Age Validation of Pacific Cod (*Gadus macrocephalus*) Using Stable Oxygen Isotopes ($\delta^{18}O$)

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THEORY

1. The $\delta^{18}O$ in otoliths is a function of temperature, an inverse relationship showing seasonal cycles may exist.
2. Microsampling of an otolith from pre first year to the outer edge, with multiple samples across any one year's growth, can follow seasonal changes throughout the life of the fish.
3. HYPOTHESIS: The number of $\delta^{18}O$ peaks (true age) should equal the estimated age (count of translucent zones), and can be used as an AGE VALIDATION. (Fig. 1)

METHODS

1. Pacific cod were collected in the eastern Bering Sea. Specimens with estimated ages up to 5 years were randomly chosen.
2. Otoliths, aged 2 to 5 years old, were sampled using a micromilling system (Fig 2). Up to 42 sequential microsamples were milled from the center to the edge of each otolith (Fig 1b).
3. Each microsample was analyzed for $\delta^{18}O$ by mass spectrometry, and results were plotted to demonstrate $\delta^{18}O$ changes during the life of the fish.
4. To confirm the relationship between $\delta^{18}O$ and temperature, otoliths aged 1 year old were microsampled and analyzed for $\delta^{18}O$ on the outer edge (reflecting capture temperature).

RESULTS

1. For specimens aged 2 to 5 years old (n = 38), most had the same number of $\delta^{18}O$ peaks as estimated age (Figs 3 and 4).
   - 1176 microsamples were milled from 40 otoliths, averaging 29 measurements of $\delta^{18}O$ per otolith.
   - Most translucent zones interpreted as annual (and counted) were associated with $\delta^{18}O$ peaks, indicating a correct age estimate.
   - The age bias plot showed agreement between ages from $\delta^{18}O$ peaks (true age) and ages from translucent growth zone counts (Fig. 4a). The probability of assigning an age less than or greater than the true age is approximately 10% (Fig. 4b).
2. The relationship between $\delta^{18}O$ in pacific cod otoliths and bottom temperature was inverse, linear, and statistically significant (Fig. 5; r^2 = 0.74, p<0.1).

CONCLUSIONS

1. The ages estimated from translucent growth zone counts were validated by the number of $\delta^{18}O$ peaks (true age).
2. There is only a small chance (10%) that ages estimated from translucent zone counts are in error by ± 1 year or more.
3. Water temperature ($T°C$) appears to be the primary factor controlling variation in $\delta^{18}O$ signatures of Pacific cod otoliths.

