Fish Ageing Services

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194 Milne Drive
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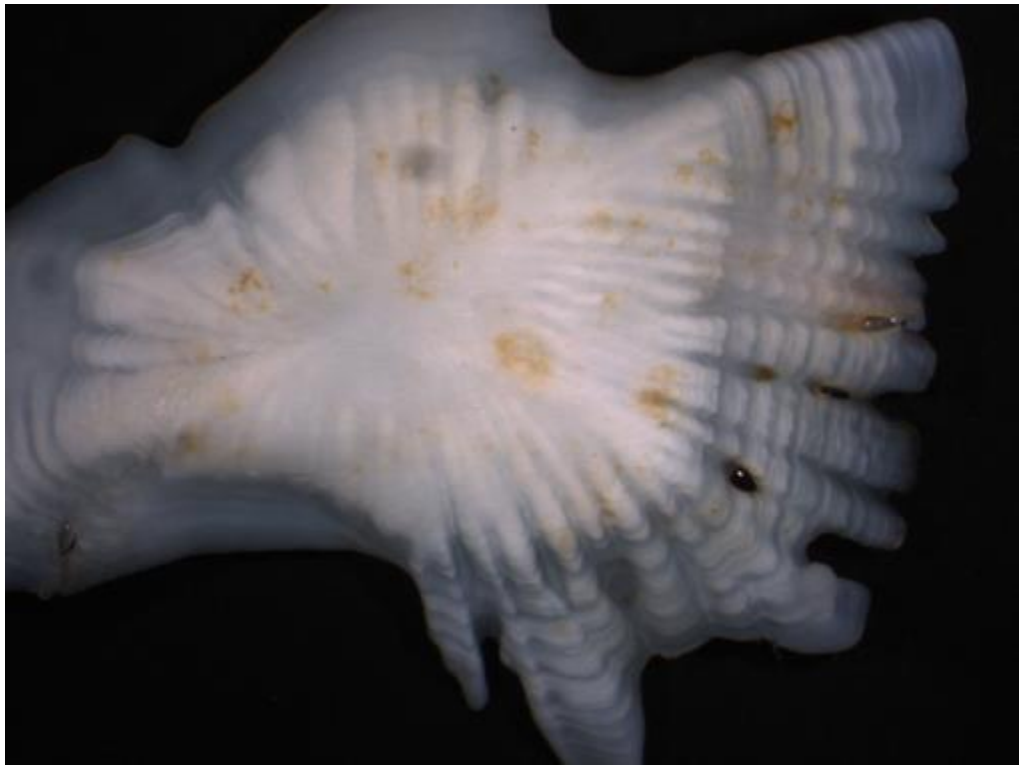
# AGEING OF ALFONSINO (*BERYX SPLENDENS*) FOR SIOFA



**Kyne Krusic-Golub - Fish Ageing Services**

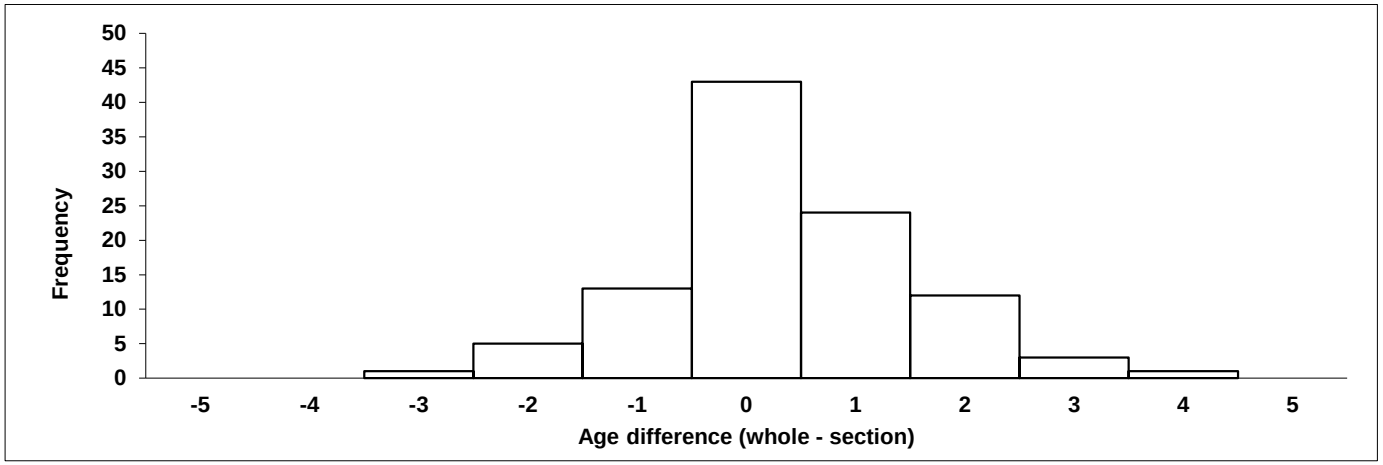
# Requirements

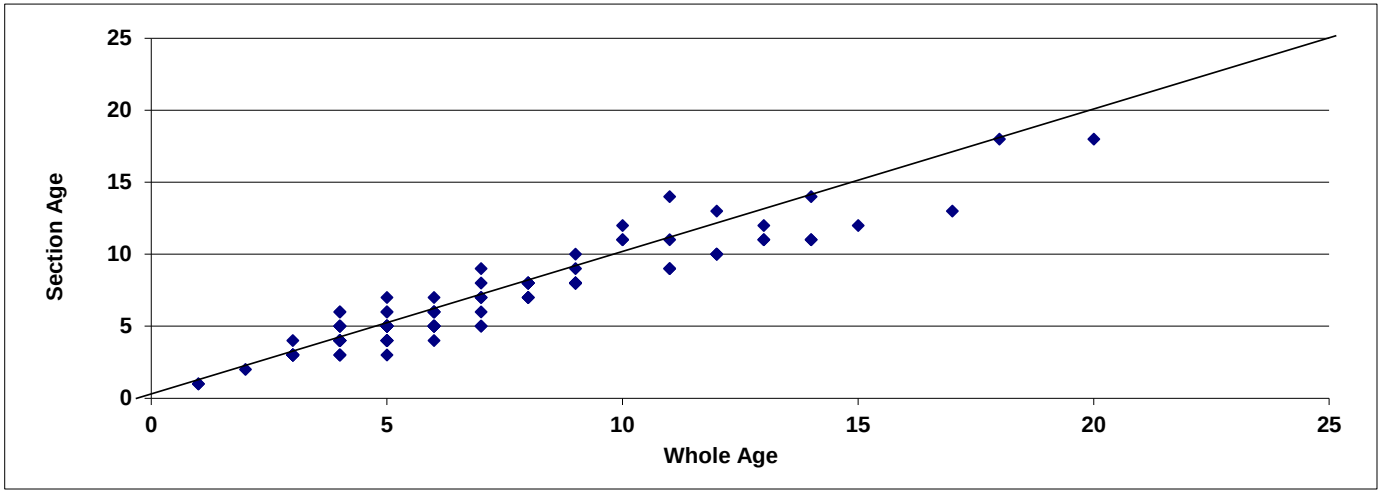
- Estimate the age of 500 Alfonsino otoliths collected from the Eastern Area
- Estimate the age of 500 Alfonsino otoliths collected from the Western Area
- Prepare a brief technical report which details the ageing methods and results of both the ageing and the QA/QC procedures.
- One of the QA/QC stipulations was that each otolith was read by 2 experienced readers

