Regulation of Calcium and Strontium Deposition on the Otoliths of Juvenile Tilapia, *Oreochromis niloticus*

Jacqueline Farrell and Steven E. Campana

MARINE FISH DIVISION, BEDFORD INSTITUTE OF OCEANOGRAPHY,
P.O. BOX 1006, DARTMOUTH, NOVA SCOTIA, CANADA B2Y 4A2

ABSTRACT. Radioisotopes of calcium and strontium were used to test for a relationship between the environmental concentrations of each element and their respective deposition rates on the otolith. On the basis of ^{45}Ca and ^{89}Sr assays, the rate of strontium deposition on the otolith was influenced by strontium concentrations in the water, while the rate of calcium deposition was affected neither by food nor water calcium concentrations. Thus, the deposition rate of strontium on the otolith at least partially reflects environmental availability, while that of calcium does not. Sources in the water contributed 75% of calcium and 88% of strontium to the sagittae of Nile tilapia, *Oreochromis niloticus*, with the remainder being provided by the diet. COMP BIOCHEM PHYSIOL 115A;2:103–109, 1996.

KEY WORDS. Calcium, strontium, otolith, radioisotope, trace element

INTRODUCTION

The interpretation of otolith chemistry is becoming increasingly popular for use in reconstructing the environmental history of fish and in discriminating among geographically distinct fish stocks (7,10,11,25). The major assumption underlying these applications is that differences in the physical and chemical environment will be reflected in differences in otolith chemical composition (6,8,11,13,37). Inherent in this assumption is that dietary contributions to elements deposited on the otolith are minor relative to those from branchial uptake. Since teleosts regulate plasma concentrations of those elements that are physiologically important (34) and are presumed to do the same for the endolymph (18), the uptake and deposition of non-essential trace elements on the otolith may not be controlled as strictly as those of physiologically important elements. Instead, concentrations of trace elements on the otolith are believed to depend on the environmental availability of these elements. Little, however, is known about either the actual source(s) or the physiological regulation of trace elements deposited on the otolith.

Much of the work in otolith trace element analysis has focused on strontium, and a number of factors affecting its deposition have been identified. These factors include salinity (17,29,41), temperature (28,38,39,40), growth rate (16,30,31), developmental and reproductive stage (16,17)

and water chemistry (10,13,25). However, information on the effects of the absolute environmental concentration of any trace element, including strontium, on its deposition in the otolith is limited. Gauldie *et al.* (12) and Tzeng and Tsai (41) found that strontium concentrations on the otolith were positively correlated with those in the ambient environment. Based on the difference in strontium content between salt and freshwater and the assumption that this difference is reflected on the otolith, Kalish (17) used otolith strontium concentrations to differentiate between the progeny of sympatric anadromous and nonanadromous salmonids. Similarly, Secor (33) examined anadromy in striped bass (*Morone saxatilis*), and Limburg (20) used otolith Sr:Ca ratios to estimate the date of movement of juvenile American shad (*Alosa sapidissima*) from freshwater into saltwater. It is not clear, however, if otolith concentrations of a calcium analogue such as strontium vary directly with strontium availability in the environment or if they are indirectly regulated in association with calcium uptake and deposition on the otolith.

Few studies have dealt with the source(s) of strontium incorporated in the otolith. Although they did not consider otoliths separately, both Berg (3) and Schiffman (32) found that water was a more important source of total strontium for goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*), respectively. Hoff and Fuiman (14) suggested that diet contributed little to otolith strontium in red drum (*Sciaenops ocellatus*) raised at a salinity of 30 ppt. A strontium-enhanced diet did, however, produce a significant increase in Sr:Ca ratio on the otoliths of American shad (*Alosa sapidissima*) held in freshwater (20). It is not known if the dietary contribution to otolith strontium is

more important for freshwater than for saltwater fish, nor is it clear what proportion of otolith strontium is actually obtained from the diet.

