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A method of stock identification based on the elemental composition of striped bass *Morone saxatilis* (Walbaum) otoliths

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Abstract: Fish otoliths usually are formed by daily increment deposits. They are primarily composed of $CaCO_3$ in the form of aragonite and a protein matrix. Inclusion of elements other than $CaCO_3$ during increment formation provides the potential to discriminate stocks based on elemental composition. Otoliths of young-of-the-year striped bass *Morone saxatilis* (Walbaum) from four tributaries of Chesapeake Bay, U.S., were examined using a scanning electron microscope equipped with an energy-dispersive X-ray analyzer. Preparation for X-ray analysis required otoliths be embedded in contaminant-free resin and ground in a frontal plane until the nucleus was reached. A dedicated microprocessor was used to identify elements and to integrate the peak areas. All data were normalized to an integral calcium peak before statistical evaluation. Elements most often included in the otoliths were: Si, Al, Cl, S, Na, K, Mn, Tc, Cr, Cu, Ra, V, and the lanthanide series elements. Stepwise discriminant function analysis demonstrated each riverine group to be distinct; $>70\%$ of all individual fish could be correctly assigned to stocks based on the composition of their otoliths. Much improved discrimination resulted from broader geographical groupings.

Key words: Chesapeake Bay; Energy-dispersive X-ray microanalysis; *Morone saxatilis*; Otolith composition; Stock identification

INTRODUCTION

Traditional methods used to identify fish stocks include tagging experiments, meristic and/or morphometric indices, and electrophoretic techniques. Recently, chemical differences in hard body parts have been used for the identification of fish stocks. This approach is based on the premise that geographically distinct populations will reflect the chemical constituents of the water in which they reside. For example, chemical differences found in scales (Lapi & Mulligan, 1981) and vertebrae (Mulligan *et al.*, 1983) have been used to identify stocks of Pacific salmon. The present study identifies stocks

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of the anadromous striped bass *Morone saxatilis* (Walbaum) from four tributaries of the Chesapeake Bay, U.S., based on otolith composition. The ability to identify stocks is of critical importance because, in theory, each must be managed separately to optimize its yield. Historically, striped bass have been an abundant, economically important species of the bay and adjoining coastal waters. A recent sharp decline in numbers has focused much effort towards improving its management.

Objectives were to develop techniques to use scanning electron microscopy (SEM) coupled with energy-dispersive X-ray analysis to determine the elemental composition of fish otoliths. Elemental information was then used to identify stocks of striped bass.

Energy-dispersive X-ray analysis has been used to identify elements in such diverse structures as cnidarian statoliths (Chapman, 1985), polychaete jaws (Valderhaug, 1985), and fish scales (Lapi & Mulligan, 1981). Advantages of this method over more conventional techniques of element analysis are expanded here from those noted by Lapi & Mulligan (1981). The method can simultaneously analyze for all elements with an atomic number ≥ 11 (Na) and ≤ 92 (U). It is nondestructive and can analyze a wide range of elemental concentrations. The technique is superior to atomic absorption methods which necessitate ashing the otolith prior to analysis and which can only analyze one element at a time. The method also compares favorably to neutron activation analysis, a destructive, expensive and lengthy procedure. Finally, the technique is better than laser-beam microprobe analysis which can only look at a small fixed area, is sensitive only over a small range of concentrations, and cannot analyze as many elements simultaneously.

Otolith composition has not been used to identify fish stocks but should give more reliable results than studies done on the chemical composition of scales, structures which can be lost and regenerated. In addition, studies of tetracycline retention indicate that components of scales can be lost due to Ca turnover (Koenings *et al.*, 1986). In contrast, otolith formation lends itself well to X-ray microanalysis. Otoliths consist of Ca carbonate crystals of aragonite laid down in a protein matrix of otolin. Their crystalline lattice structure allows inclusion of other elements during increment formation. Any trace elements held in the matrix create a permanent record of the fishes' food and surrounding water because, unlike vertebrate bone, otoliths are not reworked after a layer of bone is deposited.

