Diary of a bluegill (Lepomis macrochirus): daily δ¹³C and δ¹⁸O records in otoliths by ion microprobe

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Abstract: Otoliths provide information about an individual fish’s environment at ecologically relevant time scales. We used ion microprobe analysis to produce high-resolution δ¹³C and δ¹⁸O time series from two age-4 bluegill (Lepomis macrochirus) otoliths, which provided insight into fish behavior and otolith fractionation processes. Scanning electron microscope images revealed δ¹³C and δ¹⁸O pit diameters of 10 and 15 µm, respectively, corresponding to 1–5 and 2–9 daily increments during rapid otolith growth and 6–9 and 12–25 increments near annual otolith growth checks. Spot-to-spot reproducibility (1 SD) of the calcite standards was <0.2‰ for δ¹⁸O and <0.4‰ for δ¹³C and was small enough to resolve a change in a fish’s ambient temperature of approximately 1 °C. A whole-lake ¹³C addition experiment elevated the δ¹³C of the lake’s dissolved inorganic carbon for 56 days during the summer of 2005. Mixing model results indicated that the proportion of dietary carbon in otoliths (M) was similar for both fish (BLG-3, M = 0.45; BLG-12, M = 0.35), but the relation between M and proxies of metabolic rate differed between fish. Otolith stable isotope analysis by ion microprobe can reveal the environmental history of an individual fish and contribute to our understanding of processes that influence isotope ratio fractionation in otoliths.

Résumé : Les otolithes fournissent des renseignements sur le milieu environnant de poissons individuels à des échelles temporelles qui sont d’intérêt écologique. Nous faisons une analyse à l’aide d’une microsonde ionique qui produit une série chronologique de haute résolution des valeurs de δ¹³C et de δ¹⁸O dans deux otolithes de crapets arlequins (Lepomis macrochirus) d’âge 4, ce qui ouvre des perspectives sur le comportement de ces poissons et sur les processus de fractionation dans les otolithes. Des images au microscope électronique à balayage montrent des diamètres des fosses de δ¹³C et de δ¹⁸O respectifs de 10 et de 15 µm, ce qui correspond à 1–5 et à 2–9 incréments par jour durant la croissance rapide des otolithes et à 6–9 et à 12–25 incréments près des marques annuelles de croissance de l’otolithe. La reproductibilité d’un point à un autre (1 ET) des standards de calcite est de < 0.2 ‰ pour δ¹⁸O et < 0.4 ‰ pour δ¹³C, donc assez précise pour permettre de reconnaitre un changement d’environ 1 °C dans la température ambiante du poisson. Une addition expérimentale de ¹³C à l’échelle du lac entier a augmenté le δ¹³C du carbone inorganique dissous du lac pendant 56 jours pendant l’été de 2005. Les résultats d’un modèle de mélange indiquent que la proportion de carbone d’origine alimentaire dans les otolithes (M) est semblable chez les deux poissons (BLG-3, M = 0.45; BLG-12, M = 0.35), mais que la relation entre M et les variables de remplacement du taux métabolique est différente chez ces deux individus. L’analyse des isotopes stables de l’otolithe par microsonde ionique peut ainsi révéler le passé environnemental d’un poisson individuel et contribuer à la compréhension des processus qui affectent la fractionation des rapports isotopiques dans les otolithes.

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Introduction

Established relations between otolith precipitation rate and fish growth allow otolith layers to be interpreted as a record of environmental changes experienced by an individual fish over its lifetime, with the integrated response expressed as growth. Quantifying environmental variables that influenced growth in a continuous manner is more difficult. One must often resort to a low-frequency time series of environmental conditions that may not describe all life stages. Because the
calcium carbonate layers in otoliths are inert, they provide a record of the isotope ratios of a fish’s environment and the physical and biological processes that influence fractionation during precipitation (Campana 1999). Isotope records can potentially provide information about diet, temperature, or other environmental conditions provided that we can analyze an otolith with sufficient precision and at a temporal scale that captures relevant changes in a fish’s environment or behavior.

