Marking Striped Bass with Rare Earth Elements

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Abstract: Non-radioactive rare earth elements (REE) were evaluated as potential markers in scales of hatchery-reared juvenile striped bass *Morone saxatilis* over a 12-week feeding study. Uptake and retention levels of europium (Eu) and terbium (Tb) detected by neutron activation analyses at below 1 μg/g could be related directly to dietary concentrations of the 2 elements and duration of feeding. Decreased relative concentrations following post-feeding could be related to scale mass increases and the inherent problem with detection techniques which analyze for amounts per unit mass. We compared our 1981 study with more recent similar studies as well as studies using different applications and detection techniques for REE. Low levels of REE uptake, limited availability and high cost of detection technology, masking effects resulting during post-release growth, and potential wild-sources of REE place tagging techniques by feeding REE beyond practicability for most field fisheries studies at the present time.

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Increased recreational and commercial fishing, as well as suspected adverse localized and global impacts from man-caused environmental changes, have refocused interests of biologists on improved tagging and marking techniques to evaluate potential roles for hatchery fish. The non-radioactive rare earth elements (REE) samarium (Sm) and Europium (Eu) have been suggested by Michibuta (1981) as potential and by Kato (1985) as successful markers in enriched diets when fed to fish for 1 to 2 months. However, Sm levels in American shad (*Alosa sapidissima*) and Atlantic salmon (*Salmo salar*) were only slightly higher than in control fish 30 to 60 days after feeding with Sm-enriched diets (Zak 1984). In these 3 studies,

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questions were raised as to absorption pathways, storage sites, biological halflives of the elements, and capabilities of detection techniques.

During 1981, we evaluated the uptake and retention characteristics for rare earth elements Eu and terbium (Tb) when incorporated into experimental diets as markers being fed to fingerling striped bass. The problems encountered during and following our study, as well as the subsequent literature reviewed, caused us to re-evaluate rare earth compounds as candidate chemical marking techniques for fish. This article is intended to provide our insights about the potential uses and problems based upon our 1981 study in relation to similar studies as well as studies using REE in fish under different application and detection techniques.

Methods

Our evaluations on the uptake of Eu and Tb in scales of striped bass involved an 84-day feeding trial using four REE-enriched experimental diets (Table 1) and 2 control diets fed to 100 fingerlings (11.3 ± 2.8 g) in each of 3 replicated tanks supplied with 4 liters per minute of 20° C well water (1.37 hour exchange rate). Experimental and control diets were adjusted at 2-week intervals to maintain feeding rate at 6% of the body weight of the fish. Two fish were removed from each tank at 14, 28, and 84 days during feeding trials and at 240 and 532 days after feeding while being maintained on commercial diets. Replicate scale samples from these fish were collected and the fish were retained frozen.

Table 1. Mean relative concentrations ($\mu g/g$) of europium (Eu) and terbium (Tb) detected by neutron activation analysis in scales of striped bass sampled during (days 28 and 84) and after (days 240 and 530) feeding with 4 REE enriched and control diets.

	Day			
	28	84	240	530
Scale wts (mg)	0.08–0.11	0.22–0.28	0.19–0.94	1.80–3.81
Increase (%)		263%	230%	442%
		Diets $\mu g/g$ REE		
Control				
Eu 0.01	0.05[a]	0.26[a]	0.03[a]	0.01[a]
Tb 0.02	0.11[a]	0.27[a]	0.05[a]	0.03[a]
High Eu diet				
1. Eu 960	0.20	0.51	0.37	0.03
Tb 66	0.08[a]	0.11	0.05	0.02[a]
2. Eu 1329	0.20	0.49	0.23	0.06
Tb 58	0.10[a]	0.12	0.02	0.03[a]
High Tb diet				
1. Eu 140	0.11	0.08	0.03	0.01[a]
Tb 600	0.26	0.72	0.24	0.08
2. Eu 140	0.10	0.15	0.03	0.01[a]
Tb 755	0.19	0.57	0.13	0.03

[a]Not detected above 1-sigma error (SD); therefore values may be lower.

Neutron activation analyses (NAA) were conducted on 3 occasions by personnel of the Phoenix Memorial Laboratory Ford Nuclear Reactor at the University of Michigan, Ann Arbor. Known U.S. Geological Survey rock standards (USGS–GPS–1, USGS–G–2) and blanks were used as checks for peak energy and area for 22 elements. Spectrum detection time varied from 30 to 100 minutes. One-sigma (SD) levels, calculated as the square root for that element against the background level of all other radiation at the detection peak, varied over all scale samples. Skoog (1985) reported possible sensitivities of 5 pg for Eu and 50 mg for Tb. The actual sample composition, especially without chemical separation, as well as the blank signal affect the prior detection limits (Heydorn 1984).

