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Stable isotope ($\delta^{13}C$ and $\delta^{18}O$) and Sr/Ca composition of otoliths as proxies for environmental salinity experienced by an estuarine fish

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ABSTRACT: The ability to identify past patterns of salinity habitat use in coastal fishes is viewed as a critical development in evaluating nursery habitats and their role in population dynamics. The utility of otolith tracers ($\delta^{13}C$, $\delta^{18}O$, and Sr/Ca) as proxies for environmental salinity was tested for the estuarine-dependent juvenile white perch *Morone americana*. Analysis of water samples revealed a positive relationship between the salinity gradient and $\delta^{18}O$, $\delta^{13}C$, and Sr/Ca values of water in the Patuxent River estuary. Similarly, analysis of otolith material from young-of-the-year white perch (2001, 2004, 2005) revealed a positive relationship between salinity and otolith $\delta^{13}C$, $\delta^{18}O$, and Sr/Ca values. In classifying fish to their known salinity habitat, $\delta^{18}O$ and Sr/Ca were moderately accurate tracers (53 to 79% and 75% correct classification, respectively), and $\delta^{13}C$ provided near complete discrimination between habitats (93 to 100% correct classification). Further, $\delta^{13}C$ exhibited the lowest inter-annual variability and the largest range of response across salinity habitats. Thus, across estuaries, it is expected that resolution and reliability of salinity histories of juvenile white perch will be improved through the application of stable isotopes as tracers of salinity history.

KEY WORDS: Otolith chemistry · Stable isotopes · $\delta^{13}C$ · $\delta^{18}O$ · Sr/Ca · Salinity · Estuary · *Morone americana*

INTRODUCTION

Identification of nursery and lifetime habitat use is critical to understanding fish population dynamics, as the spatial distribution of a population influences its growth, survival, reproduction, and recruitment (Secor 1999, Beck et al. 2001). Habitat use also affects a population's response to environmental changes and fishing pressure. For example, spatial partitioning of fish in different habitats can distribute the mortality risk within a population and ultimately promote long-term persistence (Secor 2007). Otolith chemistry is a useful approach for classifying spatial behaviors of fishes at the population level, sub-population level, and finer spatial scales (Campana 1999, Thresher 1999, Campana & Thorrold 2001).

Within estuarine environments, the salinity gradient can be used as a proxy for habitat use, and several otolith chemistry tracers have been identified as proxies for salinity. Sr/Ca ratios have proved useful tracers of salinity history for many estuarine species (Secor & Rooker 2000). However, the variable nature of Sr/Ca values in freshwater sources and the uniform Sr/Ca value of oceanic sources has raised concern about the general reliability of this tracer (Kraus & Secor 2004a). Stable isotope ratios ($\delta^{18}O$ and $\delta^{13}C$) provide an alternative tracer of salinity history for species that use estuarine habitats. A number of studies indicate that $\delta^{18}O$ and $\delta^{13}C$ can serve as proxies for salinity (e.g. Lloyd 1964, Spiker 1980, Ingram et al. 1996, Fry 2002), but thus far the temporal and spatial variability of otolith stable isotope signatures has not been evaluated across the salinity gradient of an estuary.

Otolith stable isotope ratios are a function of water chemistry and isotopic fractionation that occurs during the transport of dissolved substances from water to the

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calcification front of the otolith (Campana 1999). Water chemistry is regulated by physical, chemical, and biological processes, resulting in freshwater having a unique $\delta^{18}O$ and $\delta^{13}C$ signature which differs from that of seawater. Estuaries that exhibit conservative mixing of 2 dominant end members exhibit a gradient in water chemistry that is correlated with the salinity gradient (Fry 2002).

The overall goal of the present study was to evaluate Sr/Ca, $\delta^{18}O$, and $\delta^{13}C$ as tracers of the environmental salinity experienced by an estuarine fish. The objectives were to: (1) evaluate the relationship between stable isotope values ($\delta^{18}O$ and $\delta^{13}C$) and the salinity gradient in the Patuxent River estuary as measured in water and otolith samples; (2) compare the accuracy of salinity habitat classifications based on the $\delta^{18}O$, $\delta^{13}C$ and Sr/Ca composition of the otolith ($\delta^{18}O_{otolith}$, $\delta^{13}C_{otolith}$, and $Sr/Ca_{otolith}$); and (3) compare the temporal stability of stable isotope tracers of salinity habitats based on white perch collected over 3 yr.

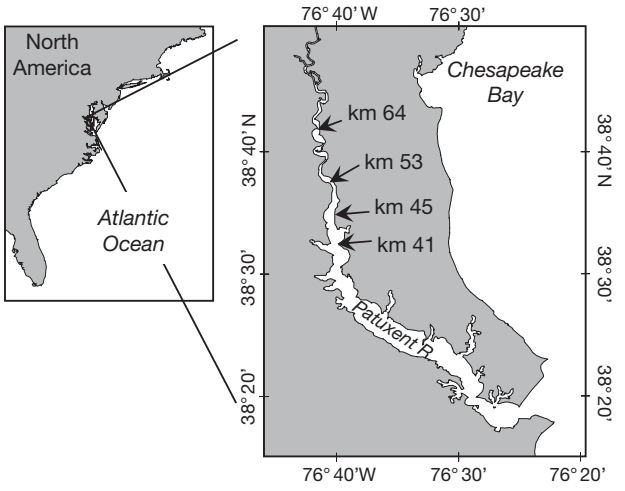

Fig. 1. Patuxent River estuary, a subestuary of the Chesapeake Bay (Maryland; Kraus & Secor 2004a). Map illustrates the location of fish collections in freshwater (river km 64), oligohaline (river km 53), and mesohaline (river km 41 and 45) regions of the estuary

MATERIALS AND METHODS

Species and study area. The white perch *Morone americana* is semi-anadromous and one of the most abundant fish in the Chesapeake Bay (Jung & Houde 2003). As young-of-the-year (YOY) juveniles, white perch use inshore estuarine areas as nursery grounds (Wang & Kernehan 1979, Setzler-Hamilton 1991), ranging from freshwater to salinities of 13 (Stanley & Danie 1983).

