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High-resolution isotope analysis of young alewife *Alosa pseudoharengus* otoliths: assessment of temporal resolution and reconstruction of habitat occupancy and thermal history

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Otoliths of age 0 year alewife *Alosa pseudoharengus* collected in different Lake Michigan habitats were microsampled, and carbon and oxygen isotope ratios ($\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$) of resulting microsamples were quantified. To assess the temporal resolution of the method, age and otolith growth rates were also estimated by counting otolith daily growth increments. Core and outer intra-otolith samples averaged 36 and 23 days, respectively. Because of the accretionary nature of otolith growth, a habitat switch by a larva occurring between 0 and 18 days post-hatch may not be recognized by this approach. Taking this temporal resolution into account, *A. pseudoharengus* habitat occupancy and thermal history in nearshore Lake Michigan, and a connecting drowned river-mouth lake were documented. Comparisons between $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ profiles, and isotope values of Lake Michigan habitats suggested that movements by individual fish between a nearshore area of Lake Michigan proper and drowned river-mouth lake habitats were rare. Some individuals evidently moved between habitats, and such movements occurred during different periods of ontogeny. Thermal reconstructions, based on $\delta^{18}O_{otolith}$ values suggested that during early life (*e.g.* first month of life) young *A. pseudoharengus* appeared to inhabit microhabitats with temperatures greater than mean epilimnetic temperatures. This study demonstrates not only the utility of intra-otolith geochemical analysis to describe the complexity of fish behaviour in fresh water but also identifies limitations of the present approach.

Key words: habitat; juvenile; otolith; stable isotopes; temperature.

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INTRODUCTION

Habitat occupancy and environmental experiences during early life have important implications for understanding recruitment dynamics of fish populations. Given that the vast majority of fishes do not survive through early life, it is particularly informative to elucidate the early life experiences of those few individuals that survive early ontogeny. By gaining a better understanding of early life habitat and thermal conditions experienced by these rare survivors, managers can target actions towards critical habitats, and researchers can develop studies (*e.g.* bioenergetics-based analyses) with appropriate appreciation of early life thermal conditions.

The invasive alewife *Alosa pseudoharengus* (Wilson) is one of the most important planktivorous fishes in the Laurentian Great Lakes, providing forage for economically valuable stocks of salmonines in Lake Michigan (Jude & Tesar, 1985; Madenjian *et al.*, 2002, 2005). In their native range, *A. pseudoharengus* are anadromous, but in Lake Michigan they use a variety of spawning and nursery habitats, including nearshore Lake Michigan, drowned river-mouth lakes, bays and tributaries (Goodyear *et al.*, 1982). These habitat types are characterized by distinct physical and biological environments, and appear to confer differential vital rates and recruitment success onto young *A. pseudoharengus* (Höök *et al.*, 2007). A more thorough understanding of how young fish use these different habitats can help elucidate recruitment processes and thereby improve management of Lake Michigan fish stocks. Given the (1) small size (Auer, 1982), (2) susceptibility to dispersive processes (Höök *et al.*, 2006) and (3) high mortality rates (Mansfield & Jude, 1986; Höök *et al.*, 2007) of young *A. pseudoharengus*, however, conventional approaches for assessing habitat utilization (*e.g.* mark7–recapture studies) are difficult and alternative methods should be developed.

Measurement of oxygen and carbon stable-isotope ratios of otoliths ($\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$) is an alternative method for assessing habitat utilization by fishes. $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ signatures are permanently imprinted in otolith carbonate, recording ambient conditions experienced by individual fishes throughout life (Campana, 1999). An otolith's oxygen isotope ratio is a function of temperature and the isotope ratio ($\delta^{18}O_w$) of ambient water (Patterson *et al.*, 1993; Thorrold *et al.*, 1997). In contrast, carbon isotopes are not deposited onto otoliths in equilibrium with dissolved inorganic carbon (DIC; $\delta^{13}C_{DIC}$) of ambient water because of a considerable contribution of metabolic carbon (Kalish, 1991; Patterson, 1998; Dufour *et al.*, 2007). Nonetheless, $\delta^{13}C_{otolith}$ values may reflect geographic differences in DIC (Thorrold *et al.*, 1997; Patterson, 1999; Solomon *et al.*, 2006). The accretionary nature of otoliths, exemplified by daily and annual microstructural increments (Campana & Nielson, 1985), and advances in mass spectrometry and micro-sampling techniques (Wurster *et al.*, 1999) enable the recovery of high-resolution isotope profiles, representing time-specific indices of environmental conditions experienced by individual fishes throughout life (Wurster *et al.*, 2005; Dufour & Gerdeaux, 2007). High-resolution intra-otolith isotope profiles have primarily been generated for adult fish species with large otoliths, but this technique also appears promising for small otoliths (Dufour *et al.*, 2005). The present study builds on Dufour *et al.*'s (2005) analysis of

$\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values of young *A. pseudoharengus* otolith cores (*i.e.* the central portion of otoliths where constituent elements were deposited during very early life), to quantify entire intra-otolith isotope profiles (*i.e.* measure isotope ratios for distinct portions of individual otoliths) from young fish captured during the autumn 2002 in different habitat types of Lake Michigan. Objectives were to determine: (1) the frequency of movement among habitat types and (2) thermal experiences of individual *A. pseudoharengus* within specific habitats. It was hypothesized: (1) that movements among habitats are rare during early life and (2) that young fish on average occupy temperatures roughly equivalent to mean epilimnetic temperatures. In addition, temporal resolution of reconstructed life-traits was examined in light of technical and biological constraints inherent to the micro-milling and isotope analysis of small otoliths. This examination highlighted limitations in the fine-scale reconstruction of early life history based on micro-milling of small otoliths.

