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Otolith oxygen isotopes measured by high-precision secondary ion mass spectrometry reflect life history of a yellowfin sole (*Limanda aspera*)

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RATIONALE: The oxygen isotope ratio ($\delta^{18}O$ value) of aragonite fish otoliths is dependent on the temperature and the $\delta^{18}O$ value of the ambient water and can thus reflect the environmental history of a fish. Secondary ion mass spectrometry (SIMS) offers a spatial-resolution advantage over conventional acid-digestion techniques for stable isotope analysis of otoliths, especially given their compact nature.
METHODS: High-precision otolith $\delta^{18}O$ analysis was conducted with an IMS-1280 ion microprobe to investigate the life history of a yellowfin sole (*Limanda aspera*), a Bering Sea species known to migrate ontogenetically. The otolith was cut transversely through its core and one half was roasted to eliminate organic contaminants. Values of $\delta^{18}O$ were measured in 10-μm spots along three transects (two in the roasted half, one in the unroasted half) from the core toward the edge. Otolith annual growth zones were dated using the dendrochronology technique of crossdating.
RESULTS: Measured values of $\delta^{18}O$ ranged from 29.0 to 34.1‰ (relative to Vienna Standard Mean Ocean Water). Ontogenetic migration from shallow to deeper waters was reflected in generally increasing $\delta^{18}O$ values from age-0 to approximately age-7 and subsequent stabilization after the expected onset of maturity at age-7. Cyclical variations of $\delta^{18}O$ values within juvenile otolith growth zones, up to 3.9‰ in magnitude, were caused by a combination of seasonal changes in the temperature and the $\delta^{18}O$ value of the ambient water.
CONCLUSIONS: The ion microprobe produced a high-precision and high-resolution record of the relative environmental conditions experienced by a yellowfin sole that was consistent with population-level studies of ontogeny. Furthermore, this study represents the first time that crossdating has been used to ensure the dating accuracy of $\delta^{18}O$ measurements in otoliths.

Otoliths, paired calcified structures found within the inner ear of fish, have been widely used to study various aspects of fish ecology in marine and freshwater ecosystems.[1,2] Consisting of calcium carbonate (usually in the form of aragonite) precipitated over a protein matrix, otoliths accrete alternating concentric layers of opaque and translucent material around their cores, typically at a rate of one pair of layers, or a single annulus, per year. The seasonal deposition and contrasting optical properties of these layers (hereafter referred to as otolith growth zones) provide time-specific markers that have long been used by biologists to estimate fish age. In recent years, the relative widths of these growth zones have been used to develop biochronologies that reflect interannual variations in climate and ecosystem productivity.[3–5]

In addition to the visual properties of otoliths, chemical components may also be employed to reconstruct fish environmental and developmental histories.[6–8] Otoliths are well suited for such applications given that they are acellular, metabolically inert, and grow from birth to death, all factors which allow the uptake and permanent retention of various elements and compounds.[1,6] Isotope ratio analyses of oxygen, carbon, nitrogen, sulfur, lead, strontium, and boron have been applied in studies of fish trophic level, diet history, behavior, migration, thermal history, age validation, stock differentiation, and metabolism.[6,9–15] Specifically, the oxygen isotope composition ($\delta^{18}O$ value) of otolith aragonite is influenced by the temperature and isotopic composition of the ambient water, but is generally thought to be unaffected by biological processes.[6,16–18] Due to the inverse relationship between $\delta^{18}O$ values and temperature, when measurements

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of otolith $\delta^{18}O$ values are correlated with visible otolith growth zones they may provide a chronological record of a fish's thermal experience over its life history.[17] Traditionally, the $\delta^{18}O$ value is measured using acid-digestion analytical methods, in which bulk samples weighing 0.01 to 10 mg are mechanically milled and homogenized into powders for analysis.

The relatively large sample mass necessary for high-precision isotope analyses in gas-source mass spectrometers is a limitation in many otolith $\delta^{18}O$ studies. Given the compact nature of otoliths, providing sufficient sample mass while maintaining high temporal resolution of measurements is often difficult or impossible. Micro-milling, a method commonly used to extract otolith material for gas-source mass spectrometry, typically requires drilling continuously along a transect parallel to growth zones in order to obtain adequate amounts of $CaCO_3$ for analysis. Thus, these conventional analytical techniques often average isotopic information across multiple seasons or years.[14] In contrast, secondary ion mass spectrometry (SIMS) has demonstrated marked improvements over conventional methods in temporal and spatial resolution of otolith $\delta^{18}O$ measurements.[12,15] In particular, recent developments at the WiscSIMS Laboratory, University of Wisconsin-Madison (Madison, WI, USA), in the analytical capabilities of the IMS-1280 ion microprobe instrument, a focused ion beam coupled with a double-focusing mass spectrometer, have allowed *in situ* analysis of otoliths on sub-annual, even daily, timescales.[12,14] The ion microprobe can analyze discrete samples that are less than one ten-thousandth of the mass required by conventional acid digestion/gas-source mass spectrometry (~1 ng vs. ≥10 μg). The increased spatial resolution of ion microprobe analyses (spot diameter = 10 μm with depth of ~1 μm) allows for finer temporal resolution of measurements while maintaining high accuracy and precision.[14,19]

The analytical capabilities of the ion microprobe are particularly advantageous for characterizing the life history of fish that undertake ontogenetic migration; changes in the temperature and $\delta^{18}O$ values of ambient water are recorded in the carbonate otolith as it grows. In this study, we analyze the otolith of a yellowfin sole (*Limanda aspera* Pallas, 1814), a common species in the eastern Bering Sea (EBS), that is the target of the largest flatfish fishery in the world.[20] Population-level studies based on commercial catches and scientific surveys have demonstrated that juvenile yellowfin sole reside in shallow waters of the inner continental shelf (<50 m) and gradually move deeper to waters of the middle and outer continental shelf (50–200 m) as they grow older.[21–23]

Recently, dendrochronology (tree-ring science) techniques were applied to the annulus widths of EBS yellowfin sole otoliths to develop an accurately dated, annually resolved biochronology.[24] Central to any dendrochronology-based study is the concept that climate induces synchronous patterns of annulus width in all individuals from a given region. These synchronous patterns, which can be thought of as growth 'bar codes', are matched among all sampled individuals using a technique called crossdating. Crossdating ensures that each annulus is assigned the correct calendar year of formation, subsequently allowing comparison of relative otolith growth with environmental variables and measures of ecosystem productivity over multi-year time scales. Given the high visual definition of their otolith growth zones and the synchronous growth pattern shared among individuals,[24] modern EBS yellowfin sole otolith annuli may be dated with a high degree of accuracy, thereby also allowing assignment of $\delta^{18}O$ measurements to the correct calendar year.

