

EVALUATION OF AN ATLANTIC BLUEFIN TUNA OTOLITH REFERENCE COLLECTION

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SUMMARY

This study evaluated age estimates from a bluefin tuna otolith collection for bias, precision and compared age estimates to the most recent growth curve (Restrepo et al. 2010). Overall, ageing error was low and within the acceptable APE and CV (10%) for quality control. However, all readers exhibited a consistent bias in ageing larger fish (CFL>180 cm) compared to the growth curve. The observed bias may be explained by the fitting of the growth model to age data, temporal variability in growth rate or edge type assignment. We conclude that this set of images can serve as a reference collection and thereby support routine production ageing of bluefin tuna.

RÉSUMÉ

Cette étude évaluait les estimations des âges d'une collection d'otolithes de thon rouge en vue d'identifier les biais, la précision et de comparer les estimations des âges avec la courbe de croissance la plus récente (Restrepo et al., 2010). Globalement, l'erreur de détermination de l'âge était faible et s'inscrivait dans les limites acceptables de APE et CV (10%) aux fins du contrôle de la qualité. Toutefois, tous les lecteurs ont fait apparaître un biais constant dans la détermination de l'âge des plus gros poissons (CFL>180 cm) par rapport à la courbe de croissance. Le biais observé peut s'expliquer par l'ajustement du modèle de croissance aux données sur l'âge, à la variabilité temporelle dans le taux de croissance ou à l'assignation du type de bord. Nous en concluons que ce jeu d'images peut servir de collection de référence et nous appuyons donc la détermination de l'âge du thon rouge de façon routinière.

RESUMEN

Este estudio evalúa las estimaciones de la edad a partir de una colección de otolitos de atún rojo para valorar el sesgo y la precisión, realizando una comparación de las estimaciones de la edad con la curva de crecimiento más reciente (Restrepo et al. 2010). En general, el error de determinación de la edad era bajo y se encontraba dentro del APE y el CV (10%) aceptables para el control de calidad. Sin embargo, todos los lectores mostraron un sesgo constante al determinar la edad de los ejemplares más grandes (CFL>180 cm) con respecto a la curva de crecimiento. El sesgo observado puede explicarse por el ajuste del modelo de crecimiento a los datos de edad, a la variabilidad temporal en la tasa de crecimiento o a la asignación del tipo de borde. Se concluye que este conjunto de imágenes puede servir como colección de referencia y por lo tanto apoyamos una producción rutinaria de la determinación de la edad del atún rojo.

KEYWORDS

Reference collection, Bluefin tuna, Ageing, Otolith

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1. Introduction

The International Commission for the Conservation of Atlantic Tunas (ICCAT) identified growth as a major source of uncertainty in stock assessment models for Atlantic bluefin tuna (*Thunnus thynnus*) (ICCAT 2009). A key component of the ICCAT Atlantic Wide Research Program for Bluefin Tuna (GBYP) has been to provide updated life history information for this species, specifically age, growth and catch-at-age to reduce uncertainty in stock assessment models.

Currently, catch-at-age of Atlantic bluefin tuna is determined by age slicing, a technique that divides catch length into different ages creating a catch-at-age matrix. To do this, a distribution of sizes is separated into age classes under the assumption there are distinct lengths which separate adjacent age classes. Typically, ages are separated based on the length half way between the two mean lengths at an age predicted from a growth curve. A critical assumption is equal variability in length across adjacent ages. Given the variability in growth between individuals and over time this technique can lead to age class smearing where one or more ages classes overlaps and is assigned to the incorrect age. Direct aging of hard parts (otoliths and dorsal spines) reduces this uncertainty and results in better characterization of catch-at-age providing more robust stock assessment models. Laboratories in Canada, Spain and USA have been collecting otoliths and dorsal fin spines from a broad size distribution of bluefin tuna captured by multiple gear types in the East and West Atlantic for routine estimation of size at age and individual growth rate. In an effort to standardize otolith based ageing protocols across the laboratories and evaluate the error underlying the interpretation of annuli, an Atlantic bluefin tuna ageing workshop was held at the Chesapeake Biological Laboratory in January 2013 (Secor *et al.* 2013). A key outcome of this workshop was the need to develop an annotated reference set of representative Atlantic bluefin tuna otolith samples for training purposes and to maintain consistency within and among laboratories. Ideally, a reference set would be composed of known-age samples (Campana 2001), however due to the inherent difficulty in acquiring known age samples of wild fish, many groups have developed reference sets based on consensus ages accomplished by a group of experts. Accordingly, a reference set of 100 otoliths from four laboratories (St. Andrews Biological Station, Chesapeake Biological Laboratory, University of Maine/Gulf of Maine Research Institute and Spanish Institute of Oceanography) was developed and aged by both experienced and inexperienced readers.

The objectives of this paper are (1) to evaluate age estimates from the Atlantic bluefin tuna reference set for bias and precision (2) compare age estimates from the reference collection to the most recent growth curve (Restrepo *et al.* 2010) and (3) suggest future work for production ageing of Atlantic bluefin tuna.

2. Material and methods

2.1. Reference set

A set of 100 otoliths from four laboratories (St. Andrews Biological Station, Chesapeake Biological Laboratory, University of Maine/Gulf of Maine Research Institute and Spanish Institute of Oceanography) was selected based on image quality (readability >3) (**Table 1**). Atlantic bluefin tuna were sampled over a broad size distribution (50 - 300 cm curved fork length, CFL) covering individuals across various life history stages and selected for by different gear types (**Figure 1**). Otolith sections (Y-section type) were imaged under both reflected and transmitted light (N = 200) and enhanced using Photoshop software. Image enhancement filter tools (levels and unsharp mask) were used to improve the contrast of opaque and translucent bands on the otolith section. A scale demarcating 0.75, 0.85 and 1 mm was added to images to aid in assignment of the first annulus. This scale was derived from the mean length measuring from the primordium up the ventral arm to the first annulus observed in a sample of yearling otoliths (N = 22).

Images were read by 9 experienced and 2 inexperienced readers who made single blind age readings and annotated the position of annual bands on every image using raster layers. Final age assignment consisted of counting the number of annotated annuli on each image. The readability code and edge type of each image (i.e., opaque or translucent edge) were also recorded for each otolith image (**Table 1** and **Table 2, Figure 2**).

The reference set was evaluated using two standards: (1) modal ages for each light type derived from age estimates of the 9 experienced readers using the Eltink (2000) workbook and (2) age assignments from a primary reader designated by the group.

In order to evaluate the representativeness of the reference set, modal age was compared to the total catch at age of 2011 (ICCAT 2012). Furthermore, age assignments were compared to modal age and primary reader age and analyzed for bias and precision by lighting type and reader. Age bias was evaluated through residual plots. Precision was estimated through Average Percent Error (APE) and Coefficient of Variation (CV) as follows:

$$APE_j = 100\% \times \frac{1}{R} \sum_{i=1}^R \frac{|x_{ij} - X_j|}{X_j}$$

$$CV_j = 100\% \times \frac{\sqrt{\frac{\sum_{i=1}^R (X_{ij} - X_j)^2}{R - 1}}}{X_j}$$

Where R = number of readers; x_{ij} = age estimate for reader i for otolith j ; X_j = mean age for otolith j .

Given the moderate longevity of Atlantic bluefin tuna and inherent difficulty in consistently identifying annuli, an APE and CV of 10% was determined to be reasonable in terms of quality control (Secor *et al.* 2013). Readability and edge type assignment were examined using graphical methods.

Age estimates of samples from western laboratories (St. Andrews Biological Station, Chesapeake Biological Laboratory and University of Maine/Gulf of Maine Research Institute) were compared to the most recent growth curve for the western Atlantic bluefin tuna stock (Restrepo *et al.* 2010) and age bias was evaluated through residual plots (observed – expected age) and box plots. To further explore bias, the primary reader in this study re-read 24 otolith images from Neilson and Campana (2008) used to derive the most recent growth curve along with other otoliths readings and size frequency distributions of small Atlantic bluefin tuna (Note: Neilson and Campana (2008) used 25 samples. However, we were unable to retrace one image). Age estimates from both primary readers (this study and Neilson and Campana 2008) were analyzed for differences (paired t-test), bias and precision and compared to the most recent growth curve (Restrepo *et al.* 2010).

3. Results

Modal age estimates seemed to cover older ages compared to the total catch at age of 2011 (**Figure 3**). However, the lack of older ages in the total catch at age is due to the truncation of the age data into a 16+ age group. Therefore, modal age estimates under both light types included all ages used in the 2012 bluefin stock assessment, indicating that age assignments were representative of the most recent catch-at-age.