Our understanding of processes that influence isotopic fractionation varies for the major otolith elements. Otolith δ18O is dependent on ambient water temperature and water δ18O and can be used to reconstruct the thermal history of a fish’s environment (Thorrold et al. 1997; Campana 1999). Otolith δ13C values reflect the isotope ratio of ambient water dissolved inorganic carbon (δ13CDIC) and dietary carbon (δ13CDiet), but the proportional importance of these sources is not well established (Solomon et al. 2006). Laboratory studies estimated that the dietary proportion of carbon in otoliths, denoted M, ranges from 0.10 to 0.40; however, species-specific differences and potential confounding effects of metabolic rate have limited the use of δ13C as an environmental marker within otoliths (Wurster and Patterson 2003; Wurster et al. 2005; Solomon et al. 2006).

During periods of somatic growth, daily otolith rings are typically visible and can be counted, but analytical techniques have hindered our ability to extract an isotopic time series at this scale. Micro-milling and laser ablation techniques allow analysis of larger areas or bands in otoliths, but series at this scale. Micro-milling and laser ablation techniques allow analysis of larger areas or bands in otoliths, but typically visible and can be counted, but analytical techniques have hindered our ability to extract an isotopic time series at this scale. Micro-milling and laser ablation techniques allow analysis of larger areas or bands in otoliths, but a penalty in analytical precision (1 standard deviation (SD) ≥ 0.5‰, spot diameter ~ 20 μm) of ion microprobe δ13C and δ18O analysis has limited its use with otoliths, but improved precisions (1 SD < 0.1‰) have been reported for spot diameters of 10 μm (Page et al. 2007).

We evaluated the use of an ion microprobe for analyzing stable isotopes of carbon and oxygen in a common freshwater fish otolith. We estimated the spatial resolution and precision for δ18O and δ13C and created time series for both isotope ratios over the life of two adult bluegill (Lepomis macrochirus). A whole-lake 13C addition experiment, designed to determine the terrestrial carbon subsidies to lake food webs, provided an opportunity use high-resolution time series to estimate the relative influences of different carbon sources on otolith δ13C in a natural setting.

Materials and methods

Sagittal otoliths from two age-4 bluegill (BLG-12 and BLG-3 captured in the spring and fall, respectively, of 2006) from Crampton Lake, Wisconsin, were analyzed. BLG-3 was prepared as a transverse section and BLG-12 was prepared as a sagittal plane section. Sections and calcite standard were cast in a 25.4 mm round epoxy mount and polished to a smooth, flat surface. Light and scanning electron microscope (SEM) images directed microprobe sampling and identified daily increments. Samples were cleaned with ultrapure water and coated with gold thin film.

Oxygen and carbon isotope ratios were measured by CAMECA-IMS-1280 ion microprobe at the University of Wisconsin–Madison (Page et al. 2007). The primary ion beam was 133Cs+ set at 0.6 nA for ~4 min for δ18O and at 2.5 nA for ~3.5 min for δ18O spots. Ions were extracted with 10 kV and selected using a 40 eV energy window. Two Pararady cups were used to simultaneously measure 18O and 16O, and an electron multiplier and a Faraday cup were used for 13C and 12C, respectively. Charge compensation was aided by a normal-incidence electron gun. Otolith analyses were bracketed with standard analyses to correct for instrumental and are reported in per mil (‰) notation relative to Standard Mean Ocean Water for δ18O and Pee Dee Belemnite for δ13C. Parallel sampling traverses for δ18O and δ13C followed a radius from the otolith origin, oriented across the widest portion of each yearly growth band. Carbon and oxygen data were paired where SEM images indicated analysis pits occurred within the same daily increments.

To estimate the contribution of different sources to otolith carbon, we used a steady-state mixing model to predict otolith δ13C based on daily time series of lake water δ13CDIC and bluegill δ13CDiet over the course of the isotope addition in 2005. To assign calendar dates to the daily increment counts and δ13COTolith, we assumed a ±2‰ changes in δ13COTolith between analysis pits represented the start and end of the 13C addition (Fig. 1). The daily δ13CDIC time series was taken from Pace et al. (2007). Age-4 bluegill diets were quantified each week (n = 23) based on the dry weight proportion of 16 invertebrate taxa from 8–25 individual fish diets (total n = 408). Weekly δ13CDiet was a weighted average of a diet item’s weekly measured δ13C and its proportion in the diets. Diet 13C data were linearly interpolated to correspond to δ13COTolith dates. The mixing model predicted the proportion of otolith carbon derived from the diet and water for each day in the δ13COTolith time series (BLG-3, n = 33; BLG-12, n = 54) in 2005 assuming a fractionation between sources and otolith of ~1.8‰ (Solomon et al. 2006). Lake water δ18O was determined from three integrated epilimnetic water samples collected during summer 2006.

