Analysis of Trace Transition Elements and Heavy Metals in Fish Otoliths as Tracers of Habitat Use by American Eels in the Hudson River Estuary

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ABSTRACT: Transition and heavy metals within the calcified otoliths of estuarine fishes may represent valuable tracers of environmental exposures, allowing inferences on natality, habitat use, and exposure to pollution. Accurate measurement of very low concentrations of these metals in otoliths by inductively coupled plasma mass spectrometry (ICP-MS) is often precluded by the interferences of predominant calcium matrix. We coupled a solid phase extraction procedure to an ICP-MS instrument to overcome the matrix problems and improve the limits of detection. To test this novel application and the utility of otolith transition and heavy metals as tracers of habitat use, otoliths of American eel (*Anguilla rostrata*) captured from 6 locations (George Washington Bridge, Haverstraw, Newburgh, Kingston, Athens, and Albany) throughout the Hudson River estuary were analyzed for site specific differences expected due to varying environmental exposure. Several trace elements, including Al, Bi, Cd, Co, Cu, Ga, Mn, Ni, Pb, V, and Zn, were selectively extracted from otolith solutions and preconcentrated on a microcolumn of chelating resin. The concentrations of all elements in *A. rostrata* otoliths were above the limits of detection that ranged from 0.2 ng g^{-1} for Co to 7 ng g^{-1} for Zn. Differences in the elemental composition of the otoliths among the groups were significant indicating different levels of exposure to environmental conditions. Discriminant analysis yielded an overall location classification rate of 78%. Al, Bi, Cd, Mn, Ni, and V contributed most to the discriminant function. Samples collected at George Washington Bridge showed 100% discrimination from other locations, and higher levels of many transition and heavy metals, consistent with higher exposure to these metals in the most polluted region of the Hudson River estuary.

Introduction

As fish reside or migrate through estuaries they are exposed to a range of aqueous metals and isotopes, representing complex mixtures of terrestrial, anthropogenic, and marine sources. Elements such as strontium (Sr), magnesium (Mg), manganese (Mn), and barium (Ba) occur at relatively high abundance in calcified structures of fishes and their concentrations have been used to draw inferences about past exposure to these chemicals and by extrapolation, past patterns of habitat use (Campana 1999; Thresher 1999; Secor and Rooker 2000). Fish otoliths (calcium carbonate accretions in the form of aragonite located in the ear canals of fishes) have been particularly useful in such applications. Otoliths are rather pure structures and examination of elements that might be expected to vary substantially among and within estuaries, transition elements and heavy metals, has been hindered by analytical issues of the calcium carbonate matrix, spectral and nonspectral interfer-

ences, and insufficient detection limits. We use American eels (*Anguilla rostrata*), a fish known to have long-term homing to restricted regions within estuaries (Morrison and Secor 2003), as a model system to test a novel method, solid phase extraction inductively coupled plasma mass spectrometry (ICP-MS), and evaluate its utility in distinguishing groups of eels among regions within the same estuary.

There is a growing literature that indicates that otoliths represent some but not all metals in proportion to their environmental occurrence. Throughout the lifetime of a fish, otoliths grow continually as a result of deposition of calcium carbonate crystals on a proteinaceous matrix. During this deposition, elements from resident waters pass through gills to the bloodstream and are incorporated in the calcium carbonate-protein lattice of the otolith. The chemical composition of the otolith is thought to be dependent primarily on the environmental conditions of the water mass experienced by the fish, although it may not directly correlate with the ambient water chemistry because of the influences of physiological processes (Kalish 1989; Fowler et al. 1995). In particular, uptake of metals is often proportionate to calcium

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(Ca), which inhibits uptake at high relative concentrations (Campana 1999; Bath et al. 2000; Kraus and Secor 2004). Numerous studies have demonstrated that there are consistent differences in the elemental composition of the otoliths for fish inhabiting different nursery areas (Campana et al. 1995; Secor and Zdanowicz 1998; Gillanders and Kingsford 2000; Rooker et al. 2001; Secor et al. 2001, 2002) Otolith trace metals (e.g., metals other than Ca, sodium [Na], and Sr) make up only about 1% of the total otolith mass (Thresher 1999). Other more abundant chemical constituents (> 100 ppm) include potassium (K), chlorine (Cl), phosphorus (P), and sulfur (S), but these are relatively poor tracers of environmental exposure because they are either tightly regulated in the bloodstream or because they are not well incorporated into the otolith's lattice structure (Kalish 1989; Campana 1999; Thresher 1999; Secor et al. 2001). Although Sr has seen wide application, many scientists have pursued a broader range of lower abundance elements (< 10 ppm) as tracers by using ICP-MS that has now become a standard technique in solution-based elemental analysis of fish otoliths due to its inherent high sensitivity and multielement and isotopic analysis capabilities.

Much of the work has focused on the relatively easily detectable trace metals, such as Ba, lithium (Li), Mg, Mn, and Sr, as indicators of environmental exposure and natal origins of several marine and estuarine-dependent fishes (Campana et al. 2000; Gillanders and Kingsford 1996, 2000; Thorrold et al. 1998a,b; Secor et al. 2001; Forrester and Swearer 2002). Despite the capabilities of ICP-MS and its successful applications, transition and heavy metals (which should provide important source information within estuaries), are seldom selected (or detected) as tracers of habitat use or population structure. One of the major difficulties is that there remain relatively few reliably detectable trace elements in otoliths by conventional solution-based ICP-MS (e.g., Thorrold et al. 1998a,b; Thresher 1999; Gillanders and Kingsford 2000; Forrester and Swearer 2002).

Analytical limits on measurement of transition elements and heavy metals are due in part to the extremely high levels of calcium carbonate occurring in otoliths (typically 90–98%). Not only does high Ca content in dissolved otolith solutions dramatically reduce the sensitivity of a quadrupole ICP-MS instrument, but it may also yield misleading results because of the spectral overlaps of molecular ions of Ca on the analyte of interest (e.g., $^{43}Ca\ ^{16}O^+$ on $^{59}Co^+$ and $^{44}Ca\ ^{16}O^+$ on $^{60}Ni^+$). Magnetic-sector ICP-MS instruments when operated at relatively high resolutions offer the capability to overcome such interferences but at the cost of re-duced sensitivity, resulting in inaccurate or anomalous measurements for many trace transition and heavy metals (silver [Ag], aluminum [Al], cadmium [Cd], cobalt [Co], nickel [Ni], tin [Sn], and zinc [Zn]) present at ng g^{-1} levels in fish otoliths (see Forrester and Swearer 2002). An alternative approach to improve the accuracy in quantification of trace elements in otoliths by ICP-MS is analyte enrichment via extraction procedures. We investigate a solid phase extraction method, as an approach to overcome these problems. Solid phase extraction methods have been widely applied to the analysis of saline solutions, such as seawater, because of the ability to selectively separate and preconcentrate the trace elements from interfering matrix components (Willie et al. 1998; Warnken et al. 2000). Application of this method to otoliths have been reported recently by several groups (Willie et al. 2001; Arslan and Paulson 2002, 2003) that showed novel capabilities to accurately measure trace elements concentrations in small otoliths.