Residual plots (**Figure 4**) revealed no systematic bias in age interpretation by experienced and inexperienced readers except for a slight pattern in residuals for older ages under transmitted light compared to the modal age. This slight bias would indicate that readers tend to underage older individuals compared to the modal age. Interestingly, inexperienced readers increased the variability in the trend in residuals only for transmitted light (**Figure 4**). From an individual reader perspective, residual plots (**Figure 5** and **6**) showed no systematic bias in age interpretation for a particular reader. However, it did indicate that all readers tend to have more variability for older ages.

Overall, average percent error (APE) was slightly lower for reflected light (APE = 7.30%) compared to transmitted light (APE = 7.75%), while the coefficient of variation (CV) was similar for both light types (CV_{transmitted} = 9.60%, CV_{reflected} = 9.72%). Experienced readers showed a lower APE and CV compared to all readers regardless of the light type (**Table 3**).

Overall, size at age plots for each reader and light type showed a good fit ($R^2 = 0.76 - 0.85$) to the current growth curve for the Western Atlantic bluefin tuna stock (**Figure 7**). However, residual plots (**Figure 8**), where the growth curve provided expected age, showed a strong pattern in the residuals for all readers. The variability in the residuals increased with increasing CFL. Furthermore, a boxplot of the assigned vs. expected ages (**Figure 9**) showed that for specimens larger than 180 cm (CFL), readers tended to under estimate age with regard to the expected age obtained by the growth curve. Interestingly, both the size at age plots and boxplot (**Figure 7** and **Figure 9**) indicated that reader estimated ages showed a larger inflexion in the length-age relationship compared to the Restrepo *et al.* (2010) growth curve.

Age estimates from re-reading the 24 otolith images from Neilson and Campana (2008) indicated that the primary reader of this study significantly ($P < 0.01$) under-aged compared to the primary reader of Neilson and Campana (2008). Furthermore, the size at age plots showed a poor fit with the current growth curve and the residual plot, where the growth curve provided expected age, showed a strong pattern in the residuals for each reader (**Figure 10**).

In terms of edge type and readability code, the results indicated that readers showed inconsistencies in assigning edge type and readability code to a given sample (**Figure 11** and **Figure 12**). For edge type assignment, light did not appear to be a factor as tile plots showed the same pattern under both light types. However, light type seemed to be an important factor for readability code assignment for inexperienced readers. For instance, PQ and MRS appeared to assign higher readability codes to the same samples under reflected light. On the other hand, experienced readers appeared to disagree mostly in assigning a readability of 3 or 4 to a given otolith image.

4. Discussion

The objective of this paper was to develop and assess a reference set of otoliths for Atlantic bluefin tuna to be used in future production ageing activities. A comparison of the modal age estimates to the total catch at age of bluefin tuna indicated that the reference set was representative of most recent catch-at-age. In terms of bias, overall readers showed no systematic bias compared to modal age and primary age reader. However, the results indicated a slight ageing bias, with lower age estimates under transmitted light for older fish as compared with the modal age. This slight bias was considered to be acceptable due to the low number of large individuals ($N = 6$) in the sample and the fact that not all readers exhibited this ageing bias. Furthermore, the ageing error was low among readers and within the acceptable APE and CV (10%) for quality control (Secor *et al.* 2013). The acceptable level of precision and lack bias indicate that the collection with consensus-derived ages from experts may be used as a reference set for otolith based ageing of bluefin tuna.

Age estimates by experienced and non-experienced readers were lower than those derived from the most recent Atlantic bluefin tuna growth curve (Restrepo *et al.* 2010) for individuals larger than 180cm CFL. Three sources of error were identified to explain the strong trend in residuals; (1) fitting of the growth model (Restrepo *et al.* 2010) to age data from Secor *et al.* (2008) and Neilson and Campana (2008); (2) temporal variation in growth rate of Atlantic bluefin tuna and (3) edge type assignment.

Restrepo *et al.* (2010) used age-length observations derived from otolith readings from two data sets: (1) Secor *et al.* (2008) ($n = 121$) and (2) Neilson and Campana (2008) ($n = 25$). The primary reader of the current study re-read 24 images from Neilson and Campana (2008) study and a paired t-test indicated that his ages were significantly lower ($P < 0.01$) than the primary reader of Neilson and Campana (2008) with a mean difference of 2.93 years. The main reason for this difference appears to be the assignment of the first annulus (**Figure 13**), where in the absence of a reference scale (such as was used in this study), the primary reader of Neilson and Campana (2008) consistently placed the first annuli lower down the ventral arm. While ages obtained by the Neilson and Campana (2008) study were validated using a radiocarbon method, the expected precision of the ages was $\pm 1-3$ years (Campana 2001), which includes the observed bias between the primary readers. Furthermore, when age estimates were compared to Restrepo *et al.* (2010), size at age plots for both readers showed a poor fit with the current growth curve for the Western Atlantic bluefin tuna stock and the residual plots showed a strong pattern in residuals for each reader. These results suggest that there might be issues related to the fitting of the growth model to the age data from Neilson and Campana (2008), contributing to the observed pattern in the residuals.

The age data from Secor *et al.* (2008) may also be contributing to the observed pattern in residuals, as V-sections of Atlantic bluefin tuna otoliths were used for ageing. The results of the 2013 Ageing Workshop showed that there was a significant bias between sections of the same otolith, with the Y-section resulting in age estimates that were 0.77 years higher than the V-section (Secor *et al.* 2013). However, the authors noted that any bias due to the V- section type would be expected to result in small to insignificant changes to the growth model.

Alternatively, Atlantic bluefin tuna may exhibit temporal variability in growth rates and the observed trend in residuals could be explained by the fact that the current growth curve may differ from the Restrepo *et al.* (2010) curve that was derived mainly from historic samples. Variations in growth rate have been reported for various species including southern bluefin tuna (Polacheck *et al.* 2004). Currently, decadal variation in growth rate of Atlantic bluefin tuna is being examined by European and North American laboratories as part of the BLUEAGE project (Rodriguez-Marin *et al.* 2012) and a National Oceanic and Atmospheric Administration project (NOAA).

Inconsistencies in edge type assignment exhibited by readers would directly influence assigned age as opaque bands are counted to derive age estimates, thus potentially contributing to the observed trend in the residuals. However, this was considered a lesser source of error as the magnitude of potential error from this source would be a maximum of +/-1 year. Nonetheless, it highlights the need to complete the edge type interpretation and marginal increment analysis tasks suggested at the 2013 Ageing Workshop (Secor *et al.* 2013) as it would aid readers in consistently identifying edge type.

In light of the upcoming Atlantic bluefin tuna assessment in 2015, it is important for laboratories to begin production ageing in order to provide age estimates in a timely fashion. This study showed that age interpretations for this reference set were within acceptable level of precision and that readers showed no systematic bias compared to the two standards. However, readers did show a systematic bias in ageing older fish compared to the most recent growth curve (Restrepo *et al.* 2010). It was suggested that the observed pattern in residuals might be related to the fitting of the most recent growth model or temporal variability in growth rate of bluefin tuna. Based on these results, we suggest that this collection of otolith images be used as a reference set using either modal age or primary reader age as standard and that production ageing of bluefin tuna otoliths begin using the following conventions:

1. Processing of Atlantic bluefin tuna otoliths

- Important life history information, such as age, growth and stock mixing of Atlantic bluefin tuna can be obtained from otoliths through ageing and micro-constituent analysis. In order to preserve samples for future analyses, it is recommended that only one otolith be used to obtain both age estimates and natal origin of Atlantic bluefin tuna. **Figure 14** illustrates location and thickness of sections for routine ageing (Y-section) and micro-constituent analysis.

2. Ageing of Atlantic bluefin tuna

- We suggest that routine ageing be carried out by one reader per laboratory. Prior to production ageing, readers should read the reference set one time single blind under their preferred light type. A precision level of APE and CV of 10% or lower and no bias would be acceptable to support production ageing. The reference set should also be used to monitor ageing consistency over time as well as among age readers (relative bias and precision) and for training purposes
- Identification of annuli on otolith section.
 - View sections (or images) under either transmitted or reflected light.
 - Annuli are a bipartite structure consisting of a translucent and opaque zone.
 - The ventral (long) arm is used for age estimates. The dorsal (short) arm can be used as check; however it important to remember that the dorsal arm tends to underestimate age.
 - Start annotations at the primordium and proceed to the terminal edge. The ventral arm can be divided in 3 general regions as one travels from the primordium to the terminal edge:
 - 1st region: Annuli are broad and diffuse and contain multiple translucent and opaque zones (~1-5 annuli).
 - 2nd region: Annuli are less broad and closer together (~5-10 annuli).
 - 3rd region: Annuli appear clearer and are regular in width (~ 10+ annuli).

