Version of Record: https://www.sciencedirect.com/science/article/pii/S0165783616300042
Manuscript_d4cba3b92240830258f0324de8b8bacc

Periodicity of strontium:calcium across annuli further validates otolith-ageing for Atlantic bluefin tuna (*Thunnus thynnus*)

M.R. Siskey[a*] msiskey@umces.edu, V. Lyubchich[a], D. Liang[a], P.M. Piccoli[b], D.H. Secor[a]

[a]Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, P.O. Box 38; Solomons, Maryland USA 20688

[b]Department of Geology, University of Maryland College Park, 237 Regents Drive; College Park, MD USA 20742

[*]Corresponding author.

Highlights

- Periodicity of annular growth bands was tested in Atlantic bluefin tuna otoliths
- Strontium:calcium ratios were measured across translucent and opaque zones
- A dominant periodicity of 1 strontium:calcium cycle per annulus was detected
- Annulus assignment is supported as a proxy for annual age in Atlantic bluefin tuna

Abstract

Fisheries assessments depend on accurate age assignments, particularly those for late maturing and moderately long-lived species. For Atlantic bluefin tuna (*Thunnus thynnus*), otolith-based ageing techniques have undergone tests for accuracy and precision to validate longevity and reproducibility of ageing metrics, but the periodicity of annular growth band formation remains untested. In this study, wavelength-dispersive X-ray spectrometry was used to measure strontium:calcium ratios across alternating translucent and opaque zones of the otolith. Time series analysis was subsequently employed to compare periodicity of otolith annulus formation to oscillations in measured otolith strontium:calcium (Sr:Ca). Under the assumption that Sr:Ca oscillations represented seasonal temperature changes, the hypothesized periodicity of 1 cycle per annulus was confirmed as the highest intensity periodicity, corroborating visual assignment of annuli as a proxy for annual age. Minor but significant sub-annular cycles (3 and 2 cycles per annulus) were also detected.

Keywords: otolith chemistry; annulus; strontium; time series analysis; bluefin tuna

1. Introduction

Otoliths contain alternating opaque and translucent ring-like structures (annuli; Figure 1), which have been interpreted to represent slow and fast growth segments of the year, respectively (Casselman, 1983). Operationally, otoliths are widely used in fisheries science and assessment to determine the ages of fish (Campana, 2001); however, the rate of annulus formation must first be confirmed to validate the otolith-ageing technique. A common means to evaluate the rate of annulus formation is to compare estimated and known longevity through mark-recapture, rearing, or, more recently, bomb-radiocarbon dating. However, while these techniques confirm longevity, they fail to confirm an investigator's ability to accurately assign each annulus structure observed within the otolith to a particular age. Here, we seek to confirm this relationship through an analysis of otolith strontium:calcium (Sr:Ca) oscillations for periodicity.

For Atlantic bluefin tuna (ABFT; *Thunnus thynnus*), Neilson and Campana (2008) verified longevity for a historical sample of ABFT >20 years in age via bomb-radiocarbon dating. Owing to high exploitation, ABFT now show a truncated age distribution, with few Northwestern ABFT >12 years in age (Secor et al., 2015). Further, parameterization of recruitment, mortality, growth, and maturity will all be more sensitive to age assignments of juveniles (<9 years for Northwestern ABFT) than adults. Precision in age determinations for juveniles is substantially lower than for adults due to opaque zones that are less consistently identified between investigators for the corresponding period of otolith growth (Secor et al., 2014). Therefore, there is priority to establish rates of annulus formation across all age-classes for this species.

Oscillating patterns in otolith Sr:Ca have previously been observed to fluctuate in concert with translucent and opaque zones in red emperor (*Lutjanus sebae*), and were attributed to seasonal temperature changes and fish physiology (Seyama et al., 1991). Strontium in cod (*Gadus morhua*), Japanese eel (*Anguilla japonica*), and herring (*Clupea harengus*) otoliths has been found to be inversely related to temperature, with incorporation presumably under temperature-regulated physiological control (Radtke, 1984; Radtke et al., 1990; Schneider and Smith, 1982; Tzeng, 1994). However, these studies relied on qualitative visual confirmation of synchrony between annulus assignments and Sr:Ca oscillations.

To more rigorously compare the periodicity of annulus formation against Sr:Ca oscillations, we employed time series analysis to link optical features of annuli (opaque and translucent zones) with oscillations in Sr:Ca measures. In this study, we assumed that Sr:Ca ratios would be associated with seasonal temperatures, with a period of fluctuations of one year. Using an electron probe microanalyzer (EPMA), high precision measurements of Sr:Ca were made across successive opaque and translucent zones of annuli spanning the juvenile and adult periods. These measurements of Sr:Ca were then evaluated for seasonal periodicity (Figure 1) and compared to the frequency of opaque zone formation.