In this study, we applied an online separation and pre-concentration procedure to ICP-MS analysis of trace transition and heavy metals in the otoliths of American eels captured from locations throughout the Hudson River estuary. Although the life cycle of American eels includes a marine origin, the vast majority of their life and associated fish and otolith growth occur in limited home ranges within coastal, estuarine, and freshwater environments (Helfman et al. 1987; Morrison and Secor 2003). In the Hudson River estuary, these home ranges were measured to be less than 2 river kilometers, which is similar or greater to home ranges measured for this species elsewhere (Morrison and Secor 2003). Sources of transition elements and heavy metals are well known to be heterogeneously distributed throughout the Hudson River estuary (Bopp and Chillrud 2001). We expected to observe heterogeneity among groups of American eels captured at locations spaced at 40–50 km intervals throughout the estuary.

Materials and Methods

SAMPLE COLLECTION

American eel were collected from 6 different locations along the Hudson River estuary in summer 1999. The river is tidal from its mouth at Manhattan Island to Troy Dam, 255 km upriver. The salt front is usually found near Yonkers, 25 km from the mouth of river (river km 25) in high flow months (late winter to early spring), and moves upriver to near Newburgh (river km 100) when the water flow decreases during summer months (Dovel et al. 1992). Eels were collected using standard
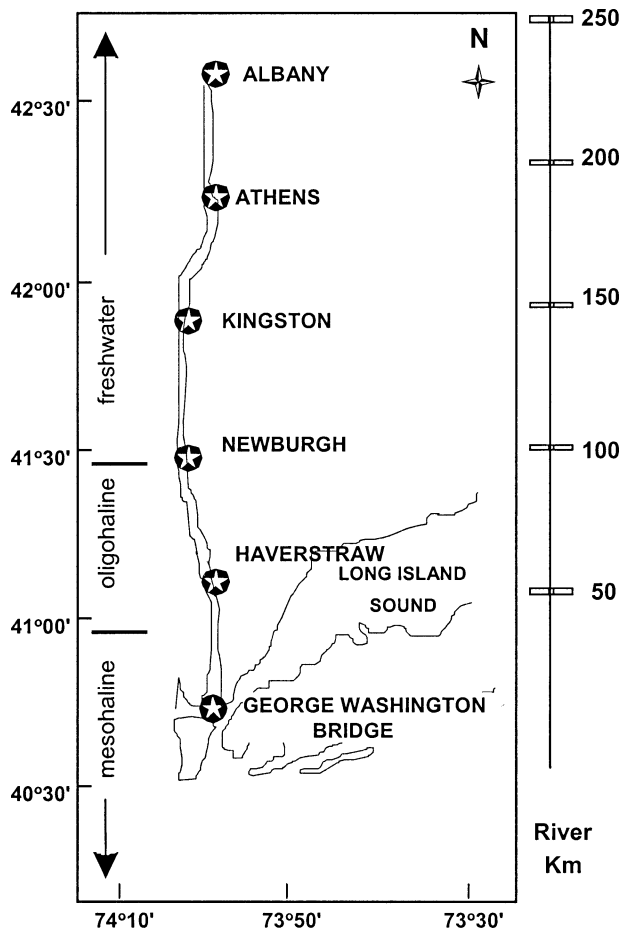
Fig. 1. Map of Hudson River estuary and sampling locations.

1-m long double funnel eel pots (13 × 13 mm mesh) that were baited with menhaden, *Brevoortia tyrannus,* and soaked overnight. The sampling locations were George Washington Bridge (GWB, river km 20), Haverstraw Bay (HAV, river km 60), Newburgh (NEW, river km 100), Kingston (KING, river km 140), Athens (ATH, river km 190), and Albany (ALB, river km 240; Fig. 1). Sizes ranged from 35.5 to 67 cm total length. Ages for this size range of fish typically range between 5 and 20 yr, and depend upon site with younger eels (< 15 yr) tending to occur at the lower two sites, and eels 10–20 yr occurring at the four up-estuary sites. Sagittal otoliths were extracted, soaked briefly (3 min) in 10% hypochlorite solution, rinsed in deionized water, dried, and stored.

SAMPLE PREPARATION

Ultrapure grade reagents were used in sample preparation and ICP-MS analysis. All containers in contact with sample or standard solutions were made of Teflon or polystyrene that had been soaked in 5% nitric acid (HNO_3) and rinsed with deionized water (18 MΩ cm^{-1}) before use. Otoliths were thoroughly decontaminated in the laboratory. They were first soaked in deionized water and then 3% hydrogen peroxide solution for 5 min to hydrate and to completely remove the remaining biological tissue on the surface, respectively. They were then immersed into 1% HNO_3 solution for 5 min to remove surface contamination and then flooded with deionized water to rinse off excess acid. They were weighed and stored in precleaned plastic vials at room temperature after air drying under a Class 100 laminar-flow hood.

The rigorous decontamination procedure follows a call by Thresher (1999) to develop consistent procedures to avoid systemic bias in otolith composition studies. Seldom can otoliths in large field applications be collected with clean-room methods. We have taken an approach that effectively eliminates surface contamination that can result from otolith extraction (Rooker et al. 2001; Secor et al. 2001). Immersion in strong acid solution results in an acidification front and we do not expect that much if any remobilization occurs within the otolith. As evidence of this, Rooker et al. (2001) found no significant differences in relative lithophilic metal concentrations between pairs of yellowfin tuna (*Thunnus albacares*) otoliths, one that remained untreated and the other receiving the decontamination procedure.

For analysis, otoliths (right and left pairs) were placed in a Teflon beaker and dissolved by concentrated HNO_3 followed by mild heating (< 80°C) until no solution remained to destroy the organic matrix. The residue was then dissolved and diluted to 10 ml by 0.1% HNO_3 in a 15-ml polystyrene centrifuge tube. Procedural blanks were concurrently prepared with same dissolution procedure. Samples of fish otolith certified reference material ([CRM] No. 22; Yoshinaga et al. 2000) obtained from the National Institute of Environmental Studies, Japan, were also dissolved similarly and analyzed for quality assurance.

PRECONCENTRATION ICP-MS ANALYSIS

Toyopearl AF-Chelate 600M (Tosohaas, Pennsylvania), a methacrylate backbone iminodiacetate chelating resin, was used for separation and preconcentration of trace elements from otolith solutions. This resin extracts a suite of trace elements (including rare earths) between pH 4.8 and 9.0 (Arslan and Paulson 2002). Retention of Ca on the resin is also affected by pH and increases as the pH of the solution passing through the column increases. Preconcentration was performed in the pH range 4.9 and 5.2 to minimize Ca retention. This was achieved by online mixing the sample so-

lution acidified to 0.1% HNO_3 with pH 9.6 ammonium acetate buffer solution (see Fig. 2).

The preconcentration column was prepared by packing the resin into a mini column of inner volume of 85 μl (1 cm long) that was push-fit into a 2 cm long column holder. Both ends of the column were tightly closed by glass frits to avoid the loss of resin. Samples were loaded onto the column by using a FIAS-400 system (Perkin Elmer, Connecticut) with a 5-port rotary flow injection valve. Pump tubings were Tygon and all other connecting tubings were Teflon (0.8 mm i.d.). A schematic representation of the preconcentration manifold built on the valve is given in Fig. 2. In step 1, sample solution and ammonium acetate buffer solution (pH 9.6) first fill the lines and then mix online prior to column loading. In step 2, buffered sample solution passes through the column for 60 s so that analytes of interest are retained on the column and the matrix elements are directed to waste. Meanwhile, 1% HNO_3 solution rinses the instrument interface. In step 3, the column is washed by deionized water for 60 s to eliminate remaining salts (e.g., Ca) and 1% HNO_3 continues to rinse the interface. In step 4, the valve is switched to elution position and the analytes preconcentrated on the column are back-flushed to the instrument by acid eluent (3% HNO_3 + 1.0 μg l^{-1} ^{103}Rh as internal standard). The column is reconditioned in step 5 by deionized water for next loading.