- Use the reference scale as a guide to identify the first annulus. This scale was derived from the mean length measuring from the primordium up the ventral arm to the first annulus observed in a sample of yearling otoliths (N = 22). The distance to the first annulus ranged from 0.73 to 1.00 mm with a mean distance of 0.86 mm.
- Count opaque growth zones. These appear dark under transmitted light and white under reflected light.
 - Crenulations (grooves along the margins) may aid in the identification of growth zones.
 - Annuli should cover the width of the ventral arm.
- Edge type assignment
 - View both the enhanced and un-enhanced version of the image. It might be helpful to view the otolith under a higher magnification.
 - Edge type should only be noted if more than 50% of the edge is visible across the width of the ventral arm.

3. Converting annuli counts to Age estimates.

- The decision whether to assign fractional ages based on when the fish was captured and assigned birth date should be deferred until marginal increment analysis and edge type interpretation tasks have been completed. For now, we suggest that age estimates be assigned based on annuli count.
- Each otolith should be interpreted through two independent readings with at least two weeks between readings:
 - If the readings differ by 1 year, the 2nd reading will be used as the final age.
 - If the readings differ by 2 or more years, conduct a 3rd reading with knowledge of the prior readings to reach a consensus final age. Otoliths where a 3rd reading is necessary should be annotated and checked for bias.
- For each otolith record: date of each reading, reader's name, section details (i.e. comments), readability (**Table 1**), edge type (**Table 2** and **Figure 2**) and assigned age for each reading.

4. Augmenting reference collection

- We suggest that the Atlantic bluefin tuna reference set be augmented every year with new annotated images.

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Table 1. Readability codes for Atlantic bluefin tuna otoliths.

<i>Readability Code</i>	<i>Definition</i>
1	Pattern present-no meaning
2	Pattern present-unsure with age estimate
3	Good pattern present-slightly unsure in some areas
4	Good pattern-confident with age estimate

Table 2. Edge type interpretation of Atlantic bluefin tuna otolith by light type.

<i>Edge type</i>	<i>Transmitted Light</i>	<i>Reflected Light</i>
Opaque	Dark Zones	White Zones
Translucent	White Zones	Dark Zones

Table 3. Precision estimates for reference set of Atlantic bluefin tuna otoliths that were interpreted by 9 experienced readers and 2 inexperienced readers (N=100).

		<i>Reflected</i>	<i>Transmitted</i>
All Readers	APE (%)	7.30	7.75
	CV (%)	9.60	9.72
Experienced Readers	APE (%)	6.68	6.90
	CV (%)	8.95	8.73

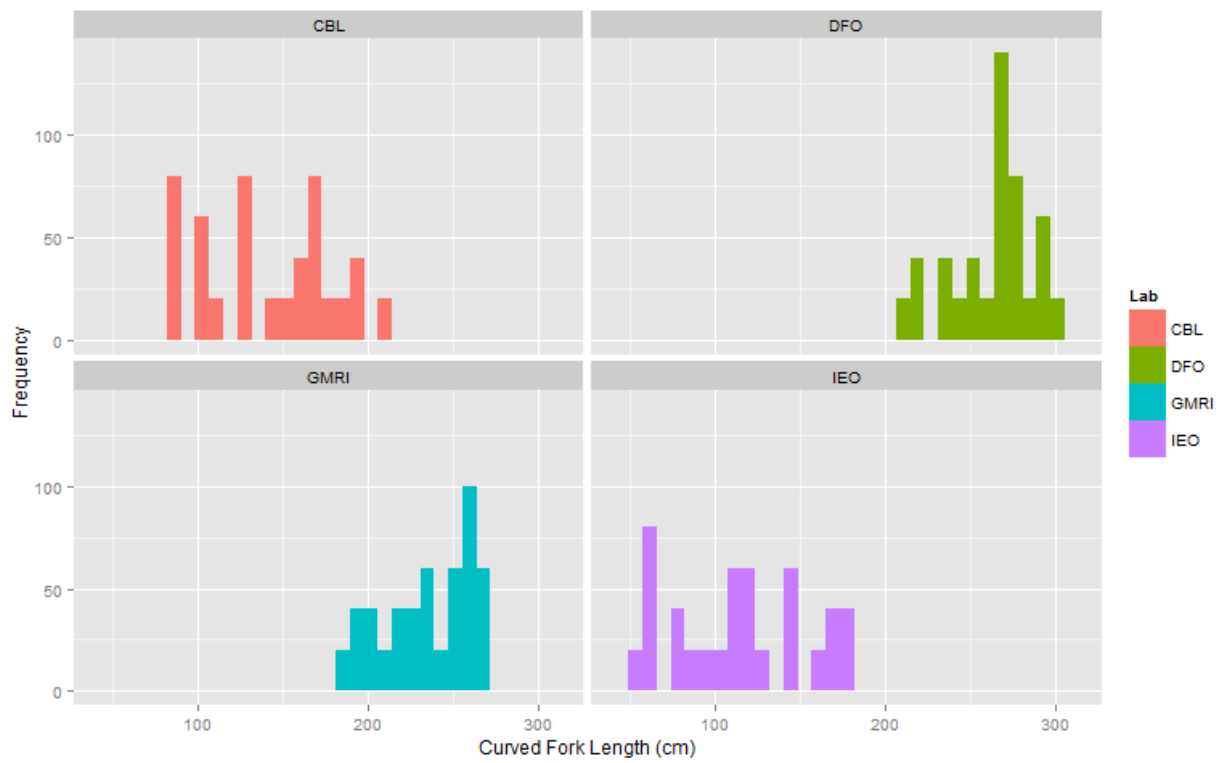
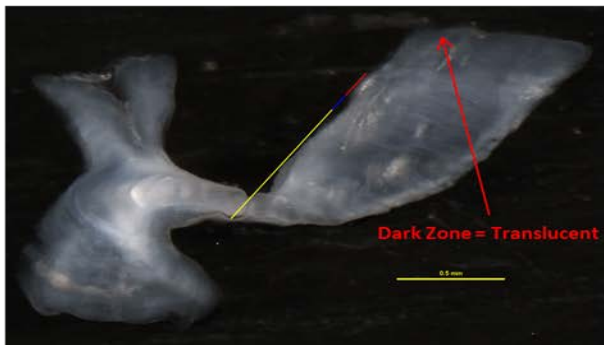
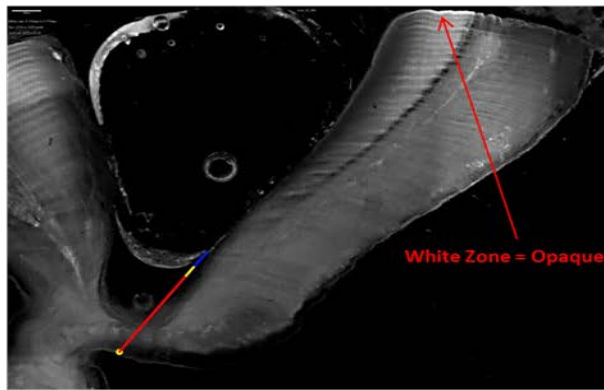


Figure 1. Length frequency plot of Atlantic bluefin tuna samples in the reference collection by laboratory (DFO =St. Andrews Biological Station, CBL= Chesapeake Biological Laboratory, UMaine/GMRI = University of Maine/Gulf of Maine Research Institute and IEO= Instituto Español de Oceanografía).

REFLECTED LIGHT



TRANSMITTED LIGHT

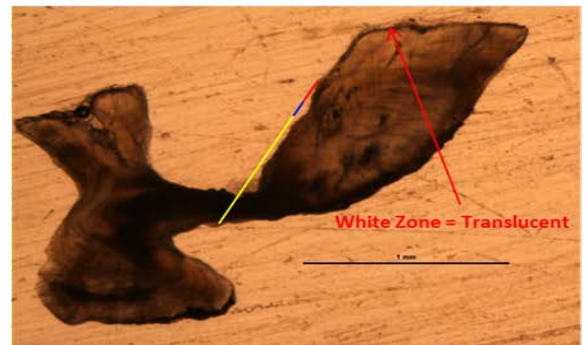
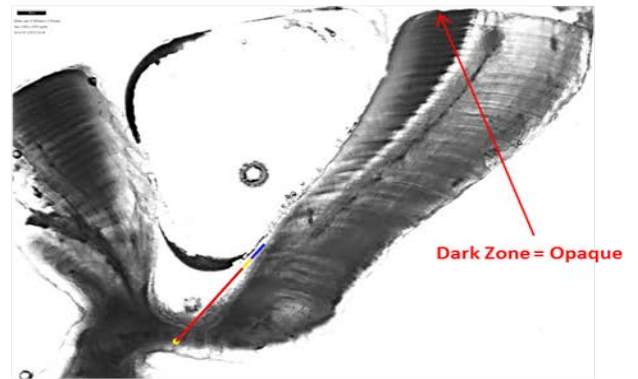


Figure 2. Photograph illustrating the edge type interpretation of Atlantic bluefin tuna otolith by light type.