The Patuxent River is a shallow, partially mixed estuary, with distinct zones of brackish and tidal freshwater (Fig. 1). The water in the river is a mixture of freshwater derived from precipitation and watershed runoff and saltwater from the main stem of the Chesapeake Bay. The salinity gradient of the Patuxent River ranges from freshwater (0) at the river head to mesohaline conditions (mean salinity range of 10 to 16) at the mouth (Ritchie & Genys 1975). The salinity gradient across the estuary is relatively stable and predictable for a given season (Ritchie & Genys 1975), with the largest deviations in salinity during spring driven by snow melt and major precipitation events.

Environmental data sources. Mean habitat-specific salinity and temperature (°C) were calculated based on monthly water quality data collected by the Maryland Department of Natural Resources (MDDNR) in 2001 and by the MDDNR and the sampling efforts of the present study in 2004 and 2005. Water quality data, i.e. water temperature (°C), salinity, dissolved oxygen (mg l^{-1}), and conductivity (µS), were collected using a hand-held YSI Model 85 Instrument. Stream-flow data ($ft^3\ s^{-1}$, 1 ft^3 = ca. 0.0283 m^3) collected by the United States Geological Survey (USGS) at the Bowie, Maryland, USA, site (USGS Code: 01594440) were used to characterize the monthly mean fluctuations in river discharge for the Patuxent River estuary in 2001, 2004, and 2005. Using an estimated median freshwater residence time of 68 d (Hagy et al. 2000), stream-flow data were averaged over the time period sampled within the otolith plus 68 d prior, encompassing a period from March 25 to September 30.

Water sample collection. Water samples were collected at sites along the Patuxent River estuary from May to September 2005. Grab samples of water were taken from littoral areas in freshwater, oligohaline, and mesohaline habitats in the vicinity of fish collection sites. Water samples were filtered through a 0.45 µm glass fiber filter using a hand vacuum pump. Vacuum filtration has the potential to subtly alter isotopic signatures in low carbonate systems; however, because dissolved inorganic carbon (DIC) is assumed to be predominantly present as HCO_3^- in the Patuxent River (based on pH conditions of 7.0 to 8.0; MDDNR), filtration was not deemed to significantly affect the isotopic values of water samples. Samples were transferred to ICHEM borosilicate glass vials (20 ml), fixed with $HgCl_2$ on site, and kept chilled on ice. Samples were refrigerated (~4°C) until the time of analysis.

Fish collection. Juvenile white perch were collected in August and September of 2001, 2004, and 2005 using a 1.2 m × 30.5 m beach seine deployed at sites along the salinity gradient. Collections occurred in freshwater (FW = salinity 0 to 1), oligohaline (OH = salinity >1 to 3), and mesohaline habitats (MH = salinity 6 to 8; Fig. 1). All white perch were counted, measured, and preserved in ethanol or frozen at the time of

capture. Sagittal otoliths from juvenile white perch were extracted, rinsed, cleaned of adhering tissue, and dried for at least 24 h.

Water sample analysis. Water samples were submitted to the University of Arizona Isotope Geochemistry Laboratory (www.geo.arizona.edu/research/iso_geoch_lab.htm) for stable isotope analysis. Water samples were equilibrated with CO_2 gas at approximately 15°C in an automated CO_2-H_2O equilibration device and analyzed for $\delta^{18}O$ using a dual inlet mass spectrometer (Delta-S, Thermo Finnegan). To measure the isotopic signature of DIC, CO_2 was generated from water samples via acidification and $\delta^{13}C$ was measured using an automated headspace sampler (Finnigan GasBench) connected to a continous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL). Values were reported as per mil (‰) relative to a standard ($\delta^{13}C_{DIC}$: Vienna Pee Dee Belemnite [VPDB] using international standards NBS-19 and NBS-18, and $\delta^{18}O_{water}$: Vienna Standard Mean Ocean Water [VSMOW]). Analytical precision measures of the mass spectrometers for $\delta^{18}O_{water}$ and $\delta^{13}C_{DIC}$ were 0.08 and 0.3‰, respectively, based on the standard deviation of repeated measures of the standard.

