Effects of microprobe precision on hypotheses related to otolith Sr:Ca ratios

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Several recent studies have used the electron microprobe to infer environmental temperature at the time of otolith formation from the concentration ratio of strontium and calcium. Sr/Ca ratios of otoliths from fish held at constant temperature or collected at known temperature were examined using atomic absorption spectrophotometry (Radtke 1984, 1989) or wavelength dispersive electron microprobe analysis (Townsend et al. 1989, Kalish 1989, Radtke et al. 1990). These studies, with the exception of Kalish (1989), concluded that there is a negative linear relation between environmental temperature and otolith Sr/Ca ratio. This relationship, coupled with assignment of age to each microprobe sample site, has been used to infer the relative temperature histories of wild-caught fish (Radtke 1984,1987,1989; Radtke and Targett 1984; Radtke and Morales-Nin 1989; Townsend et al. 1989; Radtke et al. 1990). The most ambitious application of the method used otolith Sr/Ca ratios to contrast the calculated temperature histories of different subpopulations of larval herring in the Gulf of Maine (Townsend et al. 1989).

Using the electron microprobe to calculate individual fish temperature histories from otolith Sr/Ca ratios is potentially a useful technique for fisheries biologists. However, precision of back-calculated temperature estimates should be examined in greater detail. Previous studies do not explicitly state confidence limits for mean responses or prediction limits for new observations. The scatter of points in Radtke (1989), Townsend et al. (1989), and Radtke et al. (1990) suggest that widths of 95% prediction limits may be on the order of one to several °C for most levels of Sr/Ca examined. While this might be acceptable for studies of fish which are exposed to wide variations in environmental temperature, it is of less use for species which experience more subtle temperature changes.

Future validation experiments may improve the predictive capabilities of the Sr/Ca vs. temperature relationship by examining effects of other variables. For instance, the regression model might be expanded to include growth rate (Kalish 1989) and some measure of physiological stress (Townsend et al. 1989), since these also appear to influence the Sr/Ca ratio.

However, one component of the variation not likely to change in future experiments employing the electron microprobe is the model error term associated with measurement. Usually measurement error is considered insignificant in relation to other sources of variation and is incorporated into the total error term:

$$Y = a + b*X + \epsilon_{Total}$$

where $\epsilon_{Total} = \epsilon_{Measurement} + \epsilon_{Other}$. Measurement error can be thought of as a lower bound to the variation associated with the regression model when other sources of error are minimized.

We suspect that measurement error may be nontrivial when deriving Sr/Ca vs. temperature relationships. Sr/Ca ratios associated with a 1°C change in environmental temperature were approximately 0.00013–0.00036 in previous studies (Table 1). It is difficult to evaluate the significance of these small values without more information on the analytical precision of Ca and Sr detection in fish otoliths using the electron microprobe. Of the studies cited above, only Kalish (1989) reported analytical precision for representative values of Sr and Ca. In that study, measurement error associated with Sr was 3.5% and that associated with Ca was 0.5% for an Sr/Ca ratio of 0.002.

One purpose of the present study was to examine the precision associated with measuring Sr/Ca ratios in fish otoliths, and to demonstrate how this error affects temperature estimates derived from published regressions. Our approach was to intensively sample one otolith from one fish at three beam-power densities and four counting times. By using one otolith, between-fish effects could be ignored. Within-fish Sr/Ca effects were minimized by referencing samples to the same growth zones, leaving the different analytical techniques as the primary source of variation.

Table 1
Published Sr/Ca vs. temperature relationships.

