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Diary of a bluegill (*Lepomis macrochirus*): daily $\delta^{13}C$ and $\delta^{18}O$ 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 $\delta^{13}C$ and $\delta^{18}O$ 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 $\delta^{13}C$ and $\delta^{18}O$ 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 $\delta^{18}O$ and <0.4‰ for $\delta^{13}C$ and was small enough to resolve a change in a fish's ambient temperature of approximately 1 °C. A whole-lake ^{13}C addition experiment elevated the $\delta^{13}C$ 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 $\delta^{13}C$ et de $\delta^{18}O$ 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 $\delta^{13}C$ et de $\delta^{18}O$ 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 $\delta^{18}O$ et < 0,4 ‰ pour $\delta^{13}C$, donc assez précise pour permettre de reconnaître un changement d'environ 1 °C dans la température ambiante du poisson. Une addition expérimentale de ^{13}C à l'échelle du lac entier a augmenté le $\delta^{13}C$ 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.

[Traduit par la Rédaction]

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

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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 $\delta^{18}O$ is dependent on ambient water temperature and water $\delta^{18}O$ and can be used to reconstruct the thermal history of a fish's environment (Thorrold et al. 1997; Campana 1999). Otolith $\delta^{13}C$ values reflect the isotope ratio of ambient water dissolved inorganic carbon ($\delta^{13}C_{DIC}$) and dietary carbon ($\delta^{13}C_{Diet}$), 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 $\delta^{13}C$ 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 high-frequency time series, over the life of a fish, have only been demonstrated in species with exceptionally large otoliths (Wurster and Patterson 2003; Wurster et al. 2005). Ion microprobe analyses of sulfur and strontium isotopes indicate that this method can produce time series at a resolution adequate to infer life history and movement behaviors (Weber et al. 2002, 2005). Until recently, lower precision (1 standard deviation (SD) ≥ 0.5‰, spot diameter ~ 20 μm) of ion microprobe $\delta^{13}C$ and $\delta^{18}O$ 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 $\delta^{18}O$ and $\delta^{13}C$ and created time series for both isotope ratios over the life of two adult bluegill (*Lepomis macrochirus*). A whole-lake ^{13}C 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 $\delta^{13}C$ 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 $^{133}Cs^+$ set at 0.6 nA for ~4 min for $\delta^{13}C$ and at 2.5 nA for ~3.5 min for $\delta^{18}O$ spots. Ions were extracted with 10 kV and selected using a 40 eV energy window. Two Faraday cups were used to simultaneously measure ^{18}O and ^{16}O, and an electron multiplier and a Faraday cup were used for ^{13}C and ^{12}C, respectively. Charge compensation was aided by a normal-incidence electron gun. Otolith analyses were bracketed with standard analyses to correct for instrumental bias and are reported in per mil (‰) notation relative to Standard Mean Ocean Water for $\delta^{18}O$ and Pee Dee Belemnite for $\delta^{13}C$. Parallel sampling traverses for $\delta^{13}C$ and $\delta^{18}O$ 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.

