NPRB 2301: PSS Ageing

October 2023

# Meeting Details

NPRB 2301: October Sample Discussion

Wednesday, October 4 · 10:00 – 11:00am

Time zone: America/Anchorage

Google Meet joining info

Video call link: https://meet.google.com/evi-xrjj-gwf

Or dial: ‪(US) +1 573-401-1269‬ PIN: ‪710 309 929‬#

More phone numbers: <https://tel.meet/evi-xrjj-gwf?pin=6290999497904>

# Resources

Project [Public Repository](https://github.com/CindyTribuzio-NOAA/PSS_Ageing/tree/main)

Project [Google Folder](https://drive.google.com/drive/folders/1S9ppVeVSIG6iSDOY1e9cJ0GU9jdOUmsB)

# Agenda (notes taken during meeting are in blue below)

## Updates

* FYI: National cell phone alert at x:20
* Tentative in-person meeting Jan 31st, with some option for partial day either the day before or after. Stay tuned for more details.
* Next virtual meeting in December?
* We still need a team name!!!!

## Budget Updates

AFSC budget shut down until at least Oct 25

* Some reallocation expected
* Likely cover some supplies and/or travel to make up for unexpected ARC expenses

ARC budget is nearly available?

* Some reallocation expected, need to purchase a fridge

Still getting LLNL/NPRB agreements figured out - stay tuned

## Samples

Overview of performance of sample prep so far

Dan did test peels of freezer burnt spiny dogfish

Trial runs were ok

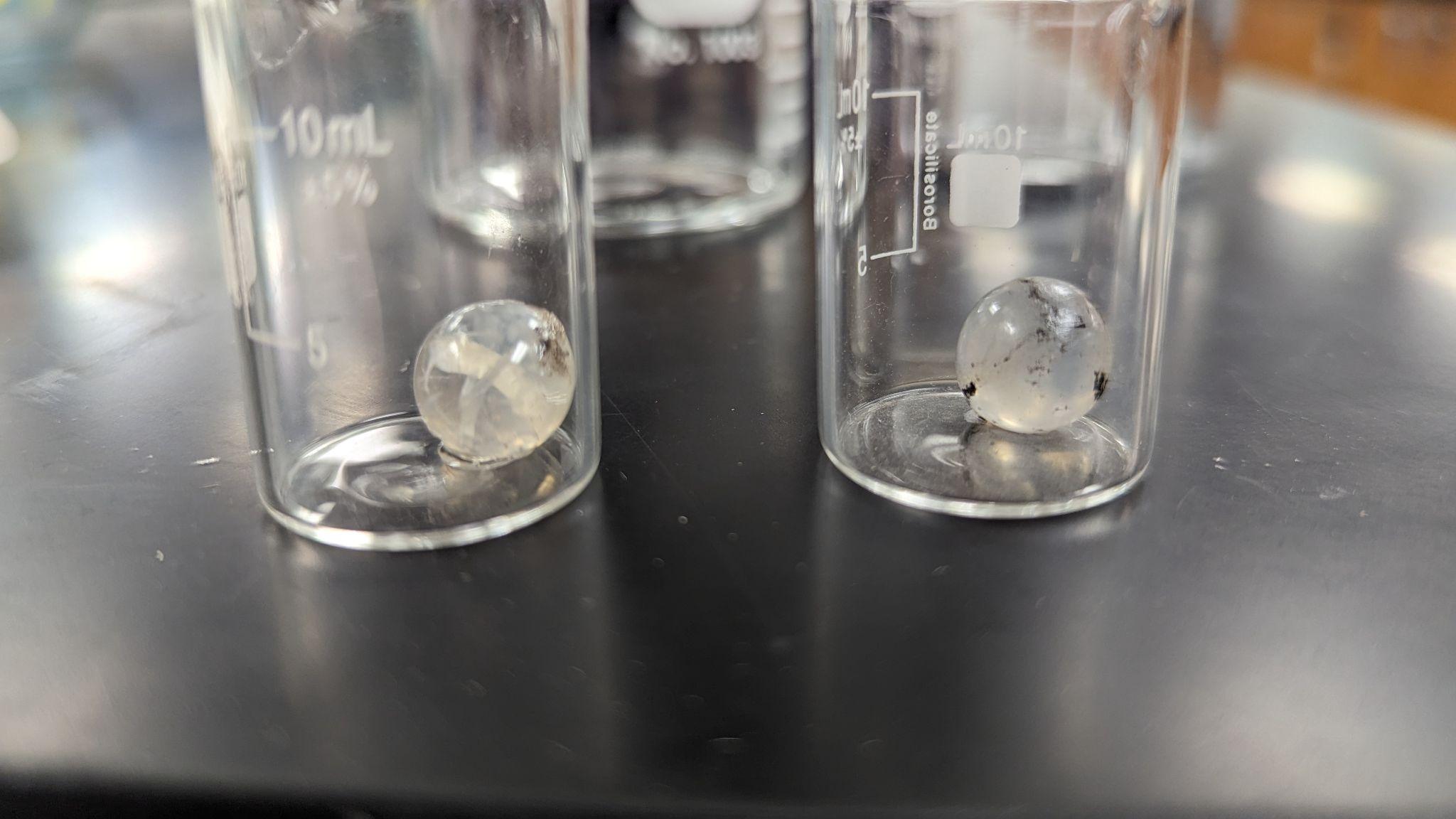


Figure 1. Lens cores?



Figure 2. Notes from Dan: there are two distinct regions of the lens, and I believe these to be the cortical portion (transparent) and the nuclear region (opaque). I recognize this from when I worked with human lenses as well, where the freeze/thaw can sometimes affect the nuclear and cortical regions of the lens differently, as the cortical region seems to "bounce-back" from the trauma and appears better at regaining its optical transparency when compared to the nuclear region. Another thing you can see is the success we had unpeeling the lens as layers (instead of having it break apart into chunks, ooze, etc). That little feather-looking thing is a layer peeling off, and you can see how cleanly and evenly the process has been working. This photo was taken after 2 decent-sized layers were already removed. The unpeeling process is really cool to watch- as you may see in the photo- these fibers are not visible within the lens until they peel away from the transparent whole of the lens. NOTE: opacity is not necessarily indicative of “good” or “bad” lenses.

Notes from discussion: Most of the samples look OK (only a couple looked compromised) and Dan is ready to go on samples for the analyses. Cindy will ship using Gold Streak and better cooler from now on (maintains freezing, prevents thawing)-future shipping should keep the samples well preserved.