Source and species examined	Equation
Radtke et al. (1990) *Clupea harengus* Atlantic herring	T = 19.172 − 2.955 * (Sr/Ca*1000) 1°C = 0.000338 (Sr/Ca) Sr/Ca range 0.002–0.0045
Townsend et al. (1989) *Clupea harengus* Atlantic herring	T = 12.6 − 2.81 (Sr/Ca*1000) 1°C = 0.000356 (Sr/Ca) Sr/Ca range 0.001–0.0045
Radtke (1989) *Fundulus heteroclitus* Mummichog	(Sr/Ca*1000) = 16.371 − 0.219 * T 1°C = 0.000219 (Sr/Ca)[a] Sr/Ca range 0.009–0.013
Radtke (1984) *Gadus morhua* Atlantic cod	(Sr/Ca*1000) = 4.19 − 0.13 * T[b] 1°C = 0.000130 (Sr/Ca)[a] Sr/Ca range 0.0028–0.0038

[a]Assumes that slope of Sr/Ca on temperature (T) will also predict T on Sr/Ca.
[b]Not stated explicitly in Radtke (1984), but later reported in Kalish (1989).

A second purpose was to determine the effect of beam exposure on the constancy of Sr/Ca ratios. This was necessary because analytical techniques, such as increasing the counting time, will improve the precision of an analysis but may reduce its accuracy through beam damage to the specimen (e.g., Smith 1986, Potts 1987). This problem is encountered in the analysis of other carbonates, but is particularly severe for otoliths, which contain organic material in addition to $CaCO_3$ (Degens et al. 1969). CO_2 is lost during electron beam exposure and, because it is not actually measured by the microprobe but assumed to occur on a 1:1 basis with cations such as Sr and Ca, concentrations of those elements will increase with increasing beam damage. However, if Sr and Ca are not fractionated from one another by beam damage, their ratio should remain unchanged. Absence of change would indicate that methods which improve precision can be implemented without affecting the accuracy of Sr/Ca ratio determinations.

Methods

Dover sole *Microstomus pacificus* is a common Pacific coast flatfish. Juvenile Dover sole 54–104 mm SL were captured by trawling off the Oregon coast on 17 March 1990 and immediately injected with oxytetracycline (OTC). Within 12 hours, fish were transferred to aquaria in Corvallis, Oregon, where they were held for up to 48 days. The OTC produced a fluorescent band which delineated growth prior to capture from subsequent growth under laboratory conditions. Only portions of the otolith formed under natural conditions (inside the OTC band) were analyzed.

An otolith from a randomly selected fish was mounted on a slide with a toluene-based medium. It was ground using 600-grit paper along the saggital plane to a level near the central primordium. The mounting medium was then melted, the otolith was removed, washed, and remounted on its opposite side with heat-setting epoxy. The second side was then ground to the central primordium and polished with a series of diamond and alumina grits, ending with 0.05 μm alumina. The specimen was cleaned ultrasonically in detergent and water between grit changes and given final rinses in water and methanol. Prior to microprobe analysis, the specimen was carbon coated.