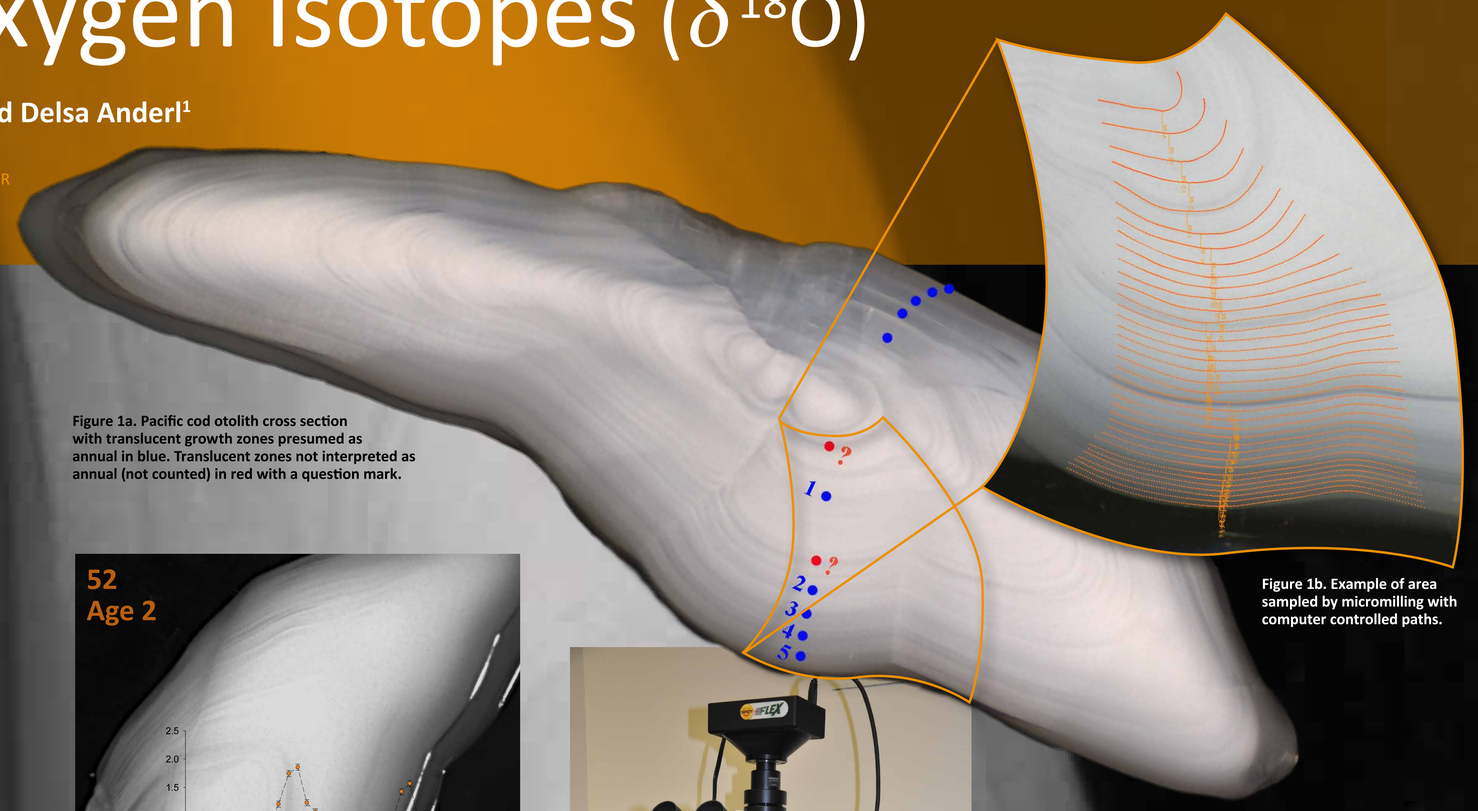
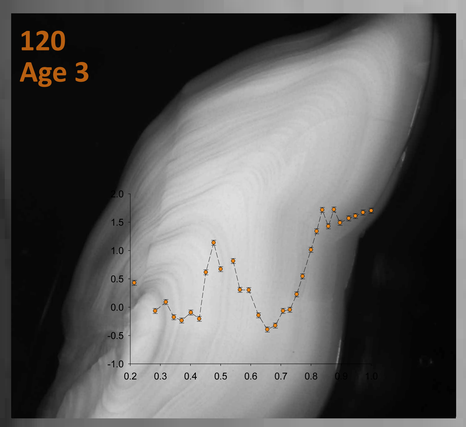
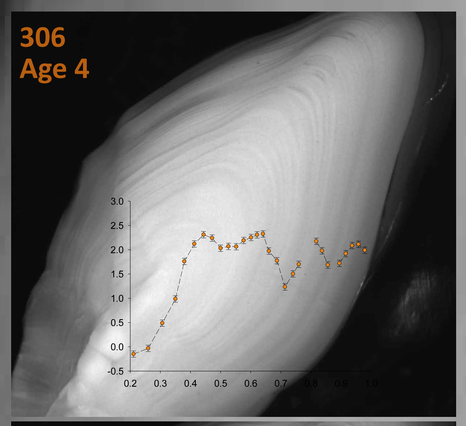


Figure 1a. Pacific cod otolith cross section with translucent growth zones presumed as annual in blue. Translucent zones not interpreted as annual (not counted) in red with a question mark.

Figure 1b. Example of area sampled by micromilling with computer controlled paths.





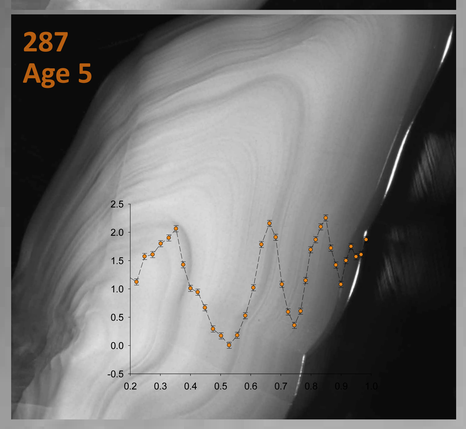


Figure 3. Example of results, one specimen for each estimated age is shown, age 2 through 5 years. Otolith images are overlaid with $\delta^{18}O$ (in ‰ VPDB) results.