A Perkin-Elmer Sciex Elan 5000 ICP-MS instrument (Perkin Elmer) with a U-5000AT ultrasonic nebulizer (Cetac, Omaha, Nebraska) attachment was used for elemental determinations. Calibration was carried out by external method using aqueous standard solutions (0.01–5 ng ml^{-1} multielements in 0.1% HNO_3). Two replicate measurements were made for each solution at pH 4.9–5.2. The remaining solution was diluted by 2-fold with deionized water (e.g., acidity reduced to 0.05% HNO_3) and reanalyzed for Mn that required a pH between 8.7 and 9.0 for quantitative determination. Internal standardization was performed by ^{103}Rh added to acid eluent. Internal standard corrected signals of five standard solutions that bracketed those of the sample were used in the calculations of elemental concentrations. Detection limits were calculated as 3 standard deviations of the mean of the procedural blanks (n = 7; Table 1). Results (mean ± SD for 5 replicate analyses) obtained from the analysis of fish otolith CRM were similar to those reported by Arslan and Paulson (2002) and Yoshinaga et al. (1999; Table 1). Precision (relative standard deviation) for two replicate measurements of otolith CRM solutions varied between 0.7% (Co) and 4.6% (bismuth [Bi]).

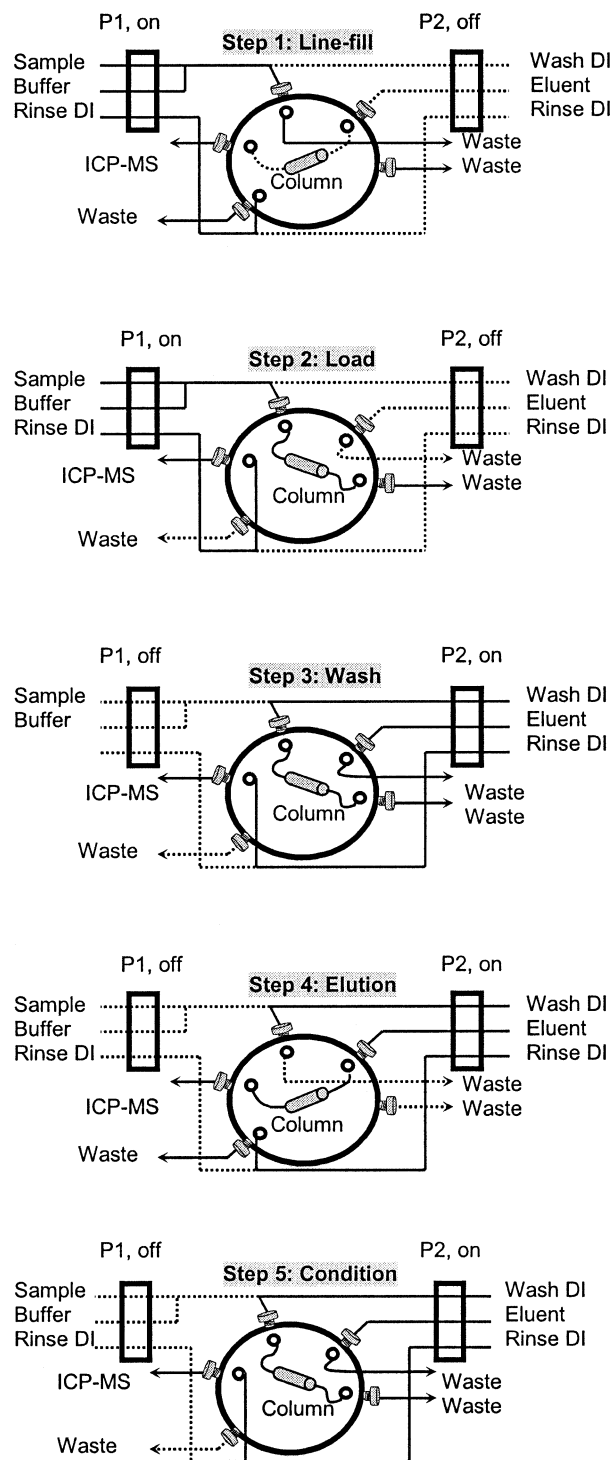The possible interferences from Ca matrix on

Fig. 2. Flow injection manifold used for online solid phase extraction of elements from otolith solutions. P1 = Pump 1; P2 = Pump 2; DI = Deionized water. P1 and P2 run at 60 rpm. Flow rates (ml min^{-1}) are: Sample = 3.2; Buffer = 1.5; Wash DI = 6.5; Eluent = 1.5; Rinse DI = 1.5.

TABLE 1. Approximate detection limits and indicative elemental concentrations in fish otolith Certified Reference Material (CRM) of emperor snapper (*Lutjanus sebae*) obtained by solid phase extraction ICP-MS. Values are mean (± SD).

| Element | Detection Limits (ng g^{-1}) | Indicative Concentrations in Fish Otolith CRM | | |
		This Study	Reported[1]	Reported[2]
^{27}Al	3.5	33 (4)	25 (3)	—
^{209}Bi	0.40	29 (5)	23 (2)	—
^{114}Cd	0.30	2.7 (0.4)	3.0 (0.4)	2.8 (0.2)
^{59}Co	0.2	2.2 (0.3)	2.8 (0.8)	—
^{63}Cu	3.2	652 (41	686 (51)	727 (7)
^{69}Ga	0.55	1.1 (0.2)	1.3 (0.3)	—
^{55}Mn	1.1	50 (6)	51 (11)	—
^{58}Ni	2.1	22 (4)	20 (3)	—
^{208}Pb	0.50	26 (3)	26 (1)	23 (3)
^{51}V	0.2	1.0 (0.1)	1.0 (0.1)	—
^{66}Zn	7.0	501 (61)	448 (69)	471 (2)

[1] Solid phase extraction and ICP-MS determination (Arslan and Paulson 2002).

[2] Liquid-liquid extraction and isotope dilution ICP-MS determination (Yoshinaga et al. 1999).

transition and heavy metals were investigated in detail elsewhere (Arslan and Pauslon 2002) by external calibration and matrix-matched calibration with the fish otolith reference material (CRM No. 22). Results of that study are summarized in Table 2 and compared with analyses of the CRM performed as part of the current study (i.e., done in the same manner as eel otolith analyses). The amount of Ca retained on the column for the otolith solutions containing about 2800–3400 µg ml^{-1} Ca was approximately 6 µg ml^{-1} at pH 5.0 and 32 µg ml^{-1} at pH 8.8. The results for ^{59}Co and ^{60}Ni that are the most prone to ^{43}CaO, ^{44}CaO, and ^{43}CaOH interferences clearly show that calcium oxide and hydroxide interferences were insignificant

and did not confound the quality of the elemental data from otolith CRM. In the case of Mn, which is virtually free of interferences of calcium oxides, the relatively high levels of Ca (e.g., 32 µg ml^{-1}) remaining in the analysis solution at pH 8.8 did not cause any significant alterations in Mn signals because the effects were accurately compensated by the internal standard element (^{103}Rh) in the acid eluent.