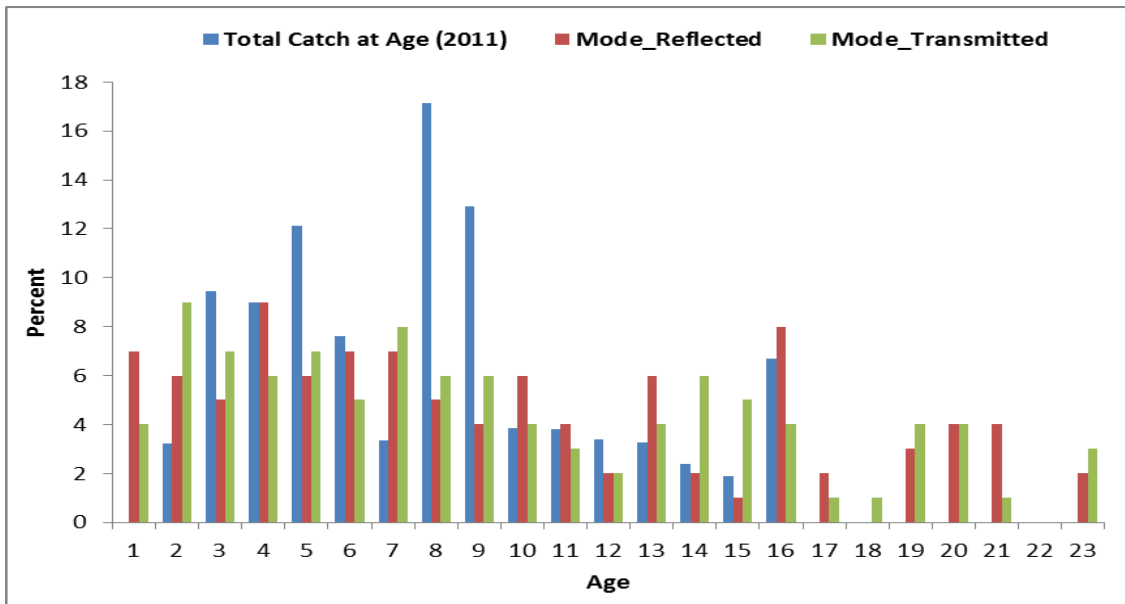


Figure 3. Proportion of ages from the Total Catch-at-Age (2011) and the reference collection (Modal age).

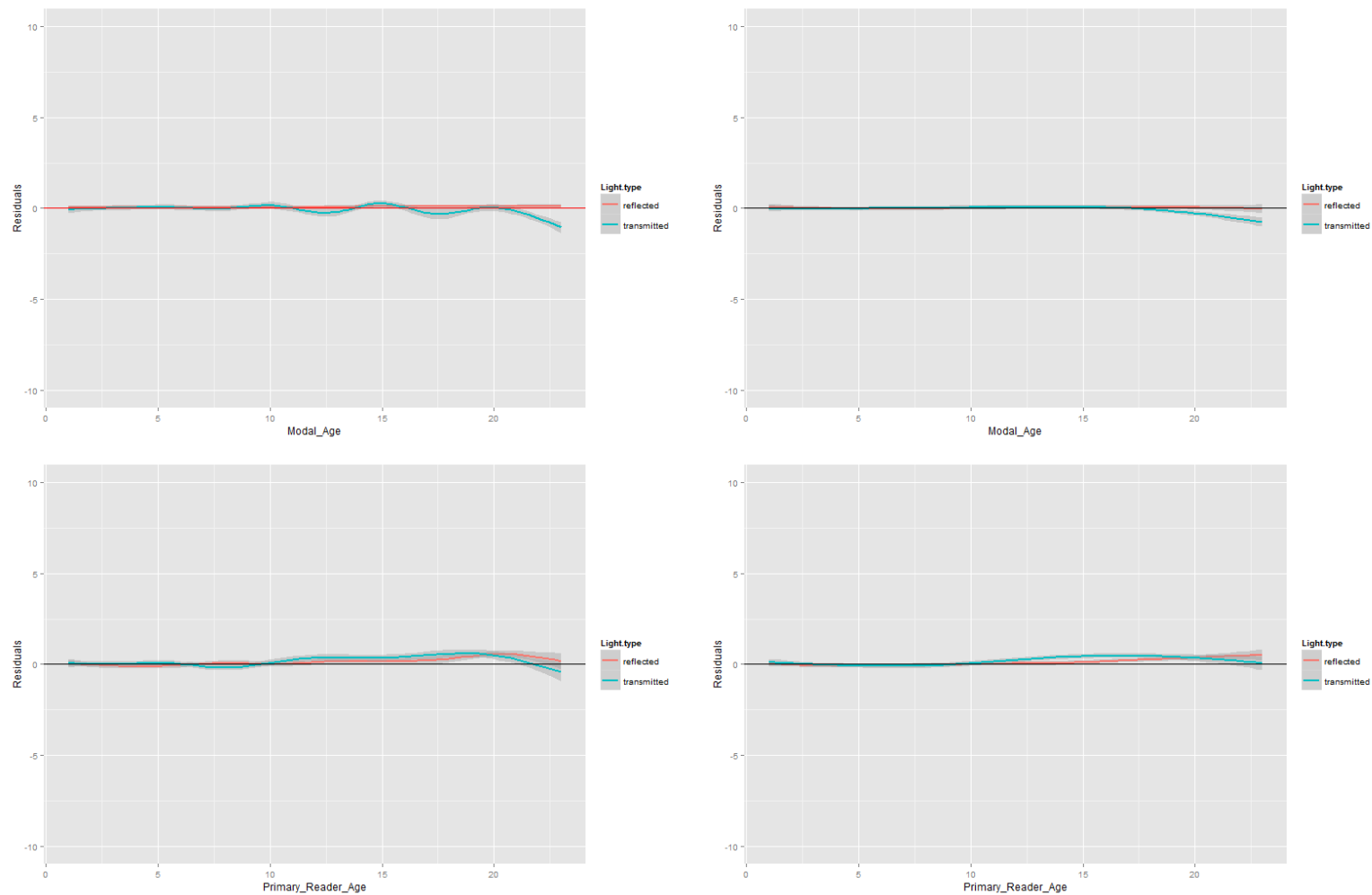


Figure 4. Residual plots of all readers (left) and experienced readers (right) vs. modal age and primary read age. (Residuals = Age estimates – Modal Age/primary reader age and light type = loess smooth line ($\alpha = 0.9$)).