MATERIALS AND METHODS

FISH COLLECTION

During September to November 2002, age 0 year *A. pseudoharengus* were captured with bottom and midwater trawls in Muskegon Lake (a drowned river-mouth lake located along the eastern shoreline of Lake Michigan), the adjacent nearshore area of Lake Michigan and Muskegon Channel (the connecting outflow from Muskegon Lake to Lake Michigan; Höök *et al.*, 2007; Fig. 1). Additional age 0 year fish from Lake Michigan were collected near Sturgeon Bay, WI, U.S.A., during October 2002 in the course of the U.S. Geological Survey's Great Lakes Science Center's annual autumn bottom-trawl survey. Upon capture, fish were immediately frozen for transport to the laboratory, where total lengths (L_T) and wet masses (M_W) were measured. Sagittal otoliths were removed and individually stored dry at room temperature for subsequent analysis. Individual otoliths were either used for ageing or isotope profiling. Thus, for some individual fish ($n = 76$) only age was estimated, for others ($n = 131$) only isotope profiles were quantified, and for a sub-set ($n = 36$) both age and isotope profile were quantified (*i.e.* from an otolith pair, one otolith for ageing and the other for isotope profiling).

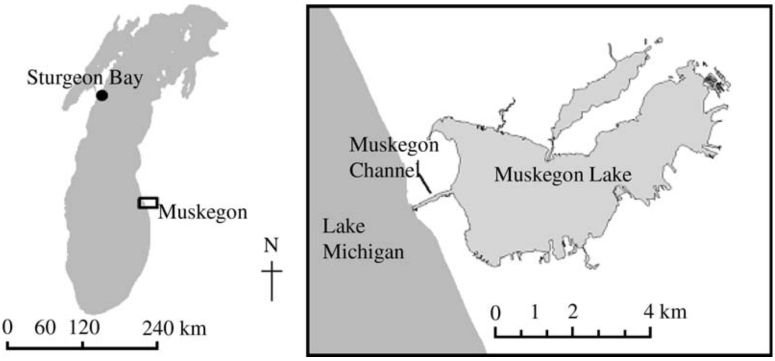

FIG. 1. Map showing the four age 0 year *Alosa pseudoharengus* collection locations in Lake Michigan during summer and autumn of 2002.

DETERMINING AGE AND INTRA-OTOLITH GROWTH RATE

Seventy-six, whole otoliths were individually embedded in epoxy and then sanded and polished until daily growth increments, and the otolith core were revealed. Ages (days from hatch to capture) were estimated by counting daily growth increments. Each otolith count was conducted at least twice by two separate researchers, and the mean count was then assigned. If counts did not agree within five increments, counts were repeated a third time and the median count was assigned. Daily otolith growth increments have previously been used as proxies for larval *A. pseudoharengus* ages, and larvae appear to begin depositing daily growth increments on their second day of life (Essig & Cole, 1986; Höök *et al.*, 2007; D. Jude, pers. comm.). Thus, to assign ages (in days) to individual age 0 year *A. pseudoharengus*, two was added to the number of growth increments counted. Estimated age at collection ranged from 53 to 127 days, while L_T ranged from 44·0 to 99·0 mm and M_W from 0·55 to 7·08 g (Table I). There was no relationship between fish size and time of capture, but size and estimated hatch date were negatively correlated ($n = 73$; $r^2 = 0{\cdot}5$; $P < 0{\cdot}05$). *Alosa pseudoharengus* captured in Lake Michigan were generally estimated to have hatched in mid to late July, while those captured in Muskegon Lake generally appeared to have hatched in early July.

For a sub-set of individuals ($n = 14$), otolith daily growth rates (*i.e.* change in length of otolith radius per day) were also estimated by counting the number of daily growth increments between the otolith core and points located at fixed distances (0·1, 0·2, 0·3 and 0·4 mm) from the otolith core. Estimates of intra-otolith growth rates suggested that, while individual otoliths grew at slightly different rates during larval and juvenile stages, these ontogenetic differences in otolith growth rates were minor and, growth closely approximated a linear function ($y = 0{\cdot}91\ x + 0{\cdot}31$; $r^2 = 0{\cdot}99$, where y is the otolith radius and x is the otolith increment number).

OTOLITH MICRO-MILLING AND TEMPORAL RESOLUTION

The computer-controlled apparatus described by Wurster *et al.* (1999) was used for micro-milling 131 otoliths. Otoliths were embedded in resin blocks for micro-milling and were cleaned in deionized water, dried, polished to reveal growth increments and glued on to glass slides. Specimens were attached to a three-dimensional micropositioning stage under a fixed high-precision dental drill. For each specimen, the centre (*i.e.* innermost point of the core; Path_c) and the outer edge (Path_e) were digitized in real-time as two series of three-dimensional co-ordinates and interpolated using a cubic spline [Fig. 2(a)]. Subsequently, an array of five to nine intermediate sampling paths (IPs) was calculated between Path_c and Path_e. The innermost part of the otolith is circular, while the edge has a more complex shape. The interpolation of otolith shape between the core and edge did not account for intra-otolith variation in shape caused by ontogentic and environmental factors [Fig. 2(a)]. Sampling path arrays served to guide three high-precision actuators, that move the sample stage relative to the fixed dental drill, permitting micro-drilling of otoliths contiguously from centre to edge at

TABLE I. Total length (L_T), wet mass (M_W) and age of age 0 year *Alosa pseudoharengus* captured during 2002 in Muskegon Lake (ML) and two areas of Lake Michigan: near Sturgeon Bay (LM–SB) and near Muskegon (LM–M) (see Fig. 1)

Origin	LM–SB	LM–M	ML
Number	23	25	29
L_T (mm) range	53–99	44–84	77–95
M_W (g) range	0·65–7·08	0·55–4·18	3·45–6·21
Age (days) range	53–100	66–99	70–127
Mean ± S.E.	82 ± 3	80 ± 2	101 ± 3

