Reassembling broken otoliths for population discrimination

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Summary
Twenty five Atlantic cod Gadus morhua otoliths were examined using eight shape measurements along with Fourier analysis of their outlines to test whether discrimination using otolith shape is affected by reassembling broken otoliths. Small differences in seven of the eight shape measurements were found between unbroken otoliths and the same otoliths after breaking and subsequently gluing together; however, none of the Fourier descriptors differed. Cluster analyses indicated that resultant morphological differences will have no impact when applying discriminant analysis.

Introduction
Since Reibisch's observation of otolith annuli in 1899, otoliths have become one of the most important tools in modern ichthyology. The initial significance of otoliths in fisheries research was due to their utility for accurate age determination. Later, other important aspects were discovered including the use of otolith morphology (Campana and Casselman 1993). In Iceland, archived otoliths are currently being used to identify different ecotypes of cod (*Gadus morhua*), previously described by Pálsson and Thorsteinsson (2003), Pampoulie et al. (2006) and Grabowski et al. (2011), based on data from Data Storage Tags (DST). These two ecotypes show different migration behaviour during feeding season. Although a relatively high number of otoliths have been retrieved from DST fish (n~420) many have been broken for age determination and thus considered unsuitable for morphological analysis. Due to the high value placed on these otoliths, it was deemed appropriate to evaluate if these otoliths could be glued back together and used in morphological analysis together with unbroken otoliths.

Material and Methods
Twenty five unbroken sagittal otoliths from cod were randomly retrieved from the archived otolith collection of the Marine Research Institute in Iceland. Each otolith was photographed whole, and then broken by hand with same technique as used during aging. Finally, all the otoliths were glued with a minimal amount of instant glue and allowed to dry before being photographed again. Eight shape measurements were extracted from the images, using ImageJ software. The outline was also automatically traced using pixel gradient of the silhouette of the otoliths, and then subjected to a Cartesian Fast Fourier Transformation to reveal 256 Fourier Descriptors of which 6 largest were used in the analysis. By repeating the photographing of each otolith six times before and after breaking, we could better detect the effect of gluing from the effect of distortion. ANOVA was used to detect whether the gluing procedure has a measurable effect on shape analysis, first with all six photographs and then with only one photograph. A Cluster Analysis was used to test whether the individual effect would influence the way otoliths are grouped according to their similarity in shape.

Results and Discussions
Seven of the eight shape measurements and none of the Fourier Descriptors were significantly different between the unbroken and glued otoliths photographs. The difference was only revealed by using the six photographs, if the standard procedure of using only one photo was applied the difference wasn't significantly detected with ANOVA.

Even though some of the shape measurements were significantly affected by gluing, the cluster analysis suggests that the magnitude of these differences would not be sufficient to influence the results from a discriminant analysis. In all cases the pairs within each 25 clusters formed on the dendrogram, are made up of the same individual otoliths with the unbroken one next to its glued counterpart (Figure 1).

This study demonstrates that cod otoliths that have been broken for age determination can in most cases be glued back together and applied in morphological analyses. Given the vast quantities of broken otoliths from cod and other species archived in repositories throughout the world, it is hoped that these findings might encourage researchers to revisit these collection and allow their true value to be realized.

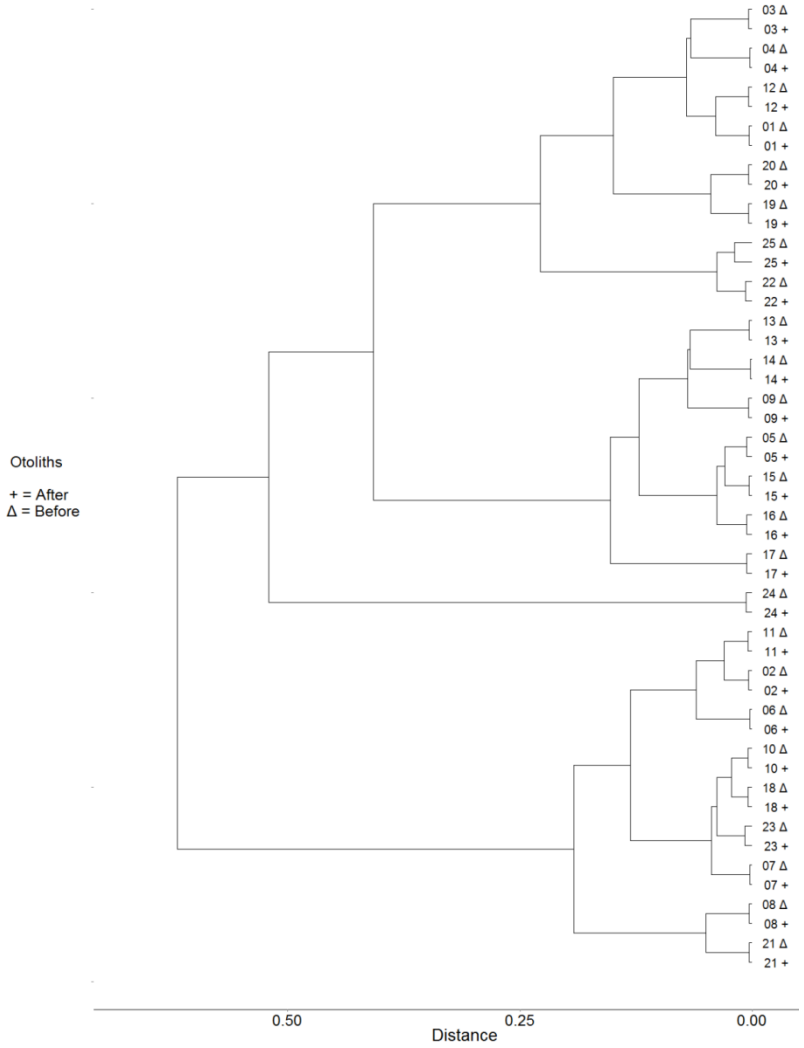

Figure 1. Dendrogram showing the results from cluster analysis on shape measurements. All individual pairs of before and after otoliths are so similar that they cluster together with a distance close to zero.

References

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