Otolith analysis. Otoliths from individuals collected in FW, OH, and MH habitats in 2001 were analyzed for $\delta^{18}O_{otolith}$, $\delta^{13}C_{otolith}$, and $Sr/Ca_{otolith}$ and otoliths from individuals collected in 2004 and 2005 were analyzed for $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ (see Table 2). The $Sr/Ca_{otolith}$ data were originally presented in Kraus & Secor (2004b), which was an analysis of divergent patterns of juvenile habitat use. Here, we used a sub-sample (N = 20) of those data that included 6 to 7 otoliths each collected from 3 salinity habitats to directly compare Sr/Ca and stable isotope tracers in 2001. In the Kraus & Secor (2004b) analysis, one otolith from each individual was transversely sectioned and analyzed for $Sr/Ca_{otolith}$ by electron microprobe wavelength dispersive X-ray spectroscopy at a series of points in a transect across the otolith section. In the present analysis, the other otolith of the pair from the fish collected in 2001 and one randomly selected otolith from each of the fish collected in 2004 and 2005 were analyzed for $\delta^{13}C_{otolith}$ and $\delta^{18}O_{otolith}$. Whole otoliths were adhered to glass microscope slides with acrylic resin and polished to a flat surface using 800 and 600 mm grit polishing paper. YOY white perch are known to transition to the juvenile period and persist in either freshwater or disperse to brackish water habitats from approximately 45 d (SD = 7 d, Kraus & Secor 2004b) onward. Otolith length at time of transition (45 d, +2 SD) was calculated on a habitat-specific basis due to observed differences in fish growth between salinity habitats and was used as a guideline in sampling otoliths. The portion of the otolith >45 d was excised using a New Wave® micromilling machine with a fine-tipped mill (6 µm). The resultant sample consisted of 2 solid peripheral pieces of calcium carbonate removed from the rostrum and post-rostrum regions of the otolith.

Otolith samples were submitted to the University of Arizona Isotope Geochemistry Laboratory for analysis. $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ were measured using an automated carbonate preparation device (KIEL-III) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252). Powdered otolith carbonate samples were reacted with dehydrated phosphoric acid under vacuum at 70°C. The resultant CO_2 was analyzed for $\delta^{18}O$ and $\delta^{13}C$, and values were reported as per mil relative to a standard (Vienna Pee Dee Belemnite [VPDB] using international standards NBS-19 and NBS-18). Analytical precision meansures of the mass spectrometer for $\delta^{13}C_{otolith}$ and $\delta^{18}O_{otolith}$ were 0.06 and 0.1‰, respectively, and based on the standard deviation of repeated measures of the standard.

Statistical analysis. Univariate analysis of variance (ANOVA) tested the null hypothesis of no significant difference in $\delta^{13}C_{DIC}$, $\delta^{18}O_{water}$, and $Sr/Ca_{otolith}$ across salinity habitats (FW, OH, and MH). Two-way ANOVA tested the null hypothesis of no significant difference in $\delta^{13}C_{otolith}$ and $\delta^{18}O_{otolith}$ across salinity habitats (FW, OH, and MH) and within a habitat between years (2001, 2004, and 2005). Tukey's pairwise mean comparisons test identified significant between-habitat differences. The accuracy with which individuals were classified to salinity habitat was evaluated on a yearly basis using linear discriminant function analysis with jackknife resampling (a 'leave-one-out' cross-validation procedure) to determine the accuracy of using all 3 tracers ($Sr/Ca_{otolith}$, $\delta^{13}C_{otolith}$, and $\delta^{18}O_{otolith}$), and combinations thereof, as predictors. Diagnostics were employed to test for normality, homogeneity of variance and covariance, and influential observations for $\delta^{13}C_{DIC}$, $\delta^{18}O_{water}$, $\delta^{13}C_{otolith}$, $\delta^{18}O_{otolith}$, and $Sr/Ca_{otolith}$. One otolith value from a fish collected in the mesohaline habitat was identified as an outlier with respect to both $\delta^{13}C_{otolith}$ and $\delta^{18}O_{otolith}$ values (–12.42 and –6.94‰, respectively); this case was removed from all statistical analyses of $\delta^{13}C$ and $\delta^{18}O$ values but is shown graphically. Statistical analyses were performed with Systat software Version 8.0 (SPSS 1998) or SAS Version 8.2 (SAS Institute 1999); $p = 0.05$ was used as a critical level of significance.

RESULTS

Species data

YOY white perch *Morone americana* collected in 2001, 2004, and 2005 ranged in length from 49 to 88 mm total length (TL). Mean TL increased with in-

creasing salinity (FW = 61.1 mm, SD = 9.0; OH = 66.6 mm, SD = 6.1; and MH = 72.5 mm, SD = 10.0). Significant differences in fish length (and consequently otolith weight) were identified between sites (ANOVA, $F_{2,17}$ = 10.69, p < 0.01) and years. Still, because all 3 tracers showed no significant correlation with fish length over the 3 yr of collection (ANCOVA, p > 0.05 for all tracers in all years), there was no need to detrend the tracer data to remove the effect of fish length in these analyses (Campana et al. 2000).

Environmental data

Over the June to September period corresponding to the timing of juvenile otolith growth sampled, mean salinity differed across sites, but within each site only varied slightly across years (Table 1). Temperature changed over the period of otolith precipitation, typically increasing from June to July, and subsequently decreasing into September. Temperature change was similar across sites, with a maximum difference of 1.6°C between sites on any particular collection date. Stream-flow data were not site specific; however, monthly trends across years showed the highest stream flow occurring in March or April, and flow rate declining into September. Overall monthly mean stream flow was lowest in 2001 compared to 2004 and 2005 (Table 1).

Strontium/Calcium

A positive and significant relationship has been previously reported between Sr/Ca_{water} values and the salinity gradient in the Patuxent River estuary (Kraus & Secor 2004a). Mean $Sr/Ca_{otolith}$ values in fish collected in 2001 exhibited an increasing trend for fish inhabiting increasingly saline environments (Table 2, Fig. 2A). Mean $Sr/Ca_{otolith}$ values were significantly different across salinity habitats ($F_{2,17}$ = 170.51, p < 0.001). Significant between-habitat differences were identified between FW and OH and between FW and MH (p < 0.001), but no significant difference was identified between MH and OH sites (p = 0.05).