2. Methods

Archived sagittal otoliths from older adult fish (age > 10 years) landed in the 1970s (N=32) were obtained from the National Marine Fisheries Service archive at the Southeast Fisheries Science Center in Miami, Florida (USA). A single otolith was randomly selected from each individual. Otoliths were embedded in Struers epoxy resin (Struers A/S, Ballerup, Denmark) and then a 2.0-mm thick section containing the core region (the inner-most portion of the otolith, pertaining to the first year of life) was cut along a transverse plane using a Buehler IsoMet saw (Beuhler, Lake Bluff, Illinois). The otolith section was then attached to a glass slide using Crystalbond 509 mounting adhesive (SPI Supplies, West Chester, Pennsylvania), and section thickness was further reduced to maximize optical clarity of opaque zones.

To reduce analytical time and expense, a power analysis was initially performed using the data from a single otolith Sr:Ca profile to determine sample size. Here, an *F* test (Hartley, 1949; Thibos, 2003) was used to assess power:

$$\frac{\bar{p}}{\qquad\qquad}$$

where $\bar{p}_1$is the estimated magnitude of annual variation, n equals the length of the time series, f is the location of the annual period in the periodogram, and *SSE* is the residual sum of squares, defined as the pooled magnitude of variations of higher frequencies. In this analysis, it was assumed that 5 measurements of Sr:Ca would be obtained per annulus across the first 10 annuli of each otolith, so that $n/2 \approx 25$. Under this assumption, the natural frequency (Van Dongen et al., 1999) equals the reciprocal of the time span ≈ 1/10, and the annual period occurs at $f_1 = 10$. For sample size calculations, power was assessed when $p_1 = 1.25\bar{p}$ and $p_1 = \bar{p}$, where $\bar{p}$ is the average variation among all periods. Accepting a Type II error of 0.2 at $\alpha = 0.05$, 26 otoliths were recommended when $p_1 = 1.25\bar{p}$ and 38 otoliths were recommended when $p_1 = \bar{p}$. This information led to an intermediate sample size of 32 otoliths.

Intra-annular oscillations in Sr:Ca were measured across opaque and translucent zones in 32 otoliths using an EPMA (JXA-8900 Superprobe, JEOL USA, Inc., Peabody, Massachusetts, USA) located in the Advanced Imaging and Microscopy Laboratory at the University of Maryland, College Park. Prior to analysis by EPMA, otolith samples were carbon-coated in a high-vacuum evaporator, and the instrument was calibrated using reference standards [calcite ($CaCO_3$) and strontianite ($SrCO_3$)]. The detection limit for Sr using the conditions employed was ~200 ppm (by weight).

Otolith Sr:Ca profiles were constructed from the core region to the periphery using 30-µm steps for the first ~8 annuli, followed by 20-µm steps for the last 4-6 annuli. This sampling design adjusted for decreasing annular width with age: annulus width for ages >8 years typically ranges 80-100 µm, and the goal was to accommodate five probe measurements per annulus. After the removal of one individual outlier point, at which the instrument measured a crack in the otolith, profiles of Sr:Ca were converted to un-equally spaced time series. Opaque bands were considered the start of an annulus, and measurement points within a cycle (opaque → translucent → opaque) were converted to fractions within each cycle.

A linear trend that was evident in the data prompted consideration of detrending (Figure 2). Elemental incorporation in otoliths has been found to vary across life history stages in several species, presumably as a result of onotgenetic changes in physiology (e.g. Kalish, 1989; Walther et al., 2010). Alternatively, this positive linear trend may have been the result of an increasing vertical and horizontal thermal niche for ABFT with age, which is well documented for this species and its congeners (Graham and Dickson, 2004; Teo et al., 2007). Exposure to colder-water habitats later in life would amplify otolith Sr according to the inverse relationship between Sr uptake and temperature. Regardless of the mechanism, the time series were detrended by extracting the linear trend in the data in order to reduce any bias attributed to variability of elemental composition across life history stages.