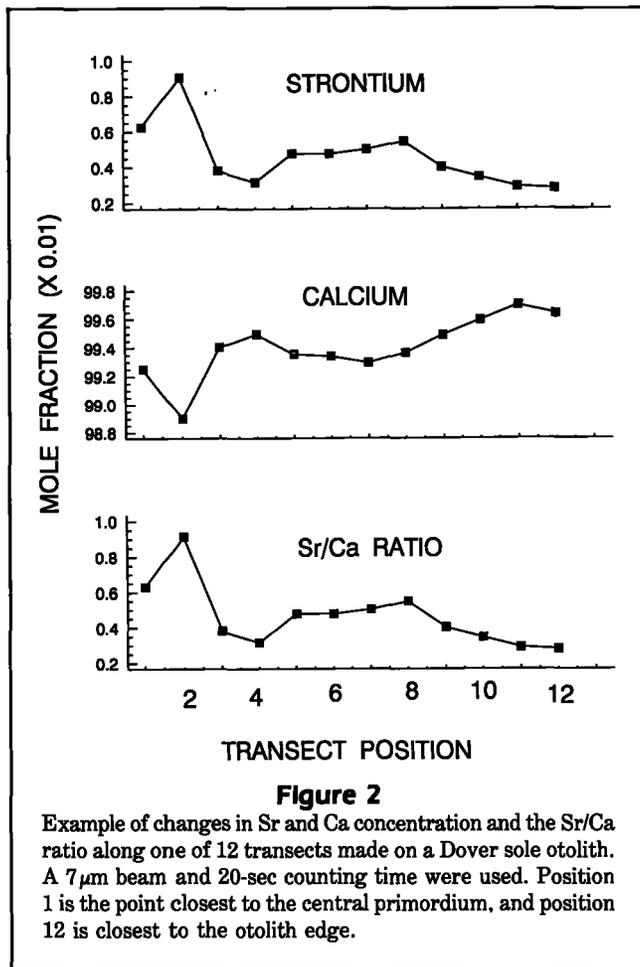## Beam power density and precision

Wavelength-dispersive electron microprobe analysis was performed with a Cameca SX-50 microprobe with a 40° beam angle. Three levels of beam-power density were obtained by varying the beam diameter while holding accelerating voltage and beam current constant at 15 KV and 20 nA, respectively. These voltage and current settings are common to most of the previous studies (R. Radtke, Hawaii Inst. Geophys., Univ. Hawaii, Honolulu 96822, pers. commun. 1990), although Kalish (1989) used a 10 nA current. Defocused beam diameters of 5, 7, and 10 μm resulted in beam-power densities of 1.019, 0.520, and 0.255 nA/μm. The most common beam diameter used in previous studies was 5 μm (R. Radtke, pers. commun. 1990), although Kalish (1989) rastered a 12.5 μm × 12.5 μm square.

Sr and Ca concentrations were calculated as normalized mole fractions (equivalent to the atomic ratios of Kalish 1989). Mole fractions are more informative than weight percentages for examination of Sr/Ca ratios, since the substitution of Sr for Ca in otolith aragonite theoretically occurs on a per-atom basis (e.g., Radtke 1989). Normalization also reduces effects of beam damage on concentrations.

Precision of elemental measurements was determined as the coefficient of variation (CV) (Williams 1987),

$$CV = \sigma_{k\text{-ratio}}/k\text{-ratio}$$

where the k-ratio is the ratio of x-ray counts from the otolith to those of the standard (i.e., the calibrated fraction of that element in the otolith) and $\sigma_{k\text{-ratio}}$ is the

standard deviation of that measurement. For a single microprobe analysis, this is calculated as

$$CV = \left\{ \left[\frac{\left[\sum\limits_{i=1}^{n} N_i^2 - \frac{\left(\sum\limits_{i=1}^{n} N_i\right)^2}{n} \right]^{0.5}}{\left(\frac{\sum\limits_{i=1}^{n} N_i}{n}\right)} \right]^2_{Standard} + \left[\frac{N_p + \left(\frac{t_p}{t_b}\right) * N_i}{(N_p - N_b)} \right]^2_{Otolith} \right\}^{0.5}$$

where n = number of samples taken on the standard,

  N_i = x-ray count (corrected for background count) from ith sample on the standard,
  N_p = x-ray count for peak wavelength of element in sample,
  N_b = x-ray counts from background wavelengths of element in sample,
  t_p = peak wavelength counting time, and
  t_b = background wavelength counting time.