STATISTICAL ANALYSIS

The within-group distribution of the elemental concentrations for Al, Bi, Cd, copper [Cu], Mn, Ni, lead [Pb], vanadium [V], and Zn were skewed. Natural logarithm (ln) transformations were performed prior to statistical analysis for these elements to meet the assumptions of normality. Co and gallium [Ga] were not ln-transformed. Otolith masses varied significantly among the fish from 6 different sampling sites (ANOVA, $p < 0.01$). Significant negative slopes ($p < 0.01$) were observed between ln-transformed elemental concentration and otolith mass for Al ($r = 0.8$), Cd (0.52), Co (0.36 for untransformed values), Cu (0.37), Ni (0.72), Pb (0.4), Zn (0.53), and V (0.42). The residuals from regressions of elemental concentrations on otolith mass were used for these elements to ensure that differences in otolith mass did not bias tests of group separation. Adjustment was not needed for Bi, Ga, and Mn.

Multivariate analysis of variance (MANOVA) was performed to test for differences in the otolith concentrations of individual elements among the sites. Sample sizes were equal for all sites ($n = 10$). Pillai trace (V) was chosen as the test statistic since it is the most robust to violations of homogeneity of covariance. Tukey's HSD test was used to identify the site differences ($\alpha = 0.05$) based on the elemental composition of the samples. Two-factor

TABLE 2. Summary of the concentrations (ng g^{-1}) from fish otolith CRM of emperor snapper (CRM No. 22) for monoisotopic and polyisotopic elements. Values are mean (± SD) for five separate determinations by solid phase extraction ICP-MS (Arslan and Paulson 2002). Calibration for Mn was performed at pH 8.8 by external method and at pH 5.0 by matrix-matched method.

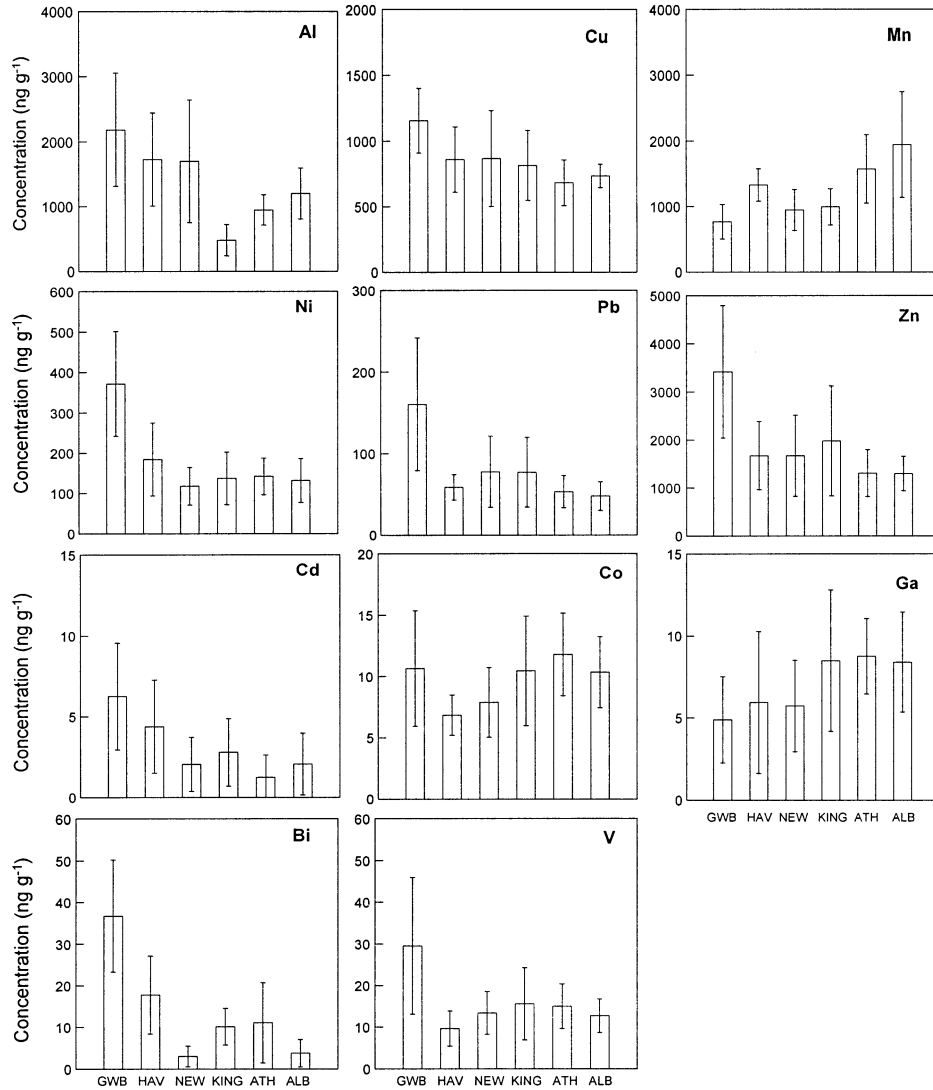| Element	Isotope	External Calibration	Matrix-matched Calibration
Al	27	25 (3)	27 (3)
Bi	209	23 (2)	28 (2)
Cd	111	2.8 (0.5)	2.9 (0.2)
Cd	114	3.0 (0.4)	2.9 (0.2)
Co	59	2.8 (0.8)	3.2 (0.7)
Cu	63	671 (52)	761 (60)
Cu	65	686 (59)	756 (66)
Ga	69	1.3 (0.3)	1.6 (0.3)
Ga	71	1.4 (0.2)	1.3 (0.5)
Mn	55	43 (8)	51 (11)
Ni	58	20 (3)	21 (5)
Ni	60	22 (5)	22 (4)
Ni	62	19 (1)	18 (3)
Pb	207	29 (2)	28 (3)
Pb	208	26 (1)	30 (2)
V	51	1.0 (0.1)	1.3 (0.4)
Zn	66	448 (69)	535 (92)
Zn	68	451 (54)	526 (88)

Fig. 3.   Average concentrations of trace transition and heavy elements in the otoliths of American eel (n = 10) across the sampling sites. Error bars are standard deviation. Total otolith mass range is: GWB = 1.71–6.54 mg; HAV = 2.82–7.49 mg; NEW = 4.74–11.48 mg; KING = 4.19–13.96 mg; ATH = 3.7–8.9 mg; ALB = 4.3–11.31 mg).

principal component analysis (PCA) was used to illustrate the affinity of all elements analyzed. Linear discriminant function analysis (LDFA) was used for multivariate classification of the groups to their locations. A forward stepwise classification procedure was used to select only those elements that contributed significantly to group classification. Relative importance of individual elements in discriminating among the groups was assessed by using the F-to-remove statistics estimated during discriminant analysis procedure (Wilkinson et al. 1996). Cross-correlation of the individual elements used in LDFA was evaluated using tolerance statistics. Tolerance estimate for an element ranges from 0 to 1 and is inversely related to the corre-

lation of that element to the others in the multivariate analysis (e.g., 0 = high correlation, 1 = low correlation).