The main objective of the present study was to assess the feasibility of ion microprobe analysis as a tool to produce a detailed signature of otolith $\delta^{18}O$ measurements, thus revealing the environmental history of an individual EBS yellowfin sole in greater detail than would be allowed by conventional analyses. An IMS-1280 ion microprobe was used to measure $\delta^{18}O$ composition in a yellowfin sole otolith *in situ* at high spatial resolution to (1) generate a precise time series of $\delta^{18}O$ measurements from discrete samples within a single yellowfin sole otolith, with dating of $\delta^{18}O$ measurements verified through crossdating; (2) reconstruct the ontogenetic history of the fish based on otolith $\delta^{18}O$ measurements; and (3) assess relative intra- and inter-annual variability in $\delta^{18}O$ measurements.

EXPERIMENTAL

Otolith collection and preparation

An otolith pair was collected from a male yellowfin sole in the southeastern Bering Sea (56.7°N, 163.4°W; bottom depth = 74 m) during a standard U.S. National Marine Fisheries Service (NMFS) bottom trawl survey in June 2009 (Fig. 1). This individual was selected for the present study due to the clarity of its otolith growth zones and its presumed old age, in order to increase the likelihood of observing the $\delta^{18}O$ signal of ontogenetic migration. The otoliths were preserved at sea in glycerol-thymol solution and returned to the laboratory where they were rinsed in distilled water and air-dried.

The left otolith was cut transversely through its core. One otolith half was placed in a small Pyrex cup and roasted in a 0.5 mTorr vacuum at 360°C for 2.5 h to eliminate organic materials that could contaminate spot samples. Roasting also allowed better contrast between opaque and translucent growth zones, a necessity for age determination.[25] The other otolith half was left unroasted for comparison of $\delta^{18}O$ values. Both otolith halves were cast in an epoxy resin, cut into separate blocks, and ground to the analytical surface using a 6 μm diamond mesh. After grinding, each otolith half was recast near the center of a 25-mm-diameter epoxy mount along with three grains of calcite standard UWC-3[26] and polished to a smooth flat surface using a sequence of 6, 3, and 1 μm diamond pastes (Fig. 2).

The total age was estimated to be 28 years from counting annuli (growth-zone pairs) visible on the roasted otolith cross-section.[25] The age at maturity for this specimen was estimated to be 7 years, based on published reports of maturity for male yellowfin sole[22] as well as the appearance of the growth zones, which became compressed after age-7 (i.e., the calendar year 1988; Fig. 2(a)), possibly due to a slowing of growth following maturity.[27,28]

To ensure dating control of annuli in the otolith examined here, we visually and statistically related its annulus widths to an existing yellowfin sole biochronology that spans 1989 through 2006, and was generated from the otoliths of 21 EBS yellowfin sole collected during the 2008 NMFS bottom trawl survey.[24] First, the growth pattern in the roasted cross-section of the $\delta^{18}O$ otolith was crossdated by visual comparison with the published yellowfin sole biochronology.[24] Next, the otolith

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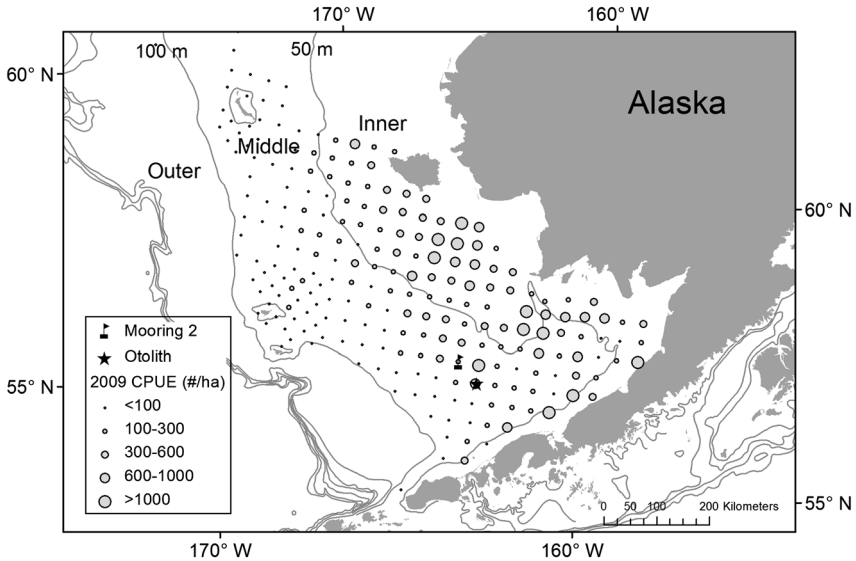

Figure 1. Summer 2009 distribution of yellowfin sole (catch per unit effort, CPUE, in number per hectare), capture location of $\delta^{18}O$ specimen (star; 74 m depth), and oceanographic buoy Mooring 2 (flag). Bathymetric contour lines (50 and 100 m) delineate oceanic domains (inner, middle, outer continental shelf). Data source: National Marine Fisheries Service eastern Bering Sea summer bottom trawl survey, 2009.

was photographed and annulus widths were measured along the dorsal side of the sulcus near the location of Transect A. The axis of measurement was parallel to the direction of growth and one complete annulus was defined as the distance from the distal side of the previous year's translucent zone to the distal side of the current year's translucent zone.[24] The widths of the first four annuli were not measured due to difficulties in fitting a measurement axis through them. The crossdating was then statistically verified using the dendrochronology program COFECHA.[29,30] In COFECHA, each of the 21 otolith measurement time series from the original biochronology as well as the otolith measurement time series from the $\delta^{18}O$ otolith values was fitted with a cubic spline with a 50% frequency cutoff set at 15 years.[31,32] The measurements were divided by the values predicted by the spline, thereby removing long-term trends including age-related growth declines, and standardizing each time series to a mean of 1. A significant correlation between the $\delta^{18}O$ values of the otolith and the average of all others corroborates that the synchronous growth pattern has been correctly identified and that each growth increment has been assigned the correct calendar year of formation.

