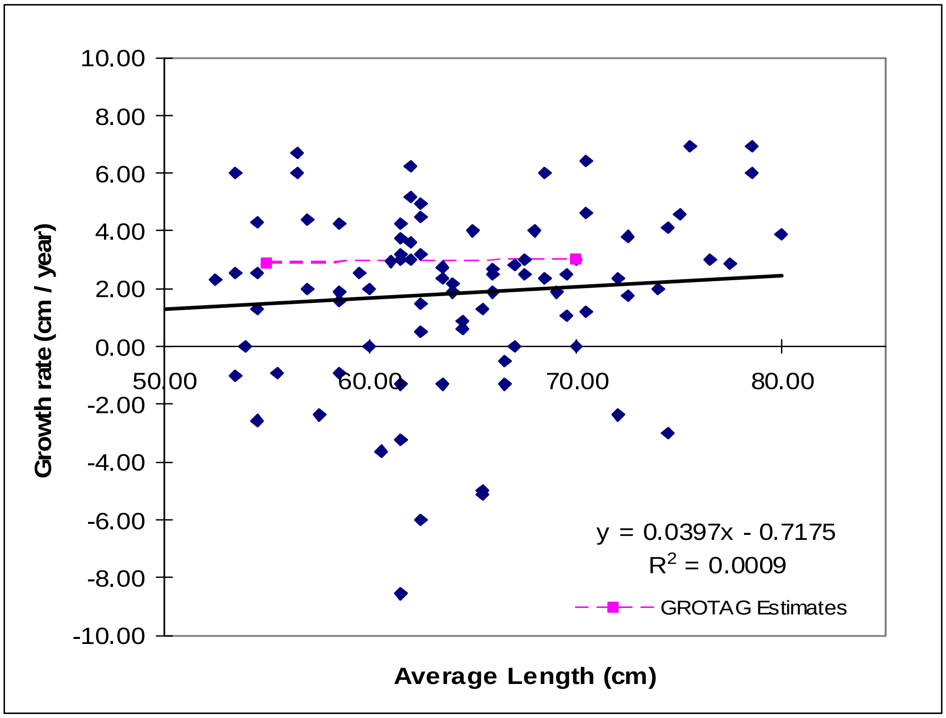


Fig. 9. Age bias plots comparing three structures (whole otoliths, otolith sections and scales) from Greenland halibut collected during a survey in NAFO Div. 0A.

Fig. 10. Age and length (cm) by sex plotted for each structure as fitted with a lowess regression .

A)



B)

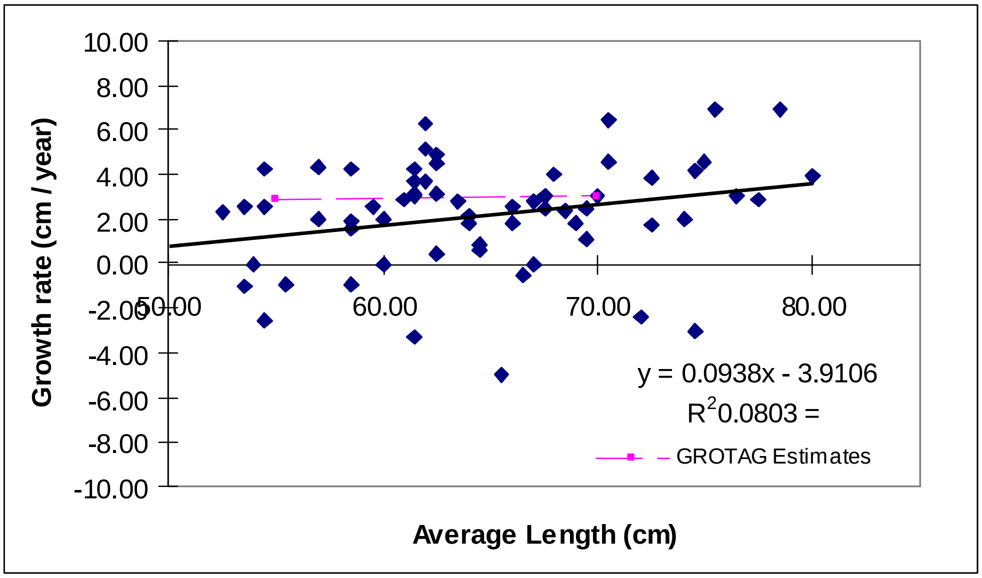


Fig. 11. Growth rate (cm/yr) and average length ((length at tagging – length at recapture)/2) for: A) all data used in the GROTAG and Gulland and Holt analysis of growth and B) data for those fish at large longer than 0.9 years. The GROTAG model estimates are also shown.

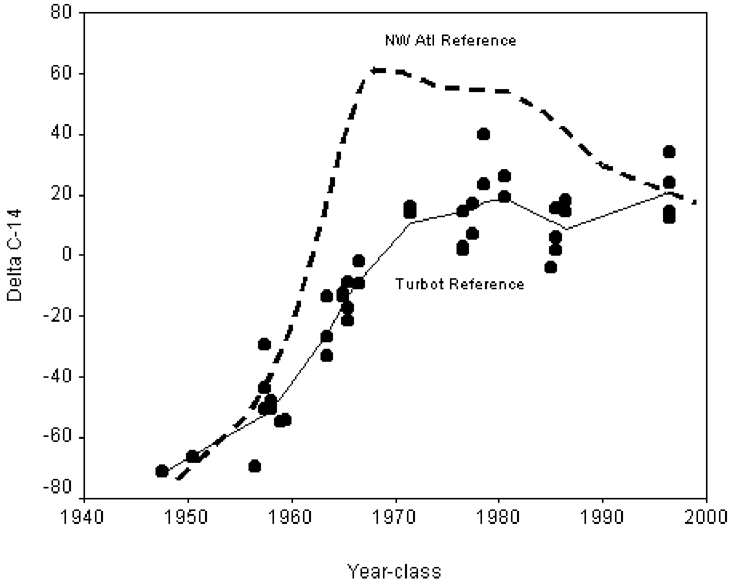


Fig. 12. Plot of Δ ^{14}C values for Greenland halibut with line fitted using a lowess regression. The reference chronology characteristic of the Northwest Atlantic (Campana et al. 2002) is also shown.