The periodicities of the detrended time series of Sr:Ca measures were identified using a Lomb-Scargle periodogram that accounts for un-equally spaced measurements (Lomb, 1976; Ruf, 1999; Scargle, 1982). Fourier series were then used for harmonic filtering to eliminate the identified periodic components and continue the search for other periodicities (Ferraz-Mello, 1981):

$$\sum\{a\ \cos(w\ t)\qquad \sin(w\ t)\}$$

where y_t are the periodicities of the detrended Sr:Ca time series at time t, $w_j = \frac{}{}$ is the angular velocity and relates to a pair of trigonometrical components that undergo j cycles over the length of the time series, and n is the length of the time series. Residual periodograms were constructed at each step to ensure that all significant periodicities were filtered out. Additionally, Hartley's F_{max} test was used to test the significance of the harmonic terms with the highest intensity (Hartley, 1949):

$$\frac{-}{\qquad\qquad\qquad\qquad}$$

where S_{max}^2 is the largest observed harmonic intensity, m is the number of harmonic terms tested, and R^2 is the residual sum of squares. The calculated F_{max} was then compared to the critical value from the ordinary *F*-distribution ($F_{\alpha/m,2,(n-2*m-1)}$). In addition, *F*-ratios for other periods were compared with the critical value to test the significance of multiple periodicities. The search for significant periods ceased when an insignificant result occurred. The denominator m ensures that the familywise error rate (the probability of making any false discoveries in the multiple testing procedure) is maintained at the level $\alpha = 0.05$.

3. Results

The Lomb-Scargle method showed evidence of significant annual periodicity in time series of Sr:Ca measurements within otoliths, with the largest peak occurring at the period of 1 annulus (or year), as represented by the opaque zone (Figure 3). Additional Fourier series related to 0.5 and 0.33 annular periods were added to remove all significant periodicities in the detrended series (Figures 3). The Hartley's *F*-test for $m = 5$ confirmed significance of the annular periodicity ($F_{max} > F_{crit}$; $F_{max} = 115.8$, $F_{crit} = 4.61$; $p < 0.001$). The *F*-ratio test of $m = 5$ periodicities found annular, bi-annular, and tri-annular periods to be significant (*F*-ratio > F_{crit}, $F_{crit} = 4.61$; Table 1).

To confirm a negative relationship of Sr:Ca with temperature, we compared the mean Sr:Ca observed in the opaque bands (presumably formed during cooler winter months) with the mean Sr:Ca observed in the translucent bands (presumably formed during warmer summer months). To accommodate the serial dependence of the observations, we bootstrapped the filtered residuals of the Sr:Ca time series (the time series remaining after extraction of the positive trend and the periodic components), and then inserted these bootstrapped values and the intercept into the model for periodic components. We repeated the bootstrapping 5000 times,each time calculating the mean Sr:Ca for opaque band (winter) and translucent band (summer) observations. As a result, we generated two distributions of the means, which did not overlap (Figure 4). The distribution of mean Sr:Ca for opaque band observations is located to the right, confirming higher otolith Sr:Ca during winter months. The 95% bootstrap confidence intervals for the means of translucent and opaque band observations were (0.00220, 0.00221) and (0.00250, 0.00257), respectively.

4. Discussion

Under the assumption that otolith Sr:Ca served as a proxy for seasonal temperature variation, we confirmed an annual rate of opaque zone occurrence in ABFT. As expected, Sr:Ca fluctuated over annular cycles at the rate of 1 cycle per annulus. Sub-annual cycles (3 and 2 cycles per annulus) were also detected but were smaller in magnitude. We do not believe these lower-intensity periodicities were due to systemic bias in assigning opaque zones, which would have led to a positive bias in age estimates. Interpretations of longevity by the reader (MRS) were first calibrated against longevity estimates confirmed through radiocarbon dating (Neilson and Campana, 2008). Further, the reader was calibrated against eight other experienced readers on the same reference set of ABFT otoliths, where overall precision of age assignment (C.V.) was estimated at 7% (Secor et al., 2014).

Sub-annular cycles in otolith Sr:Ca may relate to sub-seasonal temperature cycles and physiological processes. Radtke and Morales-Nin (1989) found “short-period” (sub-annular) cycles in otolith Sr:Ca across daily increments of juvenile Atlantic bluefin tuna otoliths and speculated that these might be associated with short-term changes in temperatures that they encountered. In addition, otolith Sr:Ca uptake is influenced by the level of free Ca in the endolymph surrounding the otolith (Mugiya, 1966; Mugiya and Takahashi, 1985). Therefore, fluctuations in otolith Sr:Ca may be associated with periods of increased calcium-binding proteins in the blood (Kalish, 1989), which cause greater incorporation of Sr into otolith carbonate. Elemental concentrations in otoliths can systematically vary in response to physiological biokinetic factors, even if ambient concentrations are kept constant. Cycles of growth and gonadal development, which are typically influenced by seasonal changes in temperature or photoperiod, can alter elemental compositions in the blood and otolith and explain an indirect linkage between Sr:Ca and temperature (Sturrock et al, 2015). Stress, physiology, condition, and reproduction can all influence the amount of calcium-binding proteins in the blood and, subsequently, otolith Sr:Ca concentrations (Kalish, 1992; Walther et al., 2010; Yamashita et al., 2000). Still, sub-annual regularity of such physiological actors remains without clear explanation.