# Other Alfonsino studies

| Study | Study Area | Structure | Annuli count | MIA or Edge Type Analysis | Birthday | Adjusted for zone formation |
|---|---|---|---|---|---|---|
| Adachi *et al.* (2000) | Izu Islands - Pacific | Whole otoliths | completed opaque | Y | Arbitrary (1st Jan ??) | N |
| Massey and Horn (1990) | NZ - Southern Pacific | Whole otoliths | completed opaque | Y | 1st August | Y |
| Anibal *et al.* (1998) | Azores - Atlantic | Whole otoliths | completed opaque | N | N | N |
| Kozolov (2014) | Azores - Atlantic | Whole otoliths | completed translucent | N? | June 1st | N |
| Lehodey and Grandperrin (1996) | New Caledonia - Pacific | Whole otoliths | completed translucent | Y | 1st January | N |
| Rico *et al.* (2001) | Macaronesian archipelagos - Atlantic | Whole otoliths | completed translucent? | Y | ? | N |
| Taniuchi *et al* . (2004) | Kato District Waters, Japan - Pacific | Sectioned otoliths | daily | N | N | N |
| Santamaria *et al.* (2006) | South West Indian Ocean | Whole otoliths | completed translucent? | N | 1st August | N |
| Macken and Krusic-Golub (2008) | East Coast Australia - Pacific | Whole otoliths | end of opaque | N | N | N |

# Consideration of preparation method Whole vs sectioned otoliths (AUS)



# Age reading protocol chosen

- The ageing process followed the one used by Massey & Horn 1990.

  1 – It was conducted on samples from the southern Pacific ocean

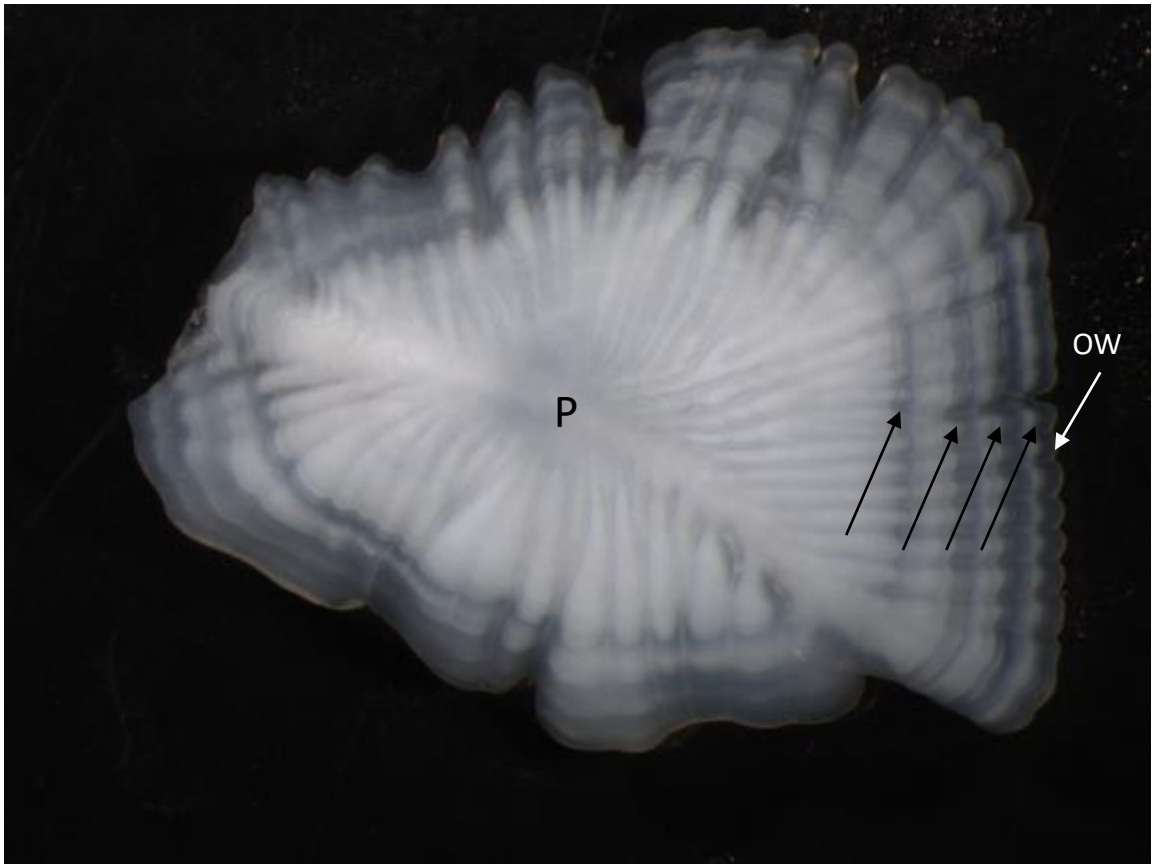
  2 – The analysis of increment formation was most complete (samples were available for all months of the year)

  3 - Peter Horn was to be the 2nd reader for this study

  4 – Method which we were most familiar with and aligned with methods used in our early Alfonsino ageing

# Method in brief

- One otolith from each pair was weighed to the nearest 0.0001g (only undamaged otoliths were weighed)
- Whole otoliths were immersed in water and illuminated with reflected light against a black background
- Magnification was set to 12.5x (Leica M125)
- Completed opaque zones were counted
- The edge of the opaque nucleus was considered to be the completion of the first annuli (approx. 9-10 months)




- Image analysis system
  - Automatically captures an image (either marked and unmarked)
  - Measures the distance between the primordium and each manually marked zone.
  - Automatically exports all the age reading data to a database including:

- Otolith Margin Classification
  - WT – Wide translucent
  - NT – Narrow Translucent
  - O – Opaque

  Note: These are the categories used routinely within FAS ageing methods and are hard wired into the ageing/imaging software. The edge classification is a little different to that used in Massey and Horn(1996) but hopefully similar in its application.