Approximate 95% confidence limits for each element measured in each sample were considered $\pm 2 * CV$, since the Poisson distribution underlying these calculations approximates a normal distribution when sample size (the number of x-rays detected by the spectrometer during an analysis) is high (Williams 1987). X-ray counts in this experiment were on the order of $10^2 - 10^3$ for Sr and $10^4 - 10^5$ for Ca. Confidence limits for the Sr/Ca ratio were also calculated as $\pm 2 * CV$, but in this case the standard deviation of the k-ratio was calculated as

$$\sigma_{Sr/Ca} = \left[\left(\frac{\sigma_{Sr}}{k\text{-ratio}_{Sr}}\right)^2 + \left(\frac{\sigma_{Ca}}{k\text{-ratio}_{Ca}}\right)^2 \right]^{0.5}.$$

Sr and Ca were analysed using the TAP (Sr L-α) and PET (Ca K-α) crystals. Background counts were taken at $\pm(0.005 * \sin \theta)$ (where θ is the angle of the spectrometer crystal when it is detecting peak counts) for the same length of time as the peak count. Due to interference with a second-order Ca K-α peak, only one background count was made for Sr. Strontianite (NMNH R10065) and calcite (USNM 136321) were used as standards.

Counting time and precision

Counting time refers to the length of time a spectrometer is collecting counts of characteristic x-rays for an element during one analysis. Counting times of 10, 20, 30, and 40 sec were compared for each beam-power density. The most commonly used counting time for both elements in previous studies was 20 sec (R. Radtke, pers. commun. 1990), although Kalish (1989) analyzed Sr at 100 sec and Ca at 20 sec. Precision was determined as with beam-power density.

Transects of twelve analyses each were made for the 12 combinations of beam power density (4) and counting time (3) (combined $N = [12*3*4] = 144$). These transects passed from an area near the central primordium to an area just inside the discontinuity created by accessory primordia (Fig. 1). This discontinuity

Figure 1

Photomicrograph of otolith from 65.7 mm SL juvenile Dover sole *Microstomus pacificus*, showing location of 12 microprobe transects used for analysis. Each circular area represents one analysis. Note hyaline area near central primordium at inner end of transects and more opaque area towards outer end. The 13th transect was an accidental repetition of the 10 μm, 30-sec transect. Bar indicates 100 μm.

Figure 2
Example of changes in Sr and Ca concentration and the Sr/Ca ratio along one of 12 transects made on a Dover sole otolith. A 7 μm beam and 20-sec counting time were used. Position 1 is the point closest to the central primordium, and position 12 is closest to the otolith edge.

Figure 3
Relation of coefficient of variation (measurement error) to elemental concentration for transects from one Dover sole otolith. Each regression represents three transects with 12 points each (*N* 36). Regression equations are presented in Table 2.

Table 2
Relationship between Sr concentration and coefficient of variation (CV) for different counting times, based on microprobe transects along the saggital plane of a Dover sole *Microstomus pacificus* otolith. Each equation was derived from three transects of twelve points each (*N* 36). Equations are in the form: $CV = \exp(A + (B*Sr \text{ mole fraction}))$. Standard errors in parentheses.

Seconds	A	B	R^2
10	1.546	−59.261	0.940
	(0.012)	(2.558)	
20	1.150	−47.156	0.938
	(0.011)	(2.076)	
30	0.941	−44.454	0.947
	(0.010)	(1.806)	
40	0.789	−42.135	0.938
	(0.010)	(1.863)	

was >100 μm inside the OTC mark. Starting and ending points for all transects were referenced to specific growth areas identified by dark continuous bands, and the remaining points were evenly spaced between these two points. Locations at the start of the transects were in a translucent area of the otolith assumed to have little organic material (Dannevig 1955), while the end points were in a more opaque area, which probably contained more organic material.

Exposure time and accuracy

Counting time and exposure time were distinguished in this experiment. Counting time is the minimum time the specimen is exposed to the electron beam, while exposure time also includes the time necessary to collect background counts and counts of other elements.

Six sequential analyses were made at each of six locations (combined *N* 36) to determine changes in elemental concentration. The locations were the start and endpoints of each 20-sec transect used for the precision analysis. Sequential analyses at each location were

made in increments of 20-sec counting times, which corresponded to exposure times of 65, 130, 195, 260, 325, and 390 sec. These exposure times were approximately twice as long as those which would result from an analysis of Sr and Ca alone, because S was also analyzed (results not reported).