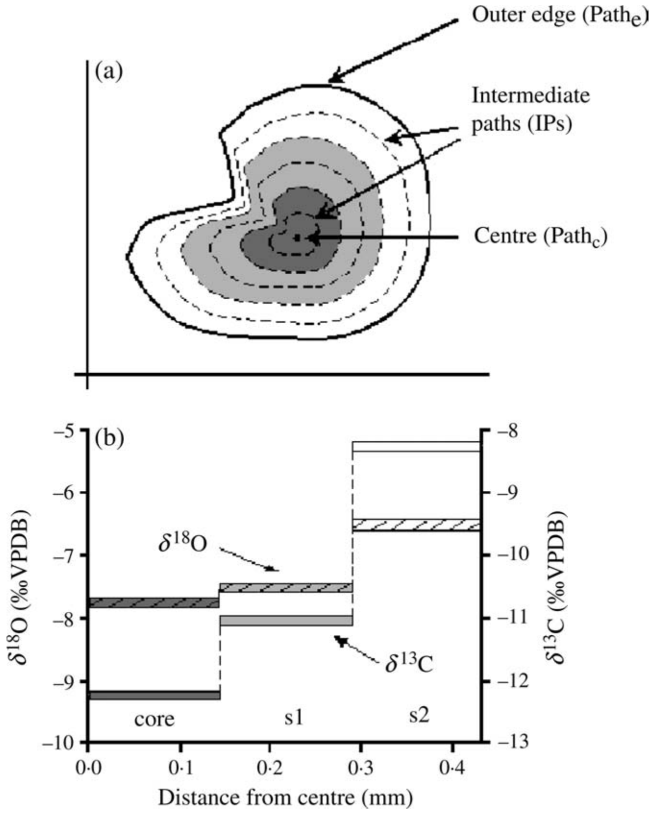

FIG. 2. (a) Micro-sampling of an age 0 year *Alosa pseudoharengus* otolith from Lake Michigan using a robotic micro-milling apparatus. The innermost part (Path$_c$) and the outer edge (Path$_e$) are digitized as two series of three-dimensional co-ordinates and interpolated using a cubic spline. Five intermediate sampling paths (IP; ----) are then calculated. The core intra-otolith sample (■) represents the early life of the fish, and is obtained by combining the powder collected during the milling of Path$_c$ with that of two following IPs. The two subsequent intra-otolith samples ▩, □) each comprise a combination of two IPs. (b) Intra-otolith variation in δ^{18}O [δ^{18}O$_{otolith}$, ‰ Vienna Pee Dee Belemnite (VPDB); ▨, ▨ and ▨] and δ^{13}C values (δ^{13}C$_{otolith}$, ‰ VPDB; ▨, ▨ and ▭) is measured along the otolith growth axis from the centre (core) at the left to the outer edge at the right.

depths ranging from 40 to 45 μm. In most cases, the powder mass resulting from one IP was insufficient for an isotope assay. As a consequence, two to five successive IPs were combined to provide the critical carbonate mass for a single isotope analysis, and two to seven intra-otolith samples were generated per otolith [*i.e.* a complete individual isotope profile consisted of two to seven measurements; Fig. 2(b)]. Henceforth, the inner most intra-otolith sample is referred to as the 'core' sample and subsequent intra-otolith samples as 'intra-otolith sample one', 'intra-otolith sample two' and so on [Fig. 2(a)]. Because of this micro-sampling procedure, the core sample of an individual fish does not specifically correspond to the larval stage, and the number of days represented by each intra-otolith sample can differ. For example, for the specimen from Lake Michigan presented in Fig. 2, two inner IPs were combined to constitute the core, two IPs were combined to form 'intra-otolith sample one', and the last two IPs were combined to form 'intra-otolith sample two' (thus, this individual's otolith profile consisted of three measurements).

Temporal resolution (*i.e.* the number of days of life corresponding to different portions of the otolith profile) was assessed by calculating the proportion of an otolith's radius represented by the radius of each intra-otolith sample. To calculate ages, the number of IPs that were combined to form a single intra-otolith sample was taken into account, as well as individual age and linear otolith growth rate.

ISOTOPE ANALYSIS OF OTOLITHS

Otolith sub-samples were stored in stainless steel cups and roasted *in vacuo* for 1 h at 200° C to remove volatiles. Isotope ratios of resulting intra-otolith samples were determined using a Finnigan MAT 253 directly coupled to a kiel-III automated carbonate preparation device (Thermo–Fisher Scientific, Waltham, MA, U.S.A.). The mass spectrometer was optimized to process CO_2 from a small (10–15 μg carbonate) amount of otolith material. The mass of intra-otolith samples was determined by comparing the amount of CO_2 generated after reacting with phosphoric acid to that of precisely weighed NBS-19 standard samples. All measurements are reported in the standard delta notation (per mil) relative to the Vienna Pee Dee Belemnite (VPDB) standard. Accuracy and precision were checked by routine analysis of a NBS-19 standard and were determined to be ±0·1‰ for both $\delta^{13}C$ and $\delta^{18}O$ values. A total of 347 intra-otolith samples were successfully analysed by mass spectrometry (Table II; note that $\delta^{13}C$ and $\delta^{18}O$ values of core samples were also presented by Dufour *et al.*, 2005). Some assays were unsuccessful due to static induced loss of powder during the collection process.

To compare otolith isotopic measurements among habitats, data were grouped by the four habitats of capture (Muskegon Lake, Muskegon Channel, Lake Michigan near Muskegon and Lake Michigan near Sturgeon Bay). Because at least one of the data sets was not normally distributed (Lilliefors test), and sample sizes were not equal, non-parametric tests were used. Kruskal–Wallis tests were used to compare $\delta^{13}C$ and $\delta^{18}O$ values among core samples ($n = 113$) and among non-core samples ($n = 234$); *i.e.* four tests total ($\alpha = 0{\cdot}05$). If isotope values differed significantly among habitats, Mann–Whitney *U*-tests were applied for pair-wise comparisons.

HABITAT OCCUPANCY AND THERMAL EXPERIENCE

Assigning a spatial origin to otolith growth (and thus habitat occupancy) is dependant on consistent differences in isotope ratios among habitats. Dufour *et al.* (2005) measured consistent mean ± S.D. $\delta^{18}O_w$ values of −8·6 ± 0·2‰ for water collected in

TABLE II. Number of micro-milled age 0 year *Alosa pseudoharengus* otoliths, generated intra-otolith sample, successful mass spectrometry assays and ranges of variation of $\delta^{18}O_{otolith}$, $\delta^{13}C_{otolith}$ (‰, VPDB) and reconstructed temperature. Fish samples originated from Lake Michigan at Sturgeon Bay (LM–SB), Lake Michigan near Muskegon (LM–M), Muskegon Lake (ML) and Muskegon Channel (MC) (see Fig. 1)

Origin	LM–SB	LM–M	ML	MC
Otoliths (*n*)	41	43	37	10
Micro-milled samples (*n*)	110	107	145	42
Successful isotopic assays (*n*)	97	88	125	38
$\delta^{18}O_{otolith}$ (‰ VPDB) range	−8·3 to −4·2	−8·4 to −5·7	−11·2 to −8·1	−11·2 to −7·9
$\delta^{13}C_{otolith}$ (‰ VPDB) range	−13·4 to −3·1	−13·3 to −7·2	−20·2 to −13·1	−19·8 to −11·4
Temperature (° C) range	16·7 to 26·5	16·7 to 28·5	15·0 to 29·8	–

VPDB, Vienna Pee Dee Belemnite.

