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Suitability of glycerin-preserved otoliths for age validation using bomb radiocarbon

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The effect of glycerin storage on the bomb radiocarbon content of otoliths was determined experimentally. Storage in either pre- or post-bomb glycerin had no detectable effect on the bomb radiocarbon content of either pre- or post-bomb otoliths. Therefore bomb-dated age validation studies need not be restricted to freshly-collected samples or dry otoliths, implying that the large numbers of glycerin-archived otoliths around the world are suitable for age validation studies using bomb radiocarbon.

Key words: age validation; isotope; otolith; preservation; radiocarbon.

INTRODUCTION

Close to one million fish otoliths are aged each year, largely in support of stock assessments and age-structured calculations such as growth and mortality rate (Campana & Thorrold, 2001). Although the accuracy of age determination methods has improved substantially since the time of Beamish & McFarlane's (1983) alarm call, the accuracy of routine ageing methods has yet to be confirmed in hundreds of species. The problem is particularly acute with long-lived fish species, for which traditional age validation methods have proven to be problematic (Campana, 2001). In such species, the use of bomb radiocarbon (^{14}C) is now recognized as one of the best possible methods of age validation (Kalish, 1993; Campana & Jones, 1998). The approach is based on the abrupt increase in global levels of radiocarbon produced by atmospheric testing of nuclear weapons in the 1950s and 1960s. The resulting radiocarbon pulse is clearly evident in assays of calcified structures which were growing at the time, and thus provides a dated marker in structures such as otoliths which produce annular growth increments.

The most sensitive years for $\Delta^{14}C$-based ageing are 1958–1965, since these are the years during which aquatic ^{14}C levels increased most rapidly. Although the

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method works well with recently-collected fishes which were old enough to have been born before 1965, the extended half-life (5715 years) of ^{14}C means that the method is equally applicable to otoliths which were collected anytime after 1965 and then stored. Otoliths are typically stored either dry, or preserved in non-acidic fluids such as glycerin or a glycerin/water mixture (Chilton & Beamish, 1982). Glycerin is used as a storage medium because of its clearing properties, which enhances the visibility of growth increments as it permeates the micro-channel architecture of the otolith over a period of a few weeks or months (Gauldie *et al*., 1998). This same property, the permeation of the otolith with the glycerin, poses potential problems with respect to the utility of glycerin-preserved otoliths for bomb radiocarbon assays. In particular, if the radiocarbon signature of the glycerin was substantially different from that of the otolith, and if sufficient glycerin was retained within the internal structure of the otolith after its removal from the storage medium, it is possible that the radiocarbon assay of the otolith would be distorted, producing an incorrect estimate of date (and age) of formation. A less likely, but still possible, situation is that the glycerin could gradually dissolve either the calcified or organic component of the otolith, thereby skewing the radiocarbon signature to the remaining, undissolved component. Either situation would disallow the use of glycerin-stored otoliths for bomb radiocarbon age validation studies, and thus greatly reduce the number of potential otolith archives which could be used for age validation studies.

The objective of this study was to experimentally determine if storage in glycerin can change the radiocarbon content of an otolith. Using a matched pair nested design, the influence of both pre- and post-bomb glycerin on the radiocarbon content of both pre- and post-bomb otoliths after soaking for a period of 4 months was assessed. The utility of worldwide archives of glycerin-preserved otoliths for bomb radiocarbon age validation studies was also investigated.

METHODS

Glycerin used to preserve and clear otoliths could conceivably change the net radiocarbon content of the otolith, depending upon the amount and radiocarbon content of the glycerin incorporated into the otolith, and the magnitude of any radiocarbon discrepancy between otolith and glycerin. Therefore, a matched pair nested design was used to test for the effect of glycerin on otolith radiocarbon content (Table I). The glycerin treatment consisted of two levels: pre-bomb (1957 or before) and post-bomb (2001). Each

TABLE I. Matched pair experimental design for the evaluation of the effect of glycerin storage on the bomb radiocarbon content of otoliths, where O_{i1} and O_{i2} are the first and second otolith from the ith fish, respectively