Secondary ion mass spectrometry

Otolith oxygen isotope ratios were measured *in situ* from 10 μm spots using the CAMECA IMS-1280[a] large radius, multi-collector ion microprobe (CAMECA, Gennevilliers, France) at the WiscSIMS Laboratory (Madison, WI, USA). Before ion microprobe analysis, the polished epoxy mount was cleaned with successive, 30-s sonication sessions in ethanol and distilled water, then dried in a vacuum oven at 40°C for 2.5 h. The epoxy mount was then gold-coated with a coating thickness of ~60 nm and imaged with a reflected-light microscope for mapping purposes.

Each ion microprobe spot was sputtered by a ~1.7 nA primary beam of $^{133}Cs^+$ ions with a total accelerating voltage of 20 kV, focused to ~10 μm diameter at the sample surface. Both the gold coating applied to the sample surface and a normal-incidence electron flood gun prevented charging of the sample surface. The primary beam sputtered a ~1-μm-deep pit over the course of each 4-min analysis, which included choosing the analysis location (1–2 min), pre-sputtering (10 s), automated centering of the secondary ion beam (90 s), and 20 analytical cycles (80 s). Secondary ions were accelerated by 10 kV into a double-focusing mass spectrometer that was configured to achieve high secondary-ion transmission.[19] The average secondary-ion intensity ($^{16}O^-$ ion) during sample analyses was 2.7×10^9 counts per second (cps). The mass resolving power for $\delta^{18}O$ measurements was 2200, which permitted separation of hydride interferences on ^{18}O; the $^{16}O^-$ and $^{18}O^-$ ions were counted simultaneously on two Faraday cups (L1 and H1, respectively) in multi-collection mode. Sample analyses were grouped in blocks of 10–15 and bracketed by eight analyses of UWC-3 calcite standard ($\delta^{18}O = 12.49$‰ Vienna Standard Mean Ocean Water (VSMOW)).[26] This standard-sample-standard bracketing technique is used to correct for instrumental mass fractionation (bias) and to calculate the spot-to-spot precision of sample analyses.[19,26,33] The average spot-to-spot precision measured during the analytical session was ±0.3‰ (2 standard deviations, S.D.).

Further description of the analytical methodology is provided elsewhere.[14,19,33] For this study, the relative values of $\delta^{18}O$ were compared, with no correction for any small differences in instrument bias for $\delta^{18}O$ values between the calcite standard and aragonite otolith. All the $\delta^{18}O$ values are reported

[a]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

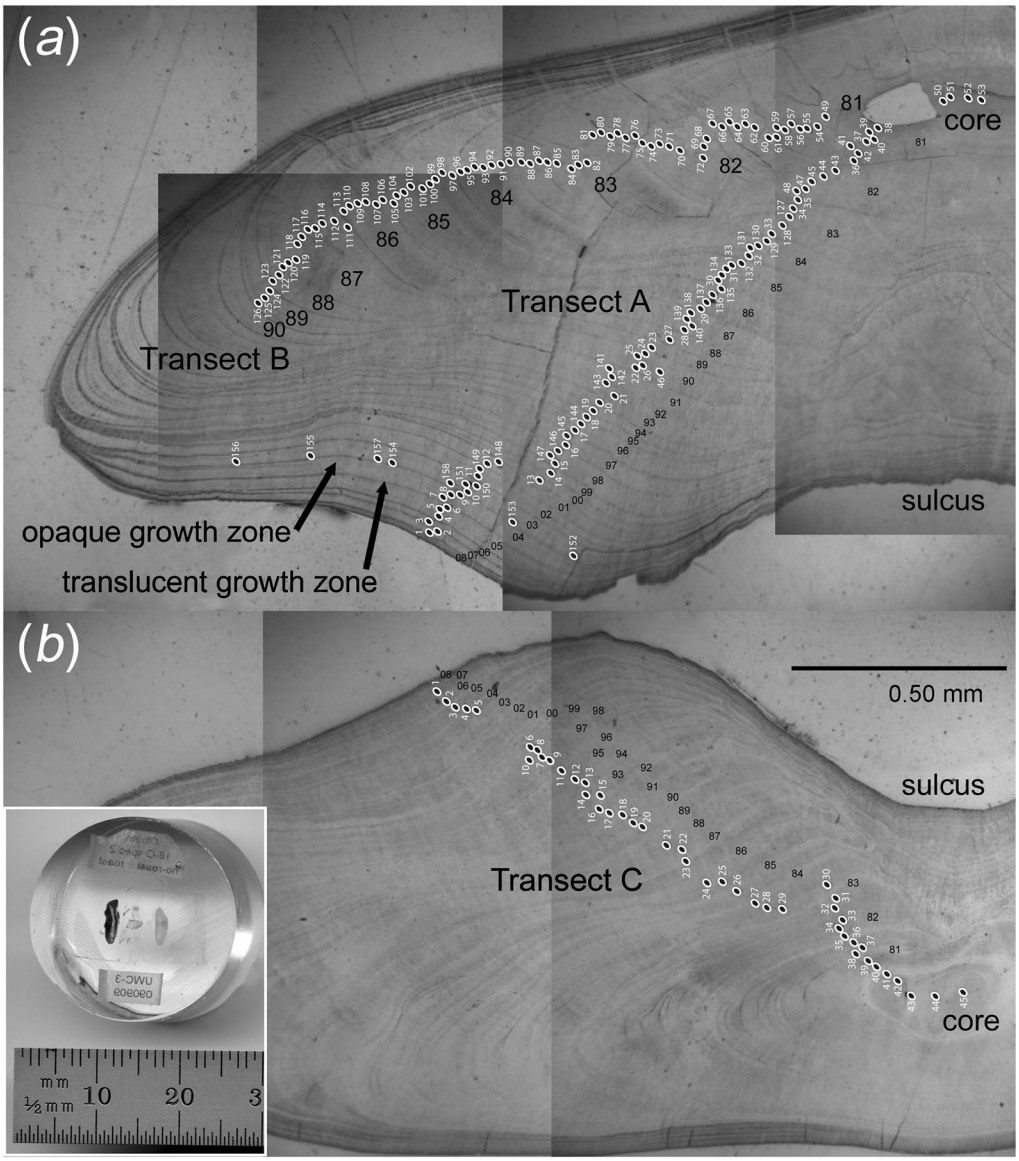

Figure 2. Composite photographs of polished surfaces of matching (a) roasted and (b) unroasted yellowfin sole otolith cross-sections labeled with $\delta^{18}O$ spot analysis locations (white numbering) and calendar year assignments (black numbering) for each transect. Inset: Roasted (darker) and unroasted otolith halves embedded in epoxy mount. Oxygen isotope ratios for each spot analysis are available in the Supporting Information.

here in permil (‰) relative to VSMOW. Raw $\delta^{18}O$ measurements and data reported on the Vienna Pee Dee Belemnite (VPDB) scale are included in the Supporting Information for this paper.