- Readability score:

  1 - Unambiguous & clear to interpret

  2 - More difficult than 1, however little doubt

  3 - Slight uncertainty, possibly zone count might differ by 1

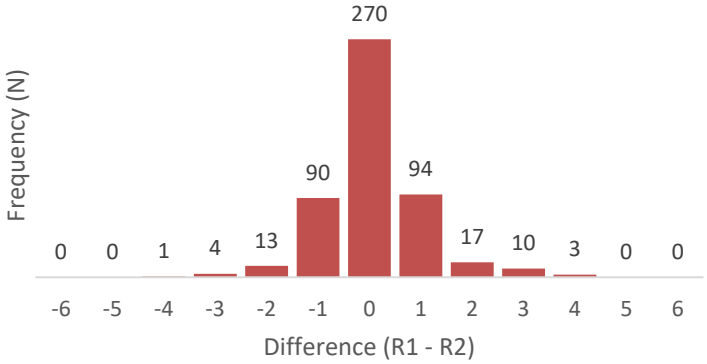
  4 - Some doubt, zone count could differ by 2 – 3 (usually only +/-1 from zone count)

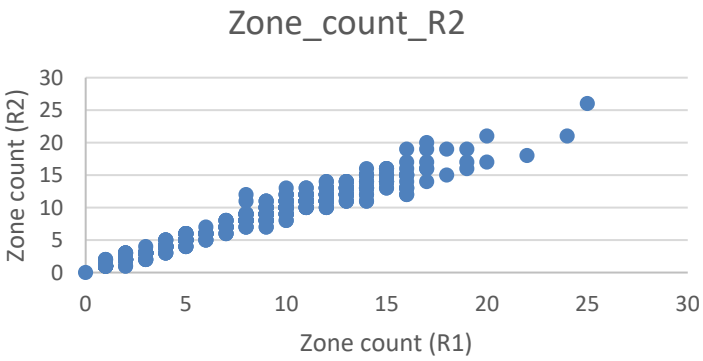
  5 - Unreadable/no sample

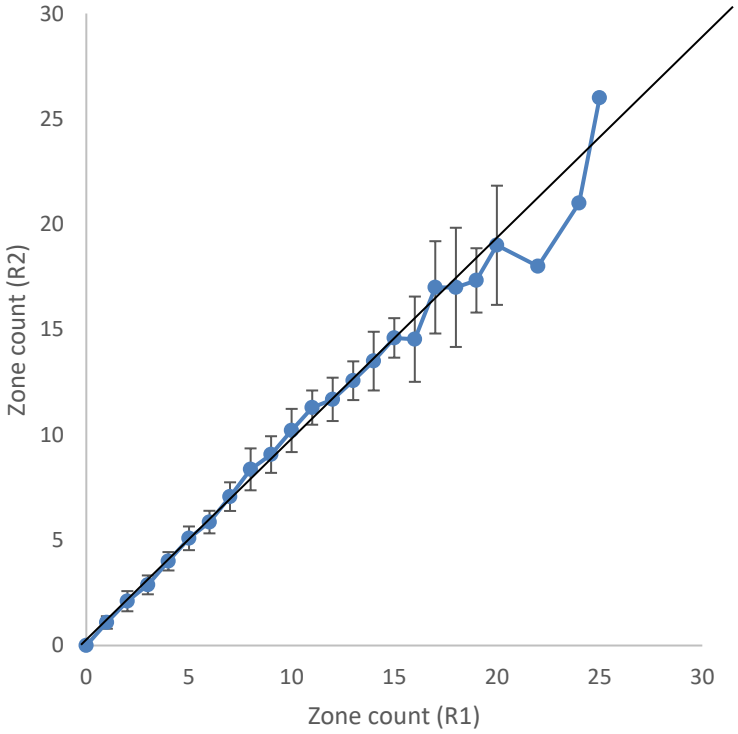
# QA/QC Used

- Age readers are required to read a testing set prior before reading a new set of otoliths
  - Must show acceptable precision and no bias
- Each otolith was required to be read by 2 readers
  - Due to COVID-19 restrictions Peter Horn was unable to do a full reading
    - Reader 1 & Reader 2 - Fish Ageing Services
    - Reader 3 (PH) - (10% re-read on supplied images)