This study complements a previous validation of otolith-ageing for this species (Neilson and Campana, 2006), which linked otolith-ageing to the absolute age of the otolith via bomb radiocarbon dating, and efforts to standardize otolith ageing techniques (Secor et al., 2014). Together, these three studies support that (1) otolith-ageing accurately captures the absolute age of Atlantic bluefin tuna, (2) readers are able to consistently resolve and identify individual annuli, and (3) formation of opaque zones occurs cyclically, consistent with seasonal cycles in Sr:Ca, an assumed proxy for temperature.

Acknowledgements

Research support came from the Bluefin Tuna Research Program of the National Oceanic and Atmospheric Administration’s National Marine Fisheries Service. Mr. Derke Snodgrass of the National Marine Fisheries Service Southeast Fisheries Center assisted in procuring archived bluefin tuna otoliths analyzed in this study.

References

Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59, 197-242.

Casselman, J.M., 1983. Age and growth assessment of fish from their calcified structures – techniques and tools. Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks 1982, 1-18.

Ferraz-Mello, S., 1981. Estimation of periods from unequally spaced observations. The Astronomical Journal 86, 619-624.

Graham, J.B., and Dickson, K.A., 2004. Tuna comparative physiology. The Journal of Experimental Biology 207, 4015-4024.

Hartley, H., 1949. Tests of significance in harmonic analysis. Biometrika 36, 194-201.

Kalish, J.M., 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. Journal of Experimental Marine Biology and Ecology 132, 151-178.

Kalish, J.M., 1992. Formation of a stress-induced chemical check in fish otoliths. Journal of Experimental Marine Biology and Ecology 162, 265-277.

Lomb, N.R., 1976. Least-squares frequency analysis of unequally spaced data. Astrophysics and Space Science 39, 447-462.

Mugiya, Y., 1966. Calcification in fish and shell fish. XI. Seasonal changes in calcium and magnesium concentration of the otolith fluid in some fish, with special reference to the zone of formation of their otolith. Bulletin of the Faculty of Fisheries Hokkaido University 32, 549-557.

Mugiya, Y., and Takahashi, K., 1985. Chemical properties of the saccular endolymph in the rainbow trout, *Salmo gairdneri*. Bulletin of the Faculty of Fisheries Hokkaido University 36, 57-63.

Neilson, J.D., and Campana, S.E., 2008. A validated description of age and growth of western Atlantic bluefin tuna (*Thunnus thynnus*). Canadian Journal of Fisheries and Aquatic Sciences 65, 1523-1527.

Radtke, R.L., 1984. Cod fish otoliths: information storage structures, in: Dahl, E., Danielssen, D.S., Moksness, E., and Solemdal, P. (Eds.), The propagation of Cod *Gadus morhua* L. Institute of Marine Research, pp. 273-298.

Radtke, R.L., and Morales-Nin, B., 1989. Mediterranean juvenile bluefin tuna: life history patterns. Journal of Fish Biology 35, 485-496.

Radtke, R.L., Townsend, D.W., Folsom, S.D., and Morrison, M.A., 1990. Strontium:calcium concentration ratios in otoliths of herring larvae as indicators of environmental histories. Environmental Biology of Fishes 27, 51-61.

Ruf, T., 1999. The Lomb-Scargle periodogram in biological rhythm research: analysis of incomplete and unequally spaced time-series. Biological Rhythm Research 30, 178-201.

Scargle, J.D., 1982. Studies in astronomical time series analysis. II. Statistical aspects of spectral analysis of unevenly spaced data. The Astrophysical Journal 263, 835-853.

Schneider, R.C., and Smith, S.V., 1982. Skeletal Sr content and density in *Porites* spp. In relation to environmental factors. Marine Biology 66, 121-131.

SCRS (Standing Committee on Research and Statistics, International Commission for the Conservation of Atlantic Tunas), 2014. Report of the 2014 Atlantic bluefin tuna stock assessment session. Madrid. Available from http://www.iccat.es/en/assess.html.

Secor, D.H., Allman, R., Busawon, D., Gahagan, B., Golet, W., Koob, E., Lastra Luque, P., and Siskey, M., 2014. Standardization of otolith-based ageing protocols for Atlantic bluefin tuna. ICCAT Collective Volume of Scientific Papers SCRS/2013/084.