Statistical analyses

The effect of elemental concentration on Sr precision (CV's) was examined with linear and nonlinear regressions. To determine if beam-power density affected Sr precision, multiple regressions containing normalized concentration and "dummy variables" corresponding to beam size were analyzed with partial-*F* tests (Neter et al. 1989:364–370). Each of the four counting times was analyzed separately.

Widths of 95% confidence intervals associated with Sr/Ca ratios were determined with linear and nonlinear regressions for each counting time. The ratios and widths of confidence intervals were then converted to temperatures using the four previously published Sr/Ca vs. temperature regressions in Table 1.

The effect of exposure time on Sr/Ca constancy was analyzed with a multiple regression containing exposure time and each location (coded as 0's and 1's) as independent variables. Locations were included to remove possible effects of initial Sr/Ca concentrations, which varied between sites. After determining that interactions and nonlinear terms did not improve a model with parallel straight lines, the common slope was compared with a slope of 0 using a t-test.

Whenever the null hypothesis could not be rejected at $\alpha = 0.05$, statistical power $(1-\beta)$ of the test was calculated as in (Neter et al. 1989:74-75). The power of a test was considered acceptable if $(1-\beta)>0.80$ (Peterman 1990).

Results

The twelve transects made under different beam conditions on the otolith of a 65.7 mm SL juvenile Dover sole exhibited consistent patterns of strontium and calcium concentrations. Sr concentrations were highest at the two innermost positions and lowest at the two outermost positions in all transects. Sr/Ca ratios mirrored the pattern of Sr. Ca concentrations were approximately 100-500 times higher than Sr concentrations. An example of one of the 12 transects (7 μm beam at 20-sec counting time) is presented in Figure 2.

Relative error of Sr measurements decreased as counting time and elemental concentrations increased, and this was best described by an exponential regression model (Fig. 3, Table 2). The coefficient of variation was 1.4-4.2% for Sr concentrations of 0.2-1.2%. When the effect of elemental concentration was removed, Sr CV's increased with decreasing beam-power density (Table 3); however, this effect was small compared with those of elemental concentration and counting time. Differences in Sr CV's attributable to beam-power density was 0.012-0.076%.

The coefficient of variation associated with Ca measurements was 0.5% for 10- and 20-sec counts and 0.4% for 30- and 40-sec counts, regardless of Ca concentration and beam-power density.

Regressions of the widths of 95% confidence intervals for Sr/Ca determinations against measured Sr/Ca ratios are presented in Figure 4 and Table 4. These regressions include only the effects of elemental concentration and counting time; the effect of beam-power density is omitted. Although relative error decreases

Table 3

Relationship between Sr coefficient of variation (CV) and beam power density, holding Sr concentration as a nonlinear covariate, based upon microprobe transects along the saggital plane of a Dover sole otolith. Each regression represents three transects with 12 points each (N 36). Equations are in the form: $CV = A_1 + A_2 Z_1 + A_3 Z_2 + B_1*(Sr$ mole fraction$) + B_2*(Sr$ mole fraction$)^2$ where A is the intercept for the 10 μm beam (0.255 nA/μm density), $A + A_2 Z_1$ is the intercept for the 7 μm beam (0.520 nA/μm density), and $A + A_3 Z_2$ is the intercept for the 5 μm beam (1.019 nA/μm density); Z_1 and Z_2 are dummy variables for the 7 and 5 μm beams; and B_1 and B_2 are fitted slope parameters. Partial-F tests indicate the significance of beam power density effects in the model. Standard errors in parentheses.