Two transects, A and B, were analyzed for $\delta^{18}O$ values in the roasted otolith cross-section (Fig. 2(a)). Transect A ran from the core of the otolith (center) to its edge parallel to the sulcus, a prominent groove on the proximal side of the otolith. This area is a standard axis used to enumerate annuli during routine age estimation.[25] Transect B was directed through the widest part of the first 10 annuli with the intent of capturing as much seasonal variability as possible during the juvenile and early adult life stages. A single transect in the unroasted cross-section (Transect C), approximately parallel to Transect A in the roasted cross-section, was analyzed to evaluate the effects of roasting (Fig. 2(b)). Whenever possible, multiple samples were acquired within a single otolith annulus to capture seasonal trends in $\delta^{18}O$ values. The integrity of the polished otolith surface was verified post-analysis through scanning electron microscopy (SEM) to ensure that no analysis pits contained contaminants or irregular surface topography that could bias the $\delta^{18}O$ values.[12] In order to evaluate variability in the measured oxygen isotope ratios in contemporaneously formed aragonite, a series of samples immediately adjacent to a single translucent growth zone in the roasted otolith cross-section was analyzed (calendar year 2003; analyses 10, 152–158; Fig. 2(a)).

RESULTS AND DISCUSSION

Otolith $\delta^{18}O$ measurements

A total of 73 spot measurements of $\delta^{18}O$ values were acquired over Transect A, which spanned the years 1981 through 2007 (Fig. 2(a)). No analyses were attempted in the growth band

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for 2008 as it was too narrow and close to the edge of the otolith. The number of 10-µm-diameter spot samples per annulus within Transect A ranged from 1 to 7. Transect B spanned the years 1981 through 1989, and the number of spot samples per annulus ranged from 3 to 23 (total n = 78; Fig. 2(a)). The linear sampling density of both Transects A and B was approximately 50 spot analyses per mm. In the unroasted half of the otolith, a total of 45 spot samples was acquired along Transect C spanning the years 1981 through 2007 (Fig. 2(b)). The number of spots per annulus in Transect C ranged from 0 to 11. The linear sampling density of Transect C was approximately 38 spot analyses per mm.

In all three transects, multiple spot samples were taken per annulus prior to estimated maturity (age-7) to assess seasonal trends in $\delta^{18}O$ values. Due to compression of the annuli following age-7, several annuli in Transect A only had one associated spot sample (years 1988, 1989, 1992–1995, 1999, 2000, and 2006), and several calendar years in Transect C (1989, 2000–2003) were not sampled for $\delta^{18}O$ values. Yellowfin sole otoliths have extremely narrow translucent growth zones, and thus most of the spot samples were taken within opaque growth zones for all three transects.

The measured values of $\delta^{18}O$ ranged from 29.0 to 34.1‰ VSMOW (Fig. 3) with average spot-to-spot reproducibility (2 S.D.) of ±0.3‰ in homogenous standards. Spot samples were checked by SEM for inclusions, porosity, and cracks; the integrity of each spot was verified and all analyses are included in the plots and interpretations presented here. Comparison of Transects A and B ($\delta^{18}O$ values averaged by year; Fig. 3, bottom panel) revealed no significant difference ($t = 0.28$, $df = 16$, $P = 0.78$), indicating that the growth axis (i.e., otolith accretion rate) did not affect $\delta^{18}O$ values. Spot samples taken immediately proximal to a single translucent growth zone to examine the variability of measured $\delta^{18}O$ values in contemporaneous growth ranged from 33.2 to 33.9‰ ($n = 8$; mean = 33.5‰, 2 S.D. = 0.4‰; Fig. 4) with nearly the same precision as attained for the UWC-3 standard.

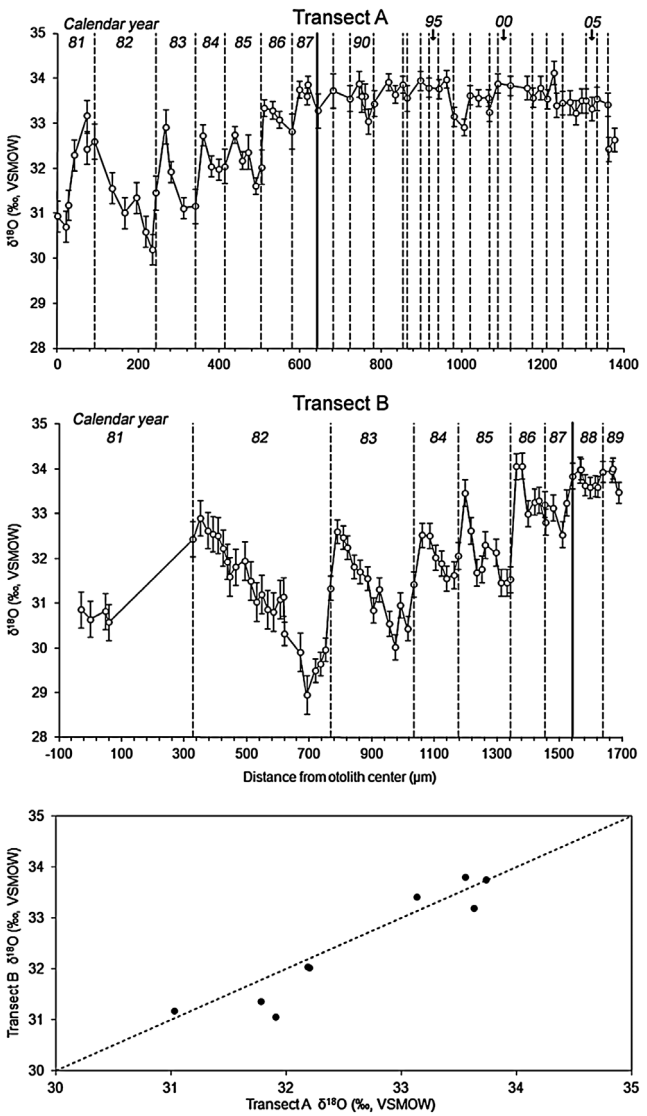

Figure 3. Measurements of $\delta^{18}O$ values (‰ VSMOW, ±2 S.D.) made at the WiscSIMS Laboratory from the otolith center to the edge of Transect A (upper panel) and Transect B (middle panel) of the roasted otolith half. Solid vertical lines indicate estimated transition between juvenile and adult phases (age-7). Dashed vertical lines indicate locations of translucent growth zones, with calendar years of formation labeled at top of each panel in italic type (1981–2007). Bottom panel shows direct comparison of $\delta^{18}O$ values between transects averaged by year. Dashed line is 1:1.

