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Spatial and temporal variation in otolith chemistry for tautog (*Tautoga onitis*) in Narragansett Bay and Rhode Island coastal ponds

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Abstract—The elemental composition of otoliths may provide valuable information for establishing connectivity between fish nursery grounds and adult fish populations. Concentrations of Rb, Mg, Ca, Mn, Sr, Na, K, Sr, Pb, and Ba were determined by using solution-based inductively coupled plasma mass spectrometry in otoliths of young-of-the year tautog (*Tautoga onitis*) captured in nursery areas along the Rhode Island coast during two consecutive years. Stable oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotopic ratios in young-of-the year otoliths were also analyzed with isotope ratio mass spectrometry. Chemical signatures differed significantly among the distinct nurseries within Narragansett Bay and the coastal ponds across years. Significant differences were also observed within nurseries from year to year. Classification accuracy to each of the five tautog nursery areas ranged from 85% to 92% across years. Because accurate classification of juvenile tautog nursery sites was achieved, otolith chemistry can potentially be used as a natural habitat tag.

Spatial and temporal variation in otolith chemistry for tautog (*Tautoga onitis*) in Narragansett Bay and Rhode Island coastal ponds

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The dependence of fish production and population dynamics on dispersal and migration among multiple habitats, referred to as "connectivity," is a critical property of marine populations. Connectivity rates determine colonization patterns for new habitats, the resiliency of populations to harvest, and can be used in the design of marine protected areas (MPAs). Quantifying connectivity rates in marine organisms is, however, extremely difficult because the natal and nursery origins of adults are almost unknown. Recently, tagging techniques with natural isotopic and elemental markers have been developed for species that were not able to be tagged or recaptured by conventional approaches. Chemical natural habitat tags in the otoliths of juvenile fish have been used to differentiate individuals from different estuarine and riverine systems (Thorrold et al., 1998a; Thorrold et al., 1998b; Gillanders and Kingsford, 2000; Gillanders, 2002b) and other types of nearshore habitats, such as estuary as opposed to rocky reef (Gillanders and Kings-

ford, 1996) and estuary as opposed to exposed coastal habitats (Yamashita et al., 2000; Forrester and Swearer, 2002). In addition, through chemical analysis of the juvenile core region of adult otoliths, natural habitat tags have been used to determine the proportion of the adult population that resided in different juvenile habitats (Yamashita et al., 2000; Thorrold et al., 2001; Gillanders, 2002a).

The tautog (*Tautoga onitis*) is an economically and ecologically important species found in the waters of eastern North America from the Gulf of Maine to North Carolina. Juvenile tautog are known to depend on shallow water habitats where they are safe from high levels of predation and can find necessary food resources (Dorf and Powell, 1997; Arendt, 1999). However, the relative importance of open coastline and enclosed bays and lagoons as nursery habitat for tautog is still poorly understood (Sogard et al., 1992). In light of the fact that the northeastern coast of the United States has experienced a major loss of its estuarine habitats

because of human alteration of the coastal zone (Bromberg and Bertness, 2005), data are needed to quantify the importance of specific coastal habitat types in sustaining tautog populations.

Our long-term goal is to investigate the utility of naturally occurring habitat tags to determine habitat linkages in Narragansett Bay and other nearby estuarine systems by juvenile tautog. This is an initial crucial step to quantify the relative contribution of estuarine habitats for the population connectivity of adult tautog.