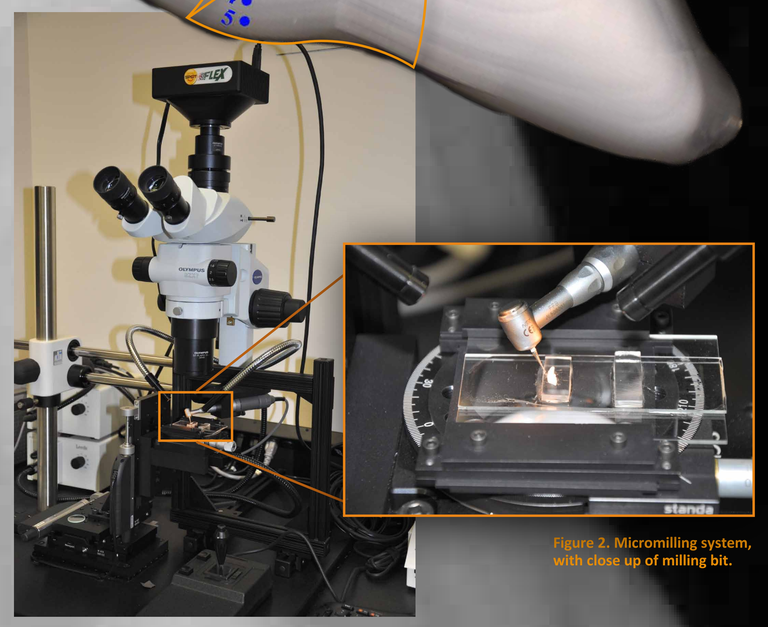


Figure 2. Micromilling system, with close up of milling bit.

δO^{18} Age (Year)

Estimatd Growth Zone Age All (Yr)	0	1	2	3	4	5	6	7
0								
1			3					
2			28	3				
3			2	20	1			
4				11	15	13	3	
5					5	8		
6				2				
7								

Age bias plot of $\delta^{18}O$ ages verses ages estimated with growth zone counts (number in each cell is count). Diagonal cells (orange) are comparisons in agreement.

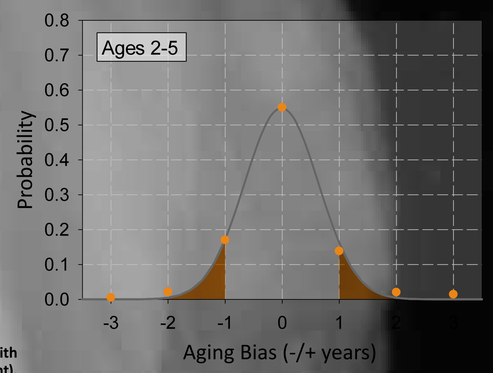


Figure 4b. Probability of assigning an age different (ageing error) from the true age by one or more years.

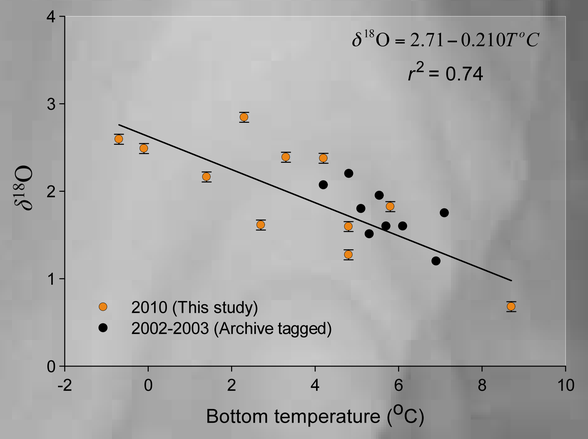


Figure 5. In 1 year old specimens, $\delta^{18}O$ (in ‰ VPDB) from otolith edges is compared to bottom temperatures ($T°C$) at capture.