Secor, D.H., Rooker, J.R., Gahagan, B.I., Siskey, M.R., and Wingate, R. W. 2015., Depressed resilience of bluefin tuna in the Western Atlantic and age truncation. Conservation Biology 29, 400-408.

Seyama, H., Edmonds, J.S., Moran, M.J., Shibata, Y., Soma, M., and Morita, M., 1991. Periodicity in fish otolith Sr, Na, and K corresponds with visual banding. Experientia 47, 1193-1196.

Sturrock, A.M., Hunter, E., Milton, J.A., EIMF, Johnson, R.C., Waring, C.P., and Trueman, C.N., 2015. Quantifying physiological influences on otolith microchemistry. Methods in Ecology and Evolution 6, 806-816.

Teo, S.L.H., Boustany, A., Dewar, H., Stokesbury, M.J.W., Weng, K.C., Beemer, S., Seitz, A.C., Farwell, C.J., Prince, E.D., and Block, B.A., 2007. Annual migrations, diving behavior, and thermal biology of Atlantic bluefin tuna, *Thunnus thynnus*, on their Gulf of Mexico breeding grounds. Marine Biology 151, 1-18.

Thibos, L.N., 2003. Fourier analysis for beginners, third ed. Digitally published by the Visual Sciences Group. http://www.opt.indiana.edu/VSG/Library/FourierBook/title.html.

Tzeng, W.N., 1994. Temperature effects on the incorporation of strontium in otolith of Japanese eel *Anguilla japonica*. Journal of Fish Biology 45, 1055-1066.

Van Donogen, H.P.A., Olofsen, E., Van Hartevelt, J.H., and Kruyt, E.W., 1999. A procedure of multiple period searching in unequally spaced time-series with the Lomb-Scargle method. Biological Rhythm Research 30, 149-177.

Walther, B.D., Kingsford, M.J., O'Callaghan, M.D., McCulloch, M.T., 2010. Interactive effects of ontogeny, food ration and temperature on elemental incorporation in otoliths of a coral reef fish. Environmental Biology of Fishes 89, 441-451.

Yamashita, Y., Otake, T., and Yamada, H., 2000. Relative contributions from exposed inshore and estuarine nursery grounds to the recruitment of stone flounder *Platichthys bicoloratus* estimated using otolith Sr:Ca ratios. Fisheries Oceanography 9, 316-327

Figure Captions

Fig. 1. Annotated Atlantic bluefin tuna otolith micrograph image showing opaque (yellow dots) and translucent zones combined to form annuli. Microprobe transects (white squares) were used to evaluate the periodicity of annulus formation.