Materials and methods

Sampling of juvenile fish

In Rhode Island, young-of-the-year (YOY) tautog of 45–64 mm fork length (FL) were sampled from three sites in Narragansett Bay: Mt. Hope Bay (MH), Gaspee Point (GP), and Rose Island (RS); and from two sites from the coastal ponds along the Rhode Island southern shore: Point Judith, lower pond (PJ), and Charlestown Pond (CP) (Fig. 1). The samples were obtained in cooperation with Rhode Island Department of Environmental Management, Division of Marine Fisheries (RIDEM), during August and September of 2005 and 2006. The sampling stations were selected to include different nursery areas and possibly different chemical backgrounds and according to information on juvenile tautog abundance from RIDEM. Average monthly surface temperatures and salinities at Gaspee Point for 2005 were 22°C and 24.9‰, and for 2006 were 20.6°C and 22.5‰. For Mount Hope Bay, average surface temperatures and salinities were 21.7°C and 27.0‰, and for 2006 were 20.5°C and 24.9‰. Data from the closest point to Rose Island showed average surface temperatures and salinities for 2006 were 17.4°C and 30.8‰. Twenty juveniles per site per year were captured for analysis. Sampled fish were kept frozen until dissection for the removal of otoliths.

Laboratory processing of samples

Before dissection, each fish was weighed (wet weight to the nearest 0.1 g) and measured (FL and standard length [SL] to the nearest 0.1 mm). Both sagittal otoliths were removed from each fish, cleaned of adhering tissue, rinsed 3× with Milli-Q–filtered (Millipore Corp., Billerica, MA) water, and allowed to dry in a class-100 laminar-flow hood. The left sagittal otolith was used for trace metal analysis and the right otolith was used for stable isotope analysis. A total of 164 otoliths were prepared for trace metal analysis. Each otolith was weighed on a Thermo Cahn microbalance (± 0.01 mg) (Thermo Fisher Scientific, Waltham, MA). Samples were then placed in acid-washed 2.5-mL snap-cap polypropylene containers. The otolith weights ranged from 0.08 to 0.34 mg and averaged 0.18 mg. Otoliths for trace metal analysis were transferred to 5-mL clean polypro-

pylene tubes and 0.2 mL of triple-distilled 17% HNO_3 was added to insure complete dissolution (in about 30 seconds). An internal thulium single-element standard spike was added (to correct for variable matrix effects during the inductively coupled plasma mass spectrometry analyses) and then the solution was diluted to 1.8 mL with triple-distilled water. This dilution resulted in a Ca concentration of approximately 40 ppm in the analyzed otolith solution.

Otolith chemistry

Elemental concentrations of YOY otoliths were determined through solution-based ICPMS at the University of Rhode Island Graduate School of Oceanography. All measurements were carried out on a Finnigan element high-resolution inductively coupled plasma mass spectrometer (HR-ICPMS) (Thermo Fisher Scientific, Waltham, MA). A procedural blank was prepared in the same manner as had been used for the other samples, but with no otolith present. The procedural blank was compared to the system blank to determine if contamination occurred during processing. System blanks were made from the same acid used for sample dissolution and were run every four samples. A drift-correction standard was prepared by gravimetrically spiking a $CaCO_3$ standard solution with the appropriate concentrations of Na, K, Rb, Mg, Ca, Mn, Ni, Cu, Zn, Sr, Ba, Co, and Pb to match the typical elemental composition of the otoliths. This drift-correction standard was analyzed every four samples to track and correct for variations in instrument sensitivity during each analytical time period. The choice of these thirteen elements for our study was based on previous studies of elemental composition of juvenile fish otoliths. Analytical results were expressed as absolute concentrations of elemental molar ratios with respect to calcium: Element:Ca ratios, expressed as units of mmol/mol or μmol/mol.

The elements that were always above detection limits (Rb, Mg, Ca, Sr, and Ba) were used for subsequent analysis. The average relative standard deviations were as follows: Rb (3%), Mg (10%), Ca (1%), Sr (1%), and Ba (5%). The limits of detection were as follows (values in ppm): Rb (0.007), Mg (0.02), Sr (0.077), and Ba (0.014). The detection limits for the whole otolith dissolution-solution–based method were calculated as three times the standard deviation of the counts per second (cps) of the isotope of interest in acid blanks divided by the sensitivity in cps/ppm of the CRM22 carbonate standard. For every isotope, these were in the sub-ppm range— a result that compares with the 3 to 2000 ppm range of the elements of interest in the sample otoliths.