Muskegon Lake, and −6·0 ± 0·2 and −6·3‰ for water in nearshore Lake Michigan near Muskegon and Sturgeon Bay, respectively. Comparing environmental and biological isotope values, and assigning a geographic origin necessitates that measured $\delta^{18}O_w$ values are transformed into potential otolith aragonite $\delta^{18}O_{otolith}$ values. For this purpose, an otolith-specific, temperature–fractionation relationship was used:

$$10^3 \ln\alpha = 18{\cdot}56(10^3 T^{-1})^{-33{\cdot}49}, \tag{1}$$

where α is the fractionation factor between water and otolith aragonite, and *T* is temperature in Kelvin (Patterson *et al.*, 1993). The relationship between α and $\delta^{18}O_{otolith}$ is:

$$\alpha = (\delta^{18}O_{otolith} + 10^3)\,(\delta^{18}O_w + 10^3)^{-1}. \tag{2}$$

Equation (1) was specifically developed for freshwater fishes in natural systems. Similar equations have been developed from fishes growing in natural and laboratory environments (Kalish, 1991; Patterson *et al.*, 1993; Thorrold *et al.*, 1997; Høie *et al.*, 2004). Temperature reconstructions based on these equations are rather precise (<1° C; Høie *et al.*, 2004), and slopes are either statistically indistinguishable or similar to that reported by Kim *et al.* (2007) for inorganic aragonite. Equation intercepts, however, can differ; for instance, temperatures calculated based on an equation presented by Thorrold *et al.* (1997) are *c.* 4° C higher than those calculated by other equations. This can lead to unrealistic temperature reconstructions for freshwater (Wurster *et al.*, 2005) and estuarine (Surge & Walker, 2005) fishes, and Equation (1) was thus preferred for this study.

While, there is clearly some variation in $\delta^{18}O_w$ values within each habitat, Dufour *et al.* (2005) demonstrated that such variation was low. Thus, to assign habitat occupancy and reconstruct thermal histories, mean habitat-specific $\delta^{18}O_w$ values were used.

A very wide potential range of growing season temperatures was assumed for juvenile *A. pseudoharengus* (from +14 to +30° C) based on environmental data and thermal tolerance of *A. pseudoharengus* (Edsall, 1970; Höök *et al.*, 2007). Using this thermal range, an array of predicted $\delta^{18}O_{otolith}$ values was calculated for each habitat: from −11·3 to −7·9‰ for Muskegon Lake, from −8·7 to −5·3‰ and from −9·0 to −6·0‰ for nearshore Lake Michigan near Muskegon and Sturgeon Bay, respectively. Thus, there is a slight overlap between the potential signatures of the different habitats.

The $\delta^{13}C_{DIC}$ values of the various habitats were not measured, but differences were predicted among Lake Michigan habitats. There is a large difference in size between Lake Michigan and drowned river-mouth lakes (*e.g.* Muskegon Lake), and a positive relationship between lake size and $\delta^{13}C$ values is expected, as reflected in planktivorous fish tissues (Dufour, 1999; Perga & Gerdeaux, 2004). A difference as large as *c.* 7‰ is suspected based on measurements of *A. pseudoharengus* otoliths (Dufour *et al.*, 2005). Because carbon isotopes in otoliths do not precipitate in equilibrium with DIC (Kalish, 1991), there is no simple relationship between $\delta^{13}C_{DIC}$ and $\delta^{13}C_{otolith}$ values. It is assumed that relatively high $\delta^{13}C_{otolith}$ values would reflect growth in Lake Michigan (higher DIC value at the base of the food chain), while relatively low $\delta^{13}C_{otolith}$ values would reflect growth in smaller habitats such as Muskegon Lake (lower DIC value).

Once geographic origin is assigned, thermal history of individual fish can be reconstructed. Ambient temperatures experienced by *A. pseudoharengus* were estimated using equation (1) and mean measured $\delta^{18}O_w$ values for the different habitats. Reconstructed temperatures were then compared to mean ± S.D. epilimnetic temperatures monitored from May to November using a Sea-bird CTD (conductivity; temperature and depth; Sea-Bird Electronic Inc., Bellevue, WA, U.S.A.) profiler in the nearshore area of Lake Michigan near Muskegon Lake and in Muskegon Lake (Höök, 2005). Thermal conditions were not reconstructed for otoliths from Muskegon Channel because no $\delta^{18}O_w$

values were measured for this habitat and because this short channel (<2 km) probably serves primarily as a migratory habitat.

RESULTS

In total, stable-isotope values for 347 intra-otolith samples from 131 individual fish were successfully determined. There was no correlation between the number of intra-otolith samples and fish size. Temporal resolutions of core and non-core intra-otolith samples were 36 ± 1 (mean ± S.E.) (range 26–49; $n = 36$) and 23 ± 1 (mean ± S.E.) days (range 11–48; $n = 73$), respectively (Table III). Variability in temporal resolution within the two categories of intra-otolith samples (core and other intra-otolith samples) was primarily attributable to the technical constraints encountered during micro-milling.