Results

The concentrations for all elements determined in the otoliths of American eel were all above the detection limits (see Table 1). Elemental concentrations varied across the sampling sites (Fig. 3). MANOVA indicated that multivariate elemental concentrations differed across the sites ($p < 0.05$). An increase was observed in the mean concentrations for Al, Bi, Cd, Cu, Ni, Pb, V, and Zn in GWB samples (lowest estuarine site), while Mn concentrations were higher in the samples from ALB
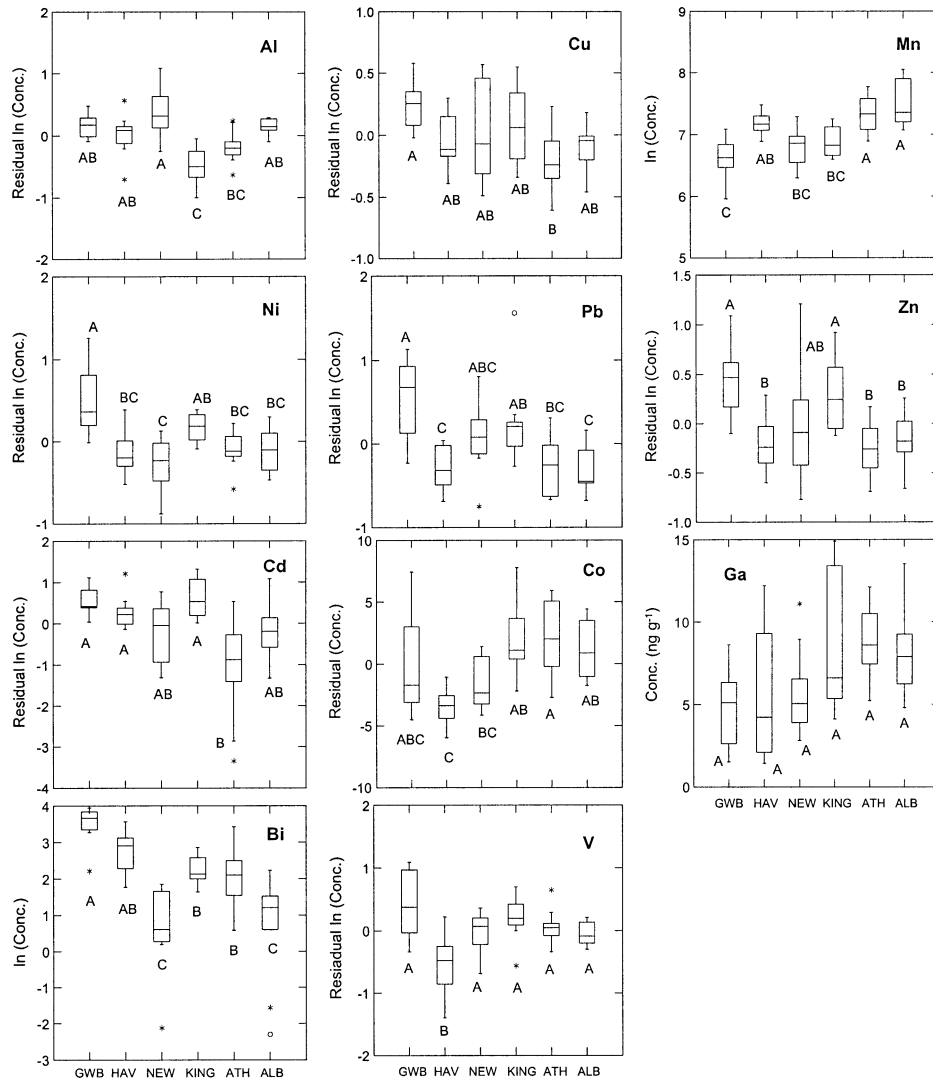
Fig. 4.   Box whisker plots of the elements measured in the otoliths of American eel. Concentrations for Al, Bi, Cd, Cu, Mn, Ni, Pb, V, and Zn are ln-transformed to meet the assumptions of normality. Residuals of transformed data are used for Al, Cd, Co, Cu, Ni, Pb, V, and Zn to compensate for mass effects.

TABLE 3.   Jack-knifed classification matrix from forward stepwise discriminant analysis of elemental data from the otoliths of American eel captured from 6 different locations along the Hudson River estuary. Al, Bi, Cd, Mn, Ni, and V are used in classification.

Site	GWB	HAV	NEW	KING	ATH	ALB	%Correct
GWG	10	0	0	0	0	0	100
HAV	0	8	0	0	2	0	80
NEW	0	0	7	0	2	1	70
KING	0	0	0	10	0	0	100
ATH	0	1	0	1	7	1	70
ALB	0	2	2	0	1	5	50
Total	10	11	9	11	12	7	78

(highest estuarine site). Tukey's HSD (multiple-comparison test) of transformed data (data corrected for skewness and mass effects) indicated that levels of Bi, Ni, Pb, and Zn were significantly higher for GWB than for ALB samples ($p < 0.01$, Fig. 4). Mn was the only element that showed significantly lower concentrations at GWB in comparison to ALB ($p < 0.001$).

Linear discriminant analysis using all elements showed a clear separation among the locations of different salinity (e.g., saltwater, oligohaline, and freshwater groups), while there was an overlap between the locations of similar salinity groups (e.g., HAV and NEW, ATH and ALB) that were separated moderately (Table 3). The forward stepwise procedure indicated that Al, Bi, Cd, Mn, Ni, and
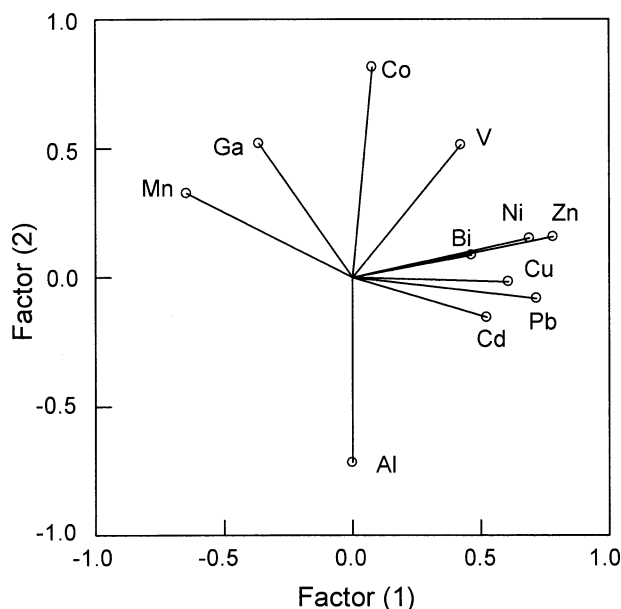
Fig. 5.   Principal component factor loadings plot of the first two eigenvectors for the elements determined in the otoliths of American eel.
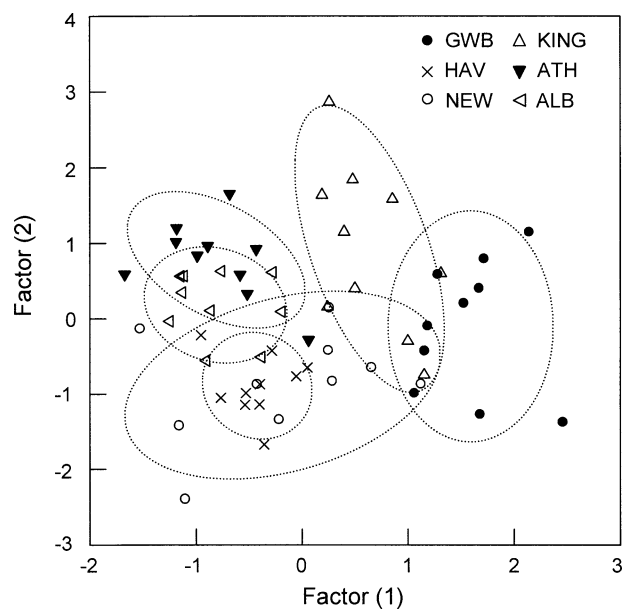

Fig. 6.   Canonical variable plot from the discriminant analysis of elemental fingerprints in the otoliths of American eel. All elements are used in discriminant analysis. Ellipses are 95% confidence intervals for each group.

