SEDAR 80- WP03: Photographic Guide to Extracting, Handling, and Reading Otoliths from Balistes Triggerfish Species

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All images by V Shervette

Photographic Guide to Extracting, Handling, and Reading Otoliths from *Balistes* Triggerfish Species

**Starting just after fish samples have been measured and processed for any additional purposes

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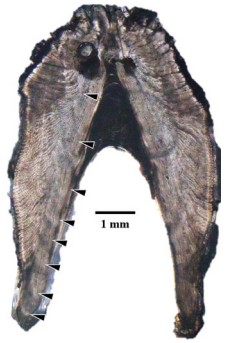
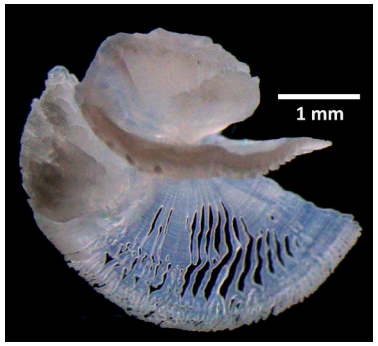
Why Bother with Triggerfish Otoliths?





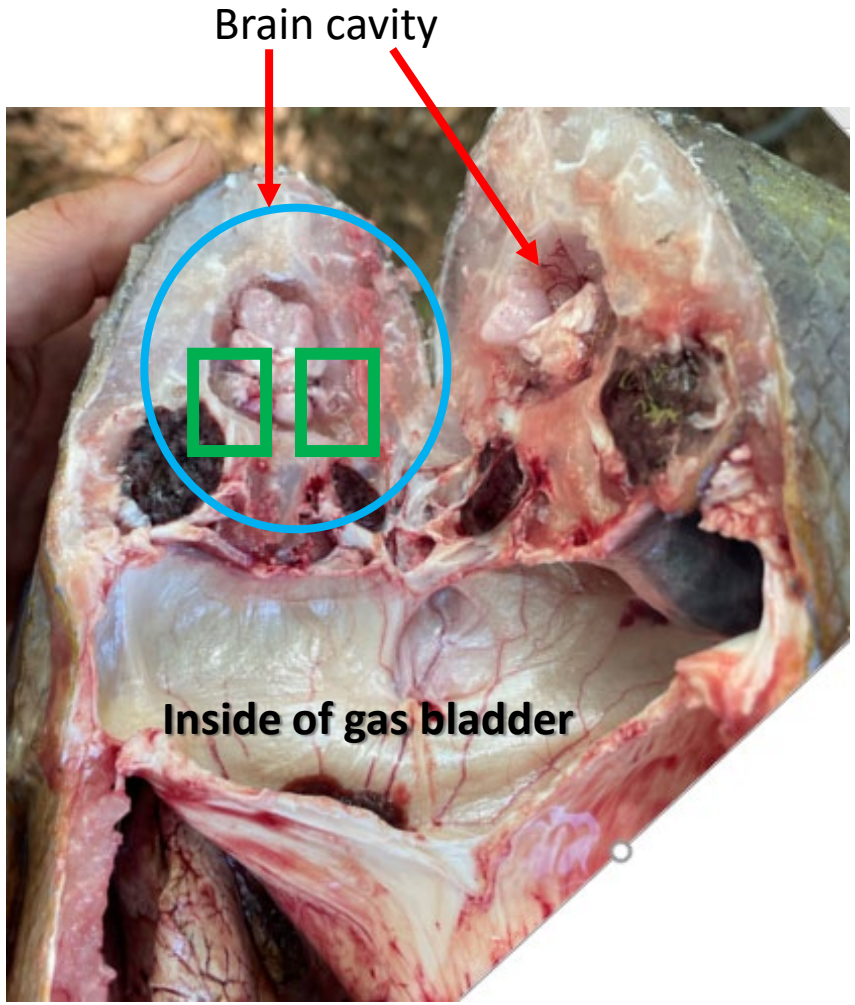
- **Accuracy and Precision**: Bomb radiocarbon was used to directly validated our otolith age estimation method for queen triggerfish[1] and conclusively documented that triggerfish sagittal otoliths provide more accurate and precise age estimates relative to dorsal spines. Our findings support concurrent work on gray triggerfish that demonstrated sagittae provided accurate[2] and more precise[3] age estimates compared to spines.
- **Population Parameters:** The tendency for triggerfish dorsal spine-derived age estimates to underestimate age results in inaccurate population growth and age-at-maturity estimates. Additional complications can result from age estimates obtained with a method/structure that underestimates true age. For queen triggerfish fishery-dependent samples[1], spine-based age estimates resulted in distinct differences in population age structure: (1) age range was truncated compared to the otolith-based population age structure; and (2) proportion of individuals in younger age classes was higher for spined-based age estimates. These differences in age structure of fishery-dependent samples between spines and otoliths would lead to differences in estimates of mean age and age at full recruitment to the fishery. The systematic underestimation of age can impede understanding the population dynamics of a species and lead to ineffective management strategies.
- **Longevity:** Our work also indicates that previously reported maximum ages for *Balistes* species based on spine-derived age estimates under-represented longevity of these species; queen triggerfish otolith-based ageing extended maximum known age by nearly three-fold, 14 y from spines versus 40 y from otoliths. For gray triggerfish, maximum reported spine-based age is 15 y; maximum otolith-based age documented so far is 20 y.

[1]Shervette VR, Rivera Hernández JM (2022). Queen triggerfish Balistes vetula: Validation of otolith-based age, growth, and longevity estimates via application of bomb radiocarbon. PLOS ONE 17(1): e0262281, doi:10.1371/journal.pone.0262281
[2]Patterson WI, Shervette V, Barnett B, Allman R. (2019). Do Sagittal Otoliths Provide More Reliable Age Estimates Than Dorsal Spines for Gray Triggerfish? SEDAR62-WP-17. North Charleston, SC 2019.
[3]Shervette VR, Rivera Hernández JM, Nunoo FKE (2021). Age and growth of grey triggerfish Balistes capriscus from trans-Atlantic populations. Journal of Fish Biology 98(4): 1120-1136..

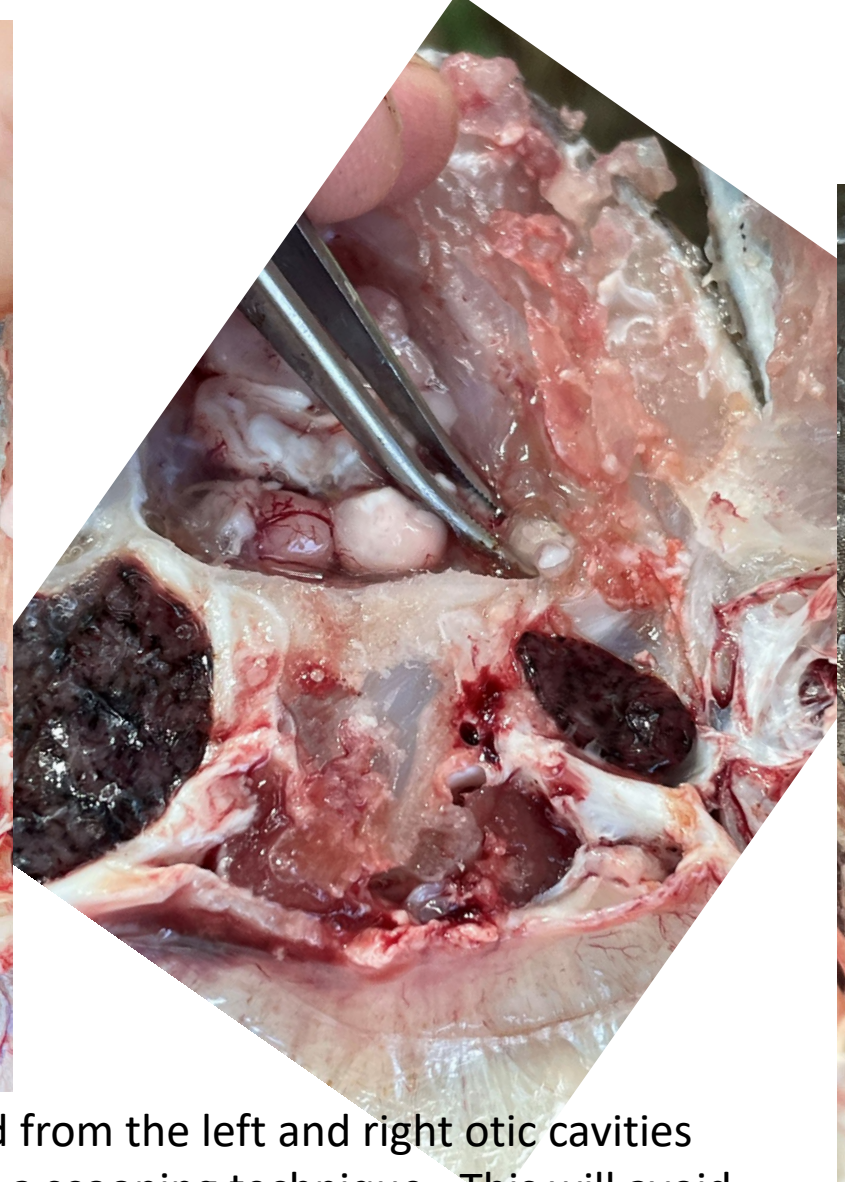
Triggerfish otolith extraction simplified