Stable carbon isotopes

Measures of $\delta^{13}C_{DIC}$ across the salinity gradient of the Patuxent River estuary over the period from May 31 to September 20, 2005 exhibited a positive and significant relationship (Fig. 3A). The overall mixing curve of aqueous $\delta^{13}C_{DIC}$ across the salinity gradient for all dates sampled was estimated with a power curve ($y = -9.74 + 2.59x^{0.46}$; $r^2 = 0.97$). Mean $\delta^{13}C_{DIC}$ values aggregated over time were significantly different across salinity habitats ($F_{2,23}$ = 17.35, p < 0.001). Significant differences were detected between all salinity habitats based on $\delta^{13}C_{DIC}$ values (p < 0.001 for all pairwise comparisons).

Across all years, $\delta^{13}C_{otolith}$ values were positively correlated with salinity (Pearson correlation coefficient: r = 0.95, n = 48; Table 2, Fig. 2B). Two-way ANOVA

Table 1. Mean environmental variables for the freshwater (FW), oligohaline (OH), and mesohaline (MH) habitats in the Patuxent River estuary in 2001, 2004, and 2005. River is measured as the distance from the river's mouth in kilometers. Mean salinity and temperature are averages for the June to September period for each year, and mean monthly stream flow is an average from March 25 to September 30 based on estimated residence time of freshwater in the estuary. (Note: 1 ft^3 = ca. 0.0283 m^3)

Year	River (km)	Habitat	Salinity		Temperature (°C)		Monthly stream flow (ft^3 s^{-1})	
			Mean	SD	Mean	SD	Mean	SD
2001	64	FW	0.2	0.1	26	1	390	217
	53	OH	2.2	1.9	27	1		
	41	MH	7.3	3.2	27	1		
2004	64	FW	0.2	0.2	25	2	402	137
	53	OH	1.7	1.7	26	2		
	45	MH	6.5	1.7	27	2		
2005	64	FW	0.5	0.8	29	2	512	400
	53	OH	2.4	2.9	29	2		
	45	MH	7.0	3.5	29	1		

Table 2. Mean otolith stable isotope ($\delta^{13}C_{otolith}$ and $\delta^{18}O_{otolith}$) and Sr/Ca values ($Sr/Ca_{otolith}$) for the freshwater (FW), oligohaline (OH), and mesohaline (MH) habitats in the Patuxent River estuary in 2001, 2004, and 2005

Year	Habitat	$\delta^{13}C_{otolith}$ (‰)		$\delta^{18}O_{otolith}$ (‰)		$Sr/Ca_{otolith}$ (mmol mol^{-1})	
		Mean	SD	Mean	SD	Mean	SD
2001	FW	−13.40	0.42	−7.07	0.13	1.26	0.27
	OH	−11.82	0.59	−6.89	0.16	3.09	0.21
	MH	−9.03	0.66	−6.33	0.23	3.39	0.18
2004	FW	−13.77	0.50	−7.48	0.02		
	OH	−12.49	0.34	−7.16	0.02		
	MH	−9.36	0.71	−7.29	0.02		
2005	FW	−13.95	0.84	−7.83	0.02		
	OH	−12.26	0.26	−7.58	0.02		
	MH	−9.66	0.31	−7.26	0.01		