Some tissues, such as scales, can be examined by energy-dispersive X-ray microanalysis simply by affixing the specimen to a C planchet because the portion to be examined is the surface of the specimen. Otoliths, on the other hand, usually have daily increments added to all surfaces so that the portion to be examined may be hidden from the electron beam. In such cases the specimen must be ground, which necessitates embedding the otoliths in an appropriate medium.

The following traits of embedding media were desirable. Most importantly, the medium should contain no elements which could interfere with analysis of the otolith. The medium should be nontoxic and easy to prepare. The polymerization time must be short so that preparation time is minimized. Medium shrinkage during polymerization should be small and predictable so that otolith orientation can be precise. The medium must be transparent so that the otolith can be seen and monitored during the grinding process. There must be good adhesion to the surface of the otolith so that it does not move during grinding. The medium must be resilient enough to absorb some of the force exerted by grinding, thus reducing the probability of shattering the otolith.

METHODS

Young-of-the-year striped bass were collected by 9.2-m beach seines at single sites on the Potomac, Patuxent, Choptank, and Nanticoke Rivers (Fig. 1) during

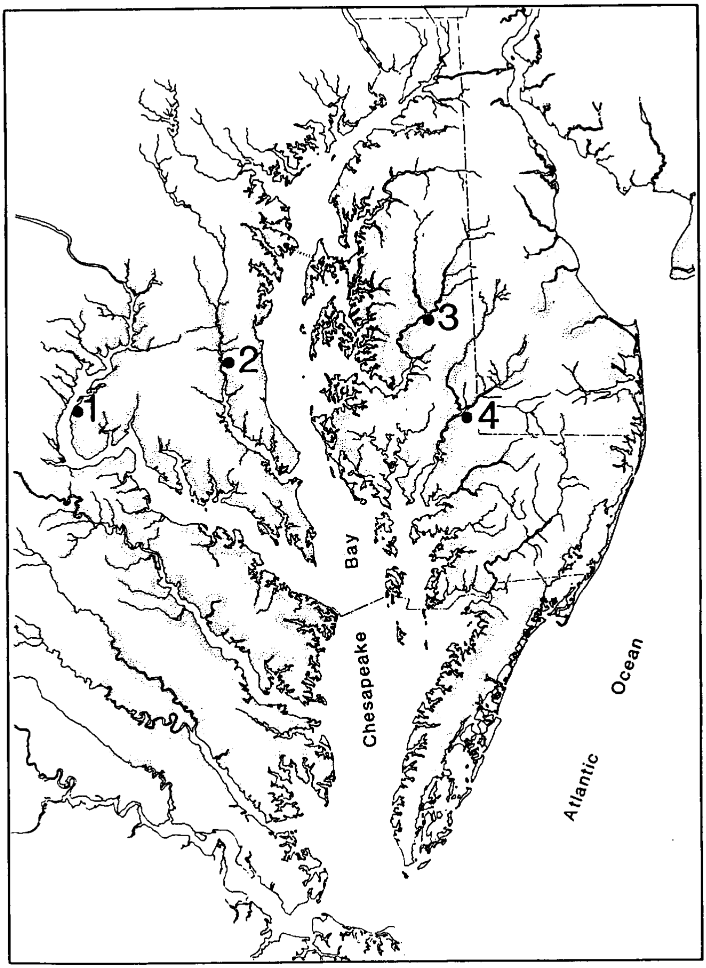

Fig. 1. Map of Chesapeake Bay, U.S. Numeral placement designates collection site on each tributary. 1, Potomac River; 2, Patuxent River; 3, Choptank River; 4, Nanticoke River.

spring–summer 1985. All fish were preserved in 95% ethanol or frozen in the field and brought back to the laboratory for processing. Sagittal otoliths were removed from 20 specimens from each study site and embedded in LR White resin (Polaron Equipment) using silicon flat molds. After curing for $\geqslant 4$ h, embedded otoliths were ground in a frontal plane with number 200, then 400 grain B carbide hand lapstones. Upon reaching the nucleus the otoliths were polished with 0.25-μm diamond paste to ensure a smooth surface. Videomicroscopy was used to monitor these processes because the large image on the monitor facilitated judging the proper degree of grinding and polishing. To expose daily increments, otoliths were etched with 25% acetic acid for 100 s at room temperature, washed in distilled water and oven dried.