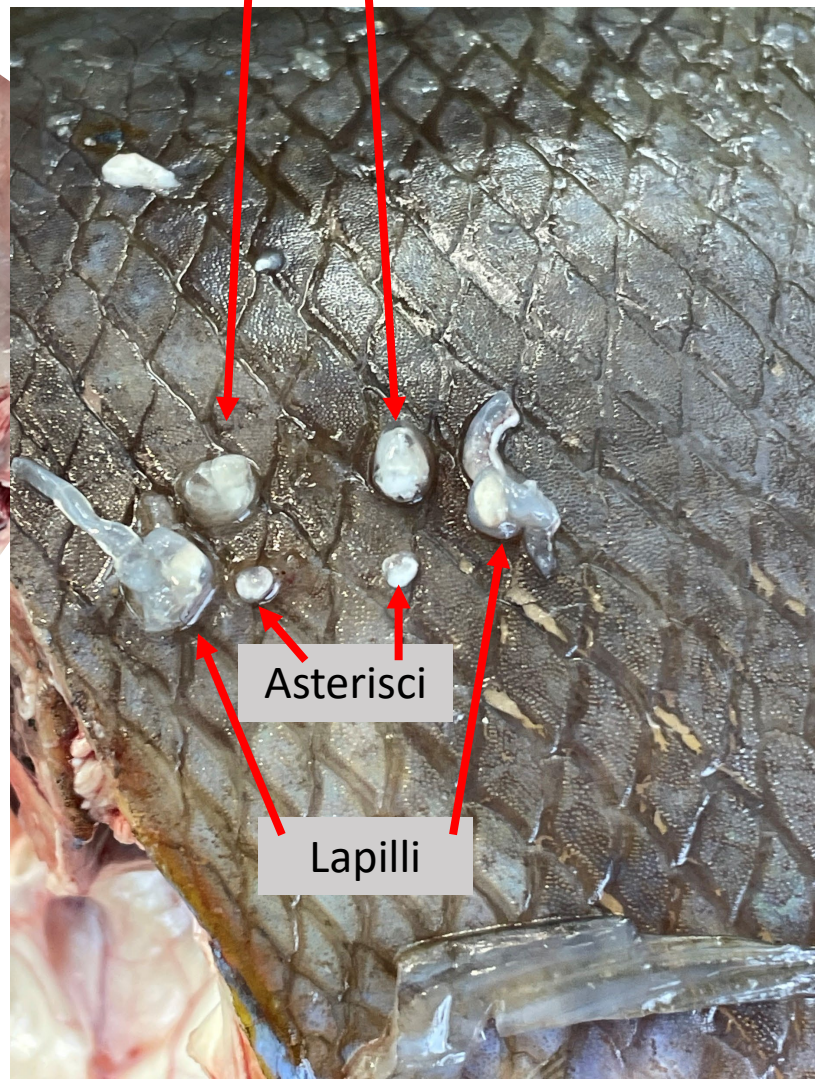
Use thumb to locate the posterior edge of the infraorbital bone as illustrated above. A large, sturdy, sharp serrated knife should be used for cutting into the cranium.

Note the location and angle of the cut relative to the position of the thumb, fish eye, and gill opening. Ensure that knife blade cuts perpendicular through the cranium – take care to avoid unintentionally cutting at even a slight angle.

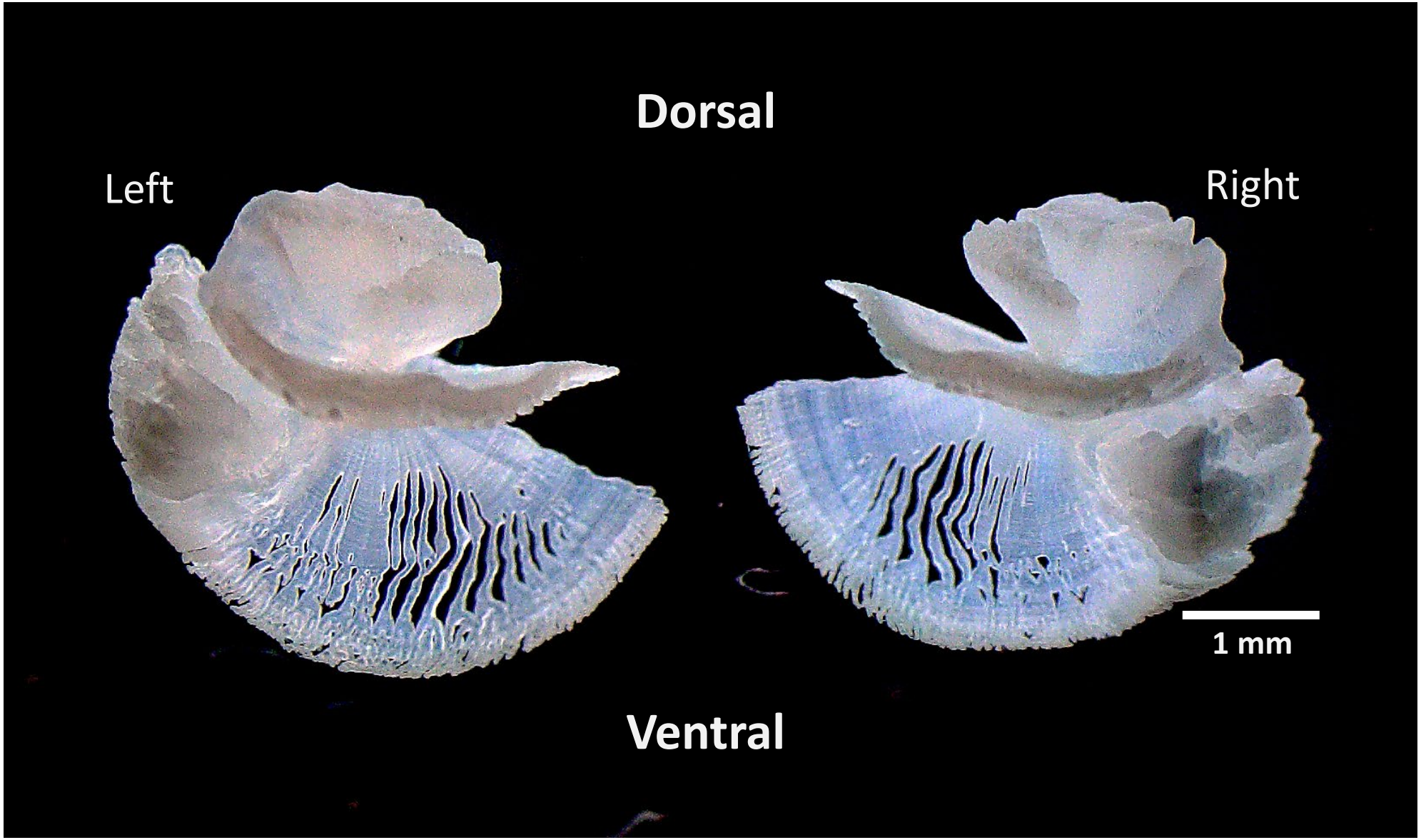


The left and right sets of otoliths are located in the ventral anterior portion of the brain cavity, tuck away in the otic cavities indicated above with the green rectangles.