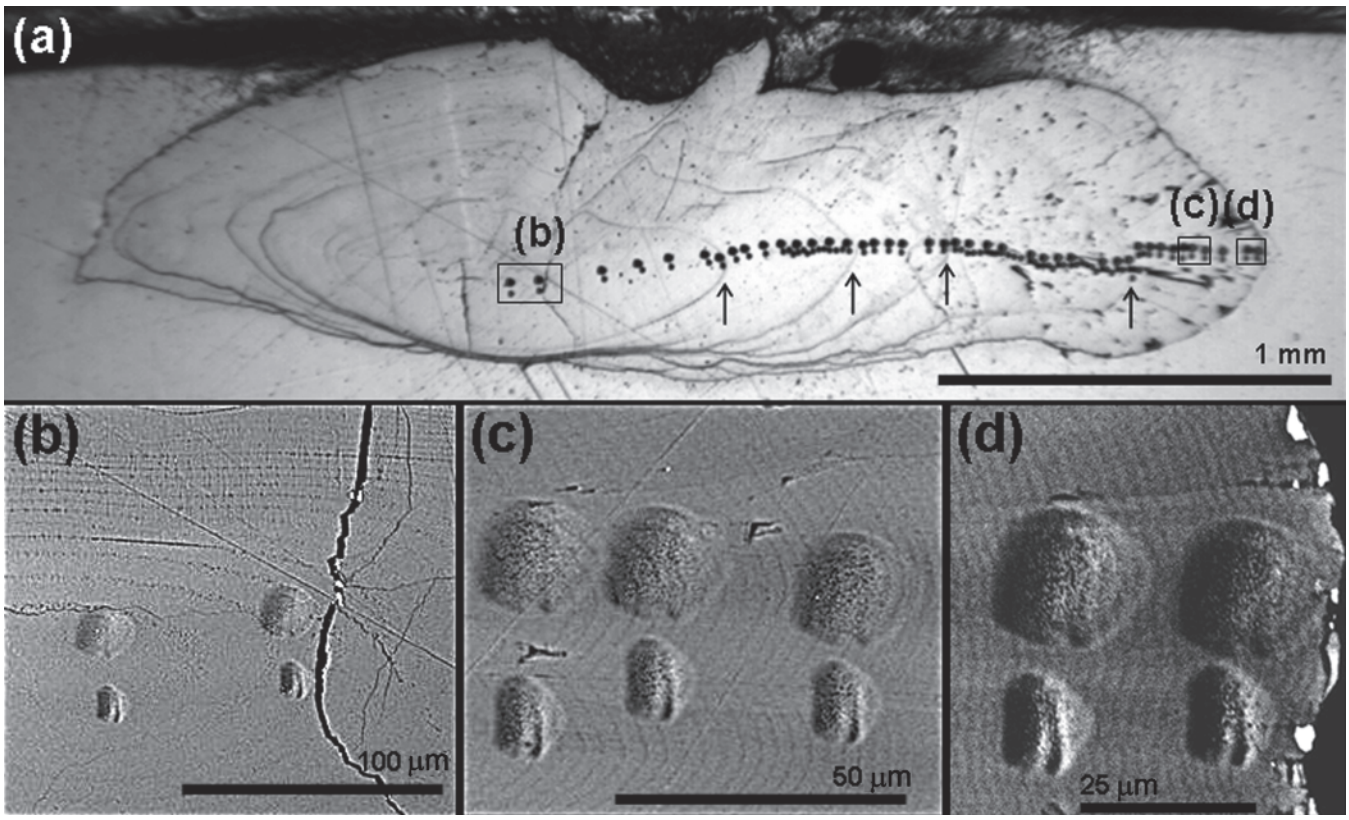
To estimate the contribution of different sources to otolith carbon, we used a steady-state mixing model to predict otolith $\delta^{13}C$ based on daily time series of lake water $\delta^{13}C_{DIC}$ and bluegill $\delta^{13}C_{Diet}$ over the course of the isotope addition in 2005. To assign calendar dates to the daily increment counts and $\delta^{13}C_{Otolith}$ series, we assumed the ~±2‰ changes in $\delta^{13}C_{Otolith}$ between analysis pits represented the start and end of the ^{13}C addition (Fig. 1). The daily $\delta^{13}C_{DIC}$ 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 $\delta^{13}C_{Diet}$ was a weighted average of a diet item's weekly measured $\delta^{13}C$ and its proportion in the diets. Diet ^{13}C data were linearly interpolated to correspond to $\delta^{13}C_{Otolith}$ dates. The mixing model predicted the proportion of otolith carbon derived from the diet and water for each day in the $\delta^{13}C_{Otolith}$ 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 $\delta^{18}O$ 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 $\delta^{13}C$ and $\delta^{18}O$, 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. 2*b* and 2*c*). 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 $\delta^{13}C$ and $\delta^{18}O$, respectively (Fig. 2*d*).