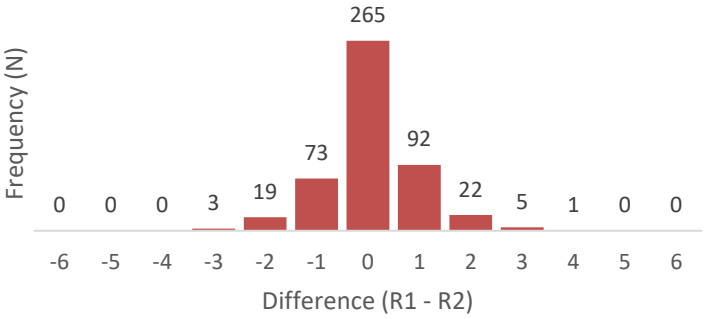
# Results QA/QC – Between Reader EAST

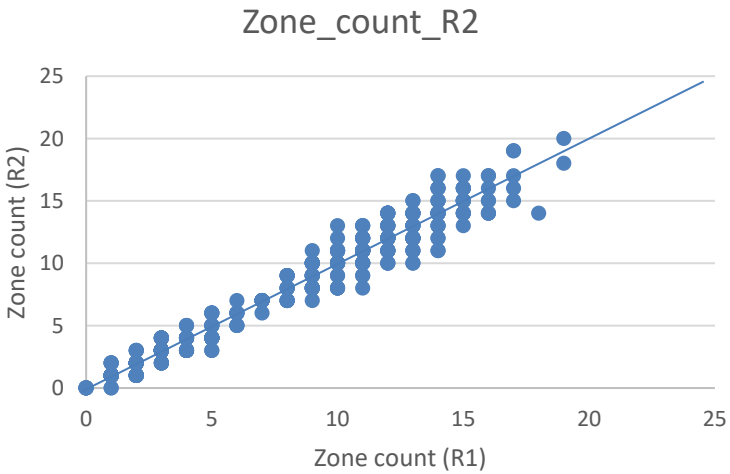


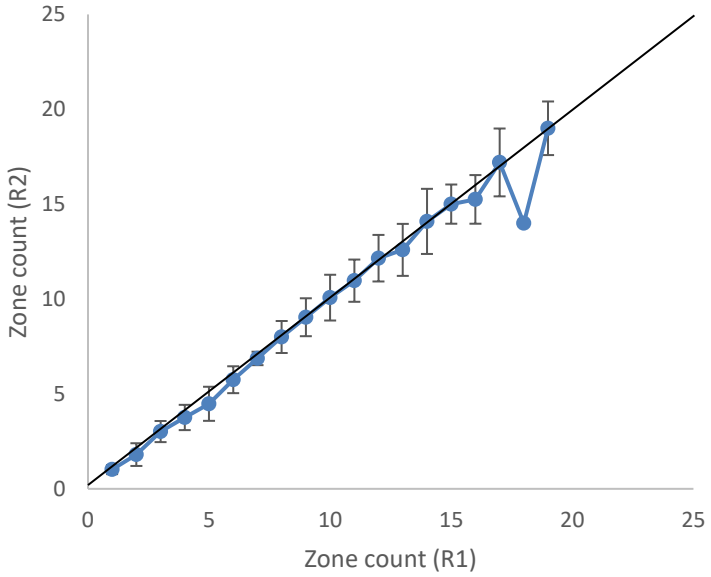



# Results QA/QC – Between Reader WEST

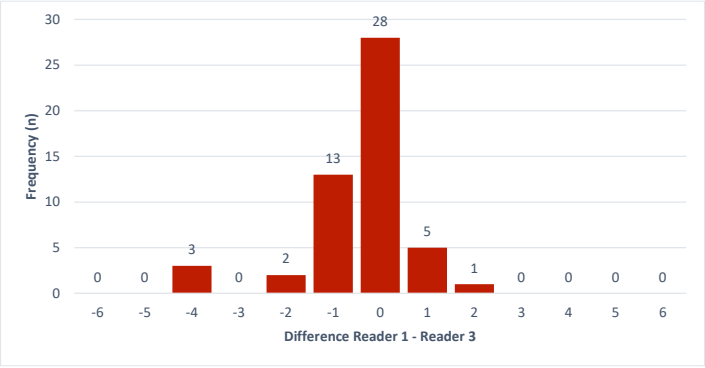


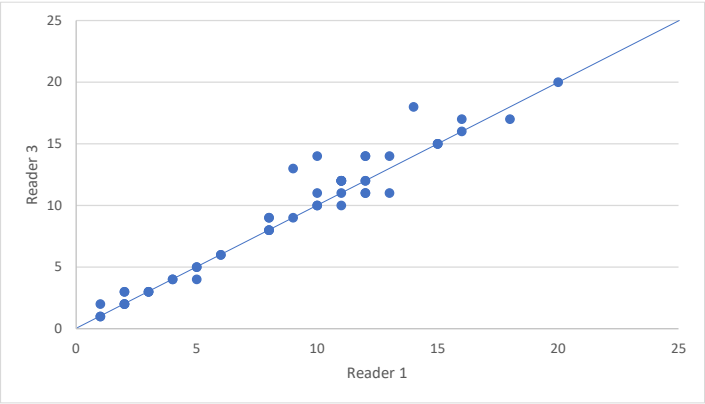



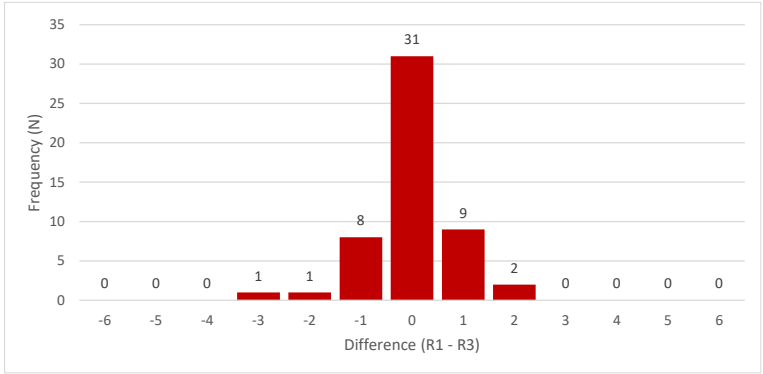
# Compared readings with Reader 3

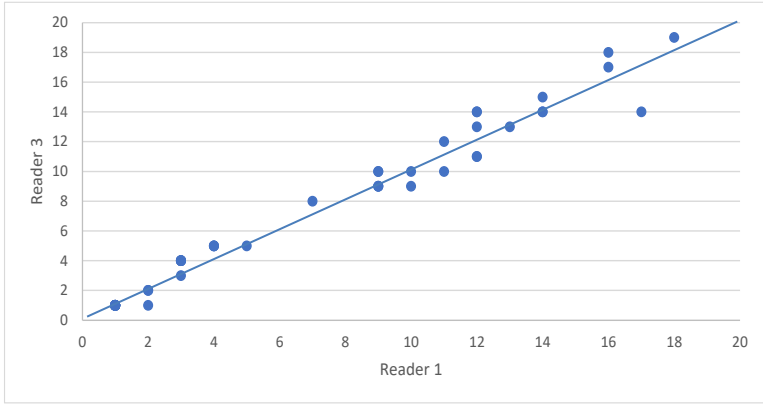
## Eastern




## Western



# Zone/Age adjustment protocols

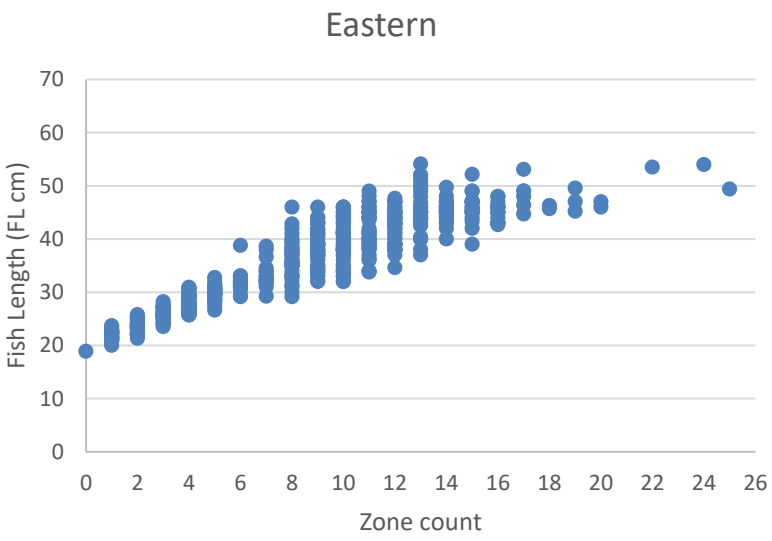
- No zone adjustment was performed on these samples (for the moment!)