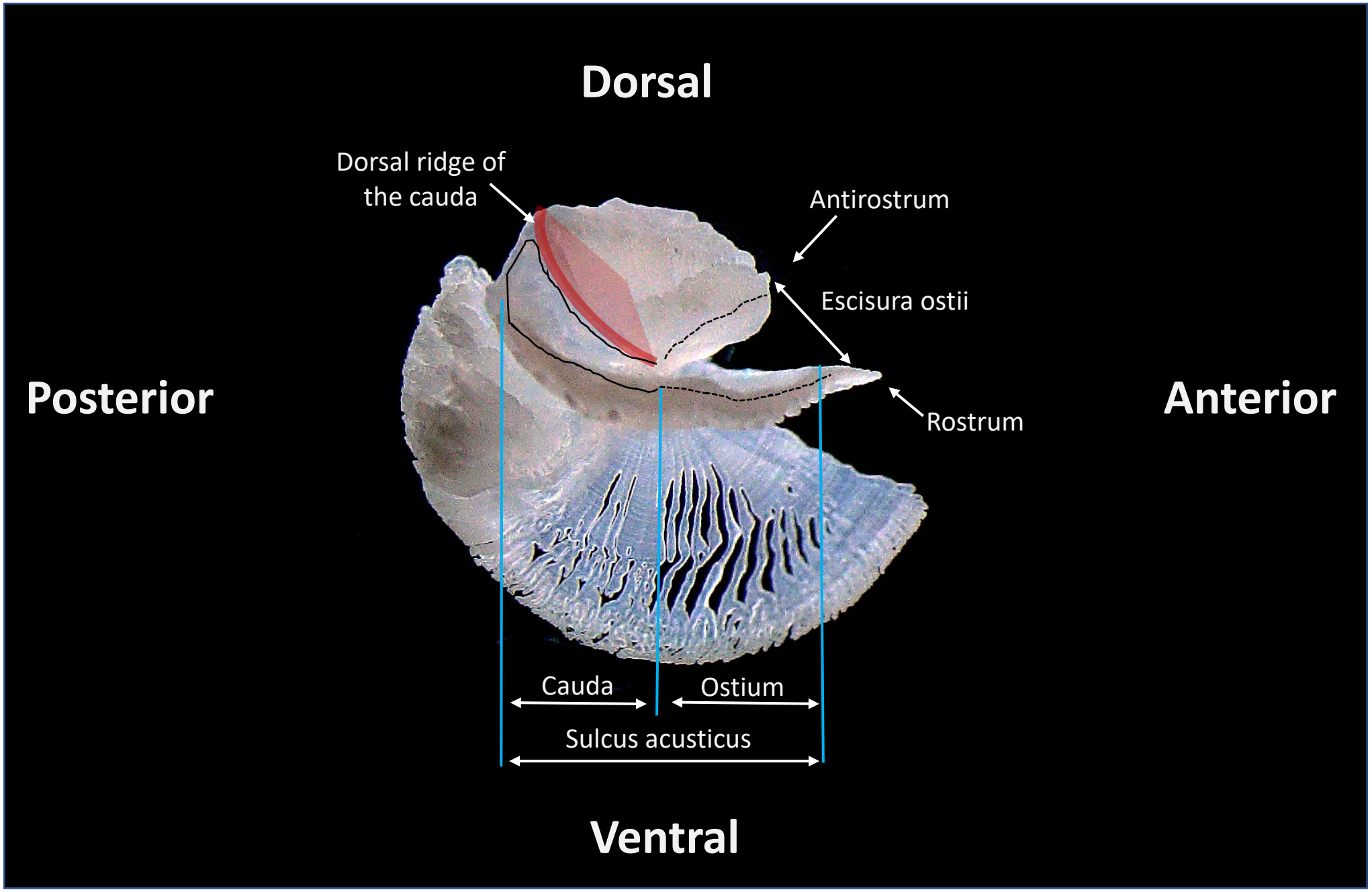




All three otoliths can be gently removed from the left and right otic cavities using fine tip curved forceps by utilizing a scooping technique. This will avoid unintentionally damaging the otoliths. After removal, otoliths can be stored in small plastic vials. We typically soak our otoliths in a mild bleach solution then gently “rinse” them by submerging in water prior to long-term storage.



Clean, intact sagittal otoliths look like the pair above and are used for estimating triggerfish age.