Stable carbon and oxygen isotopes of these otolith samples were determined at Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, by using an automated carbonate device (Kiel III) attached to a thermo Finnigan delta-plus stable isotope mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Data were expressed by using conventional δ notation
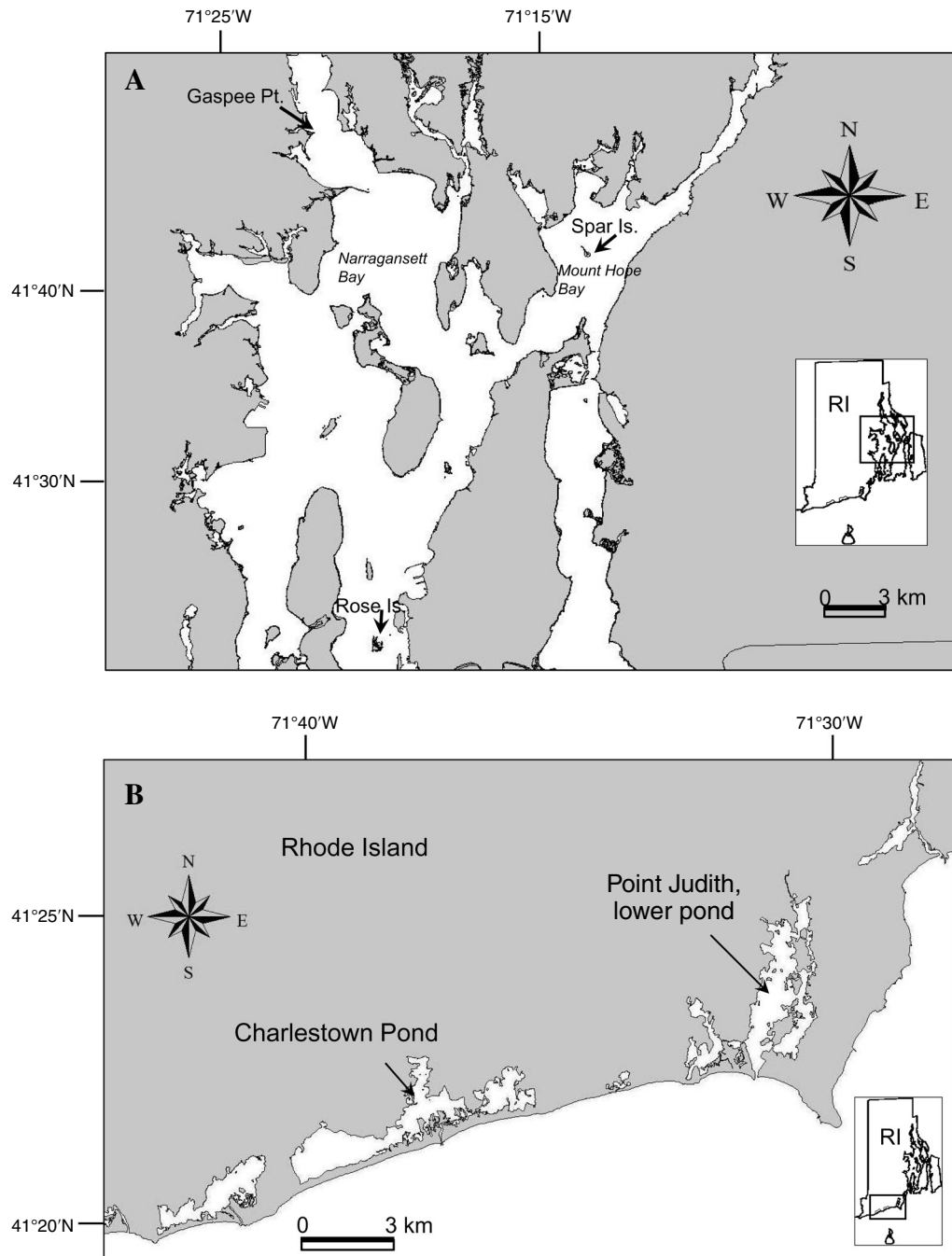
Figure 1

