# MicroCT Imaging of Otoliths from Farm-raised Atlantic Salmon (*Salmo salar L.*)

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**Aims**

Otoliths are structures (calcareous bodies) in the inner ear of all vertebrates and are important sensors for gravity, balance, movement, direction, and hearing (1). We hypothesize that abnormal formation of otoliths is involved in the development of irregular swimming patterns observed in farm-raised fish. In the current study, we used Computed Tomography (i.e., micro/nanoCT) to obtain high-resolution images of otoliths from normal farm-raised salmon for eventual comparison with those isolated from wild-caught salmon as well as from farm-raised salmon exhibiting irregular swimming patterns. Our efforts are expected to increase our knowledge of salmon development and physiology.

**Methods**

Right and left otoliths were obtained from harvested normal farm-raised salmon. Both samples were mounted in a small polypropylene plastic tube (dia. 5mm) to immobilize the samples for scanning using a SkyScan 1272 micro/nanoCT (Bruker, Kontich, Belgium). Scan conditions were as follows:

Camera setting: 1632 x 1092 pixels per image
Image pixel size: 3.75 µm
Source voltage: 80 kV
Source current: 124 µA
Frame averaging: 3
Filter: Al 0.5 mm
Image rotation: 0.2°

Images of samples were reconstructed individually with NRecon software, and arbitrarily designated Otolith 1 and Otolith 2, since the original orientation (sidedness/positioning) of the otolith within the fish was not recorded. Subsequent analysis was performed using SkyScan CTAn, CTVox, CTVol (Bruker), and VivoQuant (InVicro Inc.) software.

**Figure 1.** CTAn software was used to create 3D models of each otolith using custom processing tasks:

(1) Thresholding at 90 to 255
(2) De-speckle sweep
(3) ROI shrink-wrap, 2D space, stretch over 14 pixels
(4) 3D model, save as p3g

Each 3D model was uploaded into CTVol for comparison of morphology. Otolith 1 was

colored pink; otolith 2 was colored orange. Top pair are presumed to be facing the outside of the head, bottom is the obverse. There is a clear right/left difference between the two otoliths, However positioning within the fish (sidedness, dorsal/ventral & central/peripheral) was not noted during isolation for these preliminary scans.

**Figure 2.** Surface features of salmon otoliths.
CTVox was used to colorize each otolith to enhance the appearance of surface features. Initial colorization was based on a transfer function named “Russian Rock” from the SkyScan transfer function file.

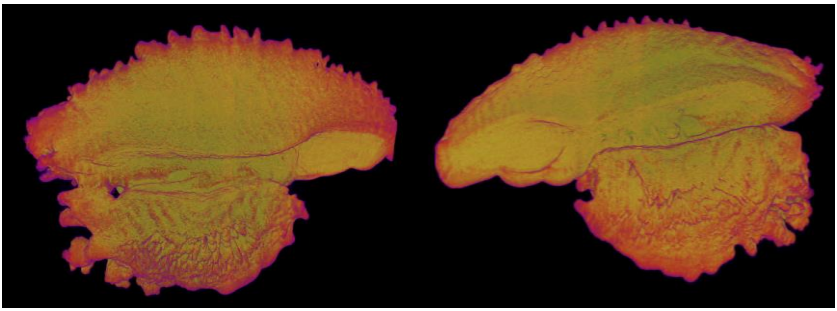
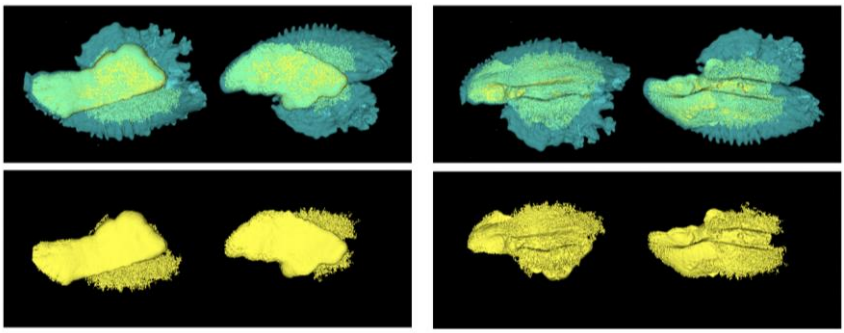


**Figure 3.** Segmentation of salmon otoliths.
*VivoQuant* imaging analysis software (InVicro Inc.) was used to segment the otoliths based on relative radiodensity. For this preliminary analysis, x-ray attenuation cut-off ranges were set arbitrarily with high and low radiodensity voxels colored yellow and cyan, respectively. The transparency of cyan was reduced to 30% to visualize core structures. Segmentation reveals a complex internal structure based on mineral density throughout the otoliths. The top images show the front and back of the otoliths, while the bottom images show high radio-dense material with low density material subtracted digitally.



**Discussion**
Each fish species has a specific otolith morphology. The variation in density along the lower section of each otolith is known to be related to seasonal changes in food availability and water temperature that result in growth spurts and alternating thin and wide spaces formed each year of growth for fish in the wild. Furthermore, morphology and the crystalline form of the calcium compounds that make up the otolith (aragonite vs. vaterite) also varies with

environmental conditions and growth rate. Interestingly, evaluating otoliths isolated from the stomach contents of fish-eating mammals, birds, and predator fish can help determine identity and age of species consumed. Farm-raised fish, which develop in highly controlled (non-cyclic) environmental conditions, are expected to exhibit differences in otolith structure and consistency compared to those of fish in the wild. For example, it is known that rapid growth rate of farm-raised salmon increases the formation of vaterite and alters the morphology of the otolith, which in turn, will likely affect environmental “sensing”, development, and behavior including swimming patterns (2, 3). Further studies involving the use of micro/nanoCT imaging to compare detailed otolith morphology of wild-caught vs. normal and abnormally swimming farm-raised salmon are currently being conducted and are expected to increase our knowledge of salmon development and physiology.

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2. Reimer T, Dempster T, Warren-Myers F, Jensen AJ, Swearer SE. High prevalence of vaterite in sagittal otoliths causes hearing impairment in farmed fish. Sci Rep. 2016;6:2045-322
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