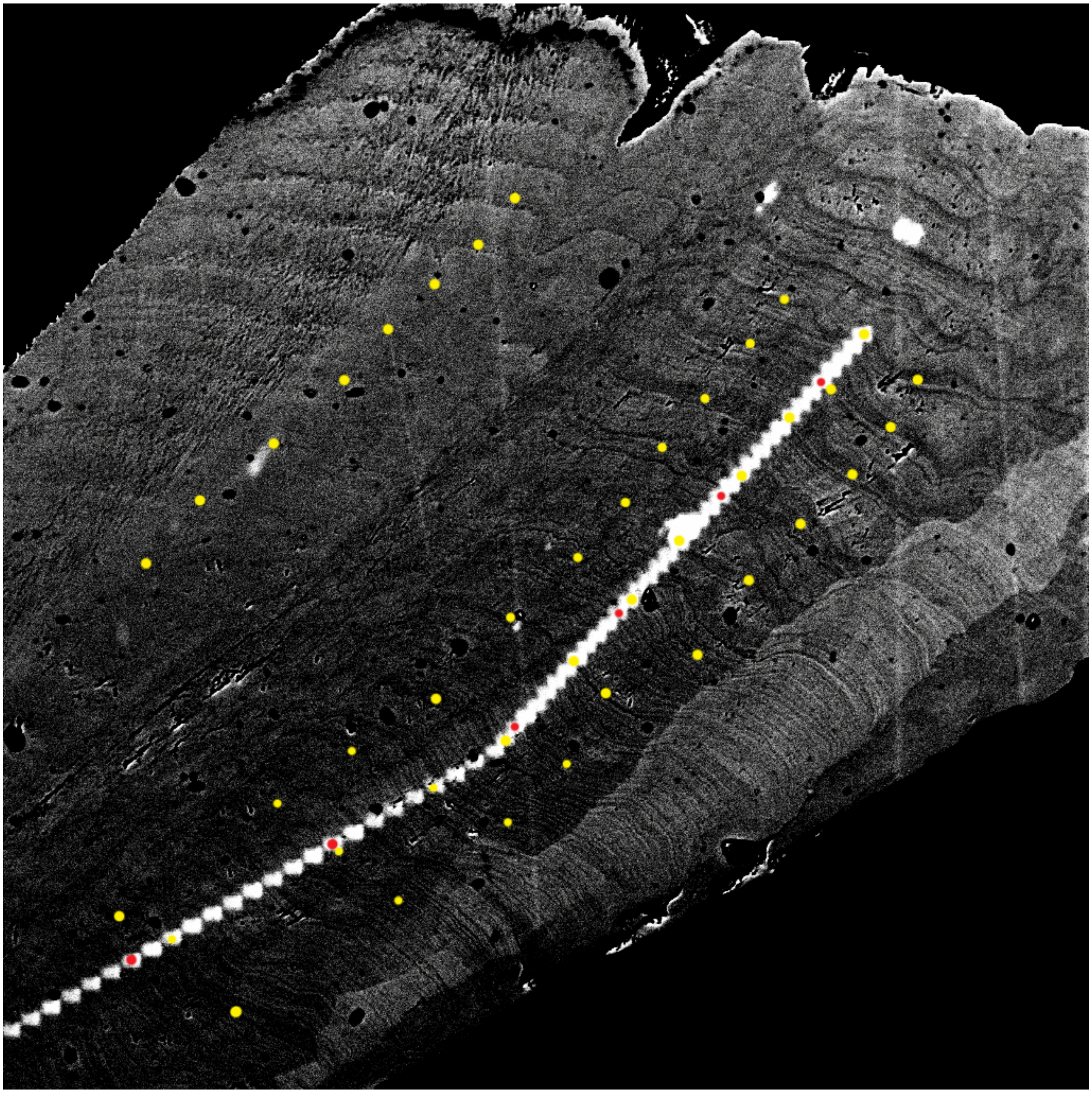


Fig. 2. Measurements of Sr:Ca along profiles of Atlantic bluefin tuna otoliths with individual outlier removed (N=31). Integers correspond to opaque zones.