(**A**) Map of tautog (*Tautoga onitis*) sampling stations in Narragansett Bay for 2005 and 2006 that were surveyed for juvenile otolith element concentrations and isotopic signatures. Sampling stations are shown in arrows. (**B**) Map of the south coast of Rhode Island showing two coastal ponds (Charlestown, Point Judith) that were surveyed for tautog for years 2005 and 2006 to determine otolith element concentrations and isotopic signatures. Sampling stations are shown in arrows.

in relation to V-PDB (Vienna Peedee Belemnite). Data were corrected for the usual isobaric interferences. The external precision (calculated from replicate analyses of an internal laboratory calcite standard) was 0.04% for $\delta^{13}C$ and 0.08% for $\delta^{18}O$.

Statistical analysis

Two-way analysis of variance (ANOVA) was used to test for differences in fish body length among stations and years. We also examined relationships between otolith

Table 1
Average size distribution of tautog (*Tautoga onitis*) collected in Rhode Island for analysis of otolith elemental concentrations and stable isotopic signatures. The numbers of fish measured at each station (*n*) to obtain average fork lengths (FL in mm) in each year are shown. Numbers in parentheses are standard errors.

Station	2005		2006	
	n	FL	*n*	FL
Gaspee Point (GP)	17	59.6 (1.2)		
Mount Hope Bay (MH)	17	59.1 (2.1)	21	63.0 (2.9)
Rose Island (RS)	20	52.1 (1.2)	17	45.3 (3.5)
Point Judith, lower pond (PJ)	18	49.4 (2.1)	19	57.3 (1.5)
Charlestown Pond (CP)	17	50.2 (3.0)	18	54.9 (2.7)

weight and otolith elemental composition and isotopic signatures with analysis of covariance (ANCOVA). If there was a significant relationship, we removed the effect of size (otolith weight used as a proxy for fish size) to ensure that differences in fish size among samples did not confound any site-specific differences in otolith chemistry. Concentrations of elements were weight-detrended by subtraction of the product of the common within-group linear slope multiplied by the otolith weight from the observed concentration (Campana et al., 2000).

To detect differences in the concentrations of particular elements and multi-element fingerprints among stations and between years, we performed ANOVA and multivariate analyses of variance (MANOVA). Pillai's trace statistic was chosen as the multivariate test statistic because it is more robust than other multivariate statistics (Wilkes's lamda, Hotelling's *T*-test) to small sample sizes, unequal cell sizes, and situations in which covariances are not homogeneous. Tukey's HSD test was used to detect *a posteriori* differences among means ($\alpha=0.05$). Before statistical testing, residuals were examined for normality and homogeneity among stations. To meet model assumptions, all analyses were performed on natural log-transformed data. We also used linear discriminant function analyses (DFAs) on tautog juvenile data to visualize spatial differences in juvenile otolith chemistry data within sites and to examine classification success for juveniles from different sites or regions. Classification success is the percentage of fish that are correctly assigned to their actual region given the information on location where the fish was collected and the chemical signature of each fish. Cross validations were performed by using jackknife ("leave one out") procedures in SYSTAT (vers. 11, Systat Software, Inc., Chicago, IL).

Results

Size distribution

Mean (FL) of juvenile tautog at stations in Rhode Island ranged from 45 to 63 mm (Table 1). There were sig-

nificant differences in mean length among stations (ANOVA, $P<0.001$) and between years (ANOVA, $P<0.05$) within Rhode Island stations. There were no significant differences in mean FL among stations within Narragansett Bay. However, in 2005, mean FL from all stations within Narragansett Bay were significantly longer than that for individuals caught in the coastal ponds (Point Judith, lower pond, Charlestown Pond) (Tukey test, $P<0.05$). In 2006; only Mount Hope Bay had fish significantly longer than those from Rose Island (Tukey test, $P<0.05$).