V contributed significantly to discrimination among groups. Jack-knifed classification matrix (group assignment excluded case being classified) assigned group membership correctly for 100% of GWB and KING groups. Classification success was 80% for HAV, 70% for NEW and ATH, and 50% for ALB groups. Overall classification success was 78%.

In the principal components analysis of all elements, the first two eigenvalues (factors) explained 82% of the total variance. Factor 1 scores received positive weightings from Bi, Cd, Cu, Ni, Pb, V, and Zn and negative weightings from Mn and Ga (Fig. 5). Factor 2 was positively associated with Co and negatively associated with Al. Factor 1 scores were positively associated with the GWB sample and negatively associated with the two most up-estuary samples (ATH and ALB; Fig. 6). Only groups sampled at ATH and HAV showed differences along Factor 2. In general, HAV, NEW, ATH, and ALB groups overlapped in principal component space, while groups from KING and GWB were more discretely clustered.

Discussion
ANALYSIS OF TRANSITION AND HEAVY METALS IN OTOLITHS

Analysis of otoliths for transition and heavy metals by solution-based ICP-MS is confounded not only by low levels of the elements, but also because of the numerous interferences caused by the high levels of Ca matrix. The predominant Ca matrix reduce the capabilities of both quadrupole, including dynamic reaction cell, and high resolution (magnetic-sector) ICP-MS instruments by changing instrument sensitivity (via suppressing or enhancing ion signals or clogging sampling interface) and by producing molecular ions of Ca that overlap with the ions of analyte element (such as ^{43}Ca $^{16}O^+$ on $^{59}Co^+$). Magnetic-sector instruments offer higher resolution over the quadrupole ICP-MS instruments but at the cost of reduced sensitivity. The limits of detection of a magnetic-sector ICP-MS operated at relatively high resolutions to overcome isobaric and molecular ion overlaps may not be very different from those of a quadrupole ICP-MS instrument because of the trade-off between sensitivity and resolution (Newman 1996). Despite the use of a high resolution instrument, for instance, difficulties were encountered in the measurement of several trace elements (Ag, Al, Cd, Co, Ni, Sn, and Zn) in the otoliths of juvenile California halibut *Paralichthys californicus* (Forrester and Swearer 2002) that therefore were not included in the statistical analysis. Dilution of sample solutions can alleviate the problems associated with the instrumental sensitivity to some extent, but not those from isobaric and molecular overlaps since the analyte-to-matrix ratio still remains the same. On the other hand, accuracy and precision may degrade substantially as the elemental concentrations approach the limits of the detection of the instrument due to the dilution. It is essential to

TABLE 4.   Review of reported otolith concentrations ($\mu g\ g^{-1}$) for selected transition and heavy metals from the literature. Included are Campana (1999) and Thresher (1999) reviews of the literature (Ref 1), this study (Ref 2), and literature published after 1999 (Refs 3–9). Concentrations are rounded to the nearest integer and order of magnitude taking into consideration reported variance statistics. References: 3. Geffen et al. (1998), 4. Hanson and Zdanowicz (1999), 5. Milton et al. (2000), 6. Forrester and Swearer (2002), 7. Sanchez-Jerez et al. (2002), 8. Elsdon and Gillanders (2003), and 9. Hanson et al. (2004).

Mn	Al	Co	Ni	Cu	Zn	Cd	Pb	Ref
1–10	1–10	0.1–1.0	1–10	1–10	1–10	1–10	1–10	1
0.5–2	0.1–3	0.005–0.02	0.1–0.5	0.5–2	1.0–5	0.001–0.01	0.02–0.2	2
							0.1–8	3
1–200			0.1–30	0.1–5				4
0.3–3				0.1–3	0.4–3	0.02–0.04	0.03–0.2	5
				0.05–5			0.004–1	6
1–30						0.005–0.05	0.05–0.5	7
4–7								8
0.5–3				0.5–1			0.1–0.2	9

eliminate matrix components without dilution by employing other means of separation (e.g., extraction) for accurate quantification of trace elements in otoliths by ICP-MS.

The results from the elemental analysis of American eel otoliths (Fig. 3) clearly show that solution-based ICP-MS when coupled to solid phase extraction procedures offers the capability for measuring small differences in otolith trace element concentrations. This is because of the substantial improvement in the limits of detection and successful removal of concomitant ions (e.g., Ca) by means of the solid phase extraction procedure. We observed no evidence of Ca interferences in our analysis of the otolith CRM (Table 1) as the values were consistent with those in performed by independent studies (Table 2).

UPTAKE MECHANISMS AND LITERATURE COMPARISONS

Several studies have now shown that uptake rates of Sr and Ba are proportional to water concentrations, but their uptake is inhibited by Ca (Mugiya et al. 1991; Farrell and Campana 1996; Bath et al. 2000; Elsdon and Gillanders 2003; Kraus and Secor 2004). The relationship between other elements, particularly transition and heavy metals, is less clear. Of these, Cu and Pb have shown positive correspondence in several studies. In rearing experiments on barramundi (*Lates calcifer*), a species that inhabits estuaries, Milton and Chenery (2001) observed elevated otolith concentrations of Cu:Ca and Pb:Ca proportional to experimental exposures. Diet enhanced in these metals did not result in increased otolith concentrations. Experiments on Mn:Ca in coastal fishes indicated that uptake in otoliths was not representative of water concentrations (Elsdon and Gillanders 2003). For a California halibut, Forrester and Swearer (2002) observed consistently higher levels of otolith Cu and Pb from fish captured in bays than in ocean environ-

ments and attributed this to higher levels of Cu and Pb in bay habitats. Barramundi captured in a Cu mine outfall did not show elevated Cu:Ca concentrations in comparison to those captured elsewhere (Milton et al. 2000).

In some instances, physiological regulation or diet rather than environmental sources may control less abundant metals (see reviews by Campana 1999 and Thresher 1999). Similar to Milton et al. (2000), Hanson and Zdanowicz (1999) sampled fish across a strong pollution gradient and observed no positive association between water and otolith concentrations of Cu, Ni, and Mn. They attributed this to metabolic regulation. Geffen et al. (1998) reported a generally positive, albeit occasionally nonlinear relationship, between exposure and otolith concentrations for Pb and Hg, which they attributed to physiological state, bioavailability, and possible toxicological response. In an examination of detritus, seagrasses, and a resident fish, Sanchez-Jerez et al. (2002) found course positive correspondence between otolith Mn and Pb and food web detritus sources.

High variability in metals including Cu, Ni, Co, Cd, chromium [Cr], Zn, Ga, Pb, and Hg concentrations were observed in past studies (Geffen et al. 1998; Hanson and Zdanowicz 1999; Milton and Chenery 2001; Forrester and Swearer 2002; Table 4), and have been attributed to measurement imprecision or lack of sensitivity (Milton and Chenery 2001; Forrester and Swearer 2002), heterogeneous distribution within the otolith (Milton et al. 2000; Milton and Chenery 2001), and possible contamination and leaching (Thresher 1999). In this study, we observed high precision ($> 95\%$) in measurements, and observed 11 transition and heavy metals all within detection limits. Through analysis of whole otoliths, we have avoided issues of heterogeneous distribution of elements, e.g., between organic and inorganic components of the otolith or between regions of the otolith (Milton

and Chenery 2001). Through rigorous surface decontamination, we sought to reduce contamination issues (see Secor et al. 2001 and Rooker et al. 2001). Although leaching (loss of analyte element) cannot be discounted, we expect that if leaching did occur, its effects were minimal (see Methods).