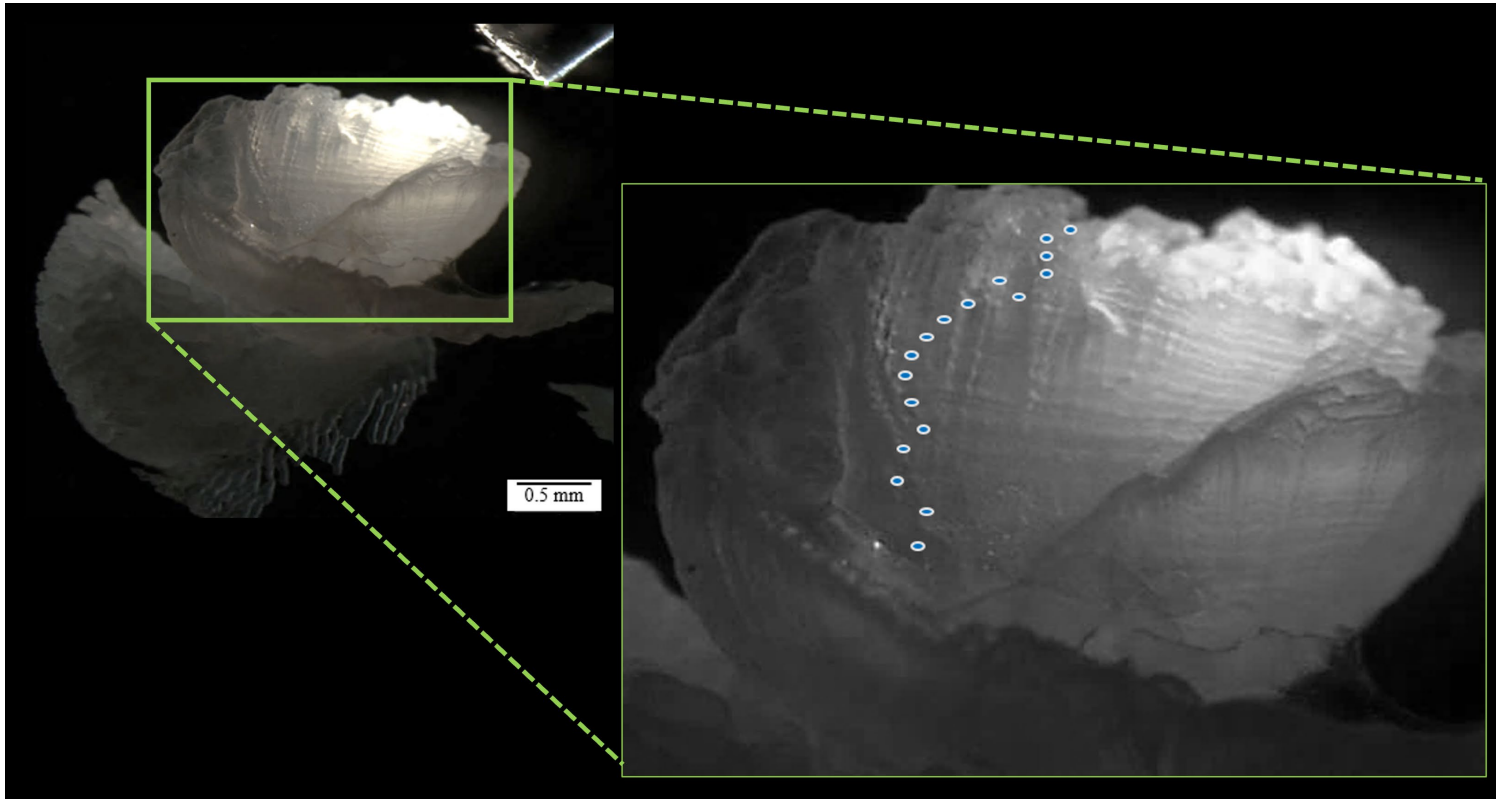


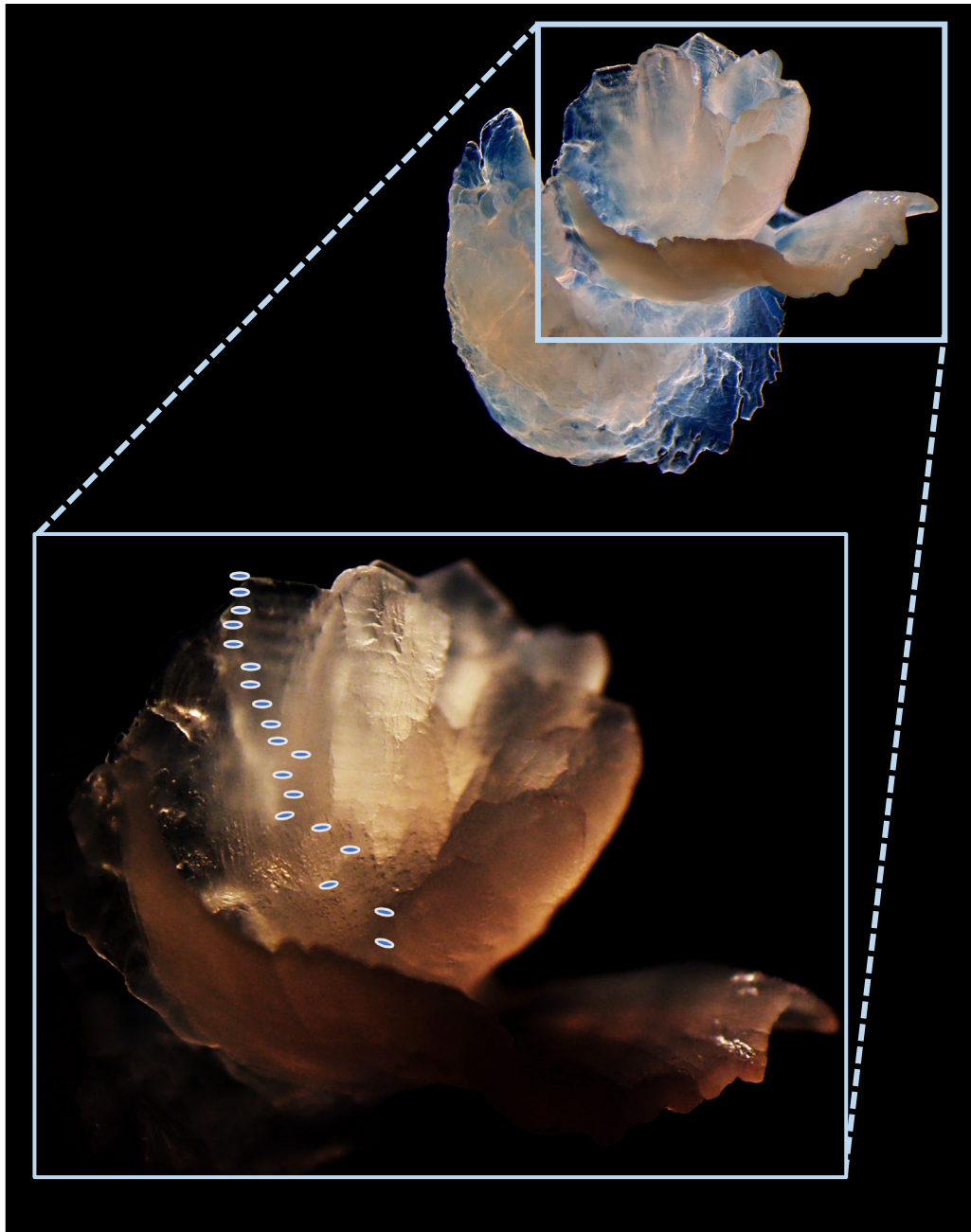
Sagittal otoliths are read using a stereo microscope with reflected light, against a black background while submerged in water at a magnification of 20-32x. Typically, we enumerate the opaque zones along the dorsal ridge of the cauda as indicated in the image by the red zone.

Light intensity is key so play around with light levels until the otolith opaque zones appear to glow.

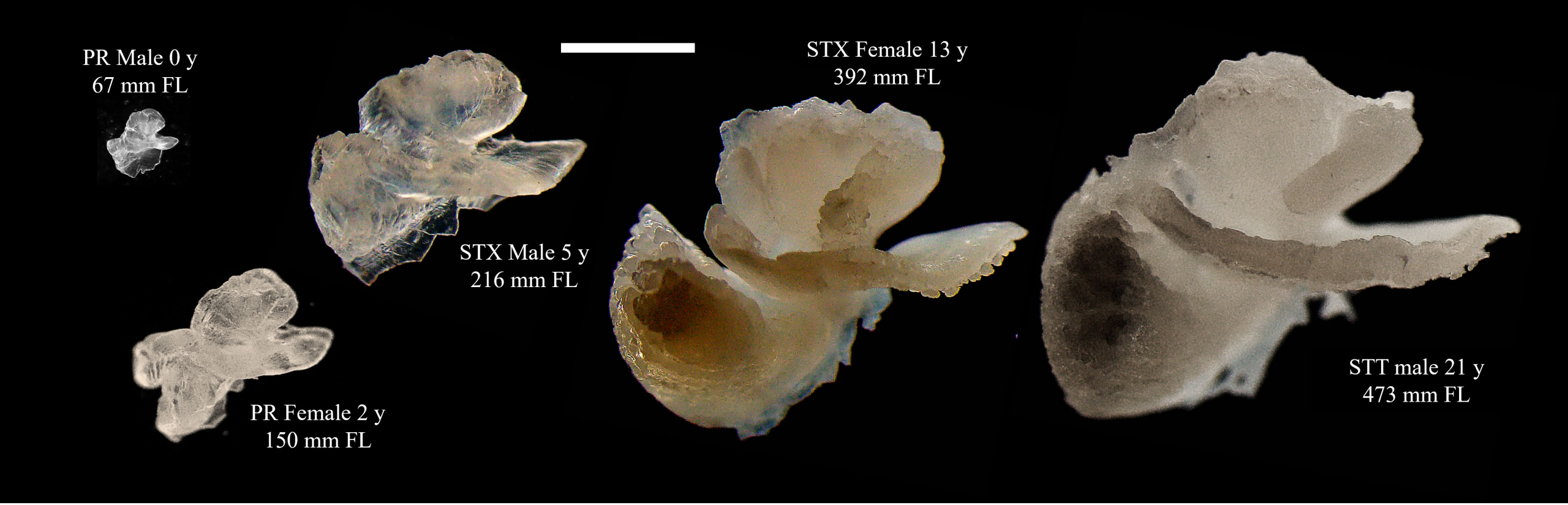
A fiber optic cable attached to the end of a light source can be used to effectively concentrate light and allow for light intensity control when trying to visualize opaque zones.