Scales of striped bass, fed high levels of Eu and Tb for 84 days, were examined by personnel of Mississippi State University Electron Microscope Center using an electron scanning microscope with x-ray spectrometer (ESM) operating at accelerating voltages of 10, 15, 20, and 30 kv and computer analyzed at 30° to the detector for 2 hours when examining whole scales as well as recent scale edge growth zones. Although Skoog (1985) suggests that electron spectroscopy information is restricted largely to surface layers, Postek (1980) indicated that x-ray production initially increased with specimen thickness. The voltage and analysis times used in our study would insure x-ray penetration and analyses to variable depths. Skoog (1985) suggested that x-ray photoelectric spectroscopy for qualitative analysis can resolve peaks leading to unambiguous identification if the element is present in concentrations greater than 0.1% (100 μg).

Secondary ion mass spectroscopy (SIMS) analysis of striped bass scales by Dr. George Morrison, Department of Chemistry at Cornell University, Ithaca, New York, was prevented by charged ion buildup on scale surfaces. Scales ashed under vacuum could not be maintained as to position nor shape when the vacuum was released; therefore, SIMS proved impractical for whole scale analyses.

Results and Discussion

Relative concentrations of Eu and Tb in striped bass scales detected by NAA increased over feeding time (day 28 and 84) in relation to the levels of REE in experimental diets (Table 1). Accuracy of values not detected above 1-sigma error cannot be verified and actual values may be much less than listed value. Differences between relative concentrations detected in scales from high REE and low REE diets was 4 to 5 times, whereas differences in diet levels were 8 to 10 fold. Detected levels remained below 1 μg/g as other researchers (Miller 1963, Anonymous 1974, Shibuya 1979, Zak 1984, Kato 1985) have generally found when examining bony substances during and following feeding trials. It does not appear possible to increase REE levels much above 1 μg/g in bones when feeding REE salts or oxides.

Retention of REE within the bony matrix as fish grow has been a matter of concern if absorped REE are to be useful longterm markers. Michibuta (1981) stated that the mechanism for the retention of Sm remains to be resolved even

though he detected by NAA more than 1 μg/g dry weight in goldfish 1 year following aquarium feeding trials. He demonstrated a relatively steady decrease in Sm concentration over the 360 days after labeling, but he did not provide complimentary growth data for the post-feeding of Sm. Kato (1985) showed that the relative decrease in Eu concentrations in chum salmon scales was proportional to body growth, and presumedly with scale growth, over the 24 days following release. Detailed analysis of scale weight changes when compared to ratios of Eu and Tb concentrations (μg/g) over time (Table 1) shows closely proportional decreases over the post-feeding samples between days 84, 240, and 530. This decrease in the relative concentrations of REE (μg/g scale) with scale growths is an inherent problem with detection techniques (NAA, MAS, etc.) which analyzes for amounts per unit mass.

Body and scale growth following release of the marked fish adds another factor in the detection of the marked fish among wild stocks. Kato (1985) is the only investigator to present comparable REE data for wild fish. We soon realized the need to evaluate background concentrations in hatchery-reared and wild striped bass from other locations. The higher levels reported (Table 2) for striped bass from Edenton Hatchery could cause separation problems for comparisons with experimental marked fish. The accuracy in detection of the Edenton samples was not as reliable since Edenton values were not detectable above one-sigma error level, but neither were any of the wild fish samples. However, only the samples from higher REE feeding level (Table 1) remained reliably detectable 1 year after the 1981 feeding trials. Even though REE in scales from adult wild stocks (Table 2) were generally 10 times below those in scales from experimental fish (Table 1), the scales were much larger from wild fish. The possibility for continued low-level uptake by wild stocks as well as unusual point-sources cannot be totally dismissed. Lapi and

Table 2. Average levels of europium and terbium measured in scales of striped bass from hatcheries (Gulf Coast Laboratory, Miss.; Edenton, N.C.; Marion, Ala.; and Welaka, Fla.) and from wild stocks (Toleda Bend, La.; Potomac River, Va.; C&D Canal, Md.; and Hudson River, N.Y.). All detection levels were below 1-sigma error by neutron activation analysis.

