

{rokbox title=[Panel of otoliths showing placement of pits on larval otoliths post-analysis via SEM imaging :: Image: Authors|
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The first

application of secondary ion mass spectrometry for the analysis of $\delta^{18}\text{O}$ in otoliths of larval fish

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Abstract: The stable isotope ratio of oxygen ($\delta^{18}\text{O}$) stored in otoliths is a common and reliable tool for studying life history events including natal origin and migratory behavior of marine fish species. This approach is useful particularly in cases where traditional tagging is impossible, such as in early larval stages. However, small amounts of available otolith material have historically precluded analysis of $\delta^{18}\text{O}$ in individual larval otoliths by traditional isotope ratio mass spectrometer (IRMS). The use of secondary ion mass spectrometer (SIMS) resolves this challenge, allowing users to obtain $\delta^{18}\text{O}$ signatures in otoliths at the scale of tens of microns. This paper presents a method for the preparation of larval fish otoliths compatible with SIMS $\delta^{18}\text{O}$ analysis through placement of larval otoliths (~ 20 to $\sim 150\ \mu\text{m}$ diameter) on a single epoxy mount and use of a Cesium ion beam, $^{133}\text{Cs}^+$, of $12\ \mu\text{m}$ in diameter with $1\ \mu\text{m}$ penetration depth to excite source larval otolith material. In this pilot study, otoliths from Atlantic bluefin tuna (*Thunnus thynnus*) larvae were used to assess the efficacy of generating quality $\delta^{18}\text{O}$ data using the newly developed preparation method for establishing spawning site baselines. The applied methods led to usable $\delta^{18}\text{O}$ signatures in $> 90\%$ of the otoliths selected for analysis. Challenges and unanticipated considerations that became apparent during the use of this method include the inverse relationship between $^{16}\text{OH}^-/^{16}\text{O}^-$, an indicator of hydrogen content, and $\delta^{18}\text{O}$, resulting in a limitation of using otoliths from preflexion larvae, as well as a statistically significant difference in $\delta^{18}\text{O}$ (-0.17‰) after storage in Type B immersion oil.

Keywords: Larval fish, Otoliths, Marine fish species, Larval stages.