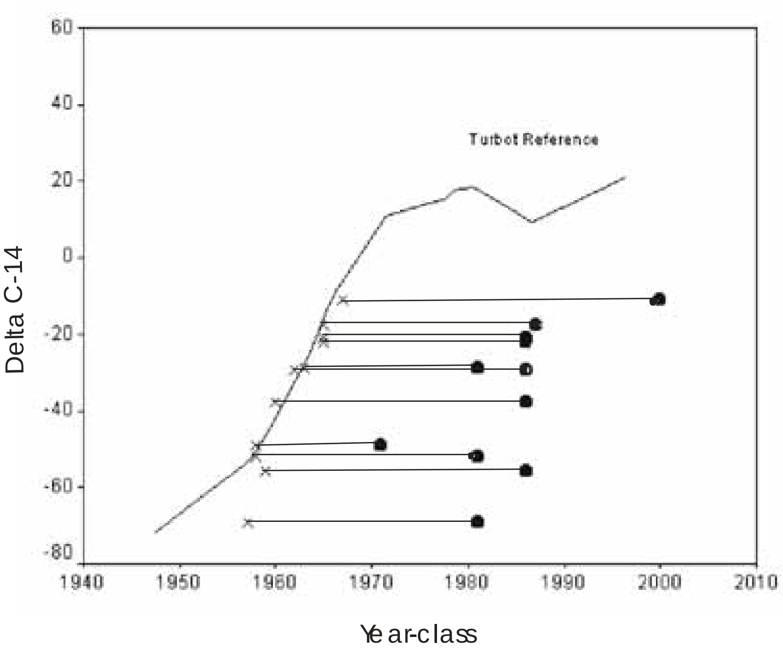


Fig. 13. Radiocarbon reference chronology for Greenland halibut in relation to 11 validation samples showing the presumed true age (x) and the year of collection (closed circle).

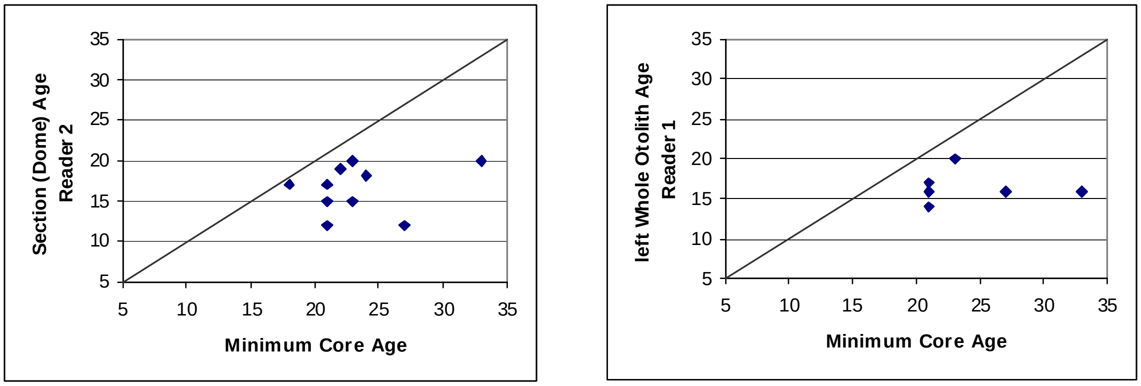


Fig. 14. Age bias plots of minimum core age from ^{14}C and a) otolith section age; b) left otolith whole age. Data found in Table 1.

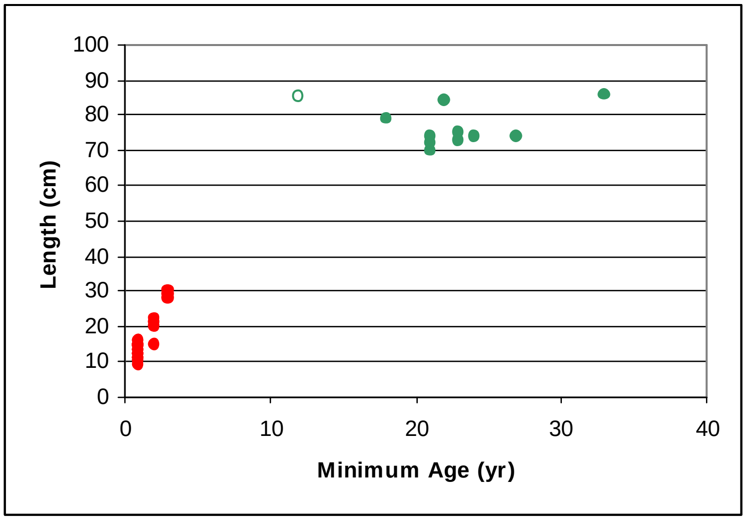


Fig. 15. Length and minimum age for samples used for reference curve (red) and validation (green). The reference sample ages are based on the whole otolith method while the ages for the validation samples are minimum ages based on the ^{14}C assay (Table 3). One of the validation samples was an 85 cm female aged at 12 years (open circle), however, this is likely an under-estimate.