	Pre-bomb otoliths		Post-bomb otoliths	
	Pre-bomb glycerin	Post-bomb glycerin	Pre-bomb glycerin	Post-bomb glycerin
Stored in glycerin	O_{11}, O_{21}	O_{31}, O_{41}	O_{51}, O_{61}	O_{71}, O_{81}
Stored dry	O_{12}, O_{22}	O_{32}, O_{42}	O_{52}, O_{62}	O_{72}, O_{82}

factor level was nested within each of the two otolith groups (pre-bomb and post-bomb) each of which contained two otolith pairs. The use of matched pairs (left and right otoliths) ensured that treatment effects were not confounded by any natural variability in otolith radiocarbon content among fishes, since one otolith of each pair was exposed to glycerin while the other remained dry as a control. Both pre- and post-bomb glycerin and otoliths were tested to ensure that depleted or enhanced radiocarbon activity in old or new glycerin sources could be detected in either old or new otolith material. Although it would have been desirable to have used a larger sample size, the high cost of AMS assays precluded the assay of more than 16 otoliths (eight treatment and eight control) in this experimental design.

Intact sagittal otolith pairs from young haddock *Melanogrammus aeglefinus* L. were used in all treatments. Otoliths were drawn from archived collections, and had been stored dry in paper envelopes since the time of collection. Pre-bomb otoliths from haddock aged 1–3 years were from collections made in 1952 and 1954 off southern Newfoundland (NAFO division 3Ps), while post-bomb otoliths were from age 1 year haddock collected off south-western Nova Scotia (NAFO division 4X) in 2001.

Post-bomb glycerin was purchased in 2001 immediately before use. Pre-bomb glycerin (40 ml) was drained from five different vials used to store Pacific halibut *Hippoglossus stenolepsis* Schmidt otoliths collected in 1957. Based on the high viscosity and yellowed nature of the glycerin that was drained, there is no reason to suspect that the glycerin used in the experiment was anything other than the original glycerin placed in the vial in 1957. It was not known if the original glycerin was diluted 1 : 1 with water, or if small amounts of thymol were added to inhibit mold, but neither process would be expected to influence the results. Samples of both batches of glycerin were subsequently assayed for radiocarbon.

The glycerin treatment consisted of storing one otolith from each pair in a glass vial filled with experimental glycerin for a period of 4 months. The matching otolith was stored dry in a separate glass vial as a control.

Upon completion of the storage period, each glycerin-preserved otolith was wiped dry with a laboratory-grade tissue wipe, brushed with an acid-washed toothbrush under a brief (<15 s) flow of Super Q water (water which had been distilled, deionized and purified through reverse osmosis), wiped dry again, then allowed to dry on paper overnight. The otolith was then once more wiped dry and weighed to the nearest mg. The intent was to remove all surface glycerin, but not to remove any of the glycerin incorporated into the microchannel architecture of the otolith. Control otoliths were treated similarly.

All otoliths were prepared for $\Delta^{14}C$ assay with accelerator mass spectrometry (AMS) by individually powdering in a cleaned mortar and pestle, then subsampling 11–12 mg for assay. Radiocarbon values were reported as $\Delta^{14}C$, which is the per mil (‰) deviation of the sample from the radiocarbon concentration of 19th century wood, corrected for sample decay prior to 1950 according to methods outlined by Stuiver & Polach (1977). All assays also reported $\delta^{13}C$ values, which were used to correct for isotopic fractionation effects in the calculation of $\Delta^{14}C$.

RESULTS

The range of $\Delta^{14}C$ values across all of the otoliths varied between -85 and 31, as would be expected for marine carbonates formed before (1957 or before) and after (2001) atmospheric testing of nuclear weapons (Fig. 1). Thus the large differences in $\Delta^{14}C$ between pre- and post-bomb otoliths were expected. $\Delta^{14}C$ (mean $\pm$ S.E.) in the pre-bomb otoliths was $-77{\cdot}2 \pm 2{\cdot}3$, while that in the post-bomb otoliths was $23{\cdot}5 \pm 1{\cdot}9$. No obvious effect due to glycerin storage was evident in any of the treatments (Fig. 1). Otolith $\delta^{13}C$ did not vary significantly among any of the experimental treatments or otolith groups ($P > 0{\cdot}10$). The mean $\pm$ S.E. $\delta^{13}C$ was $-1{\cdot}66 \pm 0{\cdot}06$ ($n = 16$).