Annual resolution of life history provided by $\delta^{18}O$ values

Mean annual $\delta^{18}O$ values generally increased from the otolith center (age-0) until approximately age-7 (Fig. 3), corresponding roughly to the age at maturity for male yellowfin sole,[22] after which $\delta^{18}O$ values were relatively stable. Because of the inverse relationship between $\delta^{18}O$ values and temperature, the observed otolith $\delta^{18}O$ values suggest that this individual experienced an overall decrease in ambient temperatures from birth until maturity. This trend reflects the gradual ontogenetic migration that yellowfin sole are known to make from shallow to deeper waters.[21–23] Similar lifetime patterns in $\delta^{18}O$ values have been documented for Pacific halibut (*Hippoglossus stenolepis*), a species also known to make an ontogenetic migration from shallow to deeper offshore waters.[34]

Sub-annual sampling was optimized in Transect B (Fig. 3), which was directed through the widest part of the first 10 annuli. Within each annulus, the highest observed $\delta^{18}O$ values corresponded to the earliest deposited part of the opaque growth zone, and translucent growth zone formation coincided with or occurred soon after $\delta^{18}O$ minima (Fig. 3). Within the juvenile portion of the otolith, sub-annual sampling revealed a recurring saw-toothed pattern of $\delta^{18}O$ values (Fig. 3). Records of bottom temperatures from the Mooring 2 buoy (57°N, 164°W) in the middle domain of the EBS (Fig. 1) share a similar saw-toothed pattern of slow warming and rapid cooling.[b] Thus, the saw-toothed pattern in otolith $\delta^{18}O$ values may reflect temperatures experienced on a small spatial scale, given that most juvenile yellowfin sole are believed to remain within the shallow waters of the inner continental shelf throughout

[b]Unpublished data (C. Ladd, Pacific Marine Environmental Laboratory, National Oceanic and Atmospheric Administration, Seattle, WA, USA).

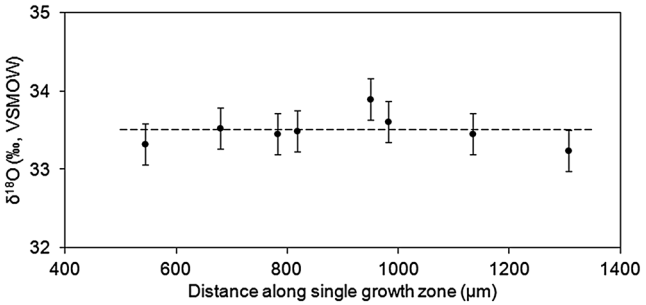

Figure 4. Measurements of $\delta^{18}O$ values made at the WiscSIMS Laboratory from individual 10-μm spots ($n = 8$) taken to evaluate intra-growth zone variation. Mean (dashed line) = 33.5‰ VSMOW; 2 S.D. (bars) = 0.4‰.

the year.[21–23] The saw-toothed pattern is probably amplified by annual slowing or stopping of otolith growth during the colder months of the year when the narrow translucent growth zones form (December–May).[35] Translucent zones in yellowfin sole otoliths are extremely narrow, and consequently most of our sampling occurred within the opaque zones, probably reflecting environmental conditions experienced in June–November. However, factors such as age, geographic region, and depth can influence individual otolith deposition rates.[36] Clearly, further studies of growth mechanisms and potential fractionation in yellowfin sole otoliths that account for these factors are warranted.

As is the case in most studies of oxygen isotope ratios in biogenic carbonate, the exact $\delta^{18}O$ value of the ambient water encountered by this yellowfin sole is uncertain. Furthermore, an oxygen isotope fractionation equation does not currently exist for yellowfin sole; other studies have demonstrated that fractionation is species-specific.[37] Thus, it is more practical to consider relative temperature changes experienced by this fish than to directly predict temperature from otolith $\delta^{18}O$ values. Below, we provide a brief outline of the variability in EBS $\delta^{18}O$ values in order to constrain our interpretation of relative temperatures from otolith $\delta^{18}O$ values.

The primary control on EBS water $\delta^{18}O$ variability is the fractional amount of runoff-sourced freshwater (negative $\delta^{18}O$ values, VSMOW) mixed into the seawater (~0‰, VSMOW). Thus, salinity is commonly used as a proxy for water $\delta^{18}O$ values. Within the Bering Sea, salinity can vary locally and seasonally.[38,39] In particular, shallow coastal areas can be influenced by meltwater or continental freshwater runoff. The annual variability of inshore surface water salinity in the southeastern Bering Sea is as much as 3 parts per thousand (ppt); salinity is at its minimum in July due to river flow.[40] The salinity is far less variable (<1 ppt) in the middle and outer domains of the EBS.[40] A decrease of 1‰ in ambient $\delta^{18}O$ values in the Bering-Chukchi Sea region would correlate to a decrease in salinity of ~1.6 ppt,[41] which is representative of the relatively shallow (<50 m) coastal waters where juvenile yellowfin sole reside. Because freshening occurred at shallow depths when water temperatures were highest, the resulting effect in the otolith would be a ~1‰ increase in the amplitude of the seasonal $\delta^{18}O$ signal.

The maximum range of $\delta^{18}O$ values observed within a single annulus was 3.9‰ (during the fish's second year), which, not taking the ambient water $\delta^{18}O$ value into account, translates into a range of experienced temperatures of approximately 18°C.[6] The bottom temperatures below pack ice in the EBS are typically around −1.8°C, the freezing point of seawater, but during the summer months temperatures increase to 10°C or greater in shallow, well-mixed nearshore areas where juvenile yellowfin sole reside.[21] A temperature range of 12°C would result in an aragonite $\delta^{18}O$ range of 2.8‰. The combined effects of temperature and the suggested 1‰ increase in $\delta^{18}O$ amplitude due to seasonal freshwater input could fully explain the variation in $\delta^{18}O$ observed in the yellowfin sole otolith. Thus, our conclusions regarding the relative importance of temperature in explaining the overall observed trends in otolith $\delta^{18}O$ values are valid.

