Pacific Sleeper Shark Eye Lens Preparation

This document details the steps to take to prepare eye lens layers for isotopic and AMS analysis. Preliminary work has demonstrated that “smaller” eyes unpeel differently from “larger” eyes in the eye layer delamination step. Smaller eyes tend to have a more gooey outer set of layers that are difficult to differentiate, and tend to result in a nuclear core and one set of layers. Larger eyes can delaminate in many layers. We hypothesize that this difference is correlated to maturity, but have little data to confirm. The majority of eyes originate from Pacific sleeper shark (PSS) < 2m pre-caudal length and are “small”.

# Labor Estimates

These estimates are still fluid, as information gets collected. Attempted to estimate high. The below processes would need to be performed at ABL.

Assumptions

* 3 layers per eye
* 170 eyes
* 3 hrs per eye for delamination
* 1 hr per layer for pre-AMS prep (spinning (n=20)/freeze drying (all)/separating isotope samples (n=220))
* 6 total hours estimated per eye
* Space and equipment availability at ABL

To date:

44 eyes have been delaminated, expect another 40 through the end of May

~100 layers are currently in solution, expect maybe another 100 layers through end of May

Timeline of needs

| Month | Task | Est Hrs |
| --- | --- | --- |
| May (assuming we can actually start in May) | Freezer inventory | 8 |
| June | pre-AMS prep | 160 |
| July | pre-AMS prep | 160 |
| August | pre-AMS prep/delamination training | 160 |
| September | delamination | 160 |
| October | delamination/pre-AMS prep | 160 |
| November | pre-AMS prep | 160 |

# Eye Layer Delamination

## These steps are currently being conducted by a collaborator at ARC. Other collaborators will need to pick up this task late summer/early fall. Total remaining sample size for summer/fall TBD

## Equipment

Ultra pure water

Stir plates(s)

Magnetic bars

Forceps

Scalpels

Beakers

Falcon tubes

Camera

Dissecting dish

Powder free gloves

Labeling notes

Each layer will be labeled with

* Species - PSS or SD (for spiny dogfish)
* Sample\_id - this is the eye specific number, which comes with the frozen eye, usually on a slip “Tribuzio Shark Sample # XX”
* Side - left or right eye
* layer number - #1 for first layer peeled away, with the highest number being the nucleus. In the case of small PSS eyes, there are only two layers, the “nucleus” and “remainder”

Examples: small PSS sample #157 would have layer numbers “PSS\_157\_L\_nucleu” and “PSS\_157\_L\_remainder”. A large PSS sample #48 which resulted in 3 layers would have layer numbers “PSS\_48\_L\_1”, “PSS\_48\_L\_2” and “PSS\_48\_L\_3” where 1 is the outermost layer and 3 is the nucleus.

## Procedure - Small Eyes

Insert work area prep steps (est 30 mins set-up time)

1. Place eyes for the day in cold water to begin defrosting. It is important to keep as cold as possible. (> 30 mins?)
2. Prep work area (~ 30 mins?)
   1. Insert steps here
3. Place eye in dissection dish and with scalpel, gently remove excess tissue from around lens. Caution against cutting into the eye itself
   1. Note: if eye shows degradation/decomposition skip to next eye
4. Place lens in beaker with XXXmL of ultra pure water and a stir bar
   1. Take image of lens with grid paper in background
   2. stir (fast/slow/med) until (insert characteristics for how to determine when “done”)
5. Pour off solution into labeled falcon tube and place in refrigerator
6. Add XX mL of ultrapure water to beaker
   1. Take image with grid paper in background
   2. Stir (fast/slow/med) until XXXXX to verify no more layers peel off
7. If no more layers peel off, then remaining is the nucleus core
   1. Pour off solution into labeled falcon tube and place in refrigerator

## Procedure - Large eyes

1. *We can fill this in later, not priority at the moment*

# Separating soluble/insoluble fibers

These steps will be conducted on a small subset of samples, ~40 layers from ~10 eyes. Processing will need to begin May/June with existing delaminated layers (as of 3/37/2024 n = ~80 layers, more coming)

## Equipment

Centrifuge with rotor for 50mL falcon tubes, capable of 20k gs at 4C *(Note from RECA - Matt Rogers: “I just realized that we have an insert for one of our centrifuges that will take 50 mL falcon tubes! I haven't used it but I will try it with water - I am pretty certain it will work. It can spin 16 tubes at once and goes up to 5000 rpm.” Using the diameter of the rotor,* [*we can convert that to rpm*](https://assets.thermofisher.com/TFS-Assets/LSG/Application-Notes/TR0040-Centrifuge-speed.pdf)*. Will the RECA centrifuge work?)*

Falcon tubes

Pipette (size? tips?)

## Procedure

1. Spin tubes at 20,000g for 30 mins at 4C
2. Pipette supernatant (which contains the water soluble crystallin proteins) into clean falcon tube
3. Rinse remaining pellet with XXmL ultra pure water and pipette rinse water into tube with supernatant (x2)
4. Resuspend pellet by adding XXmL of ultra pure water and shaking?
5. Refrigerate tubes

# Lyophilizing Layer Solutions

These steps will take the solutions created by the eye delamination, and where applicable the spinning, and dry samples to prepare for shipment to WHOI for AMS. The sample size is not set, likely 300-500 total tubes, depending on where budget falls out. Processing will need to begin May/June with existing delaminated layers (as of 3/37/2024 n = ~80 layers, more coming)

## Equipment

Freeze Dryer requirements(?)

[Scintillation vials](https://us.vwr.com/store/catalog/product.jsp?catalog_number=66022-060)

## Procedure (Cindy’s guesses until more info available)

1. Label vials to match layer labels.
2. Fill vial with solution, use multiple vials if necessary *(What is the target volume? If multiple vials are used, can they be consolidated after drying?)*
3. Dry
4. Store dry at room temperature until AMS?

# Subsample for Isotopes

A subset of dried nucleus and layers will be subsampled for further isotopic analysis. Current sample sizes are n=200 for bulk isotopes and n=20 for CSIAA *(Will these be from the same layers as the bulk isotopes?*). Processing will need to begin May/June with existing delaminated layers (as of 3/37/2024 n = ~80 layers, more coming). All isotope samples will be run by a project collaborator at WHOI/UCSC.

## Equipment

[Tin capsules](https://us.vwr.com/store/catalog/product.jsp?catalog_number=101189-554)

Weigh boats

Scale (resolution?)

scoop?

## Procedure (Cindy’s guesses until more info available)

1. Label tin capsules to match layer labels
2. Extract XX g of dried tissue from vials in previous section, place in tin capsule
   1. If multiple vials are used in previous section and result in large enough volume of sample, can replicate vial be used for isotopes?
3. Store dry at room temperature until shipping

# Things to purchase

Likely, depending on current supplies and the end sample size. Discuss with RECA to see what we need to provide to use their lab space.

Powder free gloves

falcon tubes (started with 500)

scintillation vials (started with 500)

Tin capsules (started with 250)

Scalpels

Dissecting bowls

Tube racks (maybe not)

Beakers?