Spot-to-spot reproducibility (1 SD) of the UWC-1 calcite standard was <0.2‰ for $\delta^{18}O$ and <0.4‰ for $\delta^{13}C$. Micromilling for otoliths isotope analysis has been reported to attain similar precision and temporal resolution, but only from exceptionally large otoliths (Wurster and Patterson

Fig. 1. (*a*) Reflected light image of a transverse-sectioned saggitae otolith after ion microprobe analysis from a 4+-year-old bluegill (BLG-3), *Lepomis macrochirus*, collected from Crampton Lake, Wisconsin, in September 2006. The surface is coated with gold thin film, and analysis pits appear as uniform dark spots. $\delta^{18}O$ pits are along the top of the sampling traverse and are slightly larger (15 μm) than $\delta^{13}C$ pits (10 μm). Arrows indicate interpretations of annual growth bands. (*b*) Scanning electron microscope (SEM) image (backscattered electron mode) of $\delta^{18}O$ and $\delta^{13}C$ analysis pits at the otolith origin where daily growth increment widths are 10–40 μm. (*c* and *d*) SEM images from two points within the last year of growth illustrate how daily increment width, an indicator of fish growth rate, influences the number of days incorporated in an analysis.



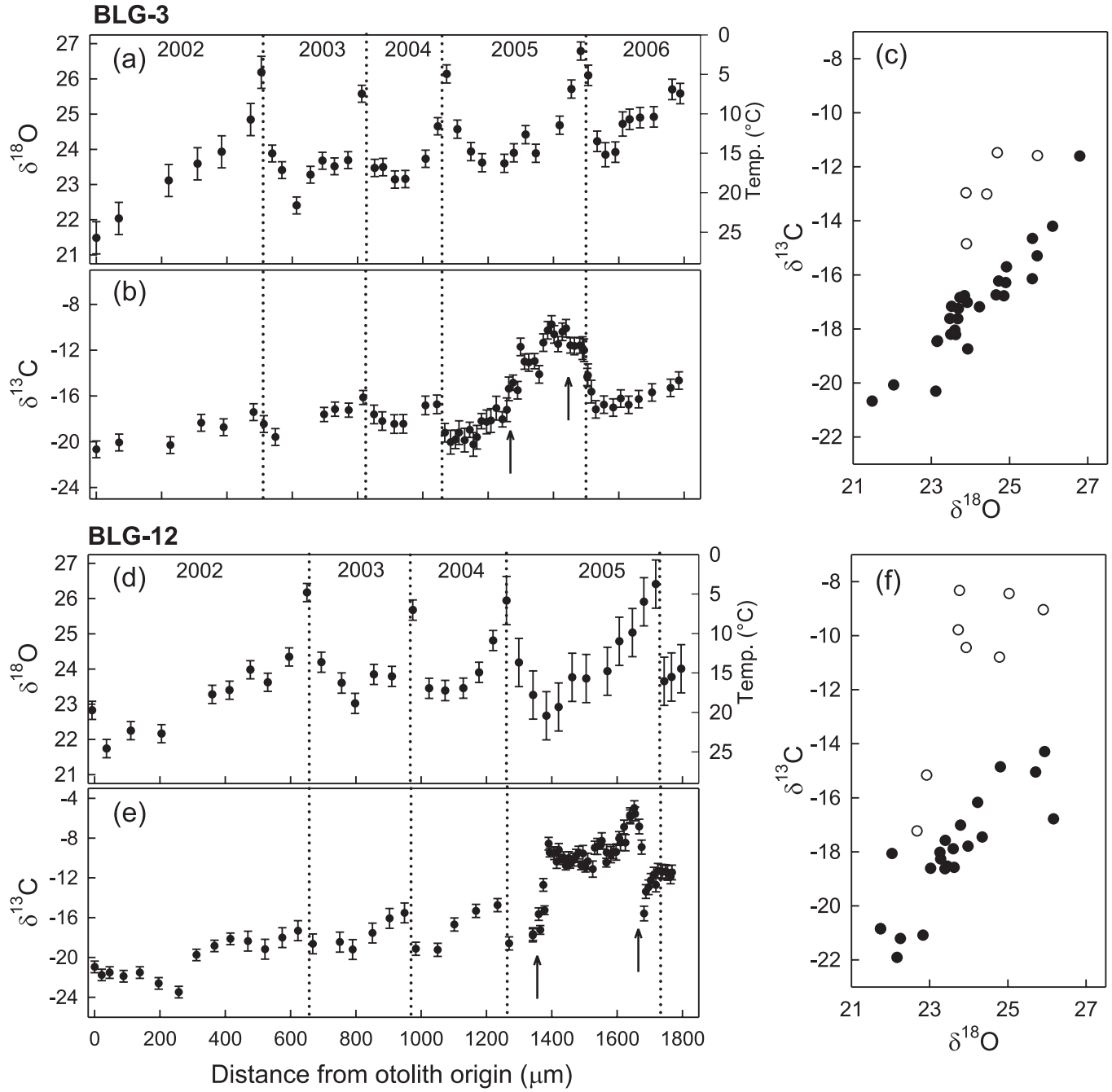
2003; Wurster et al. 2005). Furthermore, micro-milling requires 20–40 μg of $CaCO_3$ per analysis and may include otolith material from layers beneath the surface of the sample, but the ion microprobe requires less than 500 pg of $CaCO_3$ per analysis and the shallow pit depth (<2 μm) avoids inclusion 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 $\delta^{18}O$ 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 $\delta^{18}O$ value of an otolith and thus the precision of our $\delta^{18}O$ measurements (SD = 0.22‰) are sufficient to resolve fish temperature changes of ~1 °C (Thorrold et al. 1997). Using the lake water $\delta^{18}O$, 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 $\delta^{18}O$, it was not possible to accurately estimate fish temperature across the growing season; however, values of $\delta^{18}O$ change slowly in lake water and short-term temperature changes resolved by the 15 μm ion microprobe beam are meaningful. In both fish, $\delta^{18}O$ 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 ^{13}C addition quickly elevated and maintained the lake's mixed-layer $\delta^{13}C_{DIC}$ 12‰–30‰ over the 56 days (Pace et al. 2007), whereas the average 4-year-old bluegill $\delta^{13}C_{Diet}$ 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 $\delta^{13}C_{Otolith}$ corresponded well to the 56-day experiment and provided a way to align