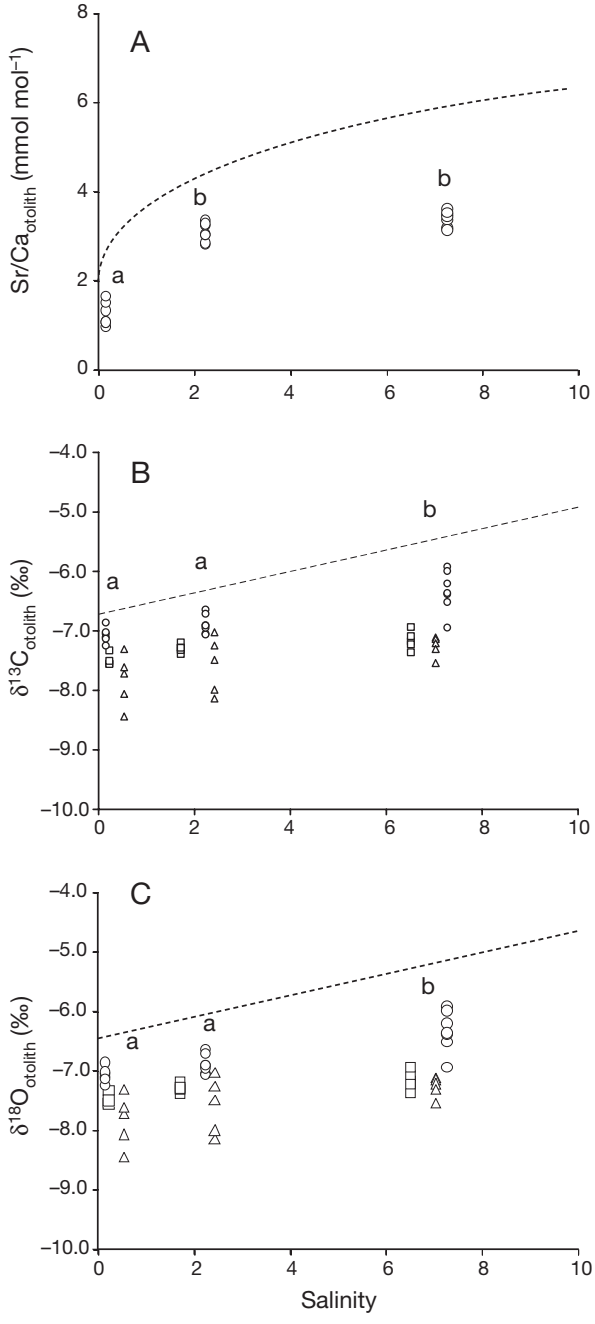

Fig. 2. (A) Otolith Sr/Ca ($Sr/Ca_{otolith}$) values from fish collected in freshwater, oligohaline, and mesohaline habitats in the Patuxent River estuary in 2001 (○). (B) Otolith stable carbon ($\delta^{13}C_{otolith}$) and (C) oxygen ($\delta^{18}O_{otolith}$) values from fish collected in freshwater, oligohaline, and mesohaline habitats in the Patuxent River estuary in 2001 (○), 2004 (□), and 2005 (△). Significant pairwise differences are denoted by different lowercase letters. Dashed trendlines indicate the relationship between Sr/Ca_{water}, $\delta^{13}C_{DIC}$, $\delta^{18}O_{water}$ and the salinity gradient. Trendlines are included to illustrate the isotopic disequilibria between water and otolith tracer chemistry. $\delta^{18}O_{otolith}$, $\delta^{13}C_{otolith}$, and $\delta^{13}C_{DIC}$ values were reported relative to a standard (Viennna Pee Dee Belemnite [VPDB] using international standards NBS-19 and NBS-18), and $\delta^{18}O_{water}$ values were standardized to the VPDB scale

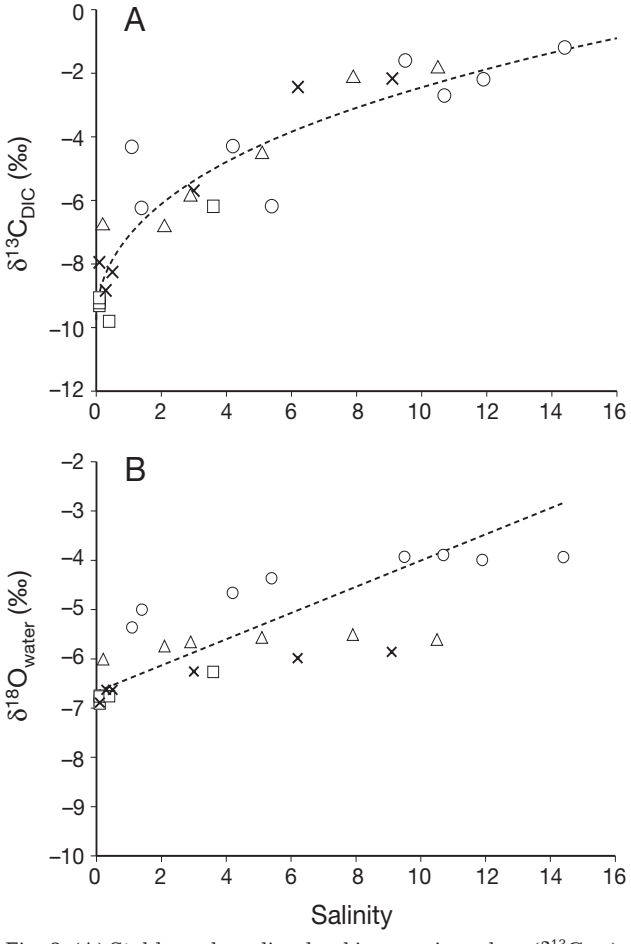

Fig. 3. (A) Stable carbon dissolved inorganic carbon ($\delta^{13}C_{DIC}$) and (B) stable oxygen ($\delta^{18}O_{water}$) values of water samples collected at sites stratified along the salinity gradient of the Patuxent River estuary in 2005 (May to September). Data were aggregated and fit with a power function (dashed line) in the case of $\delta^{13}C_{DIC}$ and a linear function (dashed line) for $\delta^{18}O_{water}$. Collection date is indicated by symbols: May 31 (□), June 6 (×), June 28 (△), and September 20 (○). Values were reported relative to a standard ($\delta^{13}C_{DIC}$: VPDB using international standards NBS-19 and NBS-18, and $\delta^{18}O_{water}$: Vienna Standard Mean Ocean Water)

indicated fish $\delta^{13}C_{otolith}$ values were significantly different across salinity habitats ($F_{2,39}$ = 239.12, $p < 0.001$) and years ($F_{2,39}$ = 9.04, $p < 0.001$), but there was no significant difference within habitats across years ($F_{4,39}$ = 0.91, p = 0.47). Significant differences were found between all sites for all years based on $\delta^{13}C_{otolith}$ values ($p < 0.01$ for all pairwise comparisons).

Stable oxygen isotopes

A positive and significant relationship was identified between $\delta^{18}O_{water}$ values and the salinity gradient

(Fig. 3B). The mixing curve for aqueous $\delta^{18}O_{water}$ was estimated with a linear fit ($y = -6.45 + 0.18x$; $r^2 = 0.82$). Mean $\delta^{18}O_{water}$ values aggregated over time were significantly different across salinity habitats ($F_{2,23}$ = 24.62, $p < 0.001$). Significant differences were detected between FW and OH ($p = 0.01$) and between FW and MH ($p < 0.001$) sites, but no significant differences were observed between OH and MH sites ($p = 0.10$).