Storage and transferring plan

* All frozen eyes currently at ABL/Juneau
* Ship in batches once funds are accessible and space allows. Very limited freezer space at ARC.
* Dan’s lab will process samples, store tubes in fridge at home until transfer to LLNL
  + Any supply needs? At least one tube per sample. Fridge purchased until sent to LLNL.

How should samples be sub-sampled?

* Eye lens layers, how many
* Size - Range of sizes will be best
* Sex - Try to stick with females (although some of our biggest fish are males). Growth curves are probably really different.
* Region - Most from EBS and WA. But some of the largest ones are off OR, AI, and SE. Sampling across the regions will work (given limitations of what’s available)

Dogfish: we have embryos, mom eyes, and spines for the ones in the table. Cindy and Beth (and Allen?) will age the spines. We have fewer than we had wanted (9/20 pairs). May allow more flexibility for additional samples in other analyses (e.g., PSS age validation part).

Dan thinks getting 4 layers per lens (especially PSS) will be doable. The core is usually easy to identify visually and by no more delamination. Sometimes there are factors beyond control (e.g., compare left and right eyes - may be hard to control for delamination at same layer of eye). Hard to control the rate of delamination. Which layer is which? Sometimes it can take a long time for the process (start and stop). Assess how many layers he can get and then combine later. Test effect of discrete vs combining the left and right against each other? May have to adjust plans depending on how the process goes. Best plan would be to get as many layers from lens as possible and then go from there.

Dan thinks there may be enough material from each layer that we could rerun AMS - could be a good way to compensate for fewer SD. Limiting factor = 50 mL falcon tube - not enough water for bigger lenses. May have to go above 50 mL (split among multiple falcon tube)-could get complicated to keep track of layers. Will need more supplies - racks, more tubes. Sharpie is OK to label tubes.

Too many layers may not be good because the samples could get too small (especially for the part separately soluble vs insoluble). Target mass ~ 200 ug. Budget is based on compact. Subsamples for amino acids will need to come from these samples too. For CSIA-AA, Taylor will check on min sample mass (may want to test to determine extraction efficiency-ideally after protein extraction that Bruce is doing). For bulk, more flexibility in sample mass (probably can be done with ~50 ug but Taylor will check) - sounds like there should be plenty of material.

Outer layer includes lens capsule and produces a lot of material- could contain embryonic material.