Portions of otolith increments 3–5, which were deposited during the early larval stage, were examined under a JEOL JSM-U3 scanning electron microscope equipped with a Tracor Northern energy-dispersive X-ray microprobe for 1000 s live time. The beam current was 215 pA ± 15%. Using this procedure the electron beam of the SEM causes emission of X-rays characteristic of individual elements within the target area of the otolith under examination. The X-rays are converted to proportional voltage pulses. A multichannel analyzer sorts the pulses and the voltage distribution is then displayed on a CRT. A microprocessor is used to store spectra, to identify elements and to quantify the peak areas. A graphite specimen holder was developed and added to the SEM. Use of this holder rather than the standard aluminum holder ensured that no spurious elements were added. The primary beam was set at 25 000 eV. All data were normalized by expressing the net elemental counts of each otolith element as a percentage of the net Ca count for that otolith. Statistical evaluation was by stepwise discriminant function analysis (Lapi & Mulligan, 1981; Cook, 1982; Meng & Stocker, 1984), using computer program BMDP 7M (Dixon *et al.*, 1981).

RESULTS AND DISCUSSION

The LR White resin was unlike traditional embedding compounds (Epon 812, Spurr's, methacrylate, etc.) in that it is totally organic except for trace levels undetectable by X-ray microanalysis. Because microprobe analysis detects only elements with atomic number ≥ 11 those composing LR White do not contaminate the analyses. Elements detected in other media included, Al, Si, Cl, and Ca. Additional advantages of the LR White medium are summarized in Table I. A typical trace from a young-of-the-year striped bass otolith is shown in Fig. 2A. Not surprisingly the elemental peaks are dwarfed by Ca, the principal component of otoliths. Fig. 2B illustrates that the medium and preparation procedures added no detectable elemental contamination to the analyses.

The elements detected from the four study sites are listed in Table II. Attention is called to the lanthanide series elements which are rarely included in estuarine sediment or water checklists although their presence has been reported in the Patuxent River

(Sigleo & Helz, 1981). Weiss (1974) noted that lanthanides act as substitutes or antagonists to Ca in many cellular reactions. This may explain their common occurrence in otoliths. The occurrence of elements in otoliths was compared among rivers. Elements, such as Al, S, Cl, and Hf, occurred consistently in all rivers while others, Ra, Tb, and Sr, showed varying degrees of occurrence (Table II). The percent of otoliths correctly classified to river of origin by discriminant analysis ranged from 60 to 80% (Table III). Elements most important in the analysis were: Na, V, Sr, Sm, Ni, and Tc. There was significant discrimination of elements in otoliths among all rivers, with the best occurring in the Patuxent while the poorest occurred in the Nanticoke. Misidentifications (e.g., three Choptank otoliths classified with those of the Nanticoke and four

TABLE I

A comparison of the four media tested. A plus (+) symbol indicates acceptable qualities while a minus (−) symbol indicates unacceptable qualities. Doubling a symbol indicates the extreme condition.

	LR White	Epon 812	Spurr's	Methacrylate
Safety and ease of preparation	+	−	−	−
Ease of otolith orientation	+	+	+	− −
Polymerization time	+	+	+	+
Shrinkage	+	+	+	− −
Transparency	+	+	+	+
Adhesion	+	+	−	+
Resilience	+	+	− −	+
Elemental contaminants	+ +	− −	− −	− −

