

# Ecological and biochemical controls on nitrogen isotopic fractionation of otolith-bound organic matter: insights from paired tissue-otolith comparisons, amino acid concentrations, and an otolith model

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The  $\delta^{15}\text{N}$  of tissue has long been used to investigate the diet and migration of fishes. Studies of past fishes and their environments would be facilitated by forms of preserved fish-native organic matter that could be analyzed for their  $\delta^{15}\text{N}$ . Otoliths are composed of calcium carbonate, as the mineral aragonite, and a small amount of organic matter (usually  $< 1\%$  by mass) that is believed to represent the residuum of an organic template for mineral precipitation. Due to their tree ring-like growth pattern, otoliths have been used for centuries to determine fish age and more recently have been analyzed for their chemical and isotopic composition. Using a highly sensitive method for measuring the nitrogen isotopic composition ( $\delta^{15}\text{N}$ ) of organic matter (OM) bound within otoliths, we have undertaken a calibration study comparing the  $\delta^{15}\text{N}$  of otoliths ( $\delta^{15}\text{N}_{\text{oto}}$ ) to that of white muscle tissue ( $\delta^{15}\text{N}_{\text{wmt}}$ ) for twenty-four teleost fish species. We developed a model for addressing the fact that otoliths integrate the whole life history of the fish whereas muscle records a shorter period of life history. We also measured the amino acid concentrations (AAs) of otolith OM, for two reasons. First, variations in AAs is a coarse measure of differences in organic matter composition across different species. Second, previous work has demonstrated systematic  $\delta^{15}\text{N}$  patterns among AAs, such that the AA composition of OM should have an effect on its bulk  $\delta^{15}\text{N}$ .

$\delta^{15}\text{N}_{\text{oto}}$  and  $\delta^{15}\text{N}_{\text{wmt}}$  were found to be highly correlated with a slope near 1. However, the differences between  $\delta^{15}\text{N}_{\text{oto}}$  and  $\delta^{15}\text{N}_{\text{wmt}}$  ( $\Delta\delta^{15}\text{N}_{\text{no-w}}$ ) varied systematically across species. This was generally a subtle effect, but both Gadiformes (e.g., cod) and Salmoniformes (e.g., salmon) were different from the others by multiple permil. Phylogeny, the concentrations of total N and amino acids, and life history were each ruled out as explanations for the observed variation in  $\Delta\delta^{15}\text{N}_{\text{no-w}}$ . Otolith size arose as a strong correlate with  $\Delta\delta^{15}\text{N}_{\text{no-w}}$ , especially with regard to the difference of Gadiformes with other species;  $\Delta\delta^{15}\text{N}_{\text{no-w}}$  was found to be lower in species producing larger otoliths. We propose that  $\delta^{15}\text{N}_{\text{oto}}$  is sensitive to isotopically fractionating degradation of the organic matrix around the growing otolith. As an example, a high  $\delta^{15}\text{N}_{\text{oto}}$  (and thus  $\Delta\delta^{15}\text{N}_{\text{no-w}}$ ) results from a large proportion of the matrix being degraded as opposed to incorporated into the otolith, which applies when otolith growth is slow and thus the otolith is small.

Across most species, however, the differences in  $\Delta\delta^{15}\text{N}_{\text{no-w}}$  are small. Moreover,  $\Delta\delta^{15}\text{N}_{\text{no-w}}$  appears to be conserved across individuals within a given species. Both of these observations bodes well for the use of  $\delta^{15}\text{N}_{\text{oto}}$  as a proxy for fish  $\delta^{15}\text{N}$ .