- EAST – the capture months were outside of those months recommended in Massey and Horn (2000). May – October

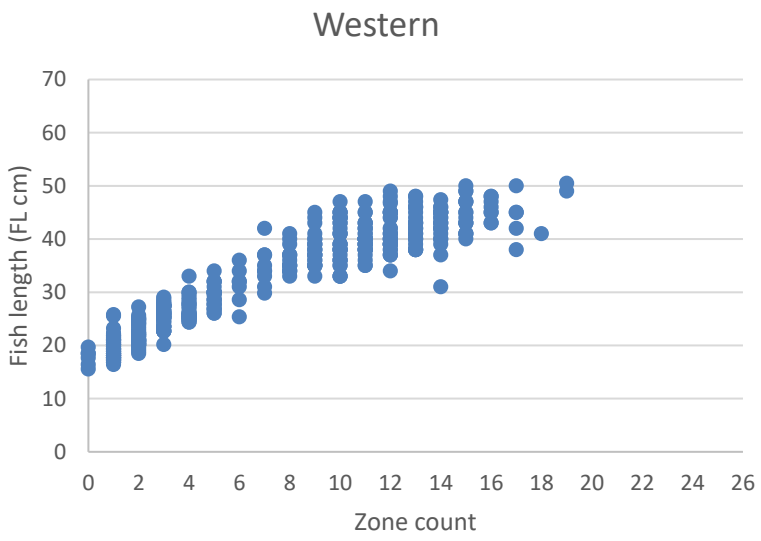
- WEST – when I closely reviewed the Massey and Horn method I was not confident that the adjustment protocol suited these samples. Therefore zone count = age

# Ageing results
# Zone count/ fish length relationship

## EASTERN

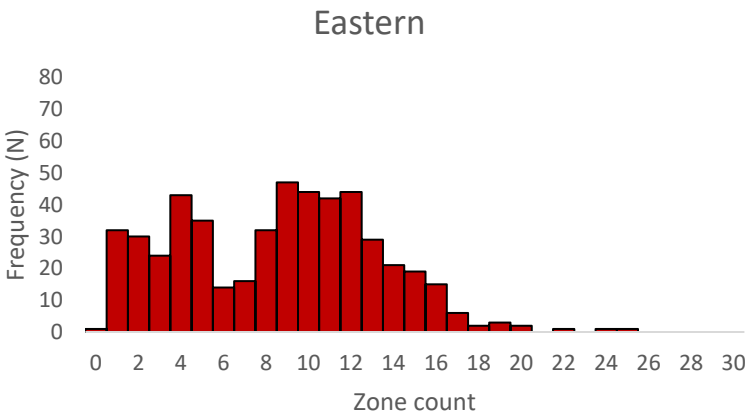



## WESTERN

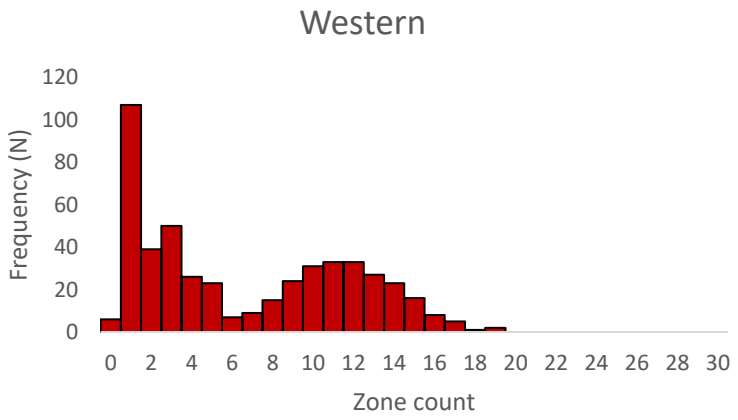


# Ageing results
# Zone count composition

## EASTERN




## WESTERN



# Considerations

Throughout the literature it is evident that variations of a similar method have been used

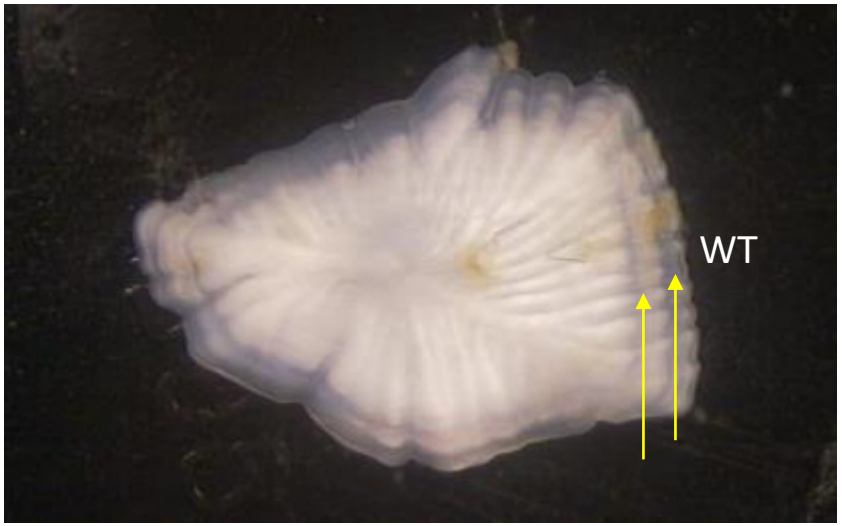
- Therefore direct comparison of age data may be complicated because not all age reading protocols were the same
  - Some studies counted completed opaque zones, others counted completed translucent zones
  - Position of the first annuli differed
  - Marginal Increment or marginal state studies were not always consistent in their approach

  Lack of information on the otolith zone formation periodicity for Indian Ocean samples.

- Santamaria study used the assumptions that:
  - Translucent zones have finished forming by July/August – *information sourced from other studies*
  - The completion of the first nucleus is approximately 9-10 months and that the subsequent (1st) translucent zone may only be formed over a few months. (Lehodey and Grandperrin)
  - If an 1st August birthdate (sourced from Massey & Horn and Adachi) is used then the biological age at the completion of the first annuli should be approximately 12 months.
- One benefit for using these assumptions is that both zone adjustment and birthday adjustment can use the same months

# Suggested protocol for future age readings

- Follow Santamaria
  - Count completed translucent zones




So this sample becomes 2+ rather than 3+ (assuming that the edge assignment is WT. If it was WO, then both counts are 2+

- Method should also include:
  - A more descriptive in the edge classification
    - TN, TW, ON,OW - width needs to relate to the width of the full annuli not just the marginal zone
  - An adjustment date of 1st August for zone formation and timing
  - If we assume that translucent zones finish forming by July/Augst the method can still use months May – October
  - Consider converting whole age to a decimal age. This might also help with the comparison of data sets from different studies.

