**THEORY**

1. The δ¹⁸O 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 δ¹⁸O 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 δ¹⁸O by mass spectrometry, and results were plotted to demonstrate δ¹⁸O changes during the life of the fish.
4. To confirm the relationship between δ¹⁸O and temperature, otoliths aged 1 year old were microsampled and analyzed for δ¹⁸O 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 δ¹⁸O peaks as estimated age (Figs 3 and 4).
   - 1176 microsamples were milled from 40 otoliths, averaging 29 measurements of δ¹⁸O per otolith.
   - Most translucent zones interpreted as annual (and counted) were associated with δ¹⁸O peaks, indicating a correct age estimate.
   - The age bias plot showed agreement between ages from δ¹⁸O 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 δ¹⁸O in pacific cod otoliths and bottom temperature was inverse, linear, and statistically significant (Fig. 5; r² = 0.74, p<0.1).

**CONCLUSIONS**

1. The ages estimated from translucent growth zone counts were validated by the number of δ¹⁸O 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 δ¹⁸O signatures of Pacific cod otoliths.