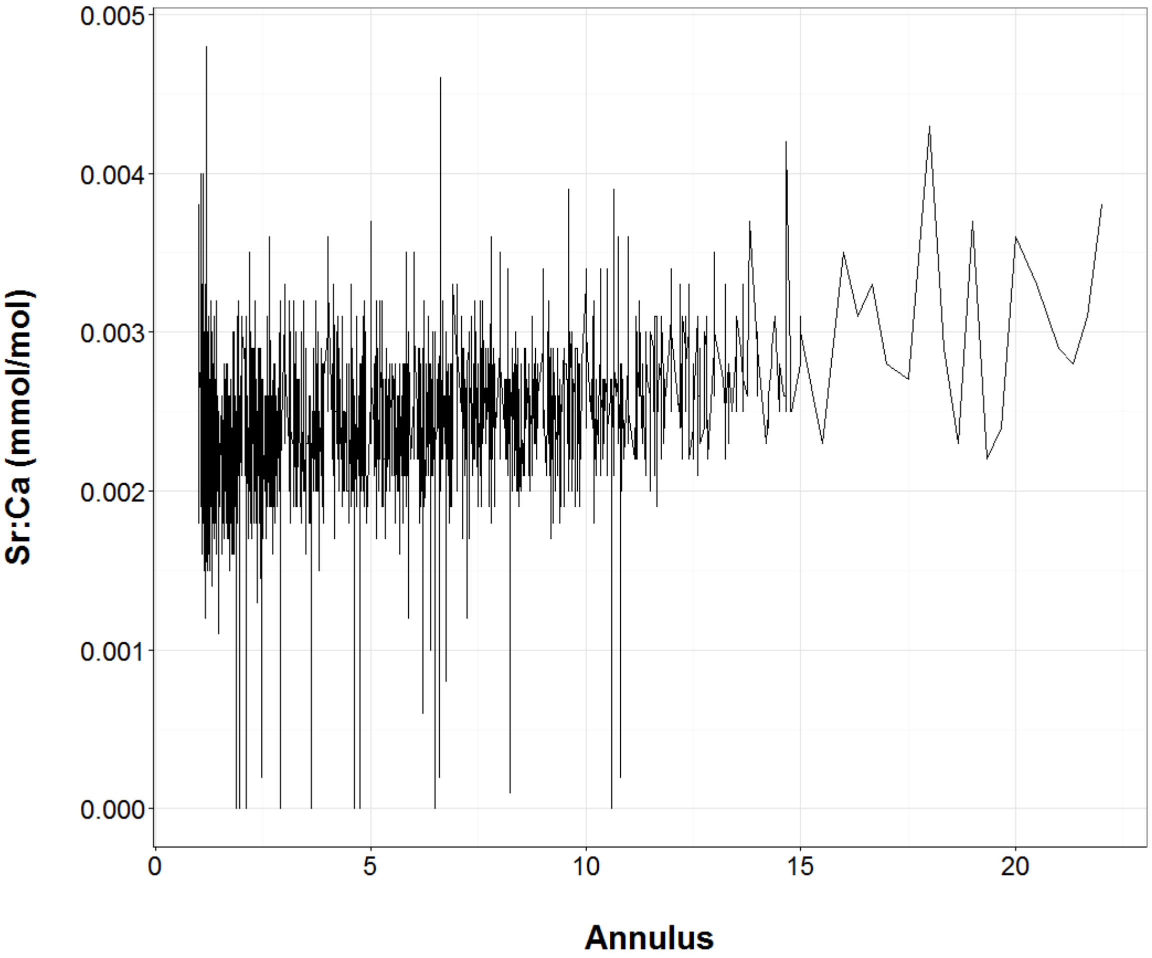


Fig. 3. Lomb-Scargle periodogram of detrended Atlantic bluefin tuna Sr:Ca time series (N=31) from otolith transects (Figure 1) showing (A) significant periodicities in the unfiltered data, (B) significant periodicities remaining after annual periodicity was filtered out, and (C) no significant periodicities remaining after annual, biannual, and triannual periodicities were filtered out. Significance is indicated by the dashed line.