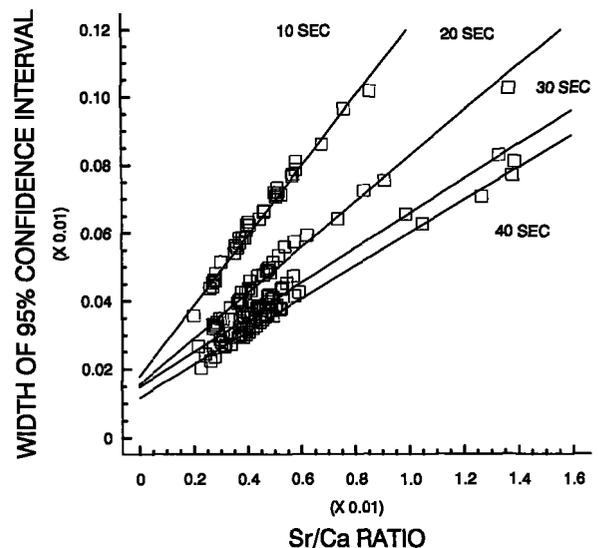| Parameter	Counts			
	10-sec	20-sec	30-sec	40-sec
A_1	4.990	3.394	2.767	2.420
	(0.101)	(0.043)	(0.051)	(0.041)
A_2	-0.013	-0.019	-0.027	-0.039
	(0.027)	(0.018)	(0.017)	(0.014)
A_3	-0.076	-0.055	-0.052	-0.053
	(0.027)	(0.018)	(0.017)	(0.014)
B_1	-389.80	-219.41	-169.07	-151.79
	(40.88)	(13.49)	(15.73)	(12.81)
B_2	17940.3	7746.5	5386.3	5229.6
	(3996.70)	(921.47)	(959.47)	(796.05)
R^2 (adj.)	0.954	0.969	0.959	0.962
$F*_{(0.05,2,31)}$	4.479	4.833	4.531	8.443
P	0.020	0.015	0.019	0.001

Figure 4

Relationship between Sr/Ca ratios and 95% confidence intervals of measurements at different counting times, based upon microprobe transects along the saggital plane of one Dover sole otolith. Each regression represents three transects of 12 points each (N 36). Regression equations are presented in Table 4.

Table 4

Relationship between Sr/Ca ratios and 95% confidence interval of measurements at different counting times, based upon microprobe transects along the saggital plane of a Dover sole otolith. Each equation was derived from three transects of 12 points each (N 36). Form of the relationship is: 95% CI = A + (B*Sr/Ca ratio). Standard errors in parentheses.

| Seconds	A	B	R^2
10	1.791E-4	0.1024	0.9861
	(9.829E-6)	(0.0021)	
20	1.551E-4	0.0669	0.9823
	(7.990E-6)	(0.0015)	
30	1.477E-4	0.0508	0.9817
	(6.579E-6)	(0.0012)	
40	1.166E-4	0.0482	0.9888
	(4.843E-6)	(0.0009)	

with increasing Sr/Ca, the actual width of the confidence interval increases. Conversion of Sr/Ca ratios and 95% confidence limits to temperatures, using the 20-sec regression and previously published temperature vs. Sr/Ca ratios, is presented in Figure 5. Confidence limits associated with calculated temperatures were 0.6–4.7°C, depending upon species, study, and temperature level.

The model which best fit the six multiple exposures is presented in Figure 6. The common slope of $-1.3*10^{-7}$ was not different from a slope of 0 ($t_{0.05,29} = 0.176$, $P = 0.86$). This experiment could have detected a change as small as $1.86*10^{-6}$ Sr/Ca per sec increased exposure (or $1.21*10^{-4}$ for each 65-sec treatment) at $\alpha = 0.05$ and $(1-\beta) = 0.90$, had such an effect existed.