# Question of longevity - Maximum Age = 25?

- The largest sample in the AUS otolith set used in the comparison between whole and sectioned otoliths was 48cm (FL) and the otolith weighed 0.4110g

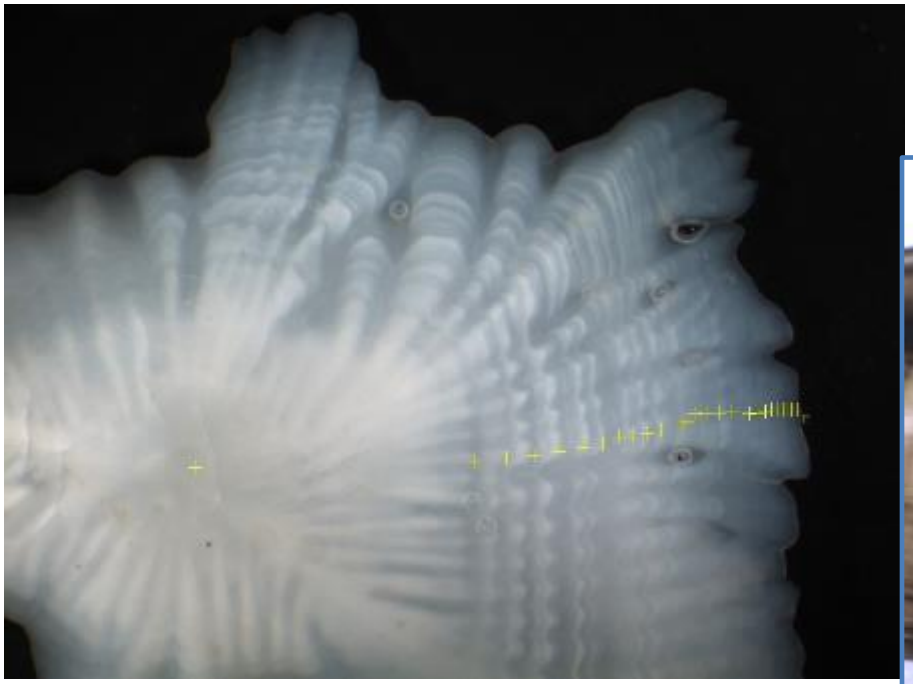
- The largest sample in the SIO otolith set was collected from a 49cm (FL) male and the otolith weighed 0.7139g

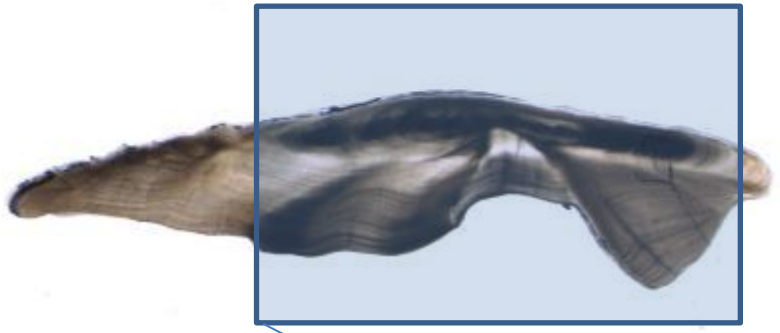
- In the current SIOFA ageing set, 32 % were larger than 40cm, compared to just 8% for the whole vs sectioned comparison set.

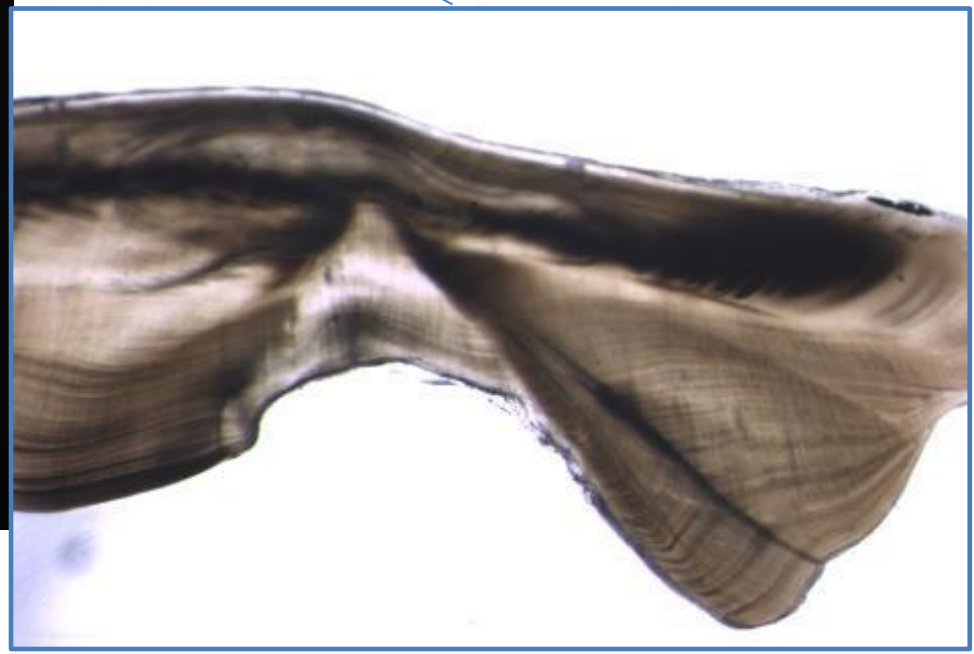
# Investigation of the 2 largest samples