The purpose of the present study was to test the assumption that otolith elemental composition reflects variations in the trace element concentrations of the environment. We first investigated the contributions made by aquatic and dietary sources to calcium and strontium deposited on the otoliths of a freshwater fish, Nile tilapia *Oreochromis niloticus*. Through comparison of an essential element (calcium) with that of a nonessential trace element and calcium analogue (strontium), we then considered the relationship between the environmental availability of each element and its deposition rate on the otolith.

MATERIALS AND METHODS

Larval *O. niloticus* were obtained from the tilapia hatchery at Dalhousie University, Nova Scotia and reared in freshwater in 38-l aquaria to approximately 3 cm standard length (SL) for the Source experiment and 2 cm SL for the Availability experiment. Ten percent of the water in each aquarium was replaced every 3 days. During the experimental period, fish were maintained at a water temperature of 28.0°C under a 13L:11D photoperiod and fed once daily with tilapia pellets (Corey Feeds, New Brunswick), unless otherwise stated.

Source Experiment

The purposes of this experiment were: (a) to determine the source(s) (aquatic and/or dietary) of otolith calcium and strontium, and (b) to estimate the relative contributions from each of these sources to otolith calcium and strontium.

To determine if water was a source of otolith calcium and/or strontium, an average of 14 fish having a mean ($\pm$ SE) SL of 3.4 $\pm$ 0.6 cm was randomly assigned to each of four aquaria. Two of these aquaria formed a 48-hr nonradioactive water treatment (control 1) and the remaining two formed a radioactive water treatment.

Water in the radioactive treatment contained 1.7 μCi $^{45}Ca \cdot l^{-1}$ and 2.5 μCi $^{89}Sr \cdot l^{-1}$. To avoid intestinal uptake of ^{45}Ca and ^{89}Sr from water-contaminated food, fish in this treatment were not fed during the exposure period.

After 48 hr, control and treatment fish were removed from their respective tanks, anaesthetized in nonradioactive water containing 0.3 g methanesulfonate salt (MS-222) $\cdot l^{-1}$ and rinsed thoroughly under running water to remove surface contamination. Campana (5) estimated that 47% of the ^{45}Ca taken up from water by coho salmon (*Oncorhynchus kisutch*) was deposited in the 12 hr following transfer from radioactive to nonradioactive water. A lag time in ^{45}Ca (and ^{89}Sr) deposition on the otolith was also presumed to occur in tilapia. Therefore fish were transferred to holding tanks containing uncontaminated freshwater for another 48 hr. Both control and treatment fish were fed

during this time. Fish were then removed from their respective tanks, anaesthetized in de-oxygenated water, killed by a blow to the head and frozen for a maximum of 48 hr prior to removal of sagittal otoliths.

To determine if food was a source of otolith calcium and/or strontium, an average of 12 fish having a mean ($\pm$ SE) SL of 3.7 $\pm$ 0.6 cm was randomly assigned to each of four replicate aquaria. Two of these aquaria formed a 48-hr nonradioactive food treatment (control 2) and the remaining two formed a radioactive food treatment.

Fish in the radioactive food treatment were fed once daily with radioactive pellets (preparation described below). Fish in the control were fed once daily with nonradioactive food, and an equivalent ration of radioactive pellets enclosed in a mesh bag was inserted in each aquarium for the duration of the feeding period. Fish were separated from the bag by a screen cage that prevented them from ingesting the radioactive pellets. This served as a control for branchial uptake of ^{45}Ca and ^{89}Sr from food-contaminated water. At the end of the exposure period, fish from both treatments were transferred to uncontaminated holding tanks for 48 hr and sacrificed as described above.