Results and discussion

We chose ion microprobe analysis pit diameters of 10 and 15 μm for δ13C and δ18O, respectively (Fig. 1). Smaller pits (<1 μm) are possible, but at a penalty in analytical precision (Page et al. 2007). Temporal resolution of individual analyses across the transect was dependent on otolith growth rate. Within the central portion of each yearly growth increment, where daily otolith growth increments were largest, analysis pits spanned 1–4 and 2–7 daily increments (Figs. 2a and 2c). For analyses adjacent to annual growth marks where growth was reduced because of cooler water temperatures, pits covered 6–9 and 12–23 or more daily increments for δ13C and δ18O, respectively (Fig. 2d).

Spot-to-spot reproducibility (1 SD) of the UWC-1 calcite standard was <0.2‰ for δ18O and <0.4‰ for δ13C. Micro-milling for otoliths isotope analysis has been reported to attain similar precision and temporal resolution, but only from exceptionally large otoliths (Wurster and Patterson 2003).
2003; Wurster et al. 2005). Furthermore, micro-milling requires 20–40 µg of CaCO₃ per analysis and may include otolith material from layers beneath the surface of the sample, but the ion microprobe requires less than 500 pg of CaCO₃ per analysis and the shallow pit depth (<2 µm) avoids inclusion of other growth zones.

Extracting a consistent and complete isotopic record from an otolith using secondary ion mass spectrometry (SIMS) requires a single, flat plane in the otolith from the origin to the edge and the ability to establish the measurement series time line. Annual growth checks were apparent in both sections, but slight concavity in the sagittal-sectioned otolith (BLG-12) required polishing beyond the origin and resulted in the first analysis not reflecting the otolith origin. A sagittal orientation provides the maximum temporal precision (Weber et al. 2005), but a transverse section provides a simple preparation technique that ensures that all growth increments are accurately represented in a single plane, preferred for ion microprobe analysis.

Time series of δ¹⁸O from micro-milled otoliths have been used to reconstruct the thermal history of fish, but low spatial resolution and destructive sampling techniques preclude the use of daily increments to assign an accurate time scale to measurements (Wurster and Patterson 2001; Wurster et al. 2005). A change in water temperature of 1 °C corresponds to a change of approximately 0.25‰ in the δ¹⁸O value of an otolith and thus the precision of our δ¹⁸O measurements (SD = 0.22‰) are sufficient to resolve fish temperature changes of ~1 °C (Thorrold et al. 1997). Using the lake water δ¹⁸O, calculated water temperatures experienced by the bluegill correspond to the seasonal water temperature patterns of Crampton Lake. Because lake water can undergo seasonal changes in δ¹⁸O, it was not possible to accurately estimate fish temperature across the growing season; however, values of δ¹⁸O change slowly in lake water and short-term temperature changes resolved by the 15 µm ion microprobe beam are meaningful. In both fish, δ¹⁸O measurements in 2003 and 2005 show a similar trend indicating that bluegill moved to cooler waters in midsummer. This may reflect a behavioral response to reduce metabolic costs from high surface water temperatures or a response to aggregations of invertebrates that have been observed near the thermocline in late summer.