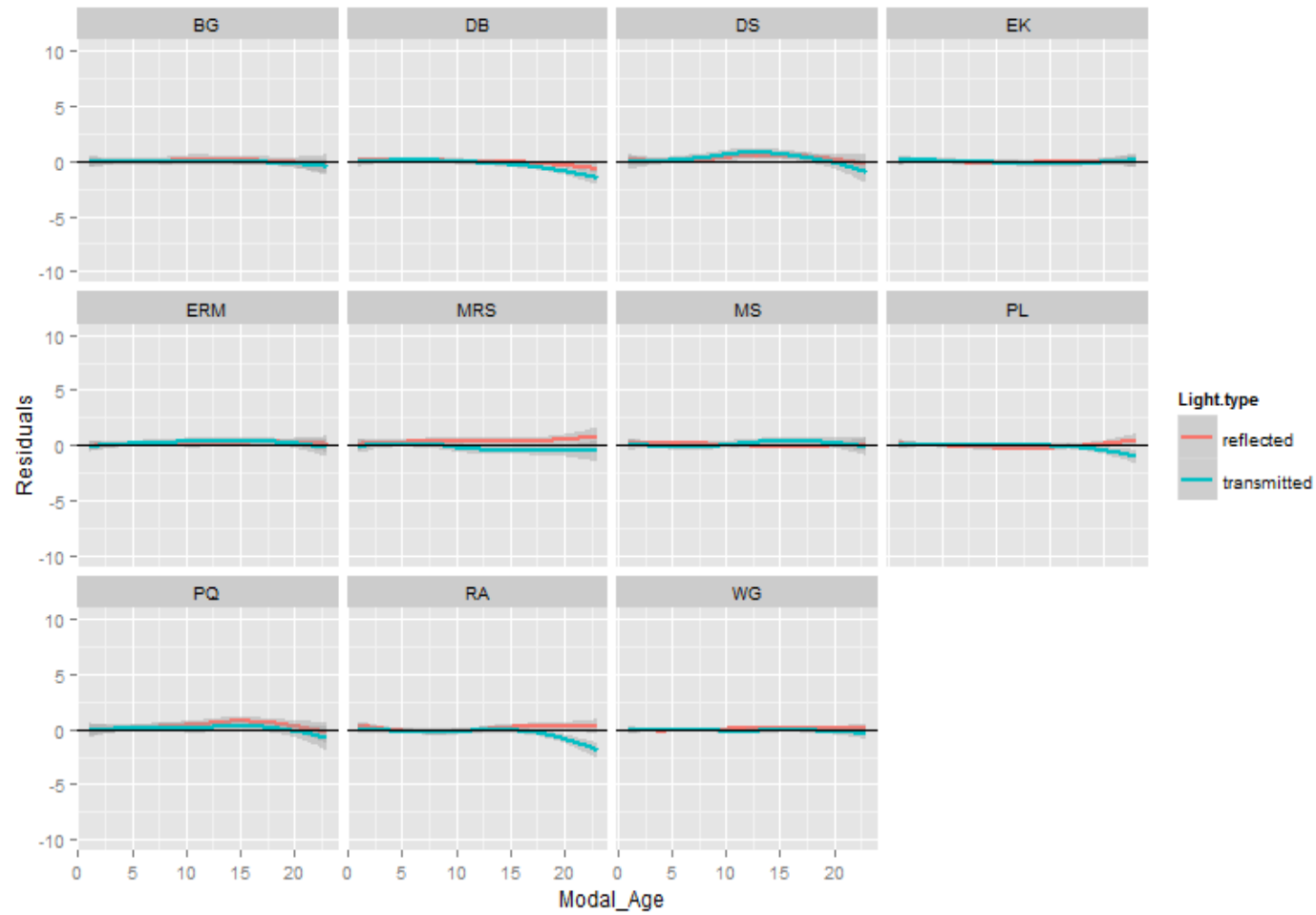


Figure 5. Residual plots of individual readers vs. modal age. (Residuals = Age estimates – Modal Age, Experienced readers: DB, BG, DS, EK, MS, RA, PL, WG & ERM, Inexperienced readers: MRS & PQ and light type = loess smooth line ($\alpha=0.9$)).

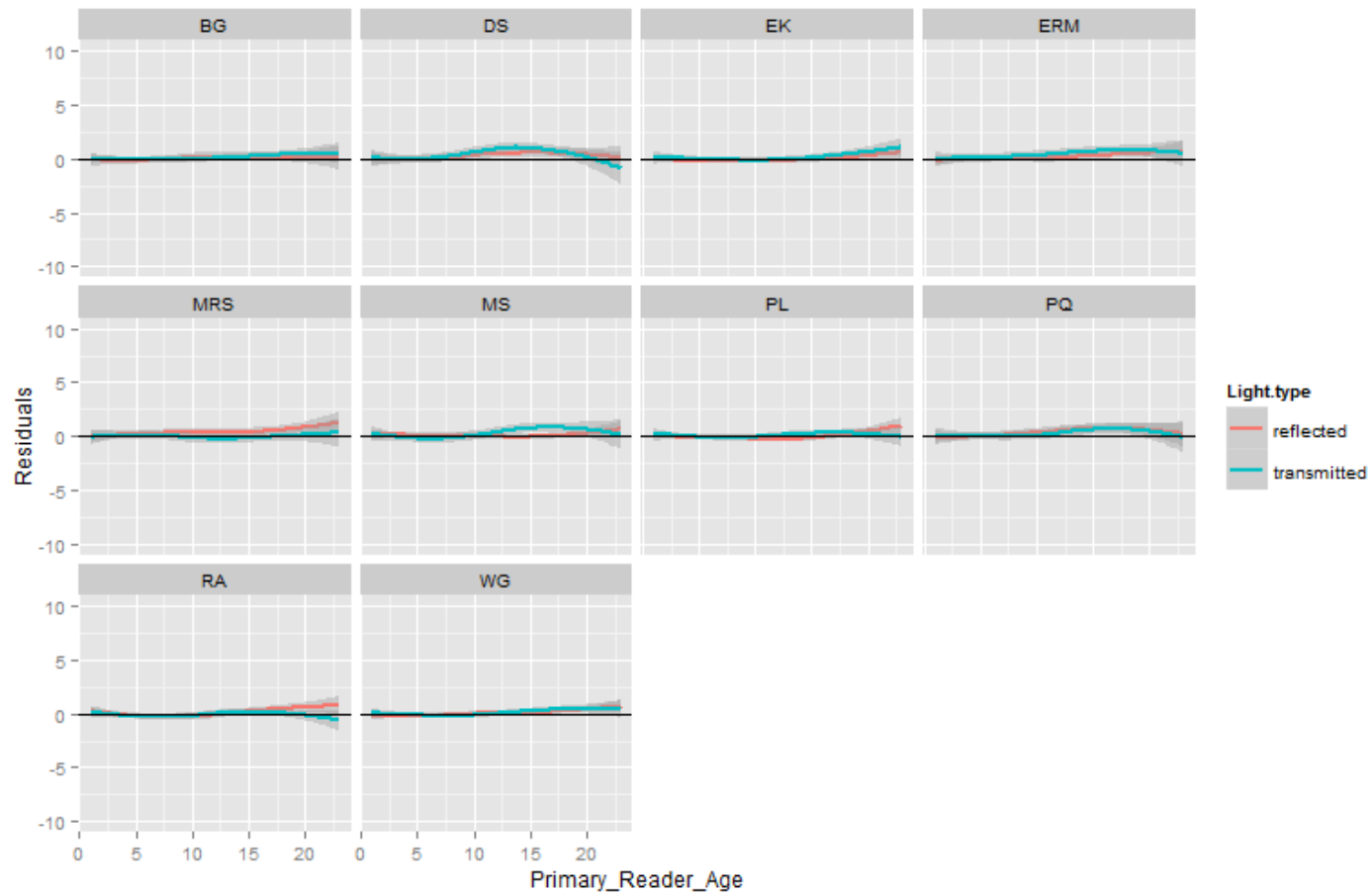


Figure 6. Residual plots of individual readers vs. Primary reader age. (Residuals = Age estimates – Primary reader age, Experienced readers: BG, DS, EK, MS, RA, PL, WG & ERM, Inexperienced readers: MRS & PQ and light type = loess smooth line ($\alpha=0.9$)).