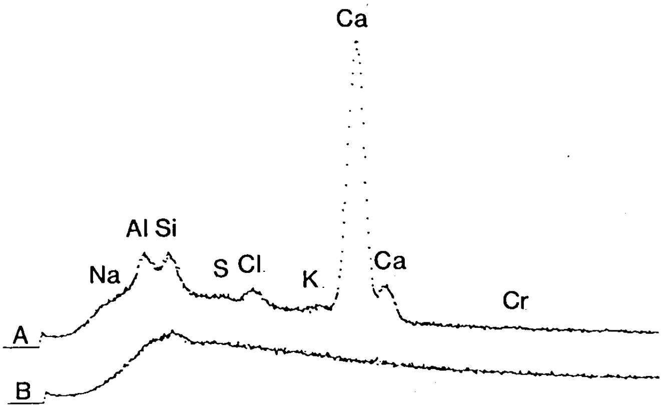

Fig. 2. A, log scale of an energy-dispersive X-ray spectra of a young-of-the-year striped bass otolith accumulated during 1000 s at 25 KeV electron beam accelerating voltage. B, LR White resin blank. The clean spectrum demonstrates the suitability of the resin, preparation procedures, and quality of instrument modifications.

Nanticoke with those of the Choptank) suggested that a broader geographical grouping of rivers might lead to improved discrimination. Data from the Choptank and Nanticoke were combined to form an Eastern Shore group and the Potomac and Patuxent were combined to form a Western Shore group. These groupings improved the classification to nearly 90% (Table III). Elements most important in the area discrimination were: Na, S, V, Fe, Ni, and Tc.

TABLE II

Elements commonly detected in young-of-the-year striped bass otoliths from the Potomac, Patuxent, Choptank, and Nanticoke Rivers. Numbers in table equal the percentage of 20 otoliths in which each element was detected. * Lanthanides.

Element	Potomac	Patuxent	Choptank	Nanticoke	Total
Si	75	85	60	70	73
Al	70	75	65	70	70
Cl	60	55	50	50	54
S	45	50	55	55	51
Na	50	45	50	35	45
K	25	35	50	30	35
Ho*	30	40	50	20	35
Yb*	35	40	30	25	33
Mn	30	15	30	20	24
Tc	35	25	20	10	23
Cr	25	30	10	20	21
Cu	15	20	30	20	21
Er*	25	15	25	20	21
Ra	20	15	5	45	21
V	30	15	10	25	20
Gd*	30	25	5	20	20
Tm*	20	15	15	30	20
Hf	20	20	20	20	20
Os	20	20	25	15	20
Zn	25	5	30	15	19
Fe	10	15	15	30	18
Bi	15	35	15	5	18
Sr	10	10	10	35	16
Ce*	5	25	10	25	16
Se	20	5	10	20	14
Hg	30	10	15	0	14
At	15	20	5	15	14
Ni	15	15	5	15	13
Pr*	20	15	5	10	13
Sm*	15	5	15	15	13
Dy*	25	0	15	10	13
Pa	15	25	5	5	13
Tb*	0	0	20	25	11
U	15	20	5	0	10

In conclusion, the technique is a simple, quick and relatively inexpensive procedure that should prove useful to identify fish stocks. The method also may provide useful information to estimate relative recruitment and rates of mixing in mixed stock fisheries. Other future applications may include evaluations of stocking programs by elemental tagging, determinations of homing accuracy by anadromous stocks and the identification of sea-run stocks of many species.

TABLE III

Classification by discriminant function analysis. $n = 80$. Elements most important in the river and area classifications were: Na, V, Sr, Sm, Ni, Tc, and Na, S, V, Fe, Ni, and Tc, respectively. Discrimination based on net elemental counts expressed as a percentage of net Ca counts for each otolith. See text for details.

Site	Correct (%)	Potomac	Patuxent	Choptank	Nanticoke
Potomac	75.0	15	1	2	2
Patuxent	80.0	0	16	3	1
Choptank	75.0	1	1	15	3
Nanticoke	60.0	4	0	4	12
Total	72.5	20	18	24	18

	Correct (%)	Western	Eastern
Western	82.5	33	7
Eastern	95.0	2	38
Total	88.7	35	45

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