The ¹³C addition quickly elevated and maintained the lake’s mixed-layer δ¹³CDIC 12‰–30‰ over the 56 days (Pace et al. 2007), whereas the average 4-year-old bluegill δ¹³CDiet increased by ~7‰ over the time period. Visual counts of daily growth increments (BLG-3, n = 59; BLG-12, n = 61) between the increase and decrease in δ¹³C otolith corresponded well to the 56-day experiment and provided a way to align
the otolith and carbon source time series (~1360 and 1650 µm; Fig. 2e). Analysis pits used to generate otolith data for the model spanned 1–4 and 2–7 daily increments for δ¹³C and δ¹⁸O, respectively. Estimates of $M$ were higher and more consistent in BLG-3 (mean = 0.45, 1 SD = 0.10, range 0.23–0.63) than in BLG-12 (mean = 0.35, 1 SD = 0.21, range 0.0–0.60). Our mean estimates of $M$ are similar to those reported from lab and field studies (0.10–0.44); how-

Fig. 2. (a and b) Time series of δ¹³C and δ¹⁸O values arranged by distance from the otolith origin in a 4+-year-old bluegill (BLG-3), *Lepomis macrochirus*. Error bars represent two standard deviations. Broken vertical lines represent the position of annual growth increments as interpreted from images. In (a), ambient water temperature (Temp.) on the right y axis in the δ¹⁸O plot is based on a constant lake water δ¹⁸O and calculated from formulas in Thorrold et al. (1997). The temperature axis provides a relative scale for the δ¹⁸O measurements as variations in lake water δ¹⁸O over time would influence the predicted temperature calculations. (b) Otolith δ¹³C measurements from 2005 were used in the mixing model, and the arrows indicate the interpreted start and end of the ¹³C addition experiment (start at 1270 µm, end at 1430 µm). (c) Relationship between δ¹⁸O and δ¹³C measured over the entire otolith transect. Open circles represent paired data during the ¹³C addition; solid circles represent values before and after ¹³C addition. (d–f) Results from an additional 4+-year-old bluegill (BLG-12), collected in the spring of 2006, are illustrated. (e) Arrows at 1350 and 1650 µm indicated the interpreted start and end of the isotope addition, respectively. Error bars and figure notations are consistent with (a) through (c).
ever, we found our range of calculated $M$ values to be greater than previously reported estimates (Wurster et al. 2005; Solomon et al. 2006), presumably because of the better temporal resolution of our data. Diets of similar-sized bluegill were used to determine the average $\delta^{13}C_{\text{Diet}}$ but varied $3\%e$–$4\%e$ between individuals by the end of the experiment and may have contributed to individual differences in estimated $M$ values. Alternatively, because $\delta^{13}C_{\text{DIC}}$ is influenced by primary production and $CO_2$ exchange with the atmosphere, $\delta^{13}C_{\text{DIC}}$ may have varied with depth within the mixed layer (Bade et al. 2004).

Wurster and Patterson (2003) observed seasonal trends in $\delta^{13}C$ similar to ours (Figs. 2b and 2e) in freshwater drum (Aplodinotus grunniens) otoliths and proposed that seasonal metabolic rate changes altered $M$. Fish metabolic rate is correlated to water temperature and we would expect a negative relationship between $M$ and otolith $\delta^{18}O$ (Wurster and Patterson 2003). We found no linear relationship between $M$ and $\delta^{18}O$ for BLG-3 ($R^2 = 0.09, p = 0.087$) but a strong, negative relationship for BLG-12 ($R^2 = 0.73, p < 0.001$). Our results partially corroborate the suggestion that $M$ changes over a season, but emphasize the importance of variability in individual fish behavior when using otoliths as an environmental recorder of water and (or) dietary $\delta^{13}C$.

In situ otolith isotope analysis by ion microprobe can provide high-resolution records that resolve environmental or behavioral dynamics at the individual level. Quantifying individual behavioral variability in populations may be a useful indicator of changes in populations. When high-frequency sampling is not possible and (or) where migratory, prey selection, and habitat choice behaviors have short-term dynamics (few days), the ion microprobe analysis of otolith $\delta^{13}C$ and $\delta^{18}O$ offers greater resolution than traditional sampling methods. Potential applications include identifying pulses of migratory prey in diets or behavioral changes associated with weather patterns, discrete thermal habitats (e.g., plumes, currents), or spawning. The increased temporal resolution and precision over current analytical techniques means that analyses to identify migration routes or ontogenetic history or provide insight into past climate are no longer restricted to only those species with unusually large otoliths (Wurster and Patterson 2001; Carpenter et al. 2003).

Pairing the capabilities of the ion microprobe with the recording capacity of otoliths offers the prospect of daily observations on fish distribution, behavior, migration, and trophic interactions.

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