- Sample 706_083_314

53.5cm and otolith weighed 0.6122 – Whole age = 22

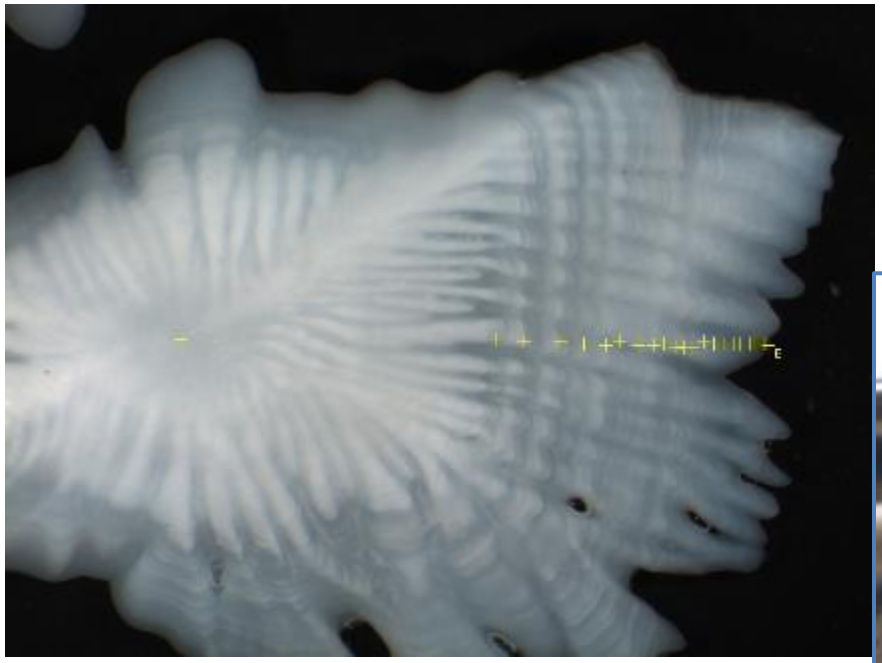


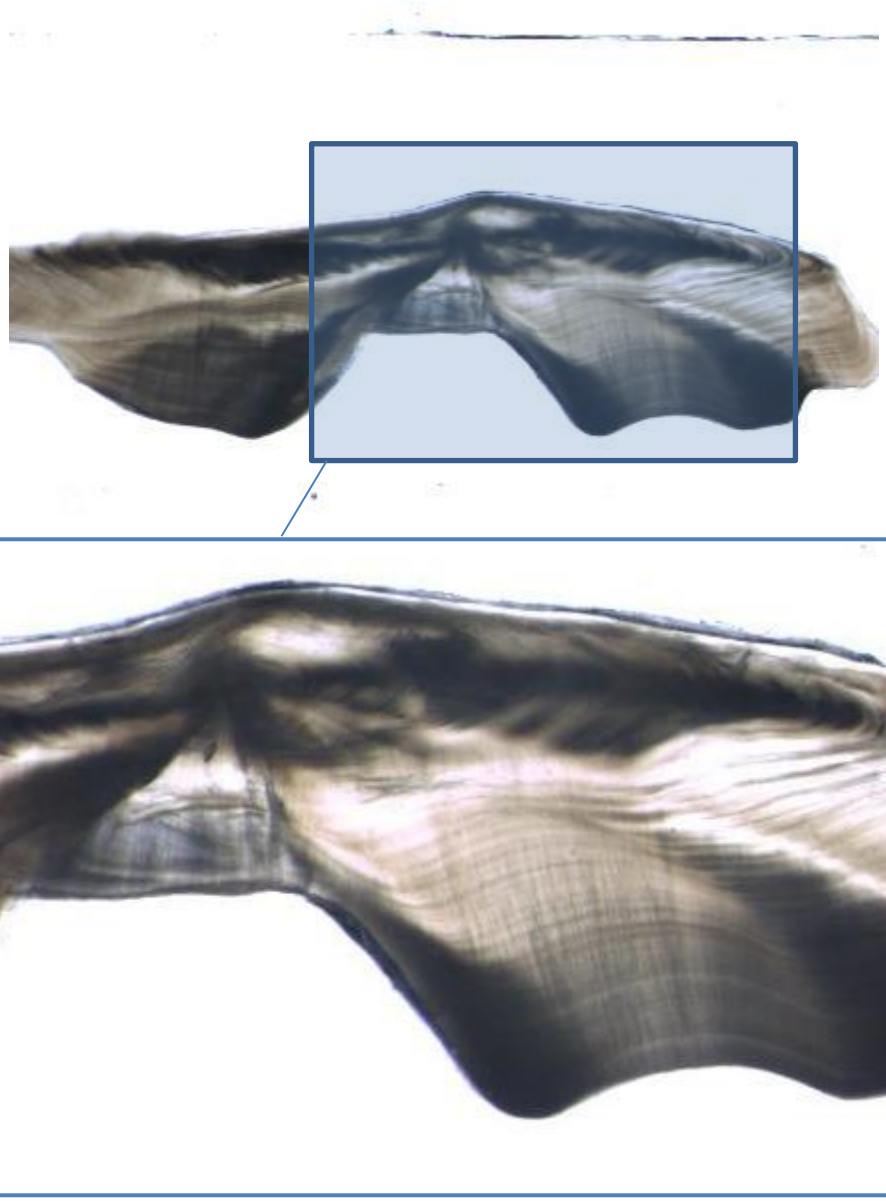




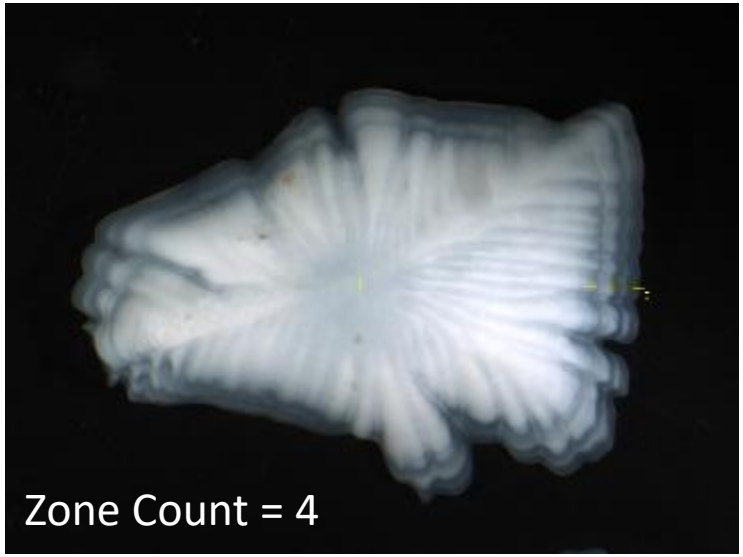
- Sample 706_083_324

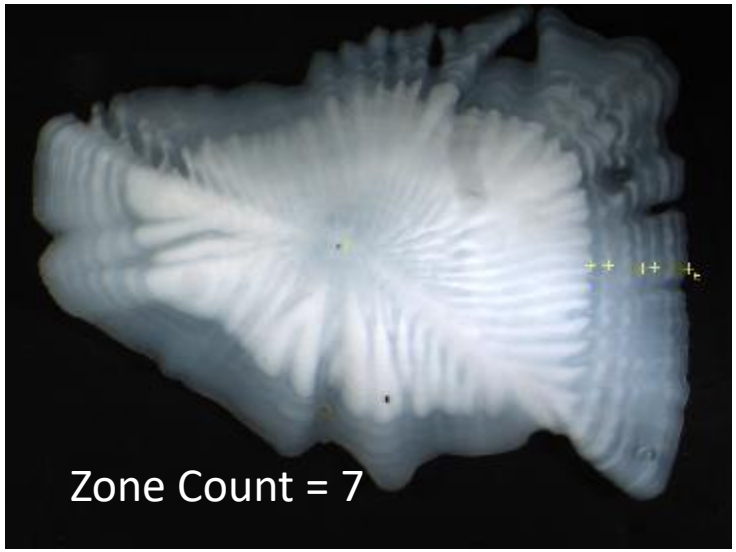
49.4cm and otolith weighed 0.7139 – Zone count = 25