Otolith chemistry

Results of MANOVA showed that the chemical signatures of trace metals and stable isotopes combined in tautog otoliths differed significantly among stations (MANOVA, $F_{18,384}=20.72$, $P<0.001$) and years (MANOVA, $F_{6,126}=9.05$, $P<0.001$) within Rhode Island. Significant interaction between station and year (MANOVA, $F_{18,384}=5.18$, $P<0.001$) implied that chemical signatures differed between years depending on the station studied. Classification success for tautog by using both trace metals and stable isotopes for stations within Rhode Island for each of the two years ranged from 85% to 92% (Table 2).

Individual elemental concentrations

In Rhode Island, one trace element (Rb) and one stable isotope ($\delta^{13}C$) showed significant relationships with the covariable otolith weight in the ANCOVA ($P<0.001$) and therefore required the effect of otolith weight be removed for subsequent ANOVA analysis. The elemental concentrations and isotope signatures varied significantly among stations (ANOVA, $P<0.001$), and between years (ANOVA, $P<0.001$) (Fig. 2). Significant interaction between station and year (ANOVA, $P<0.001$) indicated that concentration of individual elements differed between years depending on the station studied. In Rhode Island, elemental concentrations of Sr, Ba, Mg, Rb, and the stable isotopes $\delta^{13}C$ and $\delta^{18}O$ varied significantly among stations in 2005, whereas only Ba

Table 2

Classification success (as a percentage) results determined by jack-knife cross validation procedure for linear discriminant function analysis of chemical concentrations in tautog (*Tautoga onitis*) otoliths collected at Rhode Island stations in 2005 and 2006, with the use of solution-based inductively coupled plasma mass spectrometry for the combined trace metals (Sr, Ba, Mg, Rb): [Sr/Ca], [Ba/Ca], [Rb/Ca], [Mg/Ca]) and for $\delta^{13}C$ and $\delta^{18}O$ stable isotopes. Names of the stations are Gaspee Point (GP), Mount Hope Bay (MH), Rose Island (RS), Point Judith, lower pond (PJ), Charlestown Pond (CP).

	GP	MH	RS	PJ	CP	Classification success (%)
2005						
GP	14	0	0	0	1	93
MH	0	13	0	2	1	81
RS	0	0	19	1	0	95
PJ	0	0	0	16	1	94
CP	0	1	0	0	16	94
Total	14	14	19	19	19	92
2006						
GP	0	0	0	0	0	
MH	0	13	0	2	1	81
RS	0	0	14	2	1	82
PJ	0	2	0	15	0	88
CP	0	0	0	2	15	88
Total		15	14	21	17	85