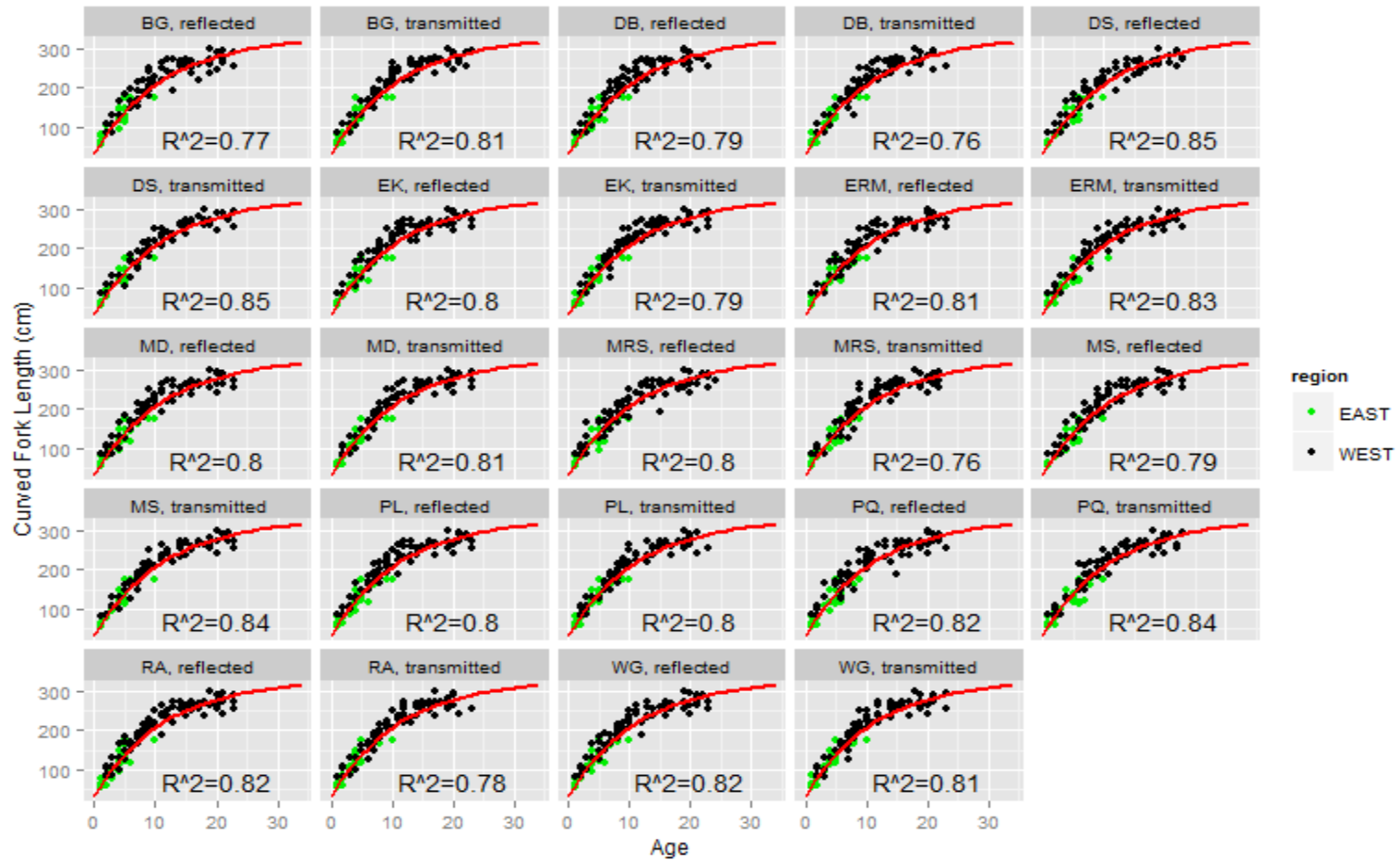


Figure 7. Estimated size-at-age by reader and light type. Each plot shows estimated ages as black (Western fish) and green (Eastern fish) dots ($N=100$) overlaying the Restrepo *et al.* (2010) growth curve (red line). (Experienced readers: DB, BG, DS, EK, MS, RA, PL, WG & ERM, Inexperienced readers: MRS & PQ and MD = Modal age).

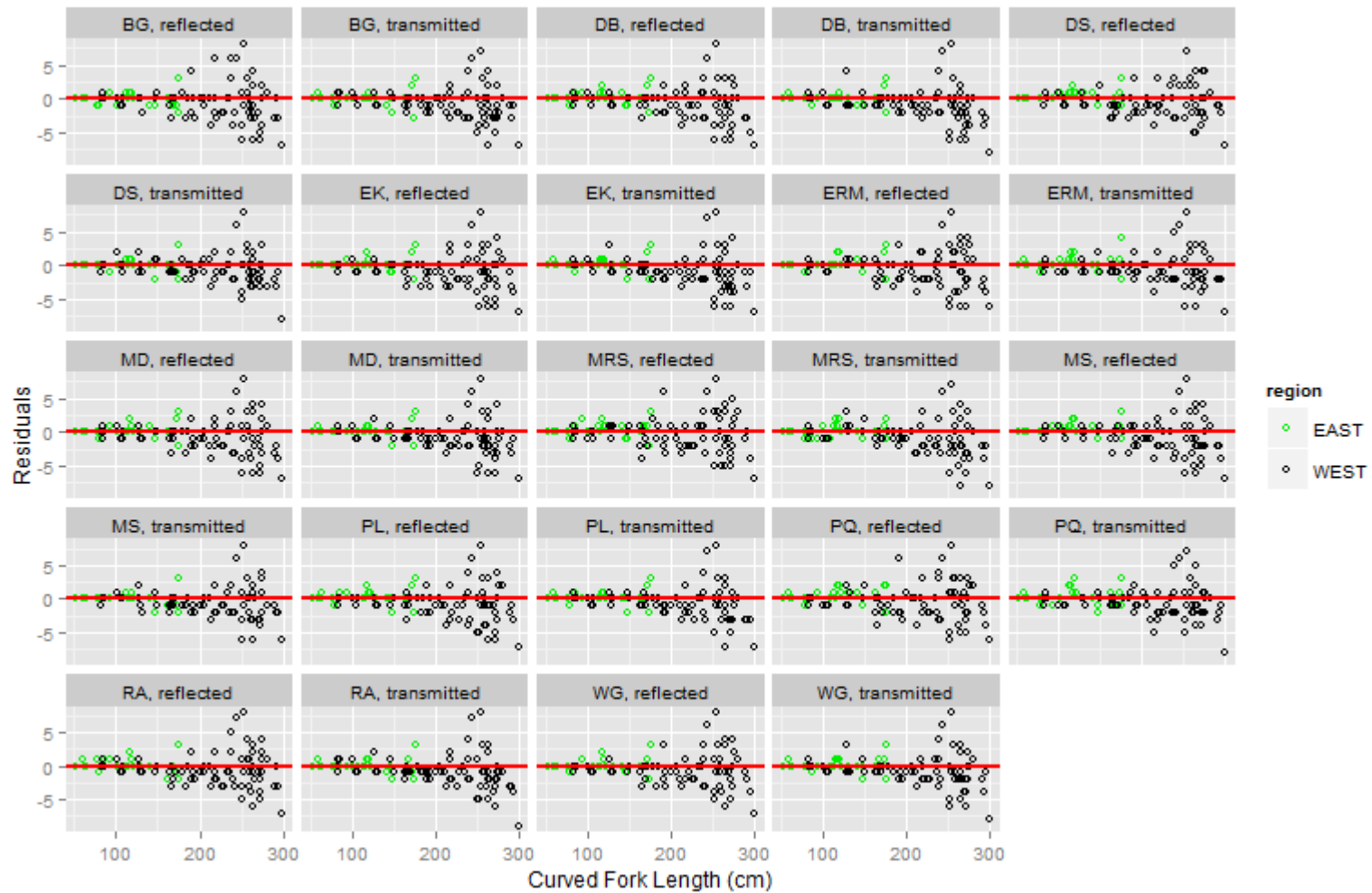


Figure 8. Residual plot for each age reader by light type. (Residuals = Age estimates – Restrepo *et al.* (2010) expected age; experienced readers: DB, BG, DS, EK, MS, RA, PL & ERM, Inexperienced readers: MRS & PQ and MD= Modal age).

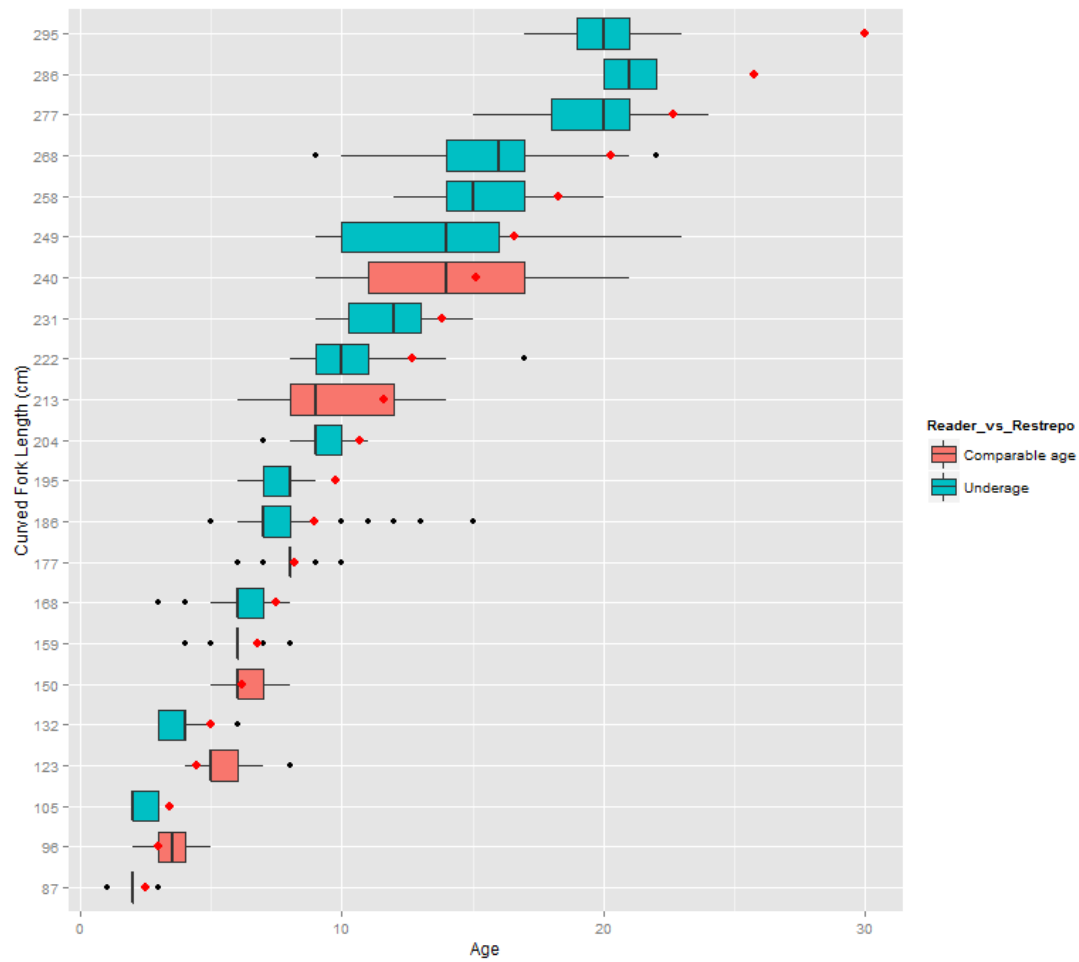


Figure 9. Box plot showing the estimated age assigned by readers compared to the expected age (red dot) from the growth curve (Restrepo *et al.* 2010). Comparable age (red box) and under ageing (blue box) were assigned based on 75% percentile (i.e. if 75% of estimated ages were under the expected age, it was considered under ageing).