HABITAT OCCUPANCY

The $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values ranged from −11·2 to −4·2‰ and from −20·2 to −3·1‰, respectively (Table II and Figs 3 and 4). Regardless of habitat of capture, most specimens displayed isotope profiles with a pattern of increasing $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values with ontogeny (Figs 3 and 4). Nonetheless, both core and non-core $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values differed among fish habitats (Kruskal–Wallis; d.f. = 3; for all tests, $P < 0{\cdot}001$). Specifically, core and non-core $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values of fish captured in Muskegon Lake and Muskegon Channel were significantly different (lower) than $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values of fish captured in Lake Michigan [Fig. 3 (a), (b); Mann–Whitney U-test; for all tests $P < 0{\cdot}001$]. There was no difference in core and non-core $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values between Muskegon Lake and Muskegon Channel fish groups (Mann–Whitney U-test, for all tests $P > 0{\cdot}05$) or between the two Lake Michigan fish groups (Mann–Whitney U-test; for all tests $P > 0{\cdot}05$). The range of variation in $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ within a habitat was consistent with ranges of predicted $\delta^{18}O_{otolith}$ and $\delta^{13}C_{DIC}$ values for Muskegon Lake and Lake Michigan [Fig. 3 (c), (d)].

TABLE III. Temporal resolution of core intra-otolith samples and outer intra-otolith samples generated by robotic micro-sampling. *Alosa pseudoharengus* samples originated from Lake Michigan at Sturgeon Bay (LM–SB), Lake Michigan near Muskegon (LM–M) and Muskegon Lake (ML) (see Fig. 1)

Origin	LM–SB	LM–M	ML
Otoliths			
n	12	9	12
Number of days represented by core intra-otolith samples			
Range	26–47	26–49	26–47
Mean ± S.E.	36 ± 2	35 ± 2	36 ± 2
Number of days represented by outer intra-otolith samples			
Range	14–42	13–42	11–48
Mean ± S.E.	23 ± 2	23 ± 4	23 ± 2

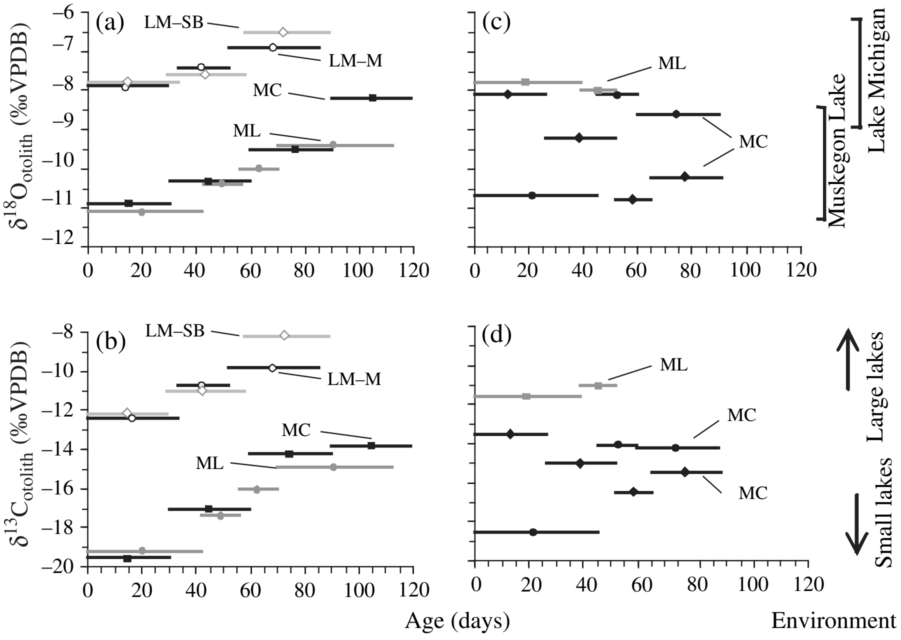

FIG. 3. Examples of (a), (c) $\delta^{18}O_{otolith}$ and (b), (d) $\delta^{13}C_{otolith}$ (VPDB, Vienna Pee Dee Belemnite) profiles (horizontal bar) of age-0 year *Alosa pseudoharengus* captured in Lake Michigan [near Muskegon Lake (LM–M) and off Sturgeon Bay (LM–SB)], Muskegon Channel (MC) and Muskegon Lake (ML) in 2002. (a), (c) The vertical bars on the second *y*-axis represent the potential ranges of $\delta^{18}O_{otolith}$ (‰, VPDB) calculated using measured $\delta^{18}O$ values of water ($\delta^{18}O_w$, ‰ VPDB) (Dufour *et al.*, 2005) and a temperature–aragonite fractionation equation established for freshwater fishes (Patterson *et al.*, 1993). (b), (d) The vertical arrows on the second *y*-axis represent trends in $\delta^{13}C$ values of the habitats (a), (b). Most of *A. pseudoharengus* display profiles with increasing $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values during ontogeny whereas (c), (d) a few individuals do not display this typical pattern of variation (c, d).

Whereas most young-of-the-year (YOY) *A. pseudoharengus* appeared to occupy the same habitat during the majority of their life (*i.e.* habitat of capture), a few individuals displayed atypical isotope profiles and evidently switched habitats during ontogeny [Fig. 3 (c), (d)]. Three individuals captured in Muskegon Channel, and one (represented by an incomplete profile) captured in Muskegon Lake exhibited atypical ontogenetic trends in $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values. For instance, the Muskegon Lake individual's $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ core values were intermediate, suggesting a switch from Lake Michigan to Muskegon Lake during the first month of life. Similarly, the intermediate $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values of intra-otolith sample one from two Muskegon Channel individuals suggested a habitat switch later in life [Fig. 3 (c), (d)]. The third individual from Muskegon Channel may have moved from Muskegon Lake to Lake Michigan during its second month of life (intra-otolith sample one) and then moved back to Muskegon Lake (intra-otolith sample two) [Fig. 3 (c), (d)].