Queen triggerfish left sagitta (below)



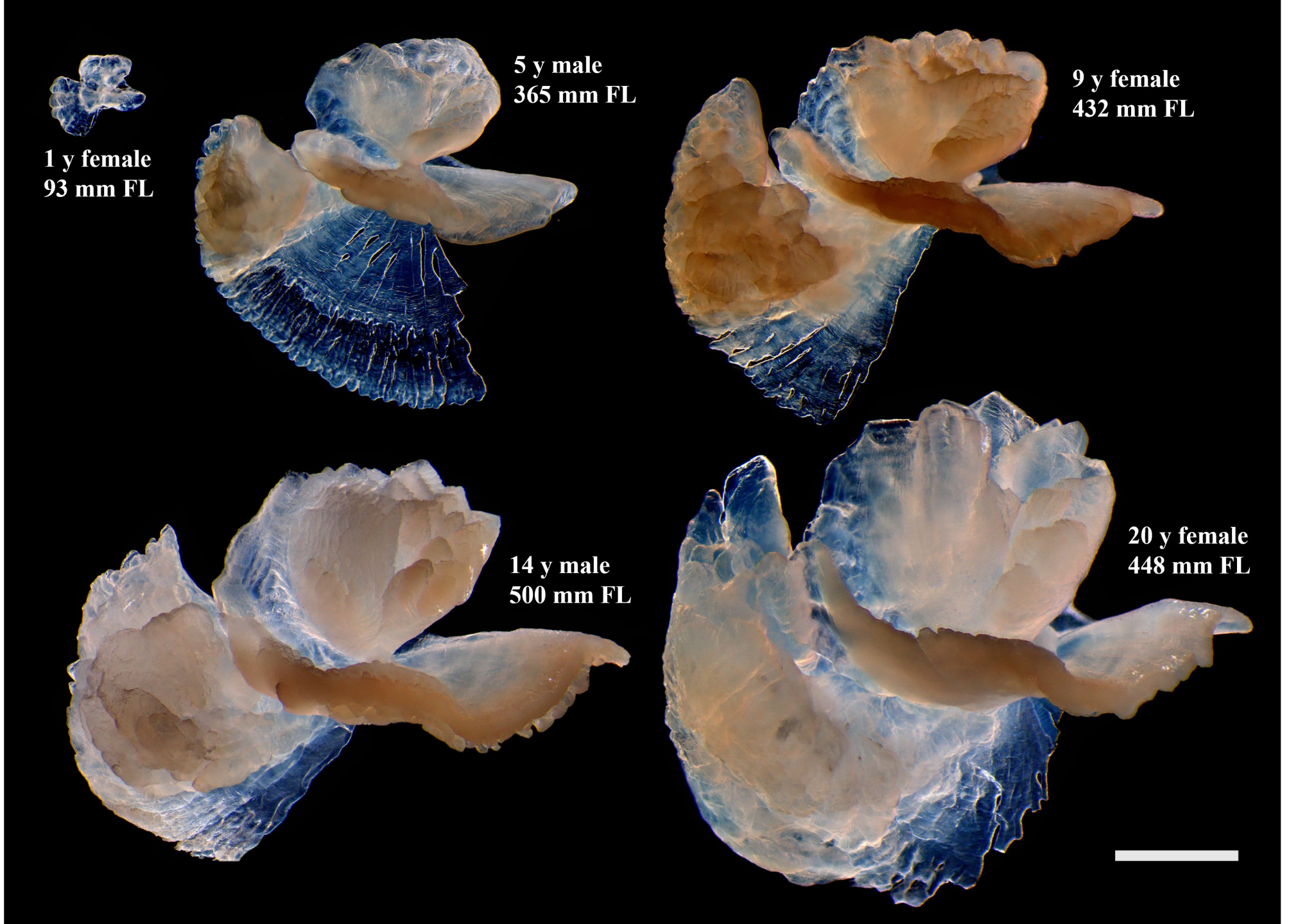


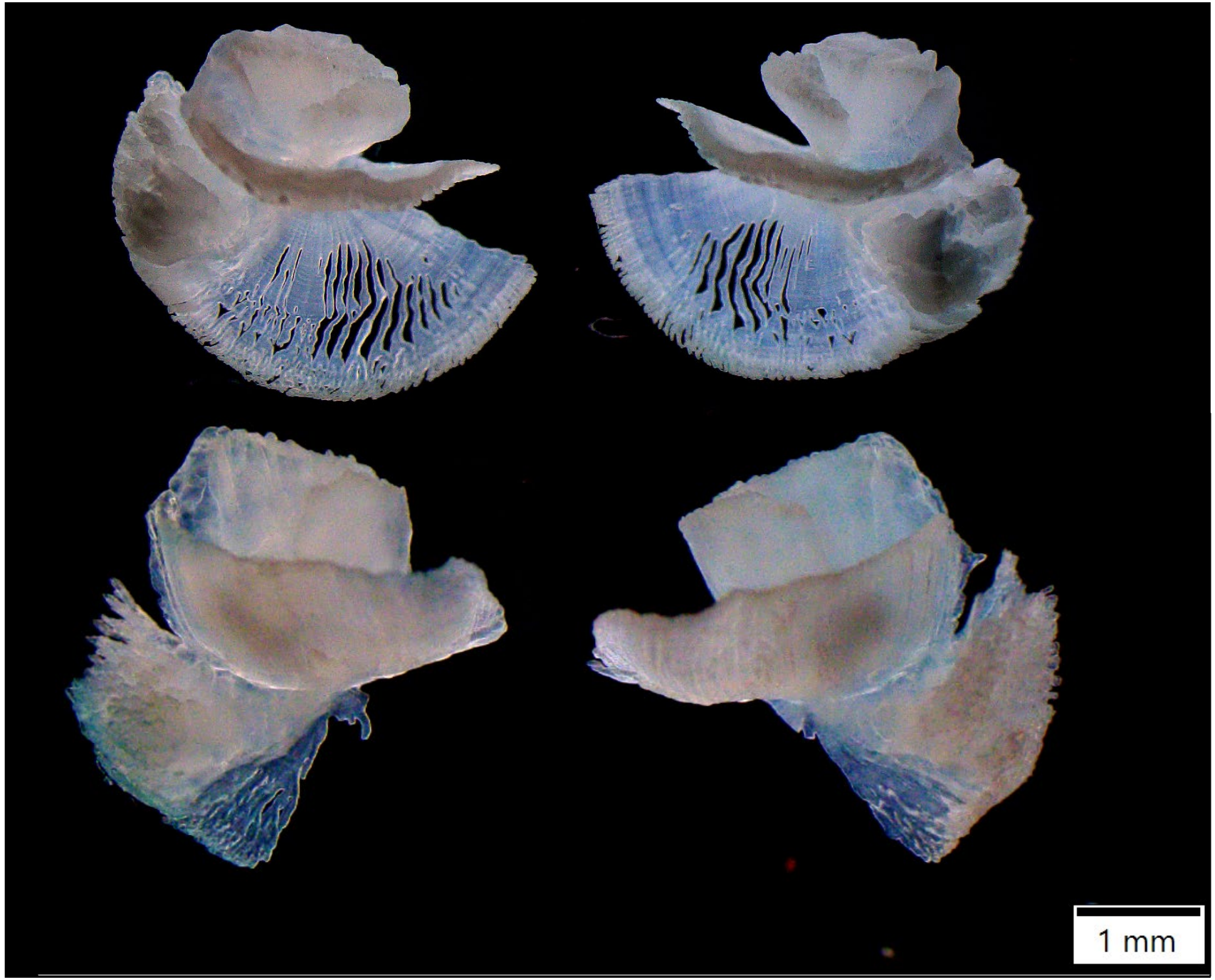
Gray triggerfish left sagitta (right): In this example, to visualize the first opaque zone, the dorsal rim of the otolith must be gently angled towards the reader to see into the funnel-like caudal area obscured by the ventral margin of the sulcular grove



Above we have provided examples for *Balistes vetula* triggerfish otoliths across a range of ages to illustrate the progression of growth and increment accumulation. Note that the fragile, plate-like formation that occurs in the anterior-ventral region of the otolith is missing from the examples – this is because it is so fragile and easily lost during the process of handling and reading triggerfish otoliths. Scale bar represents 1 mm.