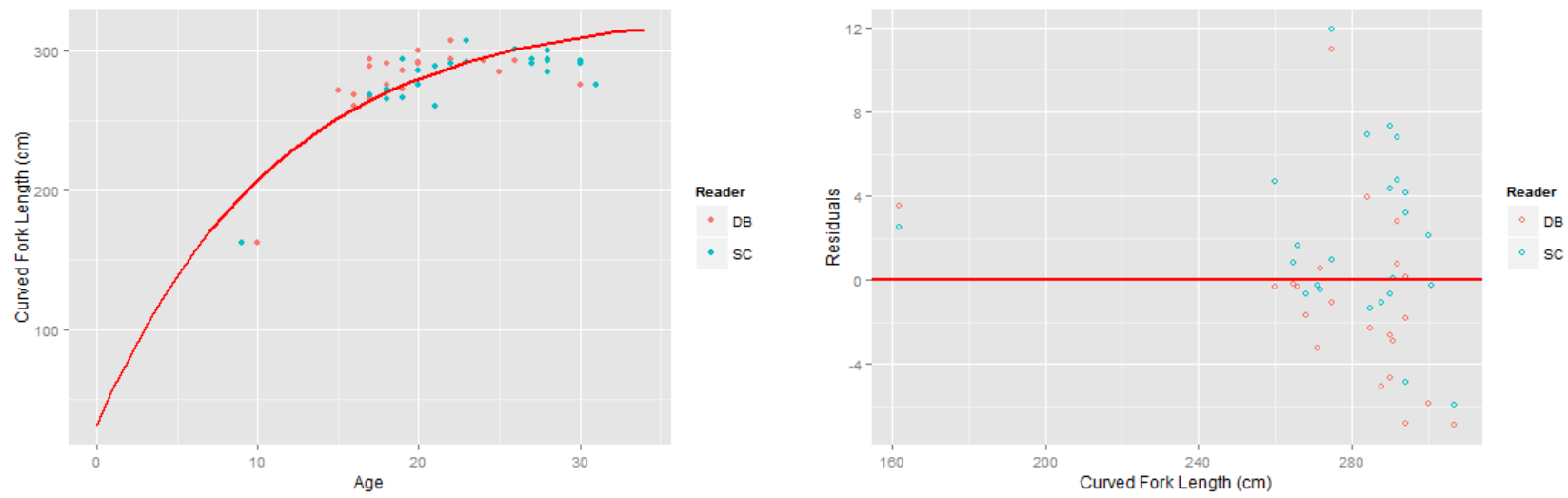


Figure 10. Estimated size-at-age (left) and residual plot (right) of the primary reader from this study (red dot; $R^2=0.16$) vs. the primary reader from Neilson and Campana (2008) (blue dot; $R^2 = 0.31$) compared to the Restrepo *et al.* (2010) growth curve (red line).

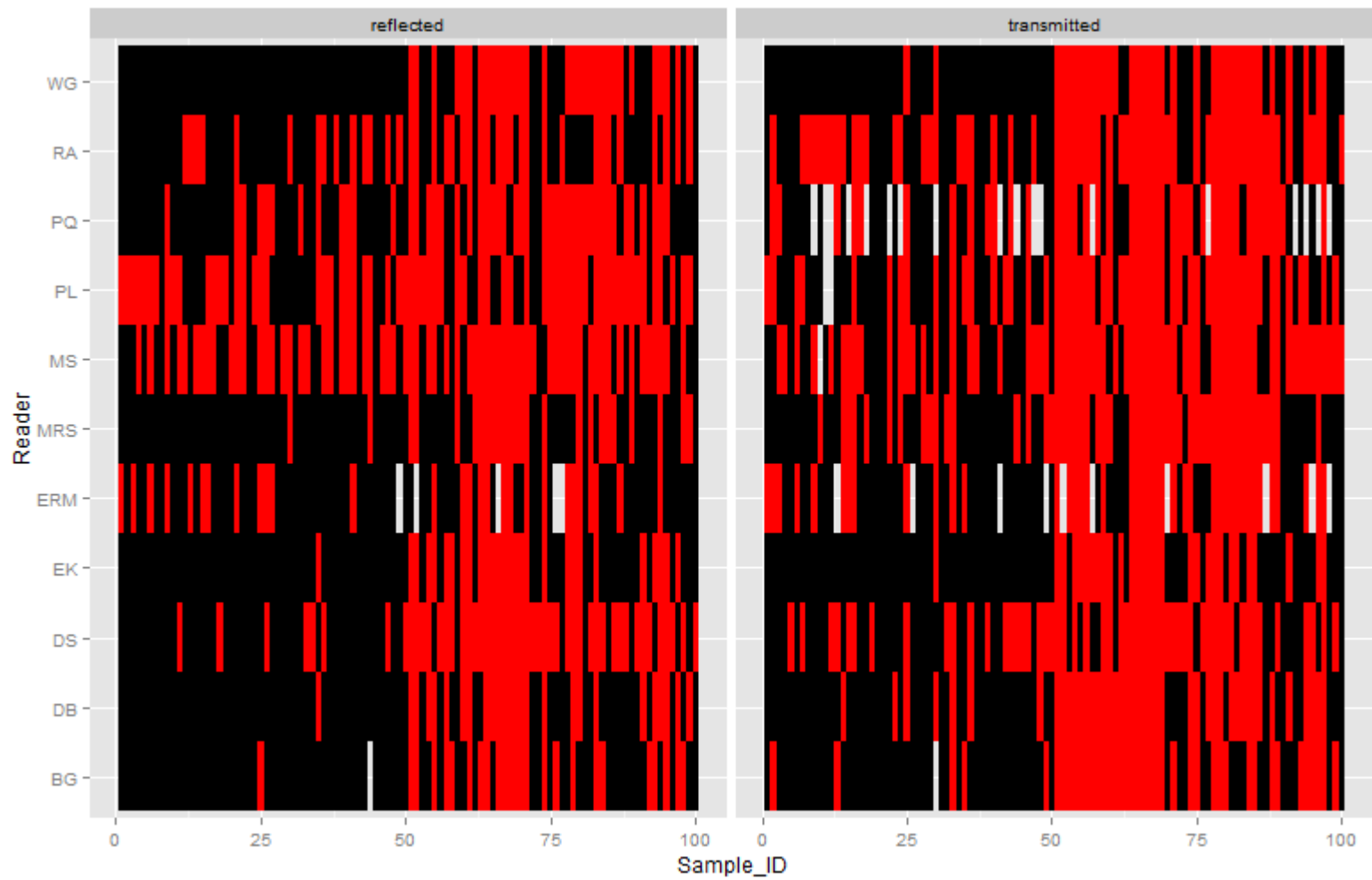


Figure 11. Tile plot of edge type assigned by each reader for a given sample by light type. (Black = Opaque, Red = Translucent, White = No data; Experienced readers: DB, BG, DS, EK, MS, RA, PL, WG & ERM and Inexperienced readers: MRS & PQ).