Can’t remove lens from solution during delamination process. One of Dan’s students is brainstorming ways to measure. Could take a picture of lens in beaker with known-size object (e.g., graph paper). Would need to figure out how accurate this would be though. Revisit whether this is feasible and adds to much time to process.

Numbering scheme - specimen ID + layer ID (and eye side)

Preference for easily sortable labeling e.g. species, spec#, sample type, layer #

E.g., sleeper shark specimen 5, eyeA, layer 3 = PSS005\_A\_L3, spiny dogfish specimen 3, embryo1, layer 1 = SD003\_E1\_L1

Potential samples:

Eye A = A

Eye B = B

Eye Left = L

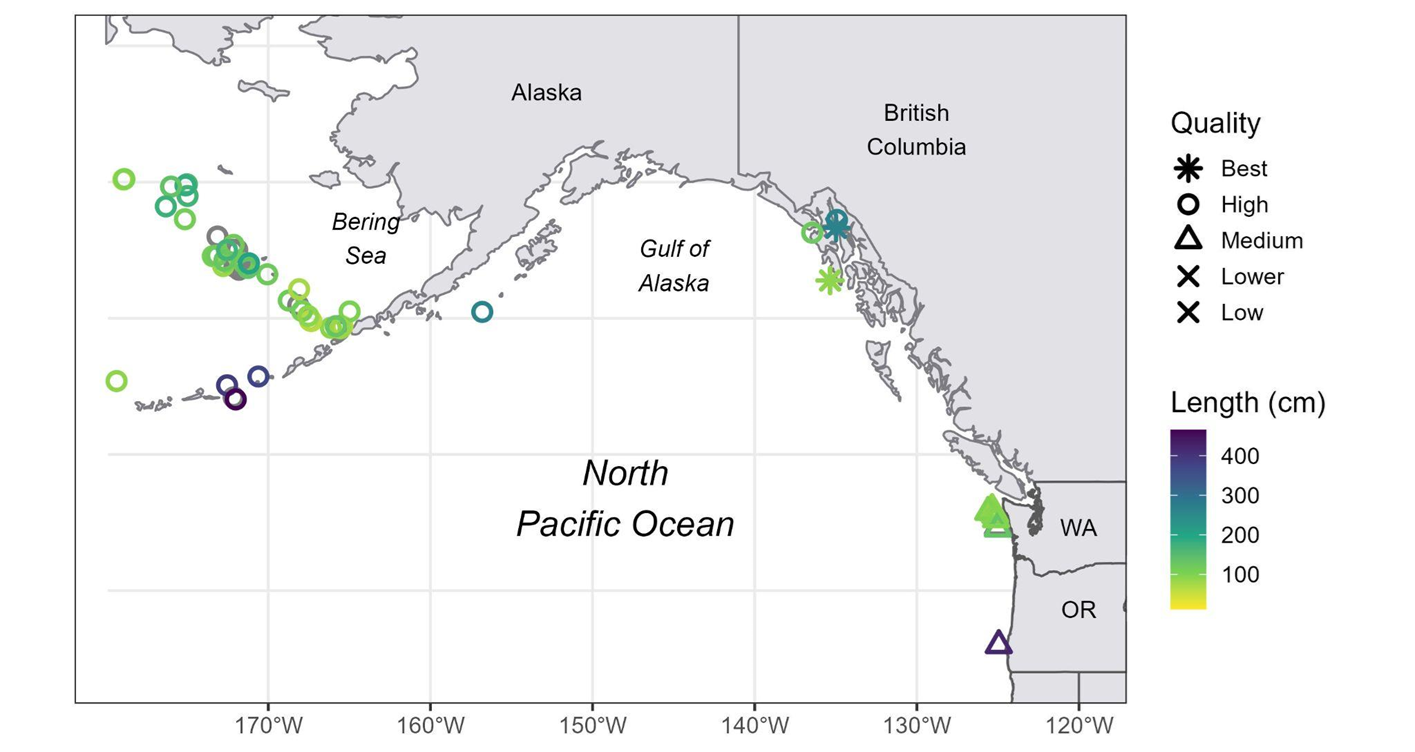
Eye Right = R

Embryo 1 = E1

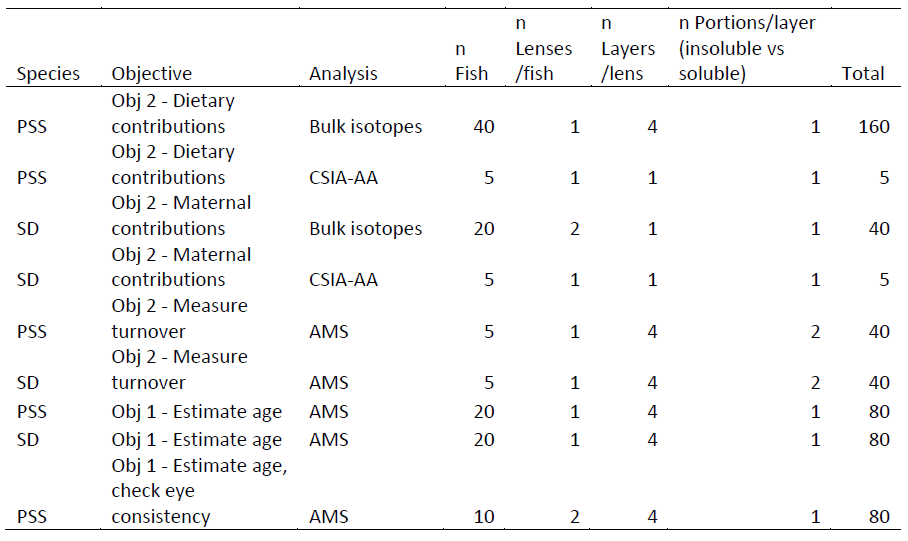
Embryo 2, etc = E2

Spine = S

Candle (effectively an embryo, but too early in gestation, we have one dogfish like this) = C



Timeline to start running samples at LLNL? Should they be in a big batch, or small batches? What is the optimal number of samples to run at LLNL at a given time?



## Dogfish samples available

What samples are in Dan’s freezers already, are they part of the below list?

Below are longline survey samples, missing length data for 2020 samples, but still digging. Is it worth collecting in the summer of 2024? Large pregnant females are getting hard to find (scary on it’s own).

| Species | Source | Length\_cm | Length Type |
| --- | --- | --- | --- |
| Squalus suckleyi | 21LLS | 91 | PCL |
| Squalus suckleyi | 21LLS | 92 | PCL |
| Squalus suckleyi | 21LLS | 91 | PCL |
| Squalus suckleyi | 21LLS | 90 | PCL |
| Squalus suckleyi | 20LLS |  |  |
| Squalus suckleyi | 20LLS |  |  |
| Squalus suckleyi | 20LLS |  |  |
| Squalus suckleyi | 20LLS |  |  |
| Squalus suckleyi | 23LLS | 84 | PCL |