Discussion

Our results confirm that measurement error associated with Sr/Ca determinations is nontrivial. At the standard counting time of 20 sec, measurement error associated with Sr/Ca determinations (expressed as 95% confidence intervals) was equal to or greater than the Sr/Ca increment representative of a 1°C temperature change in three of the four previously-published studies. Even in Townsend et al. (1989), at temperatures <4°C, measurement error was >1°C. The highest measurement error in the studies was representative of a 4.7°C temperature change. Inferred temperature differences between otolith regions or between fish should be considered in light of these values. Statistical error in the Sr/Ca vs. temperature regressions will add to the measurement error associated with temperature calculations.
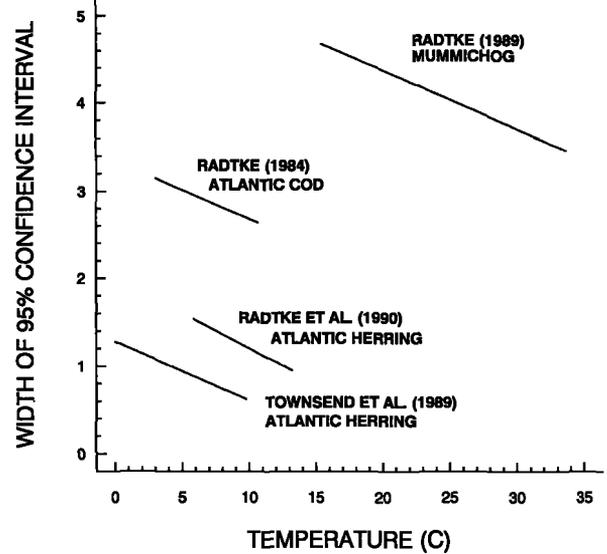
Figure 5

Relationship between back-calculated temperature estimates and 95% confidence intervals (for measurement error only) surrounding those estimates. Temperature vs. Sr/Ca conversions are from Table 1. Confidence intervals are converted from Sr/Ca confidence intervals for 20-sec counts in Table 4 and Figure 4.
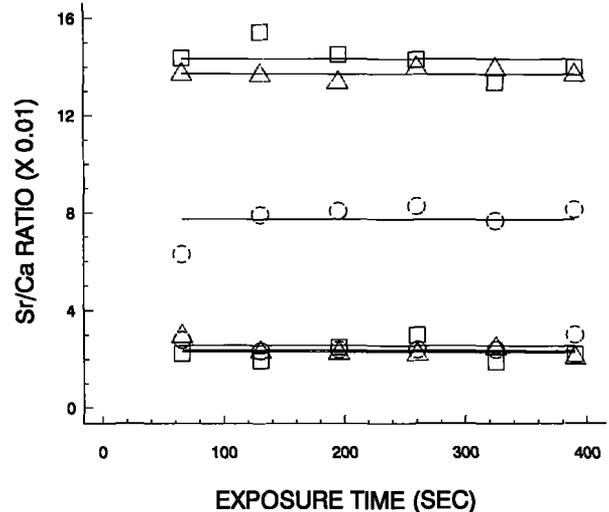
Figure 6

Relationship between Sr/Ca level and exposure time, based upon microprobe samples at six sites on one Dover sole otolith. Six sequential analyses were made at each site (N 36). The six exposure times corresponded to counting times of 20, 40, 60, 80, 100, and 120 sec. The equation describing the relationship is: Sr/Ca level = $0.0023 + (0.0054*Z_1) + (0.00026*Z_2) + (0.0114*Z_3) + (6.6 \times 10^{-5}*Z_4) + (0.012*Z_5) - (1.30 \times 10^{-7}*$ exposure time), where Z_1-Z_5 are dummy variables for locations. Adjusted $R^2 = 0.991$, $P < 0.0001$.

At least 40-sec counts would be necessary to detect a 1°C change in temperature experienced by herring (Townsend et al. 1989, Radtke et al. 1990) at all temperature levels examined in those studies. Detection of a 1°C temperature change in cod (Radtke 1984) and *Fundulus* (Radtke 1989) would require much longer counting times, beyond the range examined in this experiment.