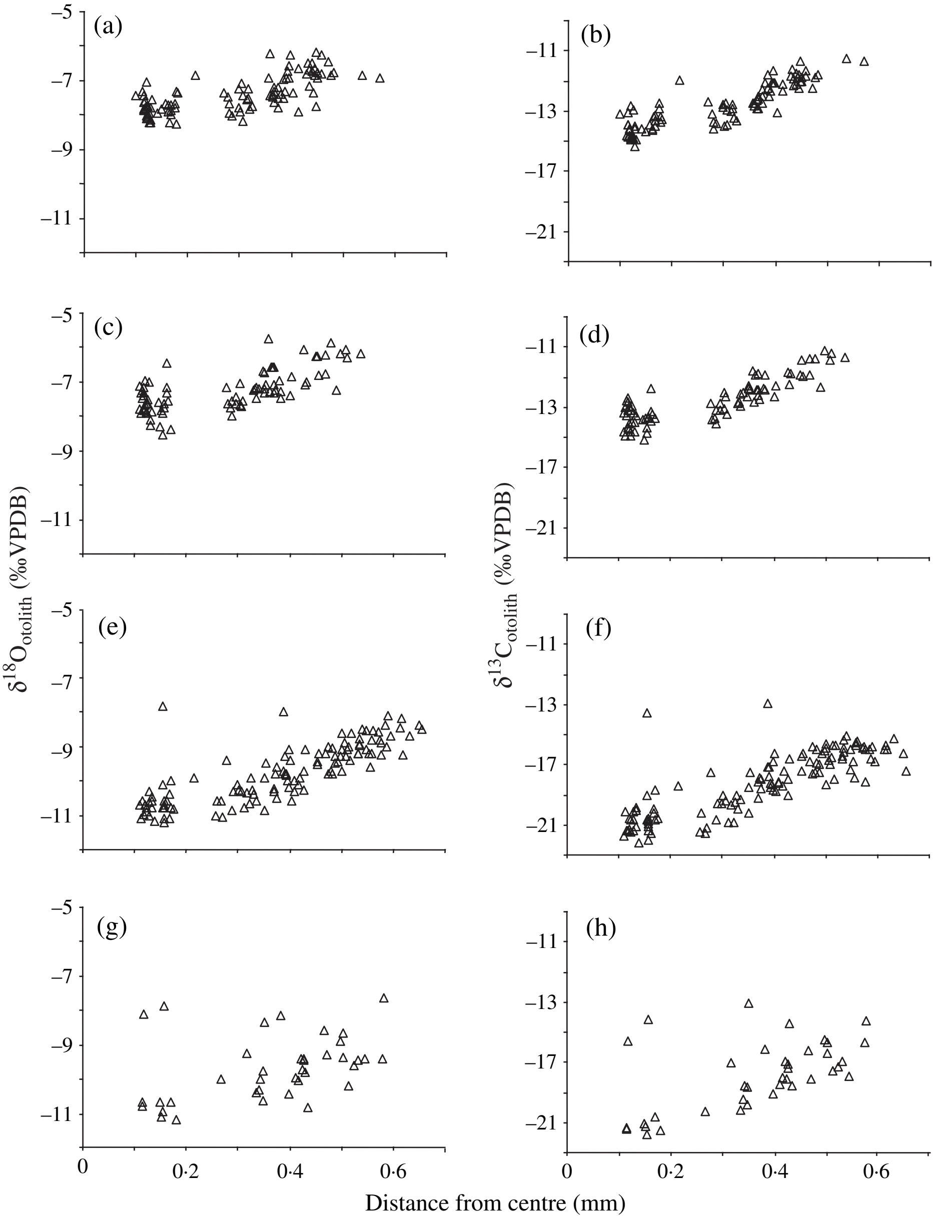

FIG. 4. Intra-otolith (a), (c), (e), (g) $\delta^{18}O_{otolith}$ (VPDB, Vienna Pee Dee Belemnite) and (b), (d), (f), (h) $\delta^{13}C_{otolith}$ values of age-0 year *Alosa pseudoharengus* captured during 2002 in Lake Michigan (a), (b) off Sturgeon Bay and (c), (d) near Muskegon in (e), (f) Muskegon Lake and in (g), (h) Muskegon Channel. The isotope value of each intra-otolith sample is plotted against mean radius length of the sample (*i.e.* the mean distance from the centre of the otolith to all material constituting the intra-otolith sample).

THERMAL EXPERIENCE

Reconstructed temperatures for all age 0 year fish identified as residents of their habitat of capture varied between 15·0 and 29·8° C for Muskegon Lake, and between 16·7 and 26·5° C and 16·7 and 28·5° C for Lake Michigan fish from Sturgeon Bay and Muskegon, respectively (Table II and Fig. 5). Reconstructed temperatures for the core intra-otolith samples were usually higher for fish from Muskegon Lake than from Lake Michigan (Fig. 5), consistent with higher mean epilimnetic temperatures in Muskegon Lake (Fig. 5). Figure 5

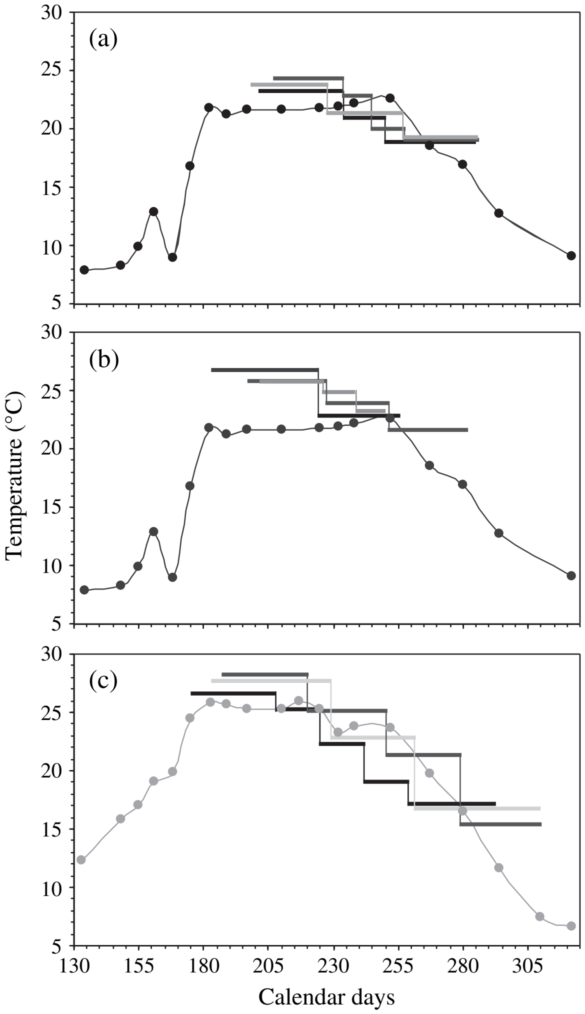

FIG. 5. Examples of intra-individual thermal history of age 0 year *Alosa pseudoharengus* captured in Lake Michigan (a) off Sturgeon Bay and (b) in the near shore area near Muskegon Lake and (c) in Muskegon Lake. For each habitat, reconstructed thermal histories of three individuals are shown. These temperature reconstructions are based on $\delta^{18}O$ values of otoliths ($\delta^{18}O_{otolith}$, ‰ Vienna Pee Dee Belemnite) and are compared to mean epilimnitic temperature for Lake Michigan near Muskegon Lake (–●–) and Muskegon Lake (–●–).