Mean $\delta^{18}O_{otolith}$ values exhibited an increasing trend for fish inhabiting FW, OH, and MH environments (Table 2, Fig. 2C). Across the years sampled, $\delta^{18}O_{otolith}$ values were positively correlated with salinity (Pearson correlation coefficient: $r = 0.49$, $n = 48$). Two-way ANOVA indicated significant differences in $\delta^{18}O_{otolith}$ of fish collected across salinity habitats ($F_{2,39}$ = 21.05, $p < 0.001$) and years ($F_{2,39}$ = 49.22, $p < 0.001$), but no difference within habitats across years ($F_{4,39}$ = 2.05, $p = 0.11$). Significant differences were identified between FW and MH and between MH and OH habitats (both at $p < 0.01$), but there was no significant difference between FW and OH habitats ($p = 0.69$).

The overall trend in $\delta^{18}O_{otolith}$ values across habitats indicated 2005 values were lower than 2001 and 2004 values (Fig. 2C). This trend was driven by the 2 lowest $\delta^{18}O_{otolith}$ values for the freshwater and oligohaline habitats. Across the 3 yr sampled, fish were primarily collected in September; however, due to a low sample size of fish from freshwater and oligohaline regions during fall 2005, we supplemented September seine samples with 2 individuals captured in August from each of these salinity habitats.

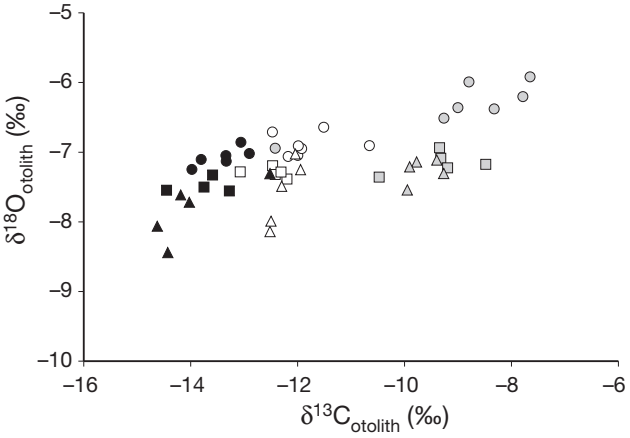

Fig. 4. Dual isotope plot with $\delta^{18}O_{otolith}$ plotted against $\delta^{13}C_{otolith}$ values from fish collected in freshwater (black), oligohaline (white), and mesohaline (gray) habitats in the Patuxent River estuary in 2001 (circles), 2004 (squares), and 2005 (triangles). Note that the absolute values of salinity vary across years for each habitat

Multivariate analyses

A strong correlation was detected between $\delta^{13}C_{otolith}$ and $\delta^{18}O_{otolith}$ from fish collected in 2001, 2004, and 2005 (Fig. 4). Across years we observed similar groupings based on $\delta^{13}C_{otolith}$ and $\delta^{18}O_{otolith}$ values by salinity habitat.

Using all 3 tracers, discriminant analysis revealed 100% correct classification of individuals collected in 2001 to known FW, OH, and MH habitats (Table 3). Analysis of various combinations of individual tracers revealed that $\delta^{13}C_{otolith}$ alone provided better discrimination of salinity (100%), than either $Sr/Ca_{otolith}$ (75%), or $\delta^{18}O_{otolith}$ (79%). In 2004, classification was improved using $\delta^{13}C_{otolith}$ alone (100%), compared to $\delta^{13}C_{otolith}$ and $\delta^{18}O_{otolith}$ (92%; Table 3). Similarly, classification of individuals collected in 2005 was improved using $\delta^{13}C_{otolith}$ alone (93%), compared to using both $\delta^{13}C$ and $\delta^{18}O$ (87%) as predictors. Pillai's trace test indicated all combinations of tracers were statistically significant in all years ($p < 0.006$), with the exception of $\delta^{18}O$ as a single predictor in 2005 ($p = 0.11$).

Table 3. Summary of results of linear discriminant function analysis with jackknife resampling (a 'leave-one-out' cross-validation procedure) to determine the accuracy of using all 3 tracers ($Sr/Ca_{otolith}$, $\delta^{13}C_{otolith}$, and $\delta^{18}O_{otolith}$), and combinations thereof, as predictors. The percent correct classification of fish to salinity habitat (FW: freshwater; OH: oligohaline; MH: mesohaline) is reported for each year and discriminant model. Otoliths were analyzed for stable isotope values over 3 yr to compare the temporal stability of stable isotopes as tracers of salinity habitats; $Sr/Ca_{otolith}$ values were analyzed for 1 yr to compare the accuracy of salinity habitat classifications based on the $\delta^{18}O_{otolith}$, $\delta^{13}C_{otolith}$ and $Sr/Ca_{otolith}$