Several metals, Co, Cd, and Pb, were substantially less abundant than would have been indicated by recent reviews by Campana (1999) and Thresher (1999). Several papers subsequent to these reviews have indicated similar ranges to those reported here and we expect that these metals occur in ng g^{-1} range rather than the previously expected μg g^{-1} concentrations (see Table 4). Differences between values reported before and after these reviews may reflect improvements in instrument accuracy and standardization. Recent studies with divergent concentration ranges to those reported here include Hanson and Zdanowicz (1999), who reported Ni and Mn values that were one to two orders of magnitude higher for values reported in this study. Geffen et al. (1998) reported Pb levels that ranged 100–800 ng g^{-1} for three coastal species, moderately higher than those reported here (50–160 ng g^{-1}). Like the current study, these studies examined fish across urban pollution gradients and should be comparable in terms of expectations for elevated metal concentrations in otoliths.

TRANSITION AND HEAVY METALS AS TRACERS OF HABITAT USE IN EEL OTOLITHS

Although incorporation of elements into otoliths is a complex process due to influences of biological and physiological factors, the significant differences observed in the elemental composition of American eel otoliths among groups separated by less than 60 km suggest that some transition elements and heavy metals in otoliths do show variability that are useful tracers of habitat use patterns in estuaries.

Metal concentrations of otoliths of eels collected near GWB that were exposed to heavily contaminated sediments showed substantially higher levels than eels collected elsewhere, and indicate that otolith metal levels may be a useful tracer of habitat use in polluted habitats. The lower Hudson River is an urban estuary with high inputs of contaminants from sewage, industry, and maritime commerce. Past industry has also contributed substantial contaminant loads that persist in the estuary. Gibbs (1994) found that the concentrations of Cd, Co, Cr, Cu, iron [Fe], Mn, Ni, Pb, and Zn in the bottom sediments of the lower 50 km of the Hudson River estuary were at maximum levels in the New York Harbor. Chillrud (1996) found a good correlation between a number of trace metals and Cu in sediments of New York Harbor region. By arguing that Cu is a good tracer of municipal wastewater effluent, he concluded that the major source of metals in the lower Hudson River estuary was the wastewater treatment plant effluent. These presumed sources were consistent with generally higher levels of Cd, Cu, Ni, Pb, and Zn found in GWB eel otoliths in comparison to other locations, but were inconsistent with lower levels of Mn observed in these eels and fairly invariant levels of Co across the Hudson River estuary. The PCA analysis also supports the interpretation that heavy metals Cd, Cu, Ni, Pb, and Zn are acting in concert (positive Factor 1 weights) in defining a lower estuarine signal. Weights for Mn and Co are very different suggesting a different source or uptake mechanism (Fig. 5).

Mn generally exhibits a nonconservative behavior and the distribution of this element in estuaries is controlled by physicochemical properties of water (e.g., pH, salinity, temperature, and dissolved oxygen) as well as by several natural processes, such as desorption from suspended matter and redox chemistry (Millward and Turner 1995). Yang and Sanudo-Wilhelmy (1998) found that the levels of dissolved Mn remained relatively constant in the Hudson River estuary over two decades since the first sampling in 1970s, but that for Cd decreased 4-fold over the same period. Their results for Mn indicate that anthropogenic inputs did not have any significant effect on Mn levels, but natural sources including up-estuary watersheds, runoff water, and sediment fluxes contributed positively to dissolved Mn. They also found that the levels of colloidal Mn declined with increasing salinity. Several other studies have also reported similar patterns for Mn with salinity in other estuaries (Sanudo-Wilhelmy et al. 1996; Ouddane et al. 1999). In this study, significant differences in the average otolith concentrations of Mn between saltwater (GWB) and freshwater (ATH and ALB) groups were consistent with these findings (Fig. 3), reflecting exposure to different Mn concentrations.

Cd may indicate behaviors expected for the suite of heavy transition elements that occurred at higher levels in GWB eel otoliths. The 4-fold decline in dissolved Cd concentration within the region of 0 to 30 km of the estuary (Yang and Sanudo-Wilhelmy 1998) is indicative of the reduction in the magnitude of the anthropogenic inputs that they attributed to 30 sewage plants in that region, but also to a battery manufacturer located at river km 100 that was closed in 1979. Cd contaminated soils, originally proximal to the plant are now transported to the lower Hudson River estuary that acts as a sink for up-estuary sources of contaminants. Similarly other metals (Ag, Cd, Cu, Pb, and Zn) and

organic contaminants (aromatic hydrocarbons) in the lower estuary are dominated by water effluent and progressive downstream transport of up-estuary contaminants (Feng et al. 1998; Baker et al. 2001). Elevated concentrations of metals and heavy metals (Al, Bi, Cd, Cu, Ni, Pb, and Zn) in eel otoliths collected near GWB are consistent with higher levels of metals expected due to local industrial and sewage sources, and hydrology and sediment dynamics in the lower estuary causing retention of local and up-estuary derived metals. We concluded that in this application, heavy metal concentrations in otoliths of eels collected at GWB were associated with exposure to those metals in the environment.

Literature Cited

Arslan, Z. and A. J. Paulson. 2002. Analysis of biogenic carbonates by inductively coupled plasma-mass spectrometry (ICP-MS). Flow injection on-line solid-phase preconcentration for trace element determination in fish otoliths. *Analytical and Bioanalytical Chemistry* 372:776–785.

Arslan, Z. and A. J. Paulson. 2003. Solid phase extraction for analysis of biogenic carbonates by electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS): An investigation of rare earth element signatures in otolith microchemistry. *Analytica Chimica Acta* 476:1–13.

Bath, G. E., S. R. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* 64:1705–1714.

Baker, J. E., W. F. Bohlen, R. Bopp, B. Brownawell, T. K. Collier, K. J. Farley, W. R. Geyer, and R. Nairn. 2001. PCBs in the Upper Hudson River: The Science Behind the Controversy. White Paper, Hudson River Foundation, New York.

Bopp, R. F. and S. Chillrud. 2001. Contaminant chronologies. *In* J. Levinton (ed.), The Hudson River Ecosystem. Hudson River Foundation, New York.

Campana, S. E., J. A. Gagné, and J. W. McLaren. 1995. Elemental fingerprinting of fish otoliths using ID-ICPMS. *Marine Ecological Progress Series* 122:115–120.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: Pathways, mechanisms and applications. *Marine Ecological Progress Series* 188:263–297.

Campana, S. E., G. A. Chouinar, J. M. Hanson, A. Fréchet, and J. Brattey. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research* 46:343–357.

Chillrud, S. N. 1996. Transport and fate of particle associated contaminants in the Hudson River basin. Ph.D. Dissertation, Columbia University, New York.

Dovel, W. L., A. W. Pekovitch, and T. J. Berggren. 1992. Biology of shortnose sturgeon (*Acipenser brevirostrum*) in the Hudson River estuary, New York, p. 187–216. *In* L. C. Smith (ed.), Estuarine Research in the 1980's. The Hudson River Environmental Society Seventh Symposium on Hudson River Ecology. State University of New York Press, Albany, New York.

Elsdon, T. S. and B. M. Gillanders. 2003. Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Marine Ecological Progress Series* 260:263–272.

Farrell, J. and S. E. Campana. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comparative Biochemistry and Physiology* 115:103–109.