compares environmental temperature data for Muskegon Lake and Lake Michigan (near Muskegon Lake) with reconstructed fish life-history temperatures for selected aged *A. pseudoharengus* specimens in a common timeframe. Within each lake, reconstructed temperatures followed the seasonal trend of variation in environmental temperature (Fig. 5). Reconstructed temperatures, however, usually exceeded mean epilimnetic temperatures for the first portion of the profiles (*i.e.* the approximate first month of the fish life), then were similar to, or lower than mean epilimnetic temperatures during later life (Fig. 5).

DISCUSSION

Young *A. pseudoharengus* otoliths were micro-milled, and $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values measured to infer habitat occupancy and thermal history. These results suggest that movements between habitats were rare. Intra-otolith isotope profiles of most individuals revealed increasing isotope ratios over ontogeny. Ambient water temperatures decreased from summer (average hatch date of fish) to autumn (time of capture; Fig. 5), and $\delta^{18}O_{otolith}$ values were negatively related to temperature. Thus, increasing $\delta^{18}O_{otolith}$ values during ontogeny are expected for individuals remaining within a single habitat. Similarly, $\delta^{13}C_{otolith}$ values should also increase with age as mass-specific metabolic rates decrease with ontogeny and temperature (Gillooly *et al.*, 2001). Variation in $\delta^{13}C_{otolith}$ values is assumed to reflect the proportion of metabolic (characterized by relatively low $\delta^{13}C$ values) *v.* environmental DIC (characterized by relatively high $\delta^{13}C$ values) over the growing season (Patterson, 1998; Høie *et al.*, 2004; Dufour *et al.*, 2007). Accordingly, $\delta^{13}C_{otolith}$ values of age 0 year *A. pseudoharengus* generally increased over the growing season (Fig. 3) reflecting decreases in mass-dependant and temperature-dependant metabolic rates.

The $\delta^{18}O_{otolith}$ values in the last portion of profiles of many Muskegon Lake fish were similar to $\delta^{18}O_{otolith}$ values in the first portion of Lake Michigan fish profiles [Fig. 3(a)] and could not be unequivocally assigned to a habitat. While, this could result from either a change of habitat (and thus a change in ambient $\delta^{18}O_w$) or ambient temperature over the growing season, relatively low $\delta^{13}C_{otolith}$ values of the last portion of most Muskegon Lake fish profiles suggest that these fish remained in Muskegon Lake throughout life and that variation in $\delta^{18}O_{otolith}$ values reflects variation in temperatures experienced over the growing season. While, movements between habitats appear to be rare, a diversity of habitat experiences is documented. Movements occurred during different periods of ontogeny, and could be complex, *e.g.* one individual appeared to move several times over the first season of growth. Movements of small larvae are expected because they are susceptible to rapid transport by water currents throughout Lake Michigan (Höök *et al.*, 2006). As young *A. pseudoharengus* grow, their swimming ability increases and they are probably able to overcome such advective forces. Thus, $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ profiles suggest that some young fish actively move between habitats during later life.

Temperature is a primary determinant of fish metabolism and growth. While other techniques exist (*e.g.* temperature recorders placed on fishes), the otolith $\delta^{18}O_{otolith}$ based thermometer is one of the only techniques, which allows reconstruction of ambient temperatures experienced by fishes from birth to death. Thermal reconstructions suggested that during very early life, age 0 year *A. pseudoharengus* inhabit water masses where temperatures exceed that of habitat-specific mean epilimnetic temperatures. Warmer temperatures calculated from otolith isotope values suggest that very young fish occupy waters close to the surface or in shallow inshore areas. Such occupation of surface and inshore waters is consistent with results of previous studies. Nash & Geffen (1991) collected larval *A. pseudoharengus* in offshore waters of Lake Michigan and found highest densities in surface waters. Collections in nearshore waters indicate that larval densities in the Great Lakes are particularly high in very shallow habitats, and that yolk-sac larvae are particularly dense in such areas (Jude *et al.*, 1981; Leslie & Timmins, 1993). The occupation of such very warm habitats may confer a growth advantage upon young *A. pseudoharengus*. While, the optimal temperature for growth is dependant upon ration and respiration, Stewart & Binkowski (1986) suggested that food consumption by YOY *A. pseudoharengus* is maximum at 25° C. Their model, however, was based on observations of fish >100 mm, and consumption rates of larval *A. pseudoharengus* are probably maximum at higher temperatures. Further, while Klumb *et al.* (2003) found that larval *A. pseudoharengus* respiration rates increase with temperature, other studies (Kellogg, 1982; McCauley & Binkowski, 1982) indicate that larvae grow and survive at temperatures $>27°$ C. Other studies suggest that annual lake wide recruitment of *A. pseudoharengus* is positively related to spring and summer temperatures (O'Gorman *et al.*, 2004; Madenjian *et al.*, 2005), and fish that occupy warm, productive habitats during early life may have a recruitment advantage (Höök *et al.*, 2007).

It is important to consider the temporal resolution of these analyses. Core and subsequent intra-otolith samples on average represent 36 ± 1 and 23 ± 1 days (mean ± S.D.), respectively. This does not imply that intra-otolith isotope values equally reflect ambient conditions during the individual days represented by an intra-otolith sample. Due to the shape and accretionary nature of otoliths, as the radius increases, the volume of aragonite deposited onto the otolith's periphery each day also increases. As a consequence, the isotope value of an intra-otolith sample is likely to be dominated by later days. The software used for digitizing and micro-milling otoliths (Wurster *et al.*, 1999) enables the calculation of the relative mass contributions of each IP. Mass-balance calculations indicate that 75% of an *A. pseudoharengus* core mass is located in the outer half of the intra-otolith sample, and thus the later days represented by a core sample exert greater influence on the measurement. For non-core intra-otolith samples, the relative contribution of the mass of the outer half is less influential. Examination of the Lake Michigan specimens shown in Fig. 2(a), (b) reveals that the outer halves of intra-otolith samples 1 and 2 represent 64 and 59% of the mass of these intra-otolith samples, respectively.