and $\delta^{18}O$ varied significantly among stations in 2006 (ANOVA, *P*<0.001) (Fig. 2). For example, $\delta^{18}O$ was highest at Rose Island at the mouth of Narragansett Bay, whereas $\delta^{13}C$ magnitudes were similar across years for all Narragansett Bay stations. Sr concentrations within Narragansett Bay and the coastal ponds also remained similar in magnitude throughout the years of study.
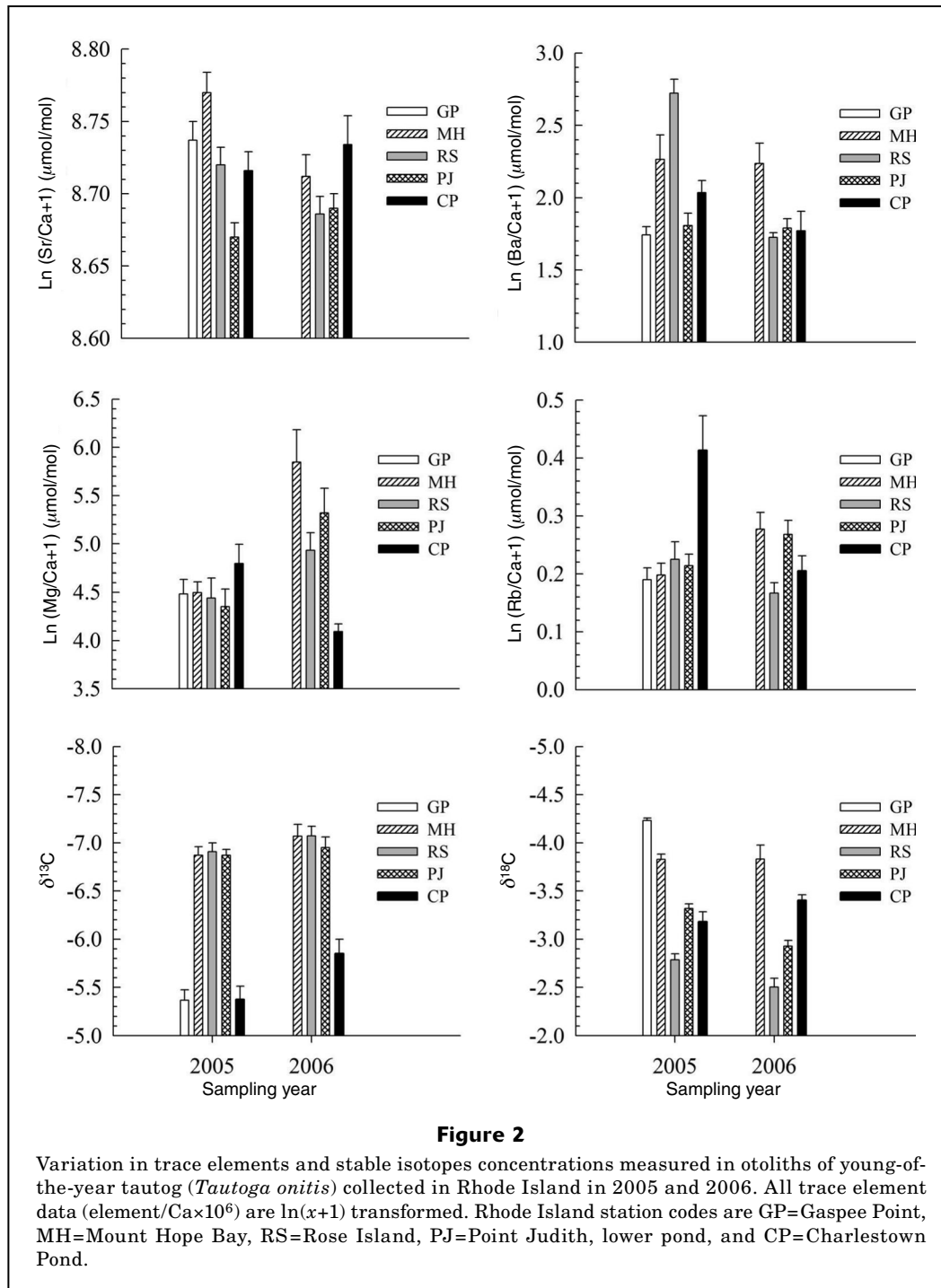
Discussion

The elemental composition of juvenile tautog otoliths varied considerably within and among estuaries and between years. We found very strong differences in the concentrations of Mg, Sr, Ba, and Rb, as well as in the stable isotopic signatures of $\delta^{13}C$ and $\delta^{18}O$, among stations within RI. High classification success rates (generally >85%) of the discriminant functions derived from trace element and stable isotope signatures together confirmed their use as an effective natural tag of the estuarine nursery area of juvenile tautog. Although most of the variance in trace element signatures was concentrated among estuaries, we also found significant differences in elemental fingerprints and stable isotopes in tautog otoliths among sites about 10 to 25 km^2 apart within Narragansett Bay resulting in 100% classification success within that water body. These data indicate that the physicochemical characteristics of specific sections of the estuaries may vary enough to generate the differences in otolith chemistry that we observed within each estuary.

