## Otoliths

### Collection

* Scotia Sea
* RRS James Clarke Ross
* 1998-2016
* JR38, JR177, JR15004 and JR16003
* Action: add map.

### Otolith Preparation

* Sagittal otoliths used.
* Otoliths removed from package and placed in petri dish filled with tap water for approx. 1 minute.
* Tissue and stuck packaging carefully removed while in water with tweezers and edge of a mounted needle.
  + Washed away.
  + Water changed between washes.
* Blotted and left to air dry.
* Mounted onto resin backing plate.
  + Struers EpoFix resin.

### Milling

* Milled using ESI New Wave Micromill.
* Outer surface, 100-200um deep.
  + Based on surface profile.

## Isotope Analysis

* Expressed permil (%o).
* Standard for C and O is Vienna Pee Dee Belemnite.
* dX = ((Ratio\_sample – Ratio\_standard)/Ratio\_Standard) \* 1000
* Analysed using Thermo Scientific Kiel IV Carbonate device coupled with a MAT253 isotope ratio mass spectrometer.

## M Calculation

* M = (d13C\_oto – d13C\_dicSW)/(d13C\_diet – d13C\_dicSW) + e
* Where δ13Coto is the δ13C value of the fish’s otolith, δ13CDIC−SW is the value for δ13C of dissolved inorganic carbon ingested by the fish through seawater, δ13Cdiet is the δ13C of the diet, and e is the isotopic fractionation factor.

### Components

* DIC – Tagliabue & Bopp, 2007
  + By location.
  + Corrected for Suess effect.
* Diet – based on d13C phyto and trophic level.
  + D13C phyto – from Magozzi et al. 2017.
  + Trophic level – from FishBase.
  + Trophic enrichment factor – 0.8 (sd 1.1) from DeNiro & Epstein 1978
* E – 0 (sd 1.8) from Solomon et al. 2006
* Repeat 10,000 times.
  + M – value with highest density.
  + Stan dev, min and max.

## Temperature Calculation

* Temperature = ((d18O\_oto-d18O\_SW) – a)/b
* Where δ18Ooto is the δ18O value of the fish’s otolith, δ18OSW is the δ18O value of the seawater, and a and b are set parameters.

### Components

* D18O\_water – Schmidt et al. 1999
  + By location and depth.
* Parameters – Hoie et al. 2004
  + a = 3.9 (sd 0.24)
  + b = -0.20 (sd 0.019)

## Statistics

* Performed on random samples of M and temperature from truncated normal distribution from outputs of calculations.
* Repeated 1000 times, got maximum density and 95% HDI.
* Kruskal-Wallis and Dunn test to look at differences in M between species.
  + Dunn.test package.
  + Bonferroni correction.
* Linear mixed-effect models to look at effects of weight and temp on M.
  + M is nested within species.
  + Use lme4 package.
  + REML.
  + lmerTest to get p-values.
* Compare d13C values for myctophids against d13C from my data and Sherwood & Rose, 2003.
* Code for M and temperature calculations, and statistical testing available in appendices.