Sources	Scale weight (mg)	Europium		Terbium	
		μg/g	mg/scale	μg/g	mg/scale
Hatcheries					
Gulf Coast	0.06	0.067	0.004	0.131	0.008
Edenton	0.31	0.367	0.114	0.639	0.198
Marion	0.40	0.053	0.021	0.092	0.037
Welaka	4.66	0.002	0.007	0.041	0.189
Wild Stocks					
Toleda Bend	31.30	0.002	0.046	0.006	0.191
Potomac River	17.47	0.003	0.236	0.007	0.129
C&D Canal	5.76	0.003	0.014	0.009	0.054
Hudson River	6.12	0.003	0.015	0.009	0.057

Mulligan (1981) used ESM differences in chemical composition of freshwater growth regions of scales from adult sockeye salmon (*O. nerka*) to classify their lakes of origin based upon elemental scale composition.

Since a simple presence or absence criteria would be highly desirable, we attempted in 1986 to learn whether concentrated Eu or Tb zones were deposited during feeding and post-feeding growth of scales as has been found in fish fed tetracyclines (Bilton 1986). Earlier NAA analyses had detected less than 1 μg/g (Table 1) Eu and Tb in the whole scales but we hoped to see whether higher concentrations could be detected by SEM near recent growth zones of scales collected on day 84. Our unsuccessful efforts, with SEM x-ray electron probe and with SIMS, indicated Eu and Tb concentrations were below milligram (0.1%) levels in recent growth zones.

Our experimental feeding trials as well as those by Kato (1985) and Zak (1984), demonstrated that feeding diets enriched with 500 to 1,000 μg/g REE salts resulted in detection at less than 1 μg/g REE depositions in scales of fish. Subsequent growth of test fish caused dilutions of those relative concentrations by increased scale mass. Kato (1985) apparently has been able to overcome this dilution and masking effect for Eu in chum salmon scales by allowing scales to cool for a 6-month period following thermal neutron flux of 8×10^{13} neutrons/cm^{2}s for 20 minutes. The longer cooling time reduced background radiation, especially from ^{45}Ca. Although we were not aware of Kato's (1985) techniques and results when securing NAA services in 1983, we cannot be assured that the small differences noted between treatments in 530-day samples (Table 1) could be considered valid following additional growth when compared with wild stocks at later time intervals (Table 2). Our failure to locate Eu or Tb concentration by SEM x-ray or SIMS techniques eliminated the possibility of a distinctive concentrated REE zone in scales from treated fish as demonstrated on vertebrae of chum salmon by Bilton (1986) following feeding of oxytetracycline in the diet for 14 to 21 days.

Although an ideal method for marking any fish is probably illusory (Laird and Scott 1978), evaluation of older as well as new techniques continues to evolve with improved technology. Wydoski and Emery (1983), mentioning metallic compounds and radioisotopes as methods of marking by immersion, injection, tattooing, and feeding presented some favorable but mostly unfavorable evaluations. The array of analytical methods available for laboratory analyses of the structural, physical, chemical, and molecular properties of biological materials have increased to a bewildering degree (Skoog 1985). Detection limits depend upon the sensitivity of the instrument, operator skills, as well as the materials being analyzed. According to Skoog (1985:5), "Data from physical measurements are always plagued by uncertainties or errors. . . . The work required in evaluating the quality of data is frequently comparable to the effort that goes into obtaining them. Ultimately, the scientist can only make a judgement as to the probable accuracy of a measurement; with experience, judgements of this kind tend to become rather harsher and less optimistic."

Our experiences over the past 5 years and in evaluating other recent studies lead us to be less than optimistic that high enough REE concentrations can be absorbed from diets and subsequently deposited in fish scales to be readily detectable in released fish several years later unless very highly specialized analytical detection facilities (Kato 1985) are dedicated to the researchers' efforts. Investigations on Tb uptake and detection of Tb in walleye (*Stizostedion vitreum*) eggs and larvae using dye-laser technology have demonstrated detection sensitivity at 1 to 10 pg (ppb) concentrations but color interferences were reported occurring in sample analyses (R. H. McWilliams, pers. commun., Fish. Res. Biol., Iowa Conser. Comm., Spirit Lake, 1985). Application of dye-laser technology would have to be tested using striped bass scales from various size fish before the possibilities for applying its increased sensitivity (Gustafson and Wright 1979) could be evaluated as applicable for the microgram per gram levels of REE retained in experimental-fed striped bass. To date, much of the newer, highly sensitive detection technology is located in specialized research laboratories dedicated to other research efforts and is not readily available to most fisheries biologists. Therefore, from our viewpoint, the ultimate potential tagging procedure of incorporating REE in bony structures of hatchery-reared fishes remains beyond practicability for most fisheries field studies.

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