In contrast to the highly variable juvenile phase, adult-phase otolith $\delta^{18}O$ values were much more stable over time. This stability could be explained in part by the temporal resolution of analysis spots in the outer annuli; the rate of otolith growth dramatically slowed after maturity, resulting in narrower annuli and potentially smoothing measurements of $\delta^{18}O$ variability. Alternatively, the $\delta^{18}O$ pattern in adult-phase annuli might have also been dampened by seasonal migrations between the inner and outer shelf; adults overwinter in outer continental shelf waters (100–270 m deep) and move to depths <100 m to spawn and feed during the summer months.[21,22,35,42] Adults appear to avoid ice cover and the colder water temperatures associated with the inner and middle shelf waters during the winter months,[21] and thus the relatively stable $\delta^{18}O$ values could reflect active seeking of less variable ambient conditions. Overall, the otolith $\delta^{18}O$ profiles reflect the cyclical variability of seasonal temperatures, slightly amplified by seasonal freshwater input (Transect B), as well as longer-term habitat changes as the fish approached maturity (Transect A). Published population-level studies of yellowfin sole ontogenetic and seasonal movements lend further support to our interpretations of the $\delta^{18}O$ pattern.[21,35]

Dating controls of $\delta^{18}O$ measurements

After detrending with cubic splines, the $\delta^{18}O$ otolith growth-increment widths (1985–2007) correlated well ($r = 0.62$, $P = 0.006$) with the average growth pattern from the 21 individuals used to develop the previously published EBS master chronology.[24] Furthermore, the detrended $\delta^{18}O$ otolith growth-increment measurements positively correlated ($r = 0.73$, $P < 0.001$) to average summer bottom temperatures recorded during trawl surveys.[43] Thus, the growth pattern of the otolith analyzed here was consistent with that of other EBS yellowfin sole,[24] providing a statistical corroboration of annulus dating,[44] and by proxy, $\delta^{18}O$ spot sample dating. Due to lingering uncertainties about otolith accretion phenology in yellowfin sole, it was not possible to date $\delta^{18}O$ spots on a sub-annual scale, and thus, $\delta^{18}O$ measurements could not be directly compared with climate indices or increment width. However, dendrochronology-based dating controls allowed direct comparison of the properties of the otolith with observational environmental records at the annual timestep. To ensure accuracy, we recommend that crossdating be a fundamental step of any sclerochronology analysis whenever annuli are sufficiently well defined for mensuration and growth is synchronous among individuals from a given species and site.

Effects of roasting

Roasting resulted in an increase of approximately 1‰ in the raw value of $\delta^{18}O$ compared with that of the unroasted sample, but did not change $\delta^{18}O$ trends (Fig. 5). Conventional

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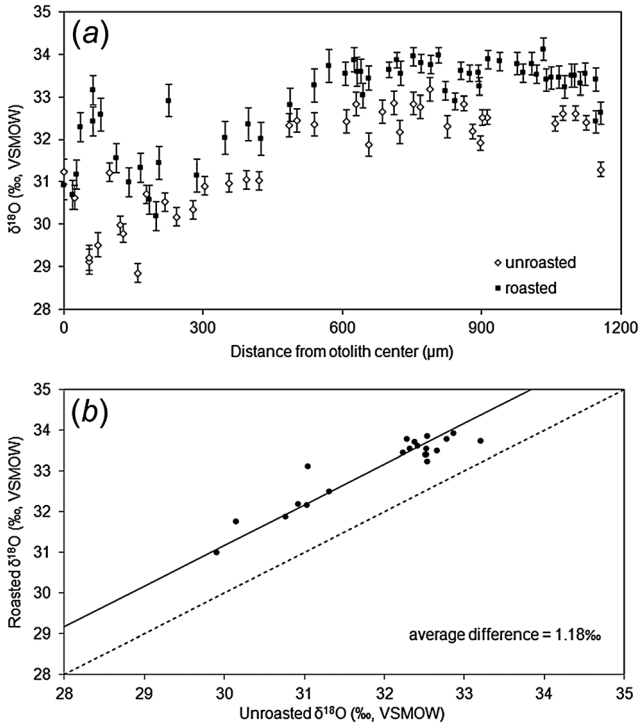

Figure 5. (a) Comparison of all spot analyses from Transect A (roasted; filled squares) and Transect C (unroasted; open diamonds) to assess the effects of roasting, which removes volatile organic contaminants, on $\delta^{18}O$ values. The unroasted data are interpreted to be biased by organic components and thus not to be accurate. Error bars represent ±2 S.D. (b) Difference between roasted and unroasted $\delta^{18}O$ values averaged by year. Dashed line is 1:1. Roasted values were on average 1.18‰ higher (solid line) than unroasted values.

acid-digestion analyses of otoliths sometimes use roasting as a means to remove organic contaminants, and a shift in measured $\delta^{18}O$ values is expected.[45] For this reason, we report the $\delta^{18}O$ values from the roasted otolith portion on the VSMOW scale. Preliminary (unpublished) *in situ* XRD analyses at UW-Madison, however, suggest that roasting converted some otolith aragonite into calcite, an unstudied effect in ion microprobe analyses. Given the fact that this partial conversion is under kinetic control, different results among samples or laboratories could potentially occur; until further study of this phenomenon is possible, roasting remains an important step in such analyses. Since roasting is an imperfect solution to the removal of organic material from otolith analyses, we avoid interpreting absolute temperatures from values of $\delta^{18}O$. Notably, however, the similar trend of $\delta^{18}O$ values in both roasted and unroasted transects (Fig. 5) suggests that our interpretations of relative temperatures from $\delta^{18}O$ values are valid.

Advantages of SIMS

The high spatial resolution of the ion microprobe allowed multiple sequential samples to be taken, often within a single year, to effectively describe the general ontogeny of an individual fish. This is the first case that we know of where so many $\delta^{18}O$ analyses have been performed for a single individual, with measurements taken sub-annually over a time span of 27 years. In contrast, most conventional $\delta^{18}O$ studies have been directed at juveniles or short-lived fish with large otoliths, possibly in part due to limitations in spatial sample resolution. Yellowfin sole otoliths are relatively small, and the individual in our study was extremely old. Had we directed our study at a species such as Pacific cod (*Gadus macrocephalus*), which has large otoliths and low longevity, even greater temporal resolution of $\delta^{18}O$ analyses could have been achieved.[14]

Future applications

The present study was designed to explore the detailed information recorded by a single otolith and while such information might prove fundamental for understanding the life cycle of one fish, it was not intended to be used to make population-level inferences. However, based on this work, future studies should be able to obtain the same level of information with far fewer analyses. Population-level studies focused on early life histories should consider transects similar to our Transect B, which maximized the number of potential $\delta^{18}O$ measurements obtainable by traversing the widest part of each annulus. Conversely, those studies interested in overall lifelong variation would be best suited by core-to-edge transects such as our Transect A. Furthermore, researchers should consider the placement of spot analyses relative to growth zone borders. Potential variation would be maximized and the number of analyses minimized by sampling the distal edge of each translucent zone and several spots evenly distributed across each opaque zone.