## DRAFT roles/expectations/manuscripts

Preferred platform for project analytics is R and our goal is to have analyses be reproducible and available on GIThub. This will not undermine our ability to publish, but does make collaboration much easier. If unfamiliar with R, use your preferred platform, but we may translate it to R at some point, especially for graphics and publication purposes.

Obj 1. Evaluate 14C values in PSS and SD eye lens cores (earliest material) and outer layers (recent material) as an indicator of age. Assess the eye lens 14C chronology by comparison with validated ages of SD. Estimate plausible ranges of age-at-length, age-at-maturity, and lifespan of PSS.

1. Do the reference 14C samples from SD eye lenses portray an accurate bomb 14C timeline?
2. Do 14C levels in eye lens cores from PSS of different sizes show evidence of formation during the prebomb, bomb-rise, or post-peak periods as an indication of birth year or temporal constraints on the age of each specimen?
3. Can accurate estimates of age be derived for PSS from eye lens 14C values and does sequential sampling work as a proxy for 14C uptake through ontogeny?

Obj 2. Investigate sources of potential variation in isotopic values (13C, 14C, and 15N) of shark eye lens protein. These include sample quality (e.g., freezing/thawing altering the structure of the eye lens), the potential for protein turnover in eye lenses, dietary and maternal contributions, and the effects of habitat, depth, and region on isotope uptake.

1. Does carbon turnover occur in shark eye lenses, and if so, would it affect the interpretation of 14C values as a temporal reference and require a correction factor? (lead - Dan)
2. What are the sources of carbon in the shark eye lens? (lead - Taylor)

Obj 3. Estimate age-related life history parameters (and associated uncertainty) used in stock assessments and test more robust stock assessment approaches. (lead - Cindy)

Obj 3 nicely ties in with ongoing projects:

* meta analysis to estimate L50 and M (Garrett Dunne UAF),
* non-target refined ORCS (nORCS) and simulation testing DLMs for PSS (Tribuzio, Cope and Free)