The temporal resolution of the analyses and accretionary nature of otolith growth have consequences for reconstruction of *A. pseudoharengus* life history affecting how long an age 0 year fish must inhabit a particular habitat to develop a distinguishable chemical signature within its otolith. It should be noted that: (1) 75% of an *A. pseudoharengus* core intra-otolith sample mass is located in the outer half of the sample, (2) core intra-otolith samples on average reflect the first 36 days of *A. pseudoharengus* life and (3) Muskegon Lake and Lake Michigan exhibit a 3·3 and a 7‰ difference in $\delta^{18}O_w$ and $\delta^{13}C_{DIC}$ values, respectively. Assuming equal ambient temperatures in both habitats, a simple mass-balance equation suggests that a switch from Muskegon Lake to Lake Michigan occurring on day 18 of life (half of the time duration that is represented by a core intra-otolith sample) would produce a *c.* 2 and 5‰ increase of $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ core values, respectively. Core isotope values of such an early migrant would be very close to that of a Lake Michigan resident. There is natural variation in isotope profiles among individuals within a habitat, and variation in $\delta^{18}O_{otolith}$ induced by a switch in habitat occurring between days 0–18 may not be recognized.

An undetected phase spent in a habitat other than that where the fish was captured would produce erroneous thermal reconstructions due to use of incorrect $\delta^{18}O_w$ values in the paleothermometry equation (1). For example, early growth in Muskegon Lake by an individual identified as a Lake Michigan resident would cause a decrease in $\delta^{18}O_{otolith}$ values (because Muskegon Lake is characterized by relatively low $\delta^{18}O_w$ values and because $\delta^{18}O_{otolith}$ and $\delta^{18}O_w$ values are positively related; Patterson *et al.*, 1993), and thereby would result in warmer temperature reconstructions than observed ambient temperatures. A long undetected period in a habitat such as Muskegon Lake, however, would result in unrealistically high reconstructed temperatures. Further, such phenomena should not positively bias temperature reconstruction for *A. pseudoharengus* caught in small habitats such as Muskegon Lake. Any period of time spent in water characterized by relatively high $\delta^{18}O_w$ values, such as Lake Michigan, should cause an increase in $\delta^{18}O_{otolith}$ values and hence should result in lower reconstructed temperatures than measured ambient temperatures. On the contrary, reconstructed temperatures were relatively high for Muskegon Lake fish compared to measured epilimnetic temperatures. Further, there is some limited within habitat variation in $\delta^{18}O_w$ values (at stream inlets for example) that can slightly confound thermal reconstruction. With these caveats, the present study suggests that during early life, young *A. pseudoharengus* occupy water masses where temperatures are slightly higher than mean epilimnetic temperature, and that such warm shallow habitats are probably important larval habitats for Lake Michigan *A. pseudoharengus*.

Most studies utilizing natural tracers in otolith carbonate to study movement and habitat utilization have involved marine or diadromous fishes (Schwarcz *et al.*, 1998; Gillanders, 2005) that occupy habitats characterized by distinctive isotope values. Freshwater habitats might exhibit relatively weak isotope variability. In the present study, isotopically distinct freshwater habitats were characterized. In particular, $\delta^{13}C_{otolith}$ values appear very promising in distinguishing freshwater habitats that present large difference in volume. Nonetheless, reconstruction of habitat utilization would have benefited from combining the analysis of $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values with other otolith geochemical tracers (Thorrold *et al.*, 2001) or the simultaneous analysis of otoliths and soft tissues (Limburg, 1998).

Intra-otolith isotope analyses of large and fast growing fishes have produced isotope profiles ranging from annual resolution (Gao & Beamish, 2003) to detailed intra-seasonal scales (Patterson, 1998; Høie *et al.*, 2004; Wurster *et al.*, 2005). Some studies have evaluated the ability to reconstruct intra-annual and interannual variations in environmental conditions. Weidman & Millner (2000) considered five to 20 sub-samples per annual growth ring of Atlantic cod *Gadus morhua* L. otoliths as an acceptable resolution for capturing the seasonal signal of the marine environment. Indeed, *G. morhua* otolith $\delta^{18}O_{otolith}$ records showed a close correspondence to the bottom-water characteristics of their capture regions in the North Atlantic. This may not be the case for habitats that present more complex or variable (in time or space) environmental conditions. Høie *et al.* (2004) applied different sampling resolutions to *G. morhua* otoliths, and as in Patterson's (1998) study, suggested that at low sampling resolution the true temperature range experienced by fish cannot be extracted (Høie *et al.*, 2004). Additionally, these authors observed an attenuation of the $\delta^{18}O_{otolith}$ signal with age, resulting in a decrease in the accuracy of reconstruction of the annual temperature amplitude, because of the ontogenetic decrease in otolith growth rate. As far as is known, however, none of these studies examine how the accretionary nature of otoliths affects the temporal resolution of core samples.

The case of *A. pseudoharengus* otoliths demonstrates that achievable temporal resolutions (several weeks to a month) may fail to characterize early movements among habitats (days of weeks). Therefore, *a priori* characterization of potential temporal resolution (dependant upon species, growth rate and environmental variability) is recommended for future research based on the micro-milling technique. New *in situ* high-precision capabilities (ion microprobes) are now being developed to quantify intra-otolith stable-isotopes ratios (Weber *et al.*, 2002; Weidel *et al.*, 2007) with high spatial resolution (a few days to weeks). Even though the analytical precision for $\delta^{18}O_{otolith}$ is still less than with classical mass spectrometry and will provide less precise thermal reconstructions, such new methodologies might provide an alternative to achieve the temporal resolution necessary to better trace potential complex and fine temporal scale early life history of fish populations such as Lake Michigan *A. pseudoharengus*.

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