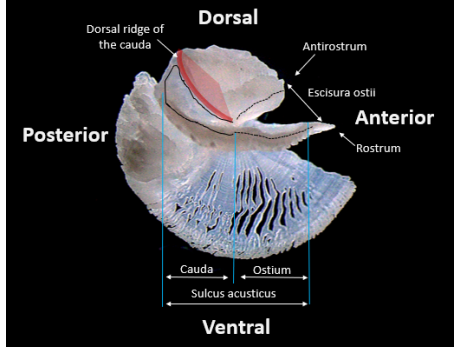
To the right are examples for *Balistes capriscus* triggerfish otoliths across a range of ages to illustrate the progression of growth and increment accumulation. Scale bar represented 1 mm.



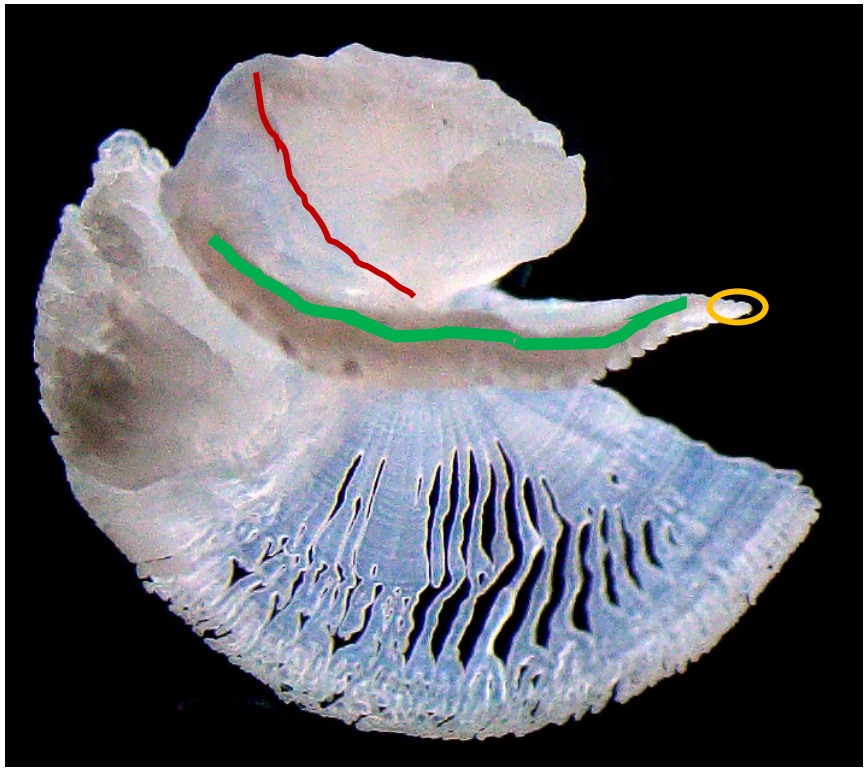


The top left and right sagittae are from a queen triggerfish (male, 500 mm FL) with an estimated age of 21 years.

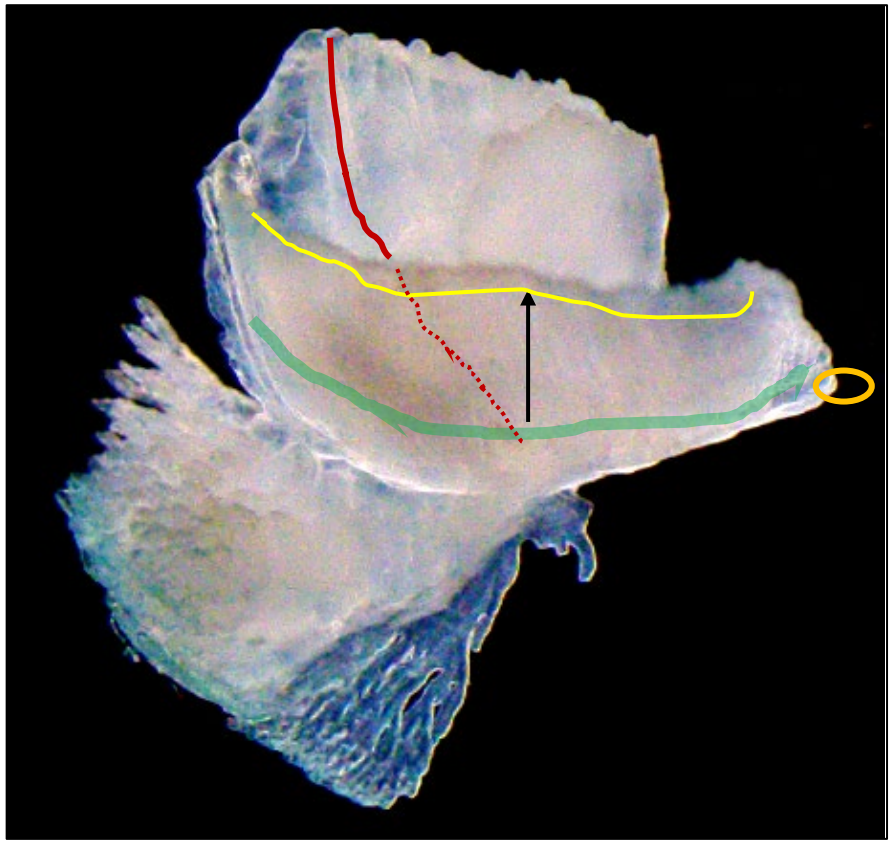
The bottom left and right sagittae are from a queen triggerfish (female, 466 mm FL) with an estimated age of 40 years.



We do not recommend sectioning all otoliths. In our investigations on queen and gray triggerfish, whole otoliths provided accurate and precise age estimates. However, sectioning may be desired for fish with estimated ages that exceed 20 years because the direction of growth for the ventral margin of the sulcus acusticus is such that the earliest increments are tucked down in the "funnelized" cauda formation and hard to visualize, even when tilting dorsal margin of the otolith towards the reader.

