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The dissolution pattern of the flattened otolith of Pacific cod using the stepwise acid dissolution method

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The inner ear of fish contains calcium carbonate ($CaCO_3$)-based crystals called otoliths, which play an essential role in hearing and balance. During otolith formation, new calcium carbonate is deposited in layers on the surface of the existing part and the part is no longer affected by metabolism. This feature means that each layer of the otolith retains the trace element ratios and isotope ratios it had at the time of formation. By analysing this preserved information, it is possible to make estimates about the environment and habitat at that time.

The stepwise acid dissolution method has been used in several studies as a technique for analysing the radiocarbon isotope ratios preserved in otoliths. In this method, phosphoric acid is used to dissolves the otolith from the outermost to the innermost layers. It has the advantage that a large amount of carbon can be collected from a single otolith, compared to the mechanical methods for the calcium carbonate sampling from each layer of the otolith.

However, this method involves dissolving the otolith in acid and the pattern of dissolution cannot be controlled minutely by the experimenter. Pacific cod (*Gadus macrocephalus*) otolith used by us is flattened and the dissolution pattern of such otoliths is not yet known. Furthermore, no direct observation of the otolith dissolution process has been made in relation to previous studies.

In this study, we dissolved three Pacific cod otoliths in phosphoric acid to directly confirm the process of otolith dissolution in the stepwise acid dissolution method. We removed each otolith from the acid one or more times during the dissolution process, weighed it and observed the change in the shape of the otolith and the layer structure exposed on the otolith surface. As the dissolution progressed and the otoliths became smaller, we polished them to check that the internal layered structure had not been destroyed by acid penetration.

As a result, we found that the serrated structures present on the outer edges of the otoliths are maintained when they are dissolved from the outside by acid. We also confirmed that acid dissolution from the outside does not destroy the inner layer structure, even microstructures such as daily rings. The validity of the stepwise acid dissolution method would be strengthened by these results. On the other hand, the otoliths were thinner as a result of acid dissolution, exposing the

more inner layers on the flat surface. This is due to the thin vertical thickness of the flattened otoliths. This observation suggests that the collected carbon may be mixed with the carbon collected from more inner layer. This carbon mixing is able to be taken into account with future work. In addition, care should be taken when using this method near the nucleus, as the final stage of dissolution results in multiple holes in the otolith.