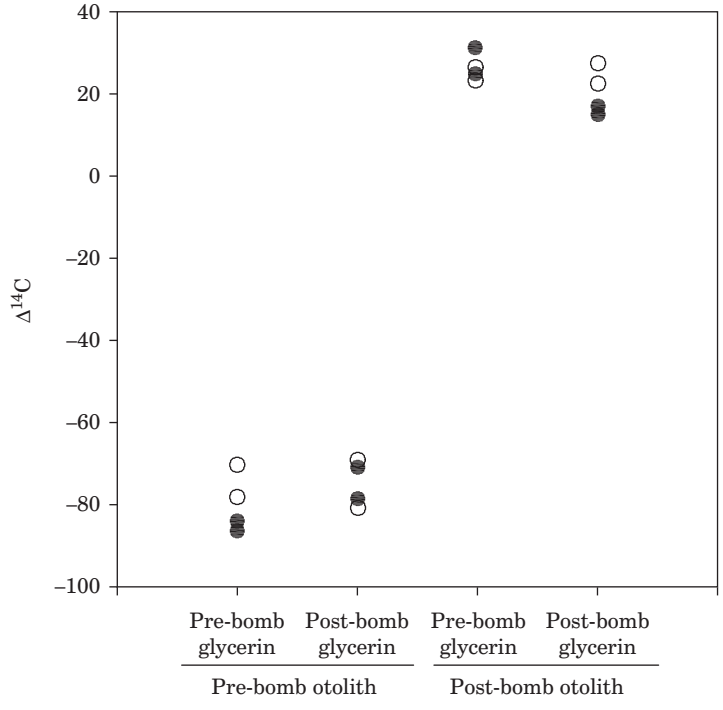

FIG. 1. $\Delta^{14}C$ content of pre-bomb (1957 or before) and post-bomb (2001) haddock otoliths preserved in pre-bomb or post-bomb glycerin. One otolith from each pair was preserved for 4 months in glycerin (●), while the other otolith was left dry (○). The S.D. of each assay point is *c.* 5.

Since the incorporation of radiocarbon into an otolith is controlled at the whole-fish level and not at the level of the individual otolith, there should be no difference in $\Delta^{14}C$ between left and right otoliths (glycerin-stored *v.* dry) other than that due to treatment effects and analytical error. Comparison of the within-fish $\Delta^{14}C$ difference indicated that there were no significant differences ($P > 0{\cdot}05$) due to either pre- or post-bomb glycerin in either pre-bomb or post-bomb otoliths (Fig. 2). Although storage in new glycerin appeared to depress $\Delta^{14}C$ slightly in post-bomb otoliths, no such effect was apparent in pre-bomb otoliths. In contrast, pre-bomb glycerin appeared to depress $\Delta^{14}C$ slightly in pre-bomb otoliths, but not in post-bomb otoliths. Thus if there was an effect due to glycerin, it was inconsistent and opposite between the two otolith groups.

Despite the small sample size of this experiment, the statistical power of these comparisons was quite high. The power to detect a $\Delta^{14}C$ increase of 15‰ due to post-bomb glycerin in the pre-bomb otoliths was 62%, while the power to detect the same increase in the post-bomb otoliths was >90%. Given a mean analytical error (S.D.) of *c.* 5 for a single radiocarbon assay, and in light of the different direction of effect in the two otolith groups, it appears that the small (and non-significant) differences evident in Fig. 2 are artifacts of natural variability and possibly a very weak treatment effect.

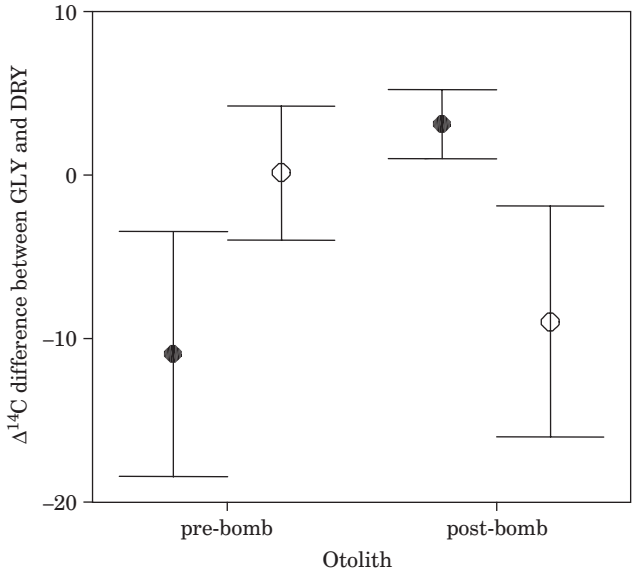

FIG. 2. Δ^{14}C differential (mean ± 2 S.E.) between glycerin-preserved (GLY) and control (DRY) otoliths using one otolith of each pair for the treatment and the other otolith for the dry control. The treatments represent pre- and post-bomb otoliths exposed to both pre- (●) and post- (○) bomb glycerin.