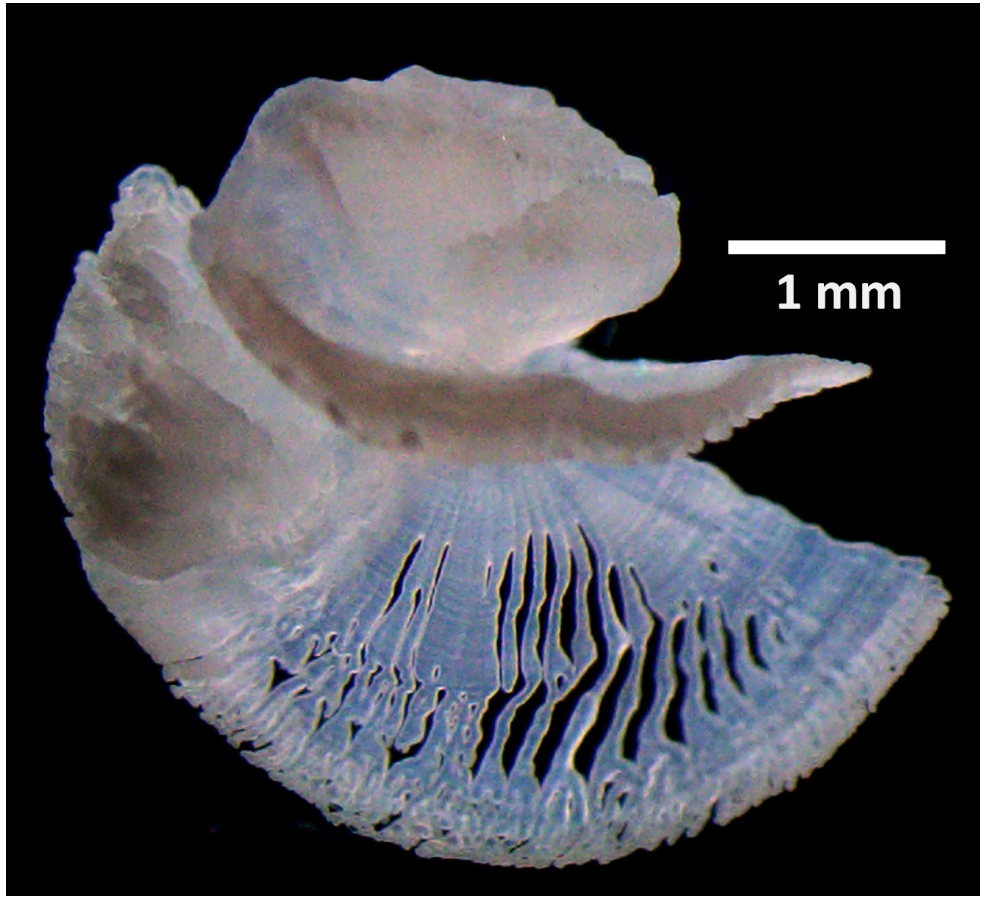


The ventral margin of the sulcus acusticus is outlined in green and the tip of the rostrum is indicated by the orange oval. The red line notes the dorsal edge of the cauda and represents the general region where we enumerate opaque zones. This otolith has 21 opaque zones.

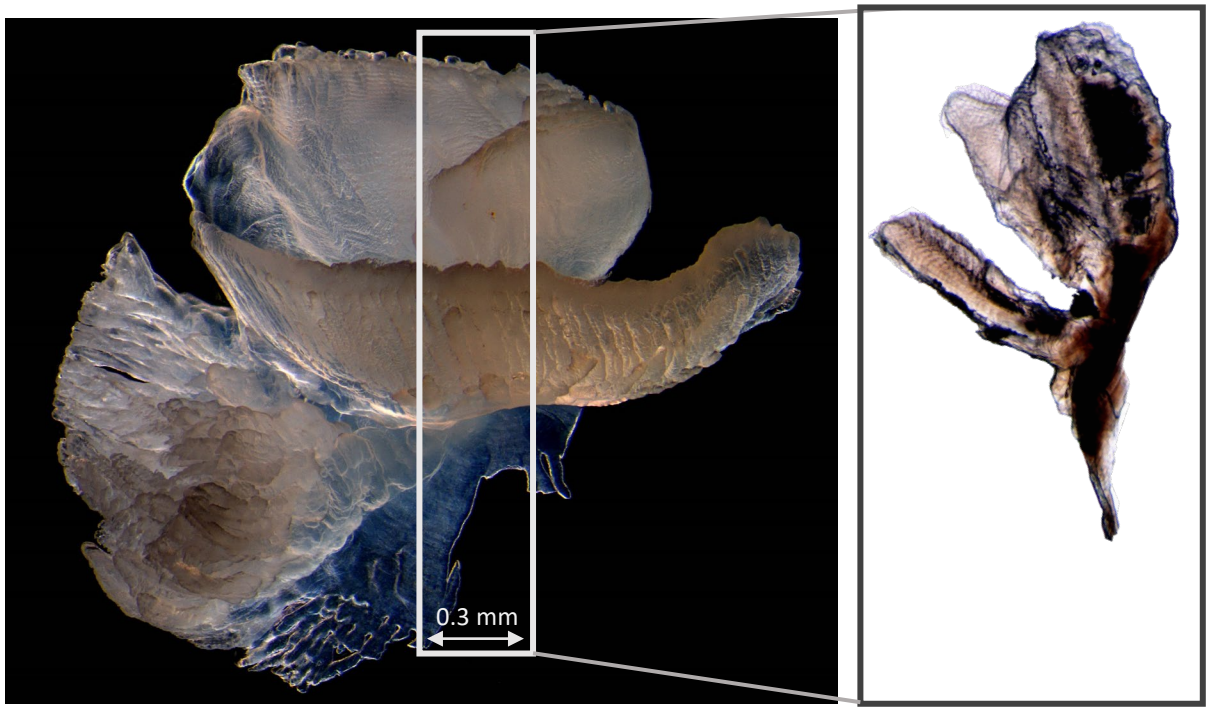


The location of the ventral margin when this otolith only contained 20 opaque zones is indicated in green and the tip of the rostrum is indicated by the orange oval. The direction of growth is indicated with the black arrow. The current ventral margin is indicated by the yellow line. The red line notes the general region where we enumerate opaque zones. Note that the growth past 20 increments now obscures the path where we usually would enumerate the earliest opaque zones (dotted red line). This otolith has 40 opaque zones.

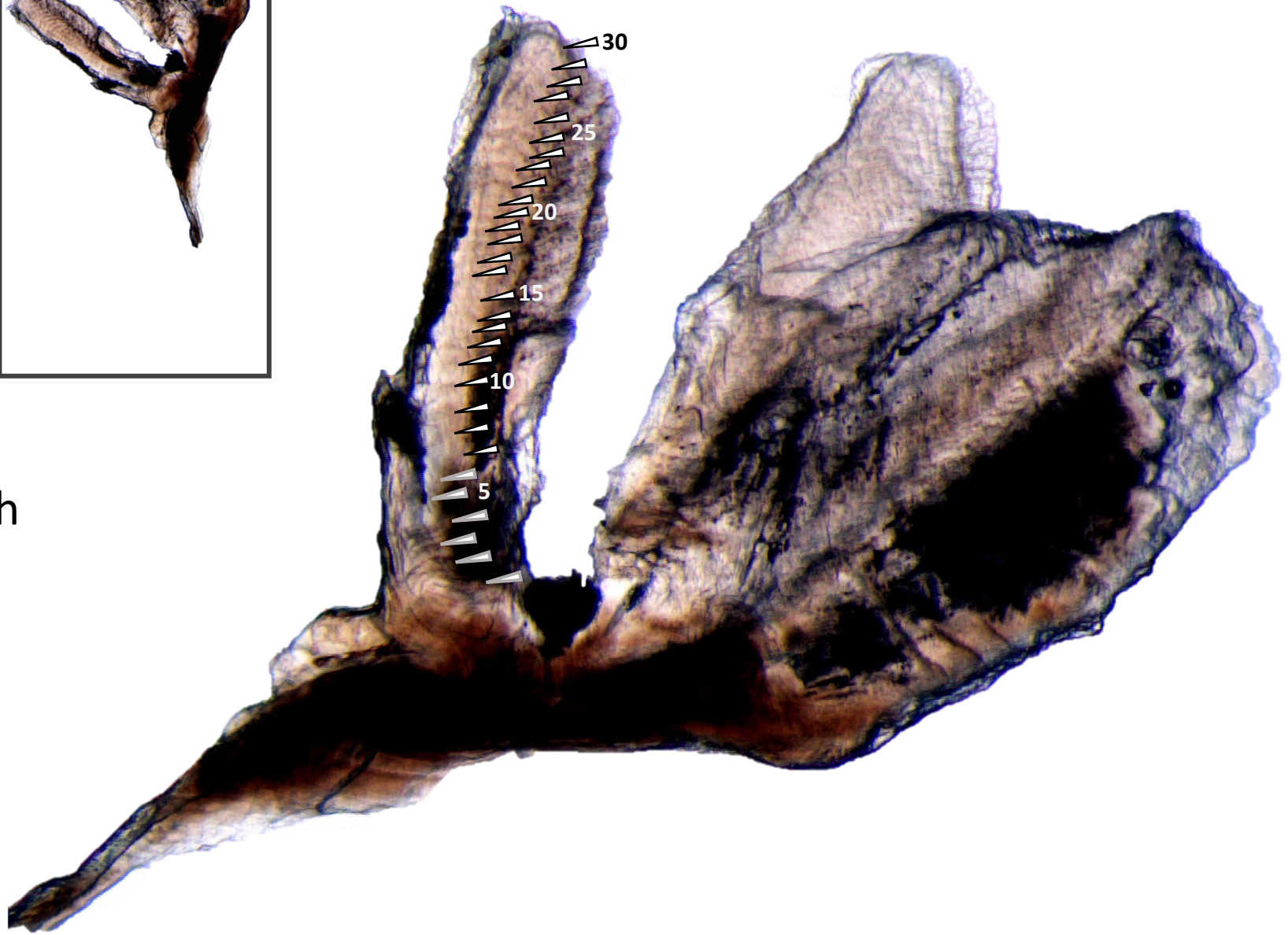
Our protocol for estimating ages of triggerfishes via opaque zone counts on whole otoliths has been validated via application of bomb radiocarbon. Triggerfish otoliths are very small and fragile so to obtain consistently readable otolith sections using the standard sectioning procedures and equipment requires extensive skill, patience, and time. However, sectioning may be useful to ensure that all opaque zones are counted by multiple readers for otoliths on which the ventral margin of the sulcus acusticus obscures visualizing the first few opaque zones.