CONCLUSIONS

Otolith $\delta^{18}O$ values obtained via ion microprobe provide an indicator of the relative environmental conditions to which a yellowfin sole was exposed during its lifetime in the EBS. The combined spatial resolution (10 μm spot diameter), sampling density (~50 discrete spots per mm in the roasted transect), and precision (0.3‰, 2 S.D.) achieved in this study reveal new information about both ontogeny of a long-lived individual and annual variability in $\delta^{18}O$ value that could not have been attained by conventional micro-mill techniques. Overall, the apparent ontogenetic and seasonal trends in observed otolith $\delta^{18}O$ values are consistent with population-level work on yellowfin sole movements.[21–23,35] Although the $\delta^{18}O$ composition of the ambient water was uncertain, evidence suggests that temperature played a primary role in controlling otolith $\delta^{18}O$ values and was largely responsible for the relative variation observed. A unique aspect of this study was the use of crossdating in conjunction with highly precise *in situ* measurements of $\delta^{18}O$ values to ensure that all growth zones and $\delta^{18}O$ spot samples were assigned the correct calendar year of formation. These results demonstrate that $\delta^{18}O$ analysis with an ion microprobe is a useful tool in hindcasting detailed life histories of individual fish. Given sufficient resources, the ion microprobe can also be used to make population-level inferences regarding large-scale migrations, often poorly understood for many species of fish.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

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REFERENCES

[1] S. E. Campana, S. R. Thorrold. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Can. J. Fish. Aquat. Sci.* **2001**, *58*, 30.
[2] H. Mosegaard, A. Folkvord, P. J. Wright. Ecological applications, in *Manual of Fish Sclerochronology*, (Eds: J. Panfili, H. de Pontual, H. Troadec, P. J. Wright). Ifremer-IRD, Brest, France, **2002**, pp. 167–178.
[3] B. A. Black, G. W. Boehlert, M. M. Yoklavich. Establishing climate-growth relationships for yelloweye rockfish (*Sebastes ruberrimus*) in the northeast Pacific using a dendrochronological approach. *Fish. Oceanogr.* **2008**, *17*, 368.
[4] A. L. Rypel. Climate-growth relationships for largemouth bass (*Micropterus salmoides*) across three southeastern USA states. *Ecol. Freshwater Fish.* **2009**, *18*, 620.
[5] B. A. Black, I. D. Schroeder, W. J. Sydeman, S. J. Bograd, P. W. Lawson. Wintertime ocean conditions synchronize rockfish growth and seabird reproduction in the central California Current ecosystem. *Can. J. Fish. Aquat. Sci.* **2010**, *67*, 1149.
[6] S. E. Campana. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Mar. Ecol.: Prog. Ser.* **1999**, *188*, 263.
[7] H. de Pontual, A. J. Geffen, Otolith microchemistry, in *Manual of Fish Sclerochronology*, (Eds: J. Panfili, H. de Pontual, H. Troadec, P. J. Wright). Ifremer-IRD, Brest, France, **2002**, pp. 245–302.
[8] S. R. Thorrold, D. C. Zacherl, L. A. Levin. Population connectivity and larval dispersal using geochemical signatures in calcified structures. *Oceanography* **2007**, *20*, 80.
[9] J. S. Edmonds, W. J. Fletcher. Stock discrimination of pilchards *Sardinops sagax* by stable isotope ratio analysis of otolith carbonate. *Mar. Ecol.: Prog. Ser.* **1997**, *152*, 241.
[10] S. R. Thorrold, C. M. Jones, P. K. Swart, T. E. Targett. Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. *Mar. Ecol.: Prog. Ser.* **1998**, *173*, 253.
[11] C. R. Weidman, R. Millner. High-resolution stable isotope records from North Atlantic cod. *Fish. Res.* **2000**, *46*, 327.
[12] B. C. Weidel, T. Ushikubo, S. R. Carpenter, N. T. Kita, J. J. Cole, J. F. Kitchell, M. L. Pace, J. W. Valley. Diary of a bluegill (*Lepomis macrochirus*): daily $\delta^{13}C$ and $\delta^{18}O$ records in otoliths by ion microprobe. *Can. J. Fish. Aquat. Sci.* **2007**, *64*, 1641.
[13] J. B. Jones, S. E. Campana. Stable oxygen isotope reconstruction of ambient temperature during the collapse of a cod (*Gadus morhua*) fishery. *Ecol. Appl.* **2009**, *19*, 1500.
[14] J. W. Valley, N. T. Kita. *In situ* oxygen isotope geochemistry by ion microprobe, in *Secondary Ion Mass Spectrometry in the Earth Sciences*, (Ed.: M. Fayek). Mineralogical Association of Canada Short Course, Toronto, Ontario, **2009**, *41*, pp. 19–63.
[15] N. N. Hanson, C. M. Wurster, E. I. M. F., C. D. Todd. Comparison of secondary ion mass spectrometry and micromilling/continuous flow isotope ratio mass spectrometry techniques used to acquire intra-otolith $\delta^{18}O$ values of wild Atlantic salmon (*Salmo salar*). *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2491.
[16] J. E. Kalish. ^{13}C and ^{18}O isotopic disequilibria in fish otoliths: metabolic and kinetic effects. *Mar. Ecol.: Prog. Ser.* **1991**, *75*, 191.
[17] S. R. Thorrold, S. E. Campana, C. M. Jones, P. K. Swart. Factors determining $\delta^{13}C$ and $\delta^{18}O$ fractionation in aragonitic otoliths of marine fish. *Geochim. Cosmochim. Acta.* **1997**, *61*, 2909.
[18] H. Høie, A. Folkvord, E. Otterlei. Effect of somatic and otolith growth rate on stable isotopic composition of early juvenile cod (*Gadus morhua* L.) otoliths. *J. Exp. Mar. Biol. Ecol.* **2003**, *289*, 41.
[19] N. T. Kita, T. Ushikubo, B. Fu, J. W. Valley. High precision SIMS oxygen isotope analyses and the effect of sample topography. *Chem. Geol.* **2009**, *264*, 43.
[20] T. K. Wilderbuer, D. G. Nichol, J. Ianelli. Yellowfin sole, in *Stock Assessment and Fishery Evaluation Report for the Groundfish Resources of the Bering Sea/Aleutian Islands Regions*. North Pacific Fishery Management Council, Anchorage, AK, **2010**, pp. 565–644.
[21] R. G. Bakkala. Population characteristics and ecology of yellowfin sole, in *The Eastern Bering Sea Shelf: Oceanography and Resources*, (Eds: D. W. Hood, J. A. Clader). US Dept Commerce NOAA, Washington DC, **1981**, pp. 553–574.
[22] T. K. Wilderbuer, G. E. Walters, R. G. Bakkala. Yellowfin sole, *Pleuronectes asper*, of the eastern Bering Sea: biological characteristics, history of exploitation, and management. *Mar. Fish. Rev.* **1992**, *54*, 1.
[23] D. G. Nichol. Effects of geography and bathymetry on growth and maturity of yellowfin sole, *Pleuronectes asper*, in the eastern Bering Sea. *Fish. Bull.* **1997**, *95*, 494.
[24] M. E. Matta, B. A. Black, T. K. Wilderbuer. Climate-driven synchrony in otolith growth-increment chronologies for three Bering Sea flatfish species. *Mar. Ecol.: Prog. Ser.* **2010**, *413*, 137.
[25] M. E. Matta, D. K. Kimura. Age Determination Manual of the Alaska Fisheries Science Center Age and Growth Program. *NOAA Professional Paper NMFS*, **2012**, *13*.
[26] R. Kozdon, T. Ushikubo, N. T. Kita, M. Spicuzza, J. W. Valley. Intratest oxygen isotope variability in planktonic foraminifera: new insights from in situ measurements by ion microprobe. *Chem. Geol.* **2009**, *258*, 327.
[27] R. I. C. C. Francis, P. L. Horn. Transition zone in otoliths of orange roughy (*Hoplostethus atlanticus*) and its relationship to the onset of maturity. *Mar. Biol.* **1997**, *129*, 681.
[28] A. J. Geffen, H. de Pontual, P. J. Wright, H. Mosegaard. Life history events, in *Manual of Fish Sclerochronology*, (Eds: J. Panfili, H. de Pontual, H. Troadec, P. J. Wright). Ifremer-IRD, Brest, France, **2002**, pp. 99–104.
[29] R. L. Holmes. Computer-assisted quality control in tree-ring dating and measurement. *Tree-Ring Bull.* **1983**, *43*, 69.
[30] H. D. Grissino-Mayer. Evaluating crossdating accuracy: a manual and tutorial for the computer program COFECHA. *Tree-Ring Res.* **2001**, *57*, 205.
[31] B. A. Black, G. W. Boehlert, M. M. Yoklavich. Using tree-ring crossdating techniques to validate annual growth increments in long-lived fishes. *Can. J. Fish. Aquat. Sci.* **2005**, *62*, 2277.
[32] B. A. Black. Climate-driven synchrony across tree, bivalve, and rockfish growth-increment chronologies of the northeast Pacific. *Mar. Ecol.: Prog. Ser.* **2009**, *378*, 37.