The radiocarbon content of the pre-bomb glycerin (±S.D.) was 10·2 ± 5·1 with a δ^{13}C of −25·3. The radiocarbon content of the post-bomb glycerin (±S.D.) was 95·4 ± 5·5 with a δ^{13}C of −28·9. Thus the glycerin values were consistent with the radiocarbon content expected of atmospheric and terrestrial sources in the year of otolith formation (Campana & Jones, 1998).

DISCUSSION

Storage in glycerin had no noticeable effect on the radiocarbon content of the otolith. The design of the experiment was such that even subtle effects should have been evident: the use of one otolith of each pair as a control insured that all but treatment effects and analytical error were eliminated, while the use of both pre- and post-bomb glycerin and otoliths maximized the likelihood that depleted radiocarbon levels in one source would be detected against the elevated backgrounds of the other source. The 4 month duration of the experiment was typical of the period used to impermeate and 'clear' otoliths in preparation for age reading (Chilton & Beamish, 1982). Despite this, no consistent effect of glycerin could be detected. Certainly, it is reasonable to expect more complete permeation of glycerin into an otolith after 40 years rather than 4 months. Nevertheless, based on typical otolith clearing rates of *c.* 1 month, an exponential rate of diffusion, and published experimental results (Gauldie *et al.*, 1998), the majority of glycerin would be expected to enter the otolith in the first 1–2 months. Indeed, the present results are consistent with expectations based on the capacity of the otolith to incorporate fluids and the carbon content of

glycerin relative to the calcium carbonate of the otolith. With a 39% carbon content by mass, glycerin contains nearly 4 times more carbon per unit mass than the otolith (10% carbon). Gauldie *et al.* (1998) reported that otolith mass increased by *c.* 1% after continuous soaking in water, which, assuming that similar quantities of glycerin were to be incorporated, would imply that 3·9% of the carbon atoms in the impermeated otolith originated from the glycerin. In fact, a somewhat lower glycerin incorporation rate is likely, given the larger size of the glycerin molecule compared to that of water. In light of the enhanced $\Delta^{14}C$ content of the glycerin compared to the otolith, the net $\Delta^{14}C$ content of the otolith would be expected to increase by *c.* 3‰ if exposed to glycerin of comparable age, shifting a post-bomb otolith from a $\Delta^{14}C$ of 23·5 to 26·3. In the most extreme case, exposure of a pre-bomb otolith to post-bomb glycerin might increase the $\Delta^{14}C$ by up to 7‰. These theoretical increases due to glycerin storage were not observed in the experiment, where glycerin tended to depress, rather than increase, the $\Delta^{14}C$ content. In any event, neither theoretical nor observed shifts in $\Delta^{14}C$ would be readily detectable against the background of natural variability and analytical error in the $\Delta^{14}C$ assay. Use of 1 : 1 glycerin-water rather than pure glycerin would further reduce any impact due to glycerin incorporation. In contrast, otolith $\Delta^{14}C$ increased by *c.* 15‰ $year^{-1}$ between 1958 and 1967 due to bomb-related production.

The present results greatly increase the potential pool of otoliths which are available for age validation studies using bomb radiocarbon. Although numbers are not available, informal conversations with fisheries laboratories around the world suggest that large numbers of archived otoliths are currently stored in glycerin. The suitability of these otoliths for bomb dating, many of which were collected decades ago, will now allow age validation of species which do not routinely attain ages of $\geq$40 years, as would be required for fresh samples. In addition, it provides access to otolith samples of young fishes collected in the 1960s, thus providing new material which can be used to prepare reference radiocarbon chronologies for unknown environments.

Although the results indicate that no detectable glycerin contamination remained after simply wiping the surface of the otolith, such a simple approach is not recommended in preparing an otolith for radiocarbon assay. Sonification in ultra-pure water is a proven method for cleansing otoliths of both surface and internal unbound contaminants (Campana, 1999), and glycerin is miscible in water. In addition, there is a possibility that multi-year immersion in glycerin might produce slightly larger effects than were observed in the present study, although the calculations based on the observed radiocarbon content of glycerin makes it difficult to imagine any immersion period that would produce more than a 1 year (15‰) deviation in otolith radiocarbon age. Nevertheless, it would appear prudent to report the $\Delta^{14}C$ content of the storage glycerin whenever glycerin-archived otoliths are assayed for bomb radiocarbon. Due to the different assays involved, the results have no implications for the suitability of glycerin-preserved otoliths in trace element assays.

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