Fig. 2. (*a* and *b*) Time series of $\delta^{13}C$ and $\delta^{18}O$ 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 $\delta^{18}O$ plot is based on a constant lake water $\delta^{18}O$ and calculated from formulas in Thorrold et al. (1997). The temperature axis provides a relative scale for the $\delta^{18}O$ measurements as variations in lake water $\delta^{18}O$ over time would influence the predicted temperature calculations. (*b*) Otolith $\delta^{13}C$ measurements from 2005 were used in the mixing model, and the arrows indicate the interpreted start and end of the ^{13}C addition experiment (start at 1270 μm, end at 1430 μm). (*c*) Relationship between $\delta^{18}O$ and $\delta^{13}C$ measured over the entire otolith transect. Open circles represent paired data during the ^{13}C addition; solid circles represent values before and after ^{13}C 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*).



the otolith and carbon source time series (~1360 and 1650 μm; Fig. 2*e*). Analysis pits used to generate otolith data for the model spanned 1–4 and 2–7 daily increments for $\delta^{13}C$ and $\delta^{18}O$, 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-

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 $\delta^{13}C_{Diet}$ but varied 3‰–4‰ between individuals by the end of the experiment and may have contributed to individual differences in estimated *M* values. Alternatively, because $\delta^{13}C_{DIC}$ is influenced by primary production and CO_2 exchange with the atmosphere, $\delta^{13}C_{DIC}$ 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. 2*b* and 2*e*) 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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