Left sagittal otolith from a queen triggerfish (male, 500 mm FL) with an estimated age of 21 years.



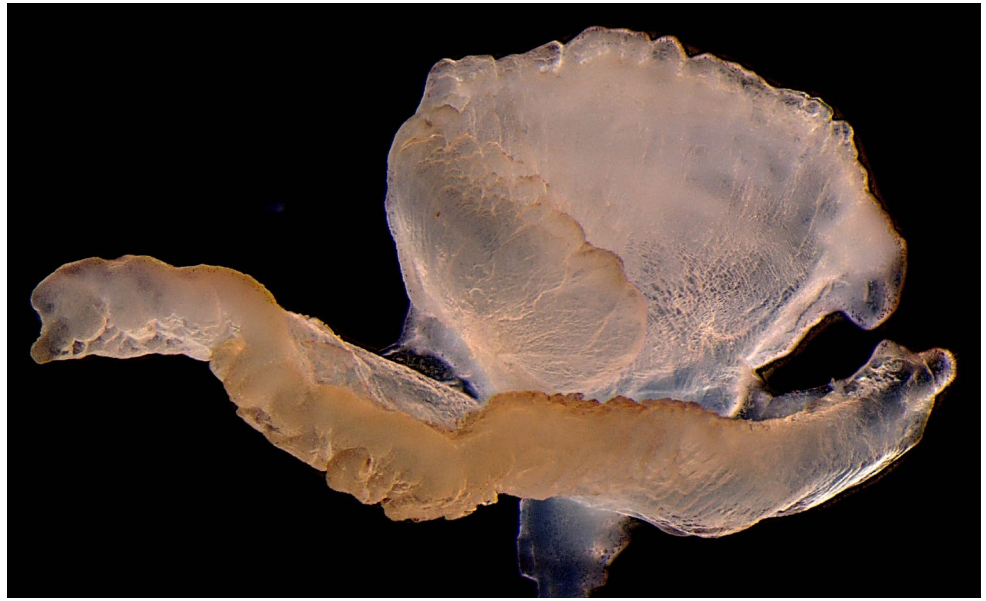
Left sagittal otolith and otolith section from a queen triggerfish (male, 525 mm FL) with an estimated age of 30 years.

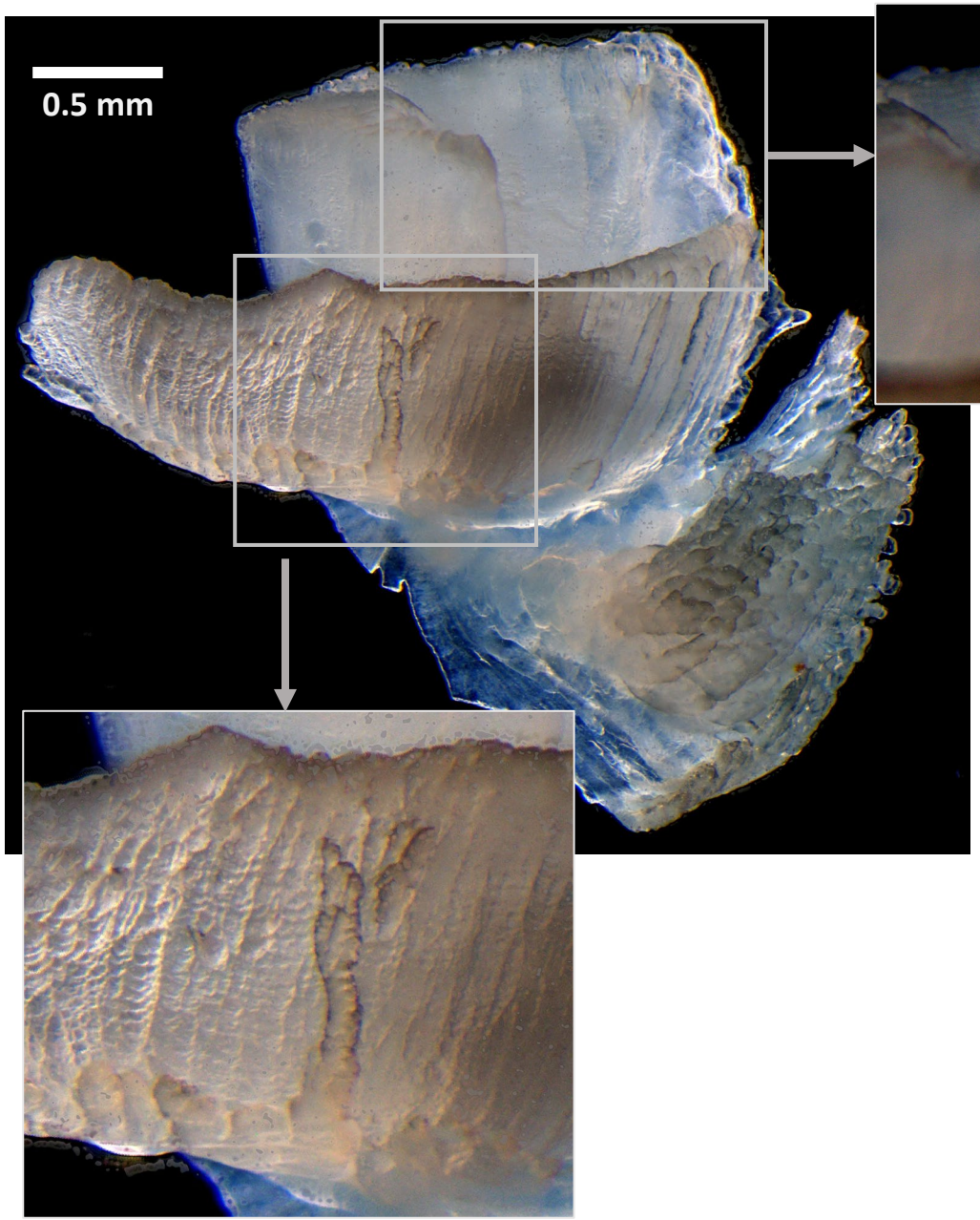


Left sagitta and otolith section from a female gray triggerfish (455 mm FL) with an estimated age of 20 years.

The maximum age documented for a gray triggerfish so far is this 20-year-old fish

Right sagitta and otolith section from a male gray triggerfish (502 mm FL) with an estimated age of 17 years.





Whole otolith and otolith section from a queen triggerfish (female, 466 mm FL) with an estimated age of 40 years.

*This is the oldest queen triggerfish aged so far (maximum documented age = 40 y)