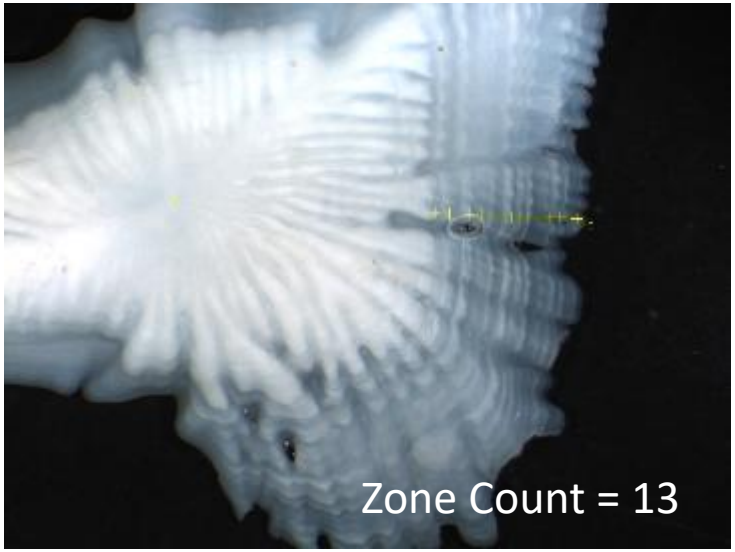




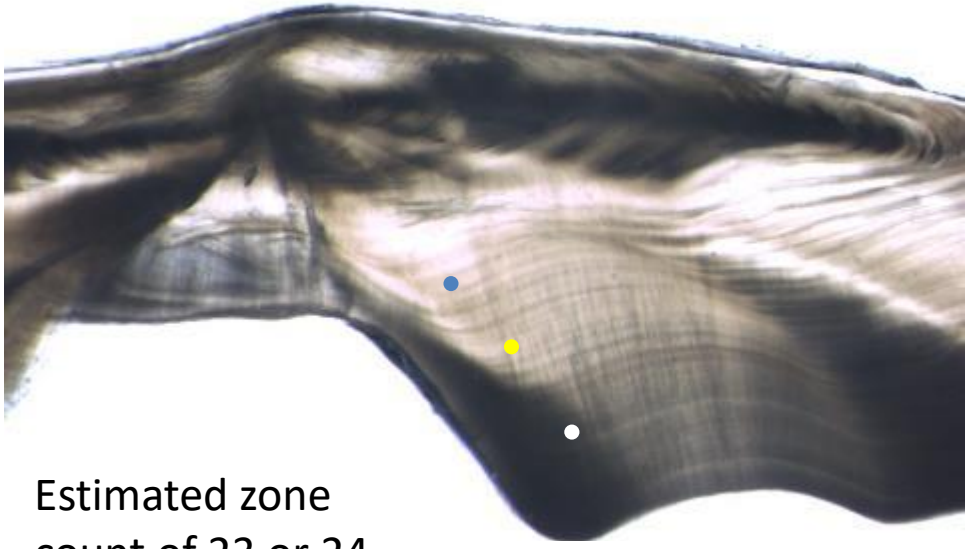
- To help with interpretation 3 samples were selected with readability 1 - Zone counts 4, 7 & 13



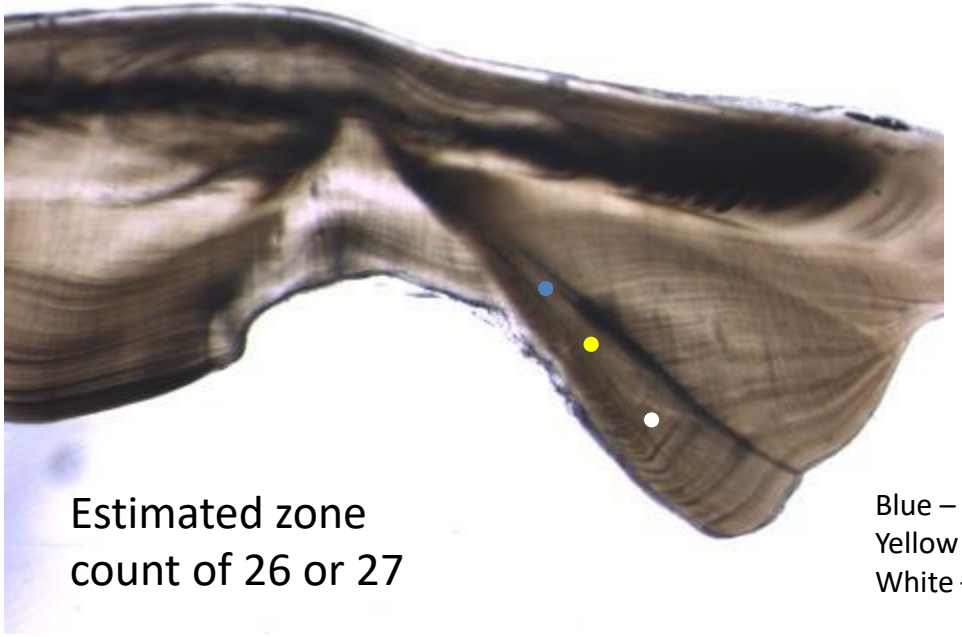




- The 2nd otolith from each pair was sectioned and the distance between the primordium and the edge was measured
- The measurements were overlayed on the images of the 2 larger otoliths



Estimated zone count of 23 or 24



Estimated zone count of 26 or 27

Blue – approximate size at age 4
Yellow – approximate size at age 7
White – approximate age at age 13

# 2 recommendations moving forward

- Verify the annual deposition of zones and determine the zone formation timing (MIA or edge type analysis) for SIO Alfonsino
- Verify longevity  (Bomb radiocarbon ??)
  - This could
    - provide an indication if age estimates from whole otoliths could be underaged
    - Provide information on ageing bias

# References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