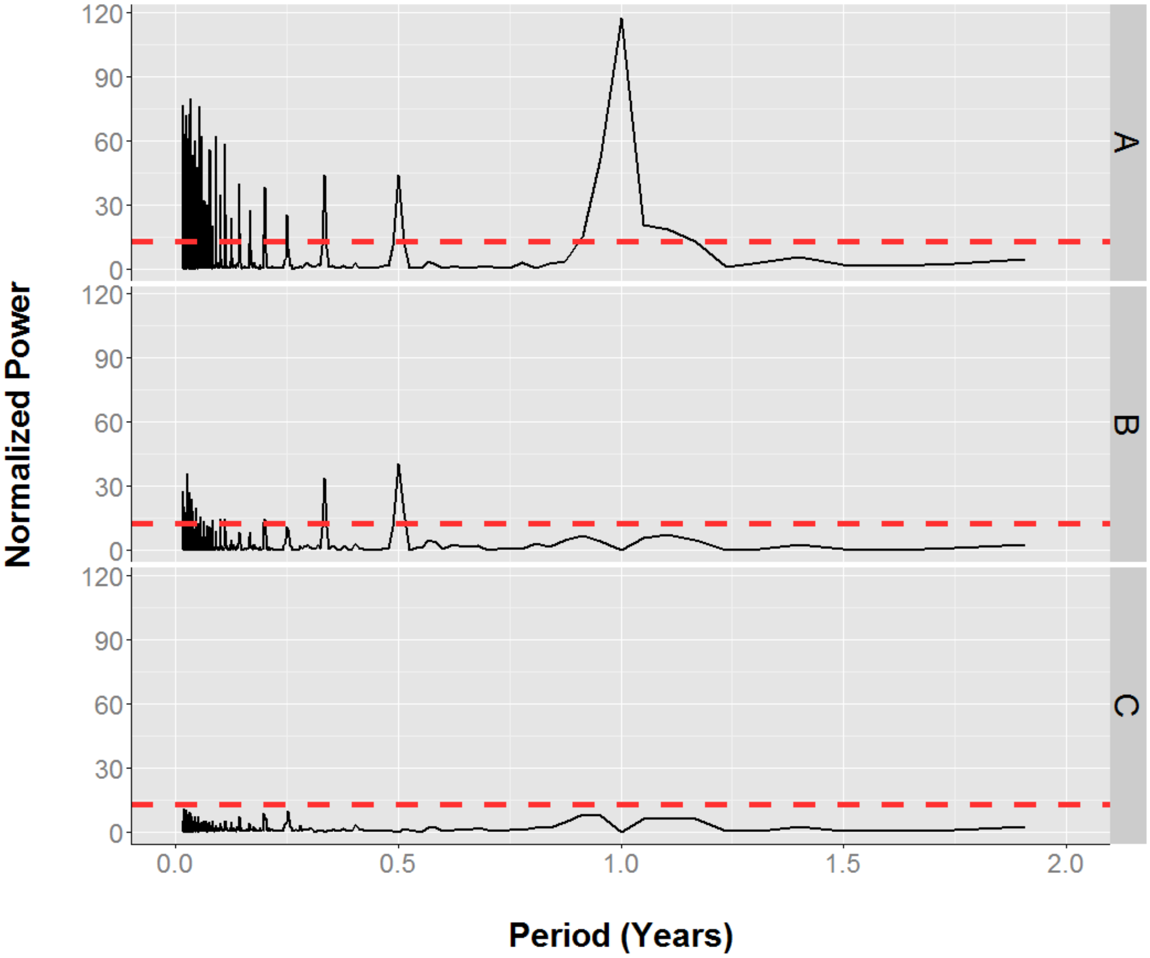
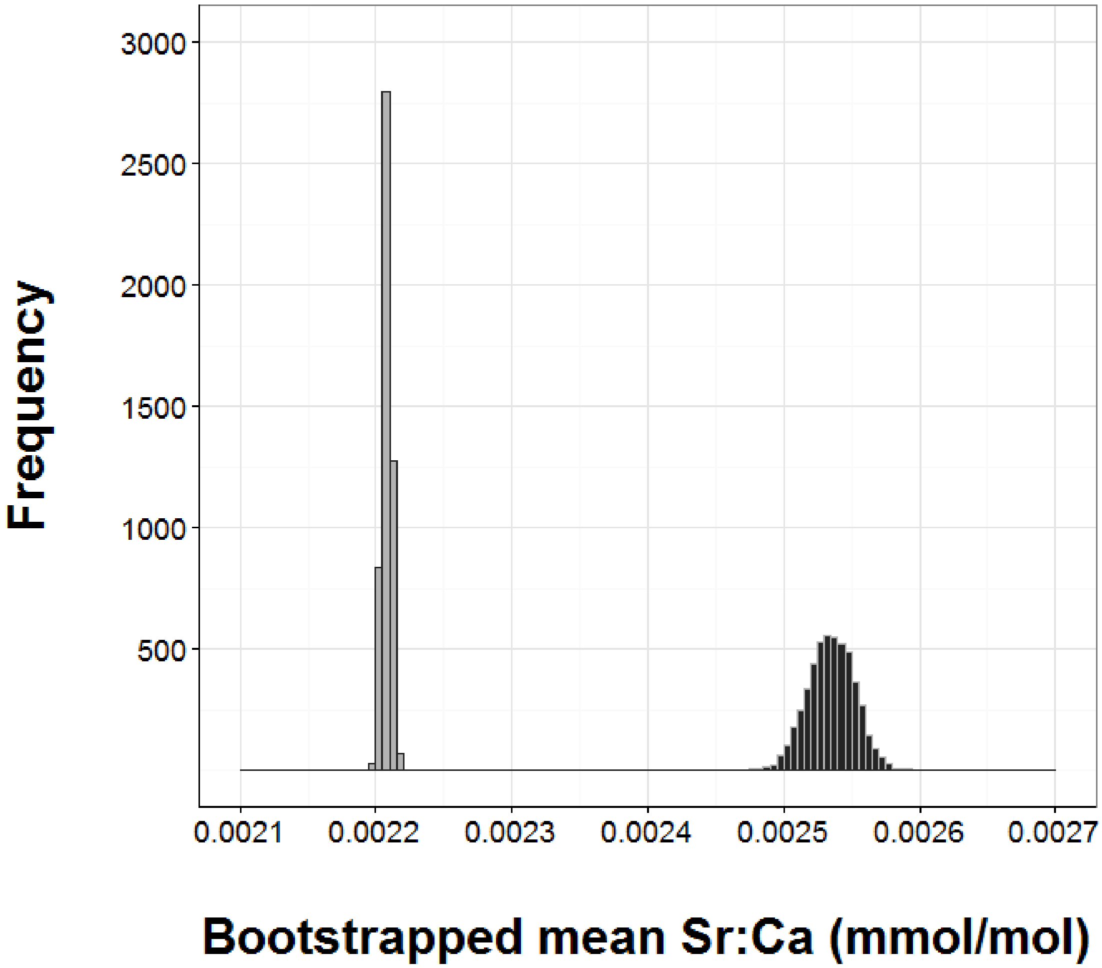


Fig. 4. Distributions of mean Sr:Ca (mmol/mol) generated by bootstrapping (5000x) residual opaque (winter; right) and translucent (summer; left) band Sr:Ca observations.



Tables

Table 1. *F*-ratio test of multiple periodicities in Atlantic bluefin tuna otolith Sr:Ca time series data. The search for significance was ended after the first insignificant (p > 0.01) result.

Periodicity (per annulus)	F-*ratio*	*Critical Value*	*p*
1	92.73	4.61	< 0.001
2	26.77	4.61	< 0.001
3	21.46	4.61	< 0.001
4	4.03	4.61	0.028
5	5.92	4.61	< 0.01