	Habitat	Sr/Ca, $\delta^{13}C$ & $\delta^{18}O$	$\delta^{13}C$ & $\delta^{18}O$	$\delta^{13}C$	$\delta^{18}O$	Sr/Ca	$\delta^{13}C$ & Sr/Ca	$\delta^{18}O$ & Sr/Ca
2001	FW	100	100	100	67	100	100	100
	OH	100	86	100	71	57	100	100
	MH	100	100	100	100	67	100	83
	Total	100	95	100	79	75	100	94
2004	FW		75	100	75			
	OH		100	100	80			
	MH		100	100	60			
	Total		92	100	72			
2005	FW		80	80	60			
	OH		80	100	20			
	MH		100	100	80			
	Total		87	93	53			

DISCUSSION

Utility of otolith tracers

Quantification of the spatial and temporal variation of otolith tracers is essential to establishing the reliability of these tools for reconstruction of the environmental history of fishes. Both $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ showed significant spatial variation across the estuarine salinity gradient and limited temporal variation. Thus, otolith stable isotope chemistry proved to be an effective tracer of salinity habitat use by juvenile white perch within the Patuxent River estuary across years. In particular, $\delta^{13}C_{otolith}$ showed the highest degree of accuracy in classifying individuals to habitat and the lowest interannual variability in the isotopic signatures of each salinity habitat. The efficacy of stable isotopes as tracers of salinity in the estuarine environment indicates distinct isotopic signatures of freshwater and saltwater end members and relative stability (within season and among years) in the mixing ratios of these 2 water masses within the estuary. Despite expected deviations due to natural processes (e.g. plankton productivity, benthic respiration, atmospheric exchange, and evaporation–precipitation) and anthropogenic input (e.g. waste water discharge), the mixing of end members appears to dominate and approach conservative mixing in this system (Spiker 1980, Taft et al. 1980, Criss 1999). Because end members will differ across estuaries, there may be some estuaries where riverine $\delta^{13}C_{DIC}$ more closely resembles oceanic $\delta^{13}C_{DIC}$, and in these cases, $\delta^{13}C$ would not be as effective a tracer. Initial analysis of end members should provide insight as to whether this tracer will prove useful in a local estuarine system.

Otolith Sr/Ca also proved useful in classifying habitat use of juvenile white perch in the Patuxent River estuary due to the distinct Sr/Ca signature of the freshwater end member and the stability of the estuarine salinity gradient (Kraus & Secor 2004a). However, recent studies have shown that the relationship between Sr concentration and salinity is not consistent due to variation in Sr/Ca values (ranging from <1 to >19 mmol mol^{-1}) in freshwater end members that can approach and exceed the relatively uniform value of seawater (Kraus & Secor 2004a). In this study, the freshwater Sr/Ca end member value in the Patuxent River estuary was in the low range, typical of most freshwater values (Kraus & Secor 2004a). Still, the use of otolith Sr/Ca values resulted in low-resolution discrimination across salinity habitats and lack of detectable difference between oligohaline and mesohaline habitats. This was due to a seawater end member that dominates even at very low salinities (salinities <3). Because the relationship between Sr/Ca and salinity is curvilinear and the majority of variation in this otolith tracer occurred only at low salinities in the Patuxent River estuary, this tracer is of limited utility in distinguishing habitat use in higher salinity habitats.

Variability in water chemistry

Water samples provided periodic snapshots of the isotopic composition across the salinity gradient of the Patuxent River estuary and thus were more variable than the time-integrated stable isotope values (~3 mo) measured in fish otoliths. The physical, chemical, and biological processes that define $\delta^{18}O_{water}$ and $\delta^{13}C_{DIC}$ in freshwater and seawater sources differ (Degens 1969, Mook & Tan 1991, Criss 1999). We identified an increasing trend over time in $\delta^{18}O_{water}$ values from May to September 2005, whereas a temporal trend in $\delta^{13}C_{DIC}$ values was not evident (Fig. 3). The seasonal trend in enrichment of water in the heavier isotope of oxygen was most likely attributable to increased evaporation as waters warmed over the summer months. Similarly, Fairbanks (1982) reported a 2‰ enrichment in $\delta^{18}O_{water}$ during the summer months in 12 east coast rivers. The small-scale variability in $\delta^{13}C_{DIC}$ values likely reflects changes in precipitation and freshwater flow (terrestrial input) in the river.

Variability in otolith chemistry

Interannual variability within habitats was greater for white perch $\delta^{18}O_{otolith}$ values compared to $\delta^{13}C_{otolith}$ values. The overall lower trend in $\delta^{18}O_{otolith}$ values in fish collected in 2005 was attributed to the influence of 4 fish collected 1 mo earlier (August) than the remaining fish analyzed in this study. The more negative $\delta^{18}O_{otolith}$ values in August-collected fish follows the observed increasing seasonal trend in $\delta^{18}O_{water}$ values, with the highest values encountered in September. Furthermore, high 2001 $\delta^{18}O_{otolith}$ values may be related to changes in the isotopic composition of the seawater end member. Because the mouth of the Patuxent River estuary is located north of the entrance of the Chesapeake Bay and south of the Susquehanna River, which contributes ~60% of the freshwater flow to the Bay (MDDNR), the freshwater flow out of this tributary affects the signature of the seawater end member of the Patuxent River estuary. Freshwater flow from the Susquehanna River was below the long-term (1985 to 2000) average for 2001 (MDDNR); these drought conditions resulted in salinities that exceeded long-term averages and a seawater end member $\delta^{18}O_{water}$ signature that was elevated compared to 2004 and 2005.