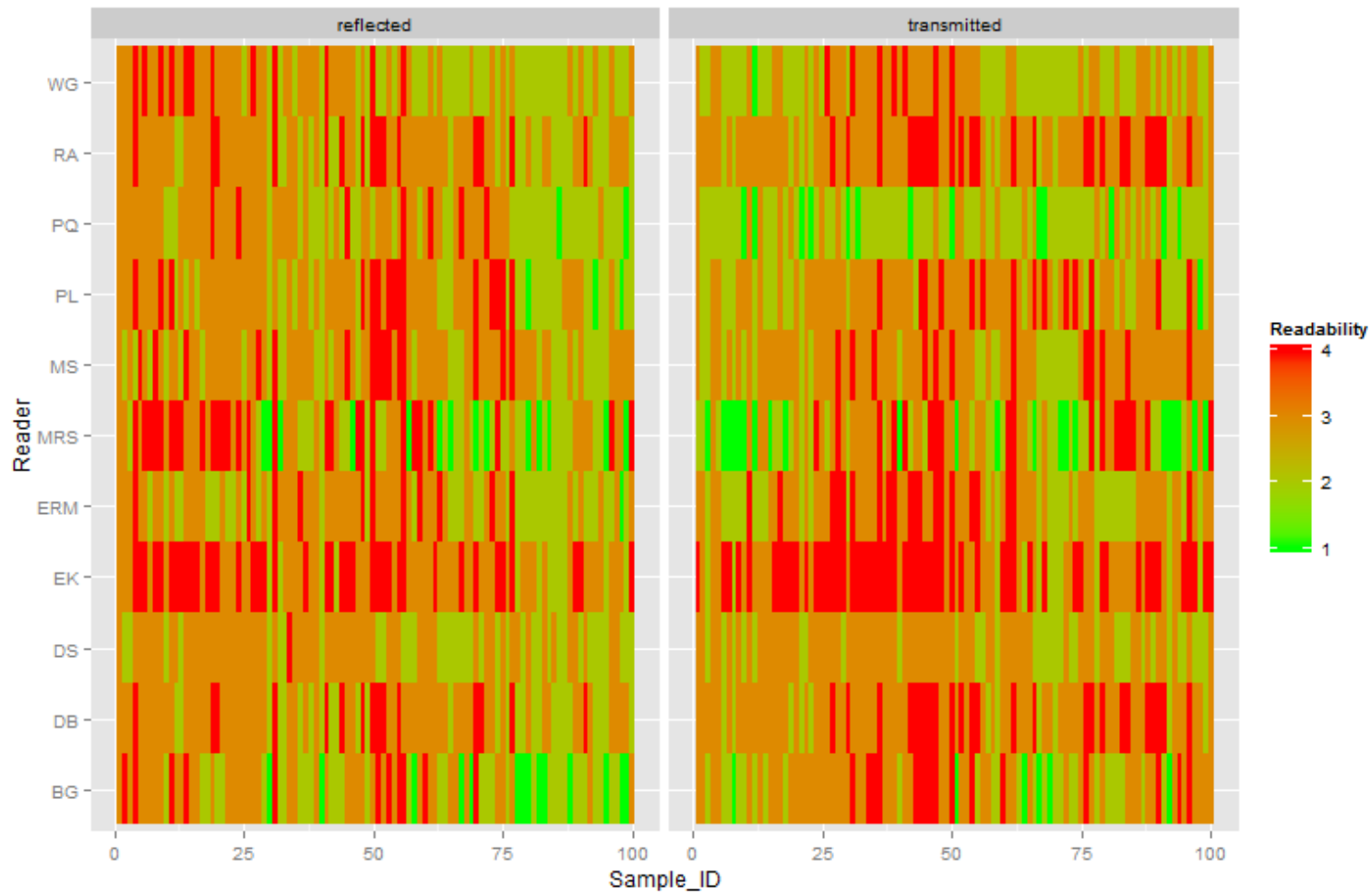


Figure 12. Tile plot of readability given by each reader for a given sample by light type. (Experienced readers: DB, BG, DS, EK, MS, RA, PL, WG & ERM and Inexperienced readers: MRS & PQ).

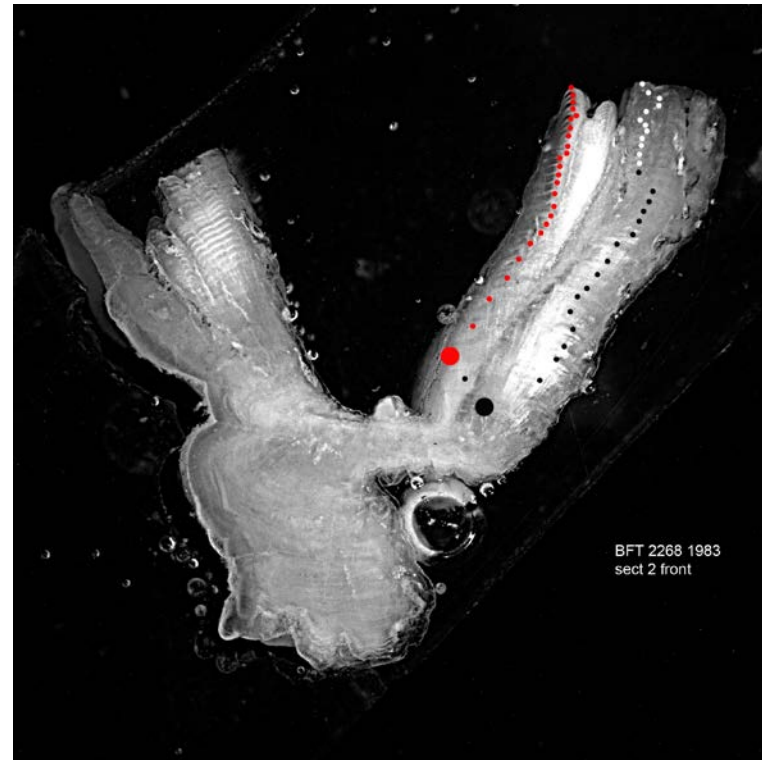
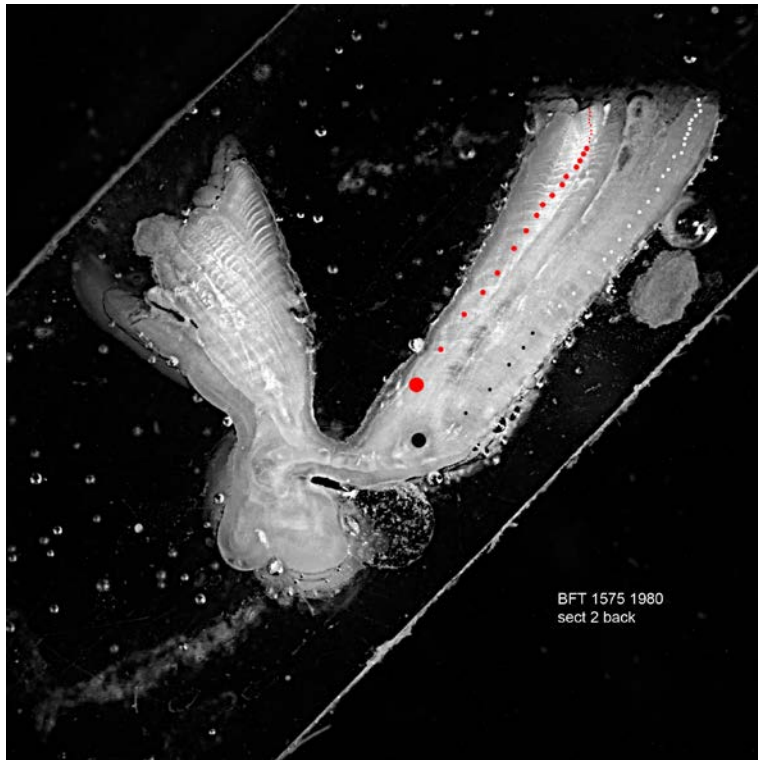


Figure 13. Photograph illustrating the placement of the first annuli of the primary reader from this study (red dot) vs. the primary reader from Neilson and Campana (2008) (black dot).

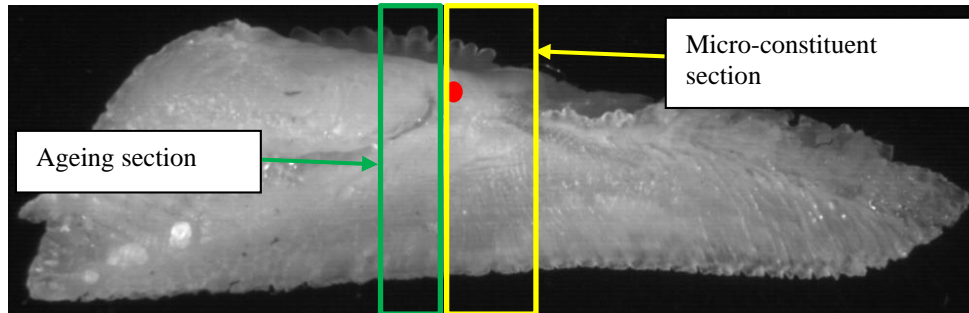


Figure 14. Location of the ageing section (section $\approx 0.80 - 0.90$ mm; polished to $0.6 - 0.7$ mm) and the micro mill section (section = 2.0 mm) on a Atlantic bluefin tuna otolith (Red dot represents the core).