Elemental fingerprints, however, should not be regarded as permanent markers of actual estuarine habitat or environment (Forrester and Swearer, 2002; Swearer et

al., 2003). Estuarine habitats are very dynamic; seawater properties and composition at a particular location can vary over tidal to annual time scales (Peters, 1999). As a result, it may be expected that the magnitude of variations in elemental fingerprints in otoliths among estuaries will not remain constant over time. The significant interannual differences we report among year classes in age-0 tautog otolith elemental signatures is similar to interannual differences in otolith chemistry reported for other marine fishes (Gillanders and Kingsford, 2000; Gillanders, 2005). Thus, interannual differences indicate that age-0 tautog elemental signatures must be analyzed on a year-class–specific basis because there were stations where concentrations were not consistent between years.

It is not surprising to see such clear differences in otolith chemical signatures among the stations sampled in Narragansett Bay. Data from RIDEM show that there were also significant differences in salinity regimes in these regions during the late spring and summer of 2005 and 2006 (H. Stoffel, and J. McNamee, unpubl. data[1]). The proximity of Rose Island station to the mouth of Narragansett Bay meant that high salinities (up to 30‰) would be observed. On the other hand, the lower-salinity stations within the upper region of Narragansett Bay are located much closer to the industrial area and watershed and therefore potentially more prone to terrestrial influences from freshwater runoff resulting in reduced salinities (20–25‰).

Figure 2

Variation in trace elements and stable isotopes concentrations measured in otoliths of young-of-the-year tautog (*Tautoga onitis*) collected in Rhode Island in 2005 and 2006. All trace element data (element/Ca×10^6) are ln(x+1) transformed. Rhode Island station codes are GP=Gaspee Point, MH=Mount Hope Bay, RS=Rose Island, PJ=Point Judith, lower pond, and CP=Charlestown Pond.

Successful discrimination between estuarine nurseries in the present study was accomplished through otolith elemental fingerprints, fulfilling one of the requirements for their possible use as natural tags (Campana et al., 2000). The estuarine nursery origin of juvenile tautog was accurately identified based on otolith elemental fingerprints and stable isotopes. Several methods based on laser ablation (Thorrold et al., 2001; Gillanders, 2002a) or micromilling techniques (Gil-

landers and Kingsford, 1996; Gillanders, 2005; Brown, 2006) could be used to determine elemental fingerprints found in the otolith cores of adult tautog for comparison with the juvenile estuarine fingerprints that we have established. We believe solution-based techniques are more suitable than microprobe techniques for analysis of tautog otolith elemental concentrations because 1) solution-based techniques tend to have higher sensitivity, accuracy, and precision compared to microprobe

techniques (Campana, 1999; Campana et al., 2000); and 2) solution-based techniques can provide more precise natural tags on fish with limited movement within habitats during their first year of life. For example, tautog have a short larval period of 15 to 20 days (Sogard et al., 1992; Dorf and Powell 1997) and once larvae have settled, they have small home range of approximately 20 meters (Able et al., 2005) during their first year of life. Thus, juvenile cores samples from age classes representing fish born in 2005 and 2006 could be extracted by micromilling procedures and their chemical elements can be analyzed by solution ICPMS. Present results are a step towards establishing juvenile movement to adult habitats, which must be examined in nursery studies (Beck et al., 2001). Identifying links between juvenile and adult habitats, and understanding connectivity between estuarine nurseries and adult populations, has the potential to aid fishery managers and aid in the management and conservation of estuarine fish nursery habitats.

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