This experiment documents the improvement in precision which is possible when otoliths are analyzed at longer counting times and higher beam power densities. Neither treatment appeared to affect the level of Sr/Ca accuracy under the range of conditions examined. Obvious burns on the otolith (Fig. 1) indicate that beam damage did occur in all of our experimental treatments, and we suspect that it also occurred in other studies using similar analytical conditions. However, whatever effect this may have had on the accuracy of the molecular weight percent concentrations for Ca and Sr, the ratio of the two elements remained constant, indicating no observable fractionation.

One implication of these results is that Sr/Ca precision can be increased, with no apparent loss of accuracy, when analyses are conducted for 40-sec rather than 20-sec counting times, and at 5μm rather than 10μm beam sizes, at an accelerating voltage of 20nA. The 5μm beam allows greater temporal resolution, which is helpful when matching the sample location to structures such as daily growth increments. These may be as small as 0.1–0.2μm, depending upon species, growth rate, and age (Campagna and Neilson 1985).

Because the level of precision may affect conclusions of studies relating otolith Sr/Ca levels to environmental temperature, it is important to know the analytical conditions under which each study is conducted. Minimal information required includes beam current and voltage, beam size, counting time for each element, standards used, and precision of measurements. This information has not been reported in sufficient detail in some of the previous studies, making interpretation difficult. The methods described in this experiment are proposed as a means of defining measurement precision in future studies of Sr/Ca ratios in fish otoliths.

Acknowledgments

Citations

Campagna, S.E., and J. Neilson
    1985 Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42:1014–1032.
Dannevig, E.H.
    1955 Chemical composition of the zones in cod otoliths. J. Cons. Cons. Int. Explor. Mer 21:156–159.
Degens, E.T., W. Deuser, and R. Haedrich
    1969 Molecular structure and composition of fish otoliths. Mar. Biol. (Berl.) 2:105–113.
Kalish, J.M.
    1989 Otolith microchemistry: Validation of the effects of physiology, age, and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132:151–178.
Neter, J., W. Wasserman, and M. Kutner
    1989 Applied linear regression models. Irwin, Boston, 667 p.
Peterman, R.M.
    1990 Statistical power analysis can improve fisheries research and management. Can. J. Fish. Aquat. Sci. 47:2–15.
Potts, P.J.
    1987 A handbook of silicate rock analysis. Chapman & Hall, NY, 622 p.
Radtke, R.L.
    1984 Cod fish otoliths: Information storage structures. *In* The propagation of cod *Gadus morhua* L. Flodevigen Rapp. 1:273–298.
    1987 Age and growth information available from the otoliths of the Hawaiian snapper, *Pristipomoides filamentosus*. Coral Reefs 6:19–25.
    1989 Strontium-calcium concentration ratios in fish otoliths as environmental indicators. Comp. Biochem. Physiol. 92A: 189–193.
Radtke, R.L., and B. Morales-Nin
    1989 Mediterranean juvenile bluefin tuna: Life history patterns. J. Fish Biol. 35:485–496.
Radtke, R.L., and T. Targett
    1984 Rythmic structural and chemical patterns in otoliths of the Antarctic fish *Notothenia larsoni*: Their application to age determination. Polar Biol. 3:203–210.
Radtke, R.L., D. Townsend, S. Folsom, and M. Morrison
    1990 Strontium:calcium concentration ratios in otoliths of herring larvae as indicators of environmental histories. Environ. Biol. Fish. 27:51–61.
Smith, M.P.
    1986 Silver coating inhibits electron microprobe beam damage of carbonates. J. Sedimentary Petrol. 56:107–108.
Townsend, D.W., R. Radtke, M. Morrison, and S. Folsom
    1989 Recruitment implications of larval herring overwintering distributions in the Gulf of Maine, inferred using a new otolith technique. Mar. Ecol. Prog. Ser. 55:1–13.
Williams, K.L.
    1987 An introduction to X-ray spectrometry: X-ray fluorescence and electron microprobe analysis. Allen & Unwin, London, 370 p.