Estimating isotopic disequilibria

In addition to understanding the underlying water chemistry, it is important to quantify the isotopic disequilibria between water and otoliths to accurately interpret $\delta^{18}O_{otolith}$ and $\delta^{13}C_{otolith}$ values (Thorrold et al. 1997). Mean habitat-specific differences between stable isotope values in the otolith and water of the estuary allowed for a coarse estimate of isotopic fractionation, recognizing that a laboratory-derived estimate would be more rigorous. Field data indicated that white perch $\delta^{13}C_{otolith}$ values were deposited in disequilibrium with $\delta^{13}C_{DIC}$ values, whereas $\delta^{18}O$ values in otoliths were deposited at near equilibrium with $\delta^{18}O_{water}$ values (Fig. 2). Otoliths were depleted in $\delta^{13}C$ by 4.65‰ (SD = 0.84) and $\delta^{18}O$ by 1.16‰ (SD = 0.46) relative to water values. The magnitude of isotopic depletion was in the range of reported values for fish otoliths in the literature ($\delta^{13}C$: −6.29, SD = 2.97 and $\delta^{18}O$: −0.87, SD = 1.01; Campana 1999).

The slight depletion of $\delta^{18}O_{otolith}$ values compared to $\delta^{18}O_{water}$ values may be attributable to temperature-dependent kinetic fractionation during otolith precipitation (Thorrold et al. 1997, Elsdon & Gillanders 2002, Høie et al. 2004). Due to the simultaneous trend of increasing temperature with increasing salinity across the Patuxent River estuary (Table 1), the effect of temperature (depletion of $\delta^{18}O$ with increasing temperature) may have also counteracted the expected influence of salinity (enrichment in $\delta^{18}O$ with increasing salinity) on $\delta^{18}O_{otolith}$ values. Although the magnitude of the influence of temperature (−0.2‰ per degree Celsius increase; Høie et al. 2004) and salinity (+0.23‰ per 1 salinity increase) on the $\delta^{18}O_{otolith}$ signature are similar, their effects are opposite. Due to the scale at which each factor changed across the estuary during a single year, the influence of temperature is likely minimal (maximum temperature difference of 1.3°C between FW and MH habitats) compared to the expected dominant effect of salinity (maximum salinity difference of 7.1 between FW and MH habitats).

Disequilibria between $\delta^{13}C_{otolith}$ and $\delta^{13}C_{DIC}$ values are primarily attributed to the incorporation of metabolically derived isotopes into the otolith (Kalish 1991a,b, Thorrold et al. 1997, Schwarcz et al. 1998). In the case of carbon, the bulk of the input to the otolith originates as DIC (65 to 80%), with a minor proportion derived from metabolic origin (20 to 35%; Kalish 1991a, Weidman & Millner 2000, Høie et al. 2003). Thus, a small contribution of an isotopically depleted metabolic source to the otolith is expected to result in $\delta^{13}C_{otolith}$ values that are more negative than $\delta^{13}C_{DIC}$ (McConnaughey 1989, Kalish 1991a,b, Thorrold et al. 1997). Direct measure of the isotopic composition of the white perch diet was not carried out; however, $\delta^{13}C$ values for white perch tissue are reported to range from −20 to −28‰ (Delaware Bay; Litvin & Weinstein 2003; Hackensack River, New Jersey; Weis 2005). Mass balance calculations support the idea that the average fractionation of white perch $\delta^{13}C_{otolith}$ could be produced by the contribution of 30% carbon from a metabolic source with a $\delta^{13}C$ value of −25.5‰ , i.e. average tissue value (−24‰) – trophic enrichment factor (1.5‰) = −25.5‰, or by a smaller percent contribution of a more negative metabolic source.

Differences in the metabolism of fish or the diet/food web between salinity habitats could potentially influence otolith stable isotope values (Kalish 1991a,b, Thorrold et al. 1997, Høie et al. 2003). Recent laboratory experiments have documented increased growth and feeding rates of white perch that disperse into brackish water habitats (L. Kerr pers. obs.). Increased metabolism has been negatively correlated with $\delta^{13}C_{otolith}$ values (Kalish 1991b, Schwarcz et al. 1998, Høie et al. 2003) and thus would have an effect opposite to salinity. The relationship between fish metabolism and $\delta^{13}C_{otolith}$, however, remains equivocal (Thorrold et al. 1997). Because $\delta^{13}C_{otolith}$ values mirror the increasing trend of $\delta^{13}C_{DIC}$ across the salinity gradient, we hypothesize that differences between habitats are driven by the salinity gradient rather than potential metabolic differences between fish in each habitat. Additionally, differences in the isotopic signature of organic matter at the base of the food web (percent autochthonous vs. percent allochthonous material) between freshwater and brackish water environments, if incorporated in the diet of white perch, could account for variability in the isotopic signature of the otolith.

CONCLUSIONS

Estuaries play an important role in early life history of many commercially important coastal fishes. Across estuaries and year classes, we expect the resolution of salinity histories of juvenile white perch will be improved through the application of stable isotopes, particularly $\delta^{13}C$, as tracers. Empirical studies examining the underlying water chemistry, uptake pathways, and the fractionation of stable isotope signatures in otoliths (Kalish 1991a,b, Thorrold et al. 1997, Høie et al. 2004, present study) and estuarine isotopic mixing models (Fry 2002) support the application of stable isotopes as tracers of salinity habitat. As we further develop our ability to accurately reconstruct past habitat use on a finer scale using otolith chemistry, these tracers will enable evaluation of the importance of the spatial distribution of juveniles within estuaries with respect to population-level dynamics and conservation objectives.

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