[33] I. J. Orland, M. Bar-Matthews, N. T. Kita, A. Ayalon, A. Matthews, J. W. Valley. Climate deterioration in the eastern Mediterranean from 200 BC to 1100 AD as revealed by ion microprobe analysis of speleothems from Soreq Cave, Israel. *Quat. Res.* **2009**, *71*, 27.
[34] Y. Gao, R. J. Beamish. Stable isotope variations in otoliths of Pacific halibut (*Hippoglossus stenolepis*) and indications of the possible 1990 regime shift. *Fish. Res.* **2003**, *60*, 393.
[35] K. Wakabayashi. Studies on the fishery biology of yellowfin sole in the eastern Bering Sea [in Japanese, English summary]. *Bull. Far Seas Fish. Res. Lab.* **1989**, *26*, 21.
[36] D. K. Kimura, D. M. Anderl, B. J. Goetz. Seasonal marginal growth on otoliths of seven Alaska groundfish species support the existence of annual patterns. *Alaska Fish. Res. Bull.* **2007**, *12*, 243.
[37] H. Høie, E. Otterlei, A. Folkvord. Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua* L.). *ICES J. Mar. Sci.* **2004**, *61*, 243.
[38] P. J. Stabeno, N. A. Bond, N. B. Kachel, S. A. Salo, J. D. Schmuacher. On the temporal variability of the physical environment over the south-eastern Bering Sea. *Fish. Oceanogr.* **2001**, *10*, 81.
[39] T. Tanaka, G. Laodong, C. Deal, N. Tanaka, T. Whitledge, A. Murata. N deficiency in a well-oxygenated cold bottom water over the Bering Sea shelf: influence of sedimentary denitrification. *Cont. Shelf Res.* **2004**, *24*, 1271.
[40] V. A. Luchin, V. A. Menovshchikov, V. M. Lavrentiev. Thermohaline structure and water masses in the Bering Sea, in *Dynamics of the Bering Sea*, (Eds: T. R. Loughlin, K. Ohtani). University of Alaska Sea Grant, Fairbanks, AK, **1999**, pp. 61–91.
[41] M. Yamamoto-Kawai, E. C. Carmack, F. A. McLaughlin, K. K. Falkner. Oxygen isotope ratio, barium, and salinity in waters around the North American coast from the Pacific to the Atlantic: implications for freshwater sources to the Arctic throughflow. *J. Mar. Res.* **2010**, *68*, 97.
[42] R. G. Bakkala. Structure and historical changes in the groundfish complex of the eastern Bering Sea. *NOAA Tech. Rep. NMFS.* **1993**, *114*.
[43] National Oceanic and Atmospheric Administration (NOAA). Bering climate: a current view of the Bering Sea ecosystem and climate. Available: http://www.bering-climate.noaa.gov/data/index.php (accessed December 4, **2012**).
[44] B. A. Black, D. C. Gillespie, S. E. MacLellan, C. M. Hand. Establishing highly accurate production-age data using the tree-ring technique of crossdating: a case study for Pacific geoduck (*Panopea abrupta*). *Can. J. Fish. Aquat. Sci.* **2008**, *65*, 2572.
[45] K. R. R. A. Guiguer, R. Drimmie, M. Power. Validating methods for measuring $\delta^{18}O$ and $\delta^{13}C$ in otoliths from freshwater fish. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 463.