Sagittal otoliths from fish in all treatments and controls were removed and rinsed in deionized water to wash off any tissue and/or surface radioactivity, then air-dried for 24 hr at room temperature. The paired sagittal otoliths from an individual fish were stored as a single sample. All otoliths were weighed to the nearest 0.01 mg, then dissolved as per Mahin and Lofberg (21). The protocol was modified slightly by substituting 1 M nitric acid for perchloric acid. Scintillation cocktail (10 mL of Aquasol-2 (New England Nuclear, Ontario)) was then added to each experimental otolith and to five replicate analytical blanks containing otoliths from fish reared in nonradioactive water.

Radioactivity on the otolith was determined using a Beckman LS 5000 CE liquid scintillation counter programmed for dual-label counting of ^{45}Ca and ^{89}Sr. Activity levels were measured as disintegrations per minute (DPM) and were automatically corrected and compensated for radioactive decay and quench during the counting period. The analytical blanks were counted first as a measure of background radiation, and their average DPM for ^{45}Ca and ^{89}Sr subtracted from each sample.

Availability Experiment

The purpose of this experiment was to determine if the deposition of calcium and strontium on the otolith reflected the environmental concentration of these elements. A factorial design involving two levels (background and enhanced) of calcium and strontium and two elemental sources (water and food) was used (Table 1). An average of nine fish having a mean ($\pm$ SE) initial SL of 1.8 $\pm$ 0.62 cm was randomly assigned to each of the two replicate aquaria per 30-day treatment.

At the end of the rearing period, fish were anaesthetized

TABLE 1. Treatment conditions for juvenile tilapia reared under background (B) and enhanced (E) concentrations of calcium and strontium in two sources: water (W) and food (F)

Treatment	Water		Food	
	Calcium (μg/L)	Strontium (μg/L)	Calcium (mg/kg)	Strontium (mg/kg)
BW-BF	7,250	18	9,270	16
BW-EF	7,250	18	79,800	280
EW-BF	125,000	2,100	9,270	16
EW-EF	125,000	2,100	79,800	280

in water containing 0.3 g MS-222-l^{-1}, sacrificed as above and frozen. Sagittal otoliths were then removed and weighed. Degens et al. (9) estimated that $CaCO_3$ composes 90% or more of otolith mass. Otolith weight was therefore used as an index of otolith calcium content. Otoliths were dissolved as above and 3.4 ml of distilled water added to each sample. A Techtron Atomic Absorption-100 spectrophotometer employing an air-acetylene flame was used to analyze otolith strontium content. The HNO_3-H_2O_2-H_2O mixture in which otoliths were dissolved was used as a blank. Signals were then converted to elemental concentrations (in ppm).

Food Preparation

Food containing background levels of calcium and strontium (9,270 ppm and 16 ppm, respectively) was made by finely grinding tilapia pellets, re-hydrating them with deionized water and blending to form a homogeneous paste. This mixture was then extruded, air-dried at room temperature for 24 hr and cut into pellets with a diameter and texture similar to those of the original Corey pellets. Food was stored frozen.

Food containing enhanced levels of calcium and strontium was prepared and stored as above. However, analytical grade $CaCl_2$.$2H_2O$ (BDH Chemicals) and $SrCl_2$.$6H_2O$ (Fisher Scientific Company) crystals were added to the ground pellets before moistening with water such that calcium and strontium levels in the final preparation were 79,800 ppm and 280 ppm, respectively. Background and enhanced elemental levels in food (Table 1) were determined by atomic absorption (AA) spectrophotometry. Food was ashed and digested with HF acid prior to analysis.

Radioactive food containing background levels of calcium and strontium was prepared and stored as above. However, aqueous $^{45}CaCl_2$ and $^{89}SrCl_2$ were added to the ground pellets such that the mixture contained 0.30 μCi ^{45}Ca and 0.43 μCi ^{89}Sr per g food.