Feng, H., J. K. Cochran, H. Lwiza, and B. J. Brownwell. 1998. Distribution of heavy metal and PCB contaminants in the sediments of an urban estuary: The Hudson River. *Marine Environmental Research* 45:69–88.

Forrester, G. E. and S. E. Swearer. 2002. Trace elements in otoliths indicate the use of open-ocean versus bay nursery habitats by juvenile California halibut. *Marine Ecological Progress Series* 241:201–213.

Fowler, A. J., S. E. Campana, C. M. Jones, and S. R. Thorrold. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS. *Canadian Journal of Fisheries and Aquatic Sciences* 52:1421–1430.

Geffen, A. J., N. J. G. Pearce, and W. T. Perkins. 1998. Metal concentrations in fish otoliths in relation to the body composition after laboratory exposure to mercury and lead. *Marine Ecological Progress Series* 165:235–245.

Gibbs, R. J. 1994. Metals in the sediments along the Hudson River estuary. *Environmental International* 20:507–516.

Gillanders, B. M. and M. J. Kingsford. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coastal reef populations of a temperate reef fish. *Marine Ecological Progress Series* 141:13–20.

Gillanders, B. M. and M. J. Kingsford. 2000. Elemental fingerprints of otoliths of fish may distinguish estuarine nursery habitats. *Marine Ecological Progress Series* 201:273–286.

Hanson, P. J. and V. S. Zdanowicz. 1999. Elemental composition of otoliths from Atlantic croaker along an estuarine pollution gradient. *Journal of Fish Biology* 54:656–668.

Hanson, P. J., C. C. Koenig, and V. S. Zdanowicz. 2004. Elemental composition of otoliths used to trace habitats of juvenile gag *Mycteroperca microlepis* along the west coast of Florida. *Marine Ecological Progress Series* 267:253–265.

Helfman, G. S., D. E. Facey, L. S. Hales, and E. L. Bozeman. 1987. Reproductive ecology of the American eel. *American Fisheries Society Symposium* 1:42–56.

Kalish, J. M. 1989. Otolith microchemistry: Validation of the effects of physiology, age and environment on otolith composition. *Journal of Experimental Marine Biology and Ecology* 132:151–178.

Kraus, R. T. and D. H. Secor. 2004. Incorporation of strontium into otoliths of an estuarine fish. *Journal of Experimental Marine Biology and Ecology* 302:85–106.

Milton, D. A., C. D. Tenakanai, and S. R. Chenery. 2000. Can the movements of barramundi in the Fly River region, Papua New Guinea be traced in their otoliths. *Estuarine Coastal and Shelf Science* 50:855–868.

Milton, D. A. and S. R. Chenery. 2001. Sources of uptake of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). *Journal of Experimental Marine Biology and Ecology* 264:47–65.

Millward, G. E. and A. Turner. 1995. Trace metals in estuaries, p. 223–245. *In* B. Salbu and E. Steinnes (eds.), Trace Elements in Natural Waters. CRC Press, Boca Raton, Florida.

Morrison, W. E. and D. H. Secor. 2003. Demographic attributes of yellow-phase American eels in the Hudson River estuary. *Canadian Journal of Fisheries Aquatic Sciences* 60:1487–1501.

Mugiya, Y., T. Hakomori, and K. Hatsutori. 1991. Trace metal incorporation into otoliths and scales in the goldfish, *Carassius auratus*. *Comparative Biochemistry and Physiology* 99:327–331.

Newman, A. 1996. Elements of ICPMS. *Analytical Chemistry* 68: 46A–51A.

Ouddane, B., M. Skiker, J. C. Fischer, and M. Wartel. 1999. Distribution of iron and manganese in the Seine river estuary: Approach with experimental laboratory mixing. *Journal of Environmental Monitoring* 1:489–496.

Rooker, J. R., V. S. Zdanowicz, and D. H. Secor. 2001. Chemistry of tuna otoliths: Assessment of base composition and postmortem handling effects. *Marine Biology* 139:35–43.

Sanchez-Jerez, P., B. M. Gillanders, and M. J. Kingsford. 2002. Spatial variability of trace elements in fish otoliths: Comparison with dietary items and habitat constituents in seagrass meadows. *Journal of Fish Biology* 61:801–821.

Sanudo-Wilhelmy, S. A., I. Rivera-Duarte, and A. R. Flegal. 1996. Distribution of colloidal trace metals in the San Francisco Bay estuary. *Geochimica et Cosmochimica Acta* 60:4933–4944.

Secor, D. H. and V. S. Zdanowicz. 1998. Otolith microconstituent analysis of juvenile bluefin tuna (*Thunnus thynnus*) from the Mediterranean Sea and Pacific Ocean. *Fisheries Research* 36:251–256.

Secor, D. H. and J. R. Rooker. 2000. Is otolith strontium a useful scalar of life-cycles in estuarine fishes? *Fisheries Research* 46:359–371.

Secor, D. H., J. R. Rooker, E. Zlokovitz, and V. S. Zdanowicz. 2001. Identification of riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerprints. *Marine Ecological Progress Series* 211:245–253.

Secor, D. H., S. E. Campana, V. S. Zdanowicz, J. W. H. Lam, L. Yang, and J. R. Rooker. 2002. Inter-laboratory comparison of Atlantic and Mediterranean bluefin tuna otoliths microconstituents. *ICES Journal of Marine Sciences* 59:1294–1304.

Thorrold, S. R., C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam. 1998a. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidisima*). *Limnology and Oceanography* 43:1826–1835.

Thorrold, S. R., C. M. Jones, P. K. Swart, and T. E. Targett. 1998b. Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. *Marine Ecological Progress Series* 173:253–265.

Thresher, R. E. 1999. Elemental composition of otoliths as a stock delineator in fishes. *Fisheries Research* 43:165–204.

Warnken, K. W., D. Tang, G. A. Gill, and P. H. Santschi. 2000. Performance optimization of a commercially available iminodiacetate resin for the determination of Mn, Ni, Cu, Cd, and Pb by on-line preconcentration inductively coupled plasma mass spectrometry. *Analytica Chimica Acta* 423:265–276.

Wilkinson, I., G. Blank, and C. Gruber. 1996. Desktop Data Analysis with SYSTAT, 1st edition. Prentice Hall, Englewood Cliffs, New Jersey.

Willie, S. N., Y. Iida, and J. W. McLaren. 1998. Determination of Cu, Ni, Zn, Mn, Co, Pb, Cd, and V in seawater using flow injection ICP-MS. *Atomic Spectroscopy* 19:67–72.

Willie, S. N., J. W. H. Lam, L. Yang, and G. Tao. 2001. On-line removal of Ca, Na, and Mg from iminodiacetate resin for the determination of trace elements in seawater and fish otoliths by flow injection ICP-MS. *Analytica Chimica Acta* 447:143–152.

Yang, M. and S. A. Sanudo-Wilhelmy. 1998. Cadmium and manganese distributions in the Hudson River estuary: Interannual and seasonal variability. *Earth and Planetary Science Letters* 160:403–418.

Yoshinaga, J., M. Morita, and J. S. Edmonds. 1999. Determination of copper, zinc, cadmium and lead in a fish otolith certified reference material by isotope dilution inductively coupled plasma mass spectrometry using off-line solvent extraction. *Journal of Analytical Atomic Spectrometry* 14:1589–1592.

Yoshinaga, J., A. Nakama, M. Morita, and J. S. Edmonds. 2000. Fish otolith reference material for quality assurance of chemical analyses. *Marine Chemistry* 69:91–97.