Water Preparation

The calcium and strontium concentrations of dechlorinated freshwater (7.25 ppm and 0.02 ppm, respectively), determined by AA, were adopted as background levels. Freshwater with enhanced levels of the two elements was prepared by adding $CaCl_2$.$2H_2O$ and $SrCl_2$.$6H_2O$ crystals to freshwater such that calcium and strontium levels were 125.0 ppm and 2.1 ppm, respectively (Table 1). Ten percent of the water in each aquarium was replaced with water of equivalent elemental concentration every 3 days.

Statistical Analysis

All data were tested for normality and homogeneity of variance by the Kolmogorov-Smirnov (Lilliefors) test and the Levene statistic, respectively. A significance level of $p \leq 0.05$ was used in all tests. Mean values are reported $\pm$ SE, unless otherwise indicated.

Mean otolith ^{45}Ca and ^{89}Sr activity of the treatment and control groups for the Source experiment were compared using approximate one-tailed t-tests based on unequal variances (35). Data from replicate tanks were not pooled.

Individual contributions to otolith calcium from aquatic and dietary sources were estimated using mean data from control 1 fish reared under background elemental levels in the Source experiment. The following calculations were used for each source:

$$C = U * Ca \tag{1}$$
$$U = (^{45}Ca \text{ DPM on otolith})/(^{45}Ca \text{ DPM in source}),$$

where U = uptake efficiency, C = calcium contribution from source (mg), and Ca = total calcium (mg) in source. The ratio of C_{water}/C_{food} was then calculated and converted to a percent contribution from each source to the total otolith calcium deposited during the experimental period. A similar set of calculations was used to estimate the percent contribution to otolith strontium from each source.

Treatment group means in the Availability experiment were compared for differences in overall growth and final otolith weight using nested one-way analysis of variance of ln-transformed data. Replicate tanks were nested within the treatment factor. Differences in otolith strontium deposition were analyzed by comparing treatment slope contrasts of final otolith Sr versus final otolith weight. Tank effects were non-significant and data from replicate tanks were pooled.

RESULTS
Source Experiment

^{45}Ca and ^{89}Sr activity on the otolith were significantly higher for fish reared in radioactive water and for those fed radioactive food than for their respective non-radioactive controls (Table 2). Since ^{45}Ca and ^{89}Sr act as tracers for their non-radioactive counterparts, these results indicate that both water and food are sources of otolith calcium and strontium in O. niloticus. However, water was the more important source of both elements, contributing 75.5% of cal-

TABLE 2. Activity of ^{45}Ca and ^{89}Sr incorporated into each pair of juvenile tilapia sagittal otoliths as a result of exposure to either radiolabelled water or food (Source experiment)

Treatment	^{45}Ca DPM	^{89}Sr DPM
Control 1 (nonradioactive water)	24.4 ± 6.2	4.6 ± 3.2
Radioactive water	4232* ± 395	1427* ± 105
Control 2 (nonradioactive food)	19.2 ± 5.9	9.0 ± 2.0
Radioactive food	187.9* ± 26.7	171.5* ± 21.1

Mean ± SE, n = 2 tanks with 13–15 fish per tank.
*Significantly different (p < 0.05) from control.

cium and 88.0% of strontium deposited on the otolith during the experimental period.

Availability Experiment

After the one-month experimental period, there were no significant differences in final SL between treatment groups, indicating that neither calcium nor strontium concentrations affected growth. Mean growth was 0.85 ± 0.07 cm over 30 days, giving a mean SL of 2.7 ± 0.66 cm at the end of the experiment.

There were no significant differences in the final otolith weight (used as a proxy for calcium content) between treatment groups, indicating that the rate of calcium deposition on the otolith was not affected by the calcium concentration in either the water or the food (Fig. 1). Mean otolith weight was 0.5528 ± 0.0353 mg at the end of the experiment. Therefore, calcium deposition on the otolith appears

FIG. 2. Concentrations of strontium in the otolith after one month of rearing in background (B) and enhanced (E) concentrations of calcium and strontium in two sources: water (W) and food (F). The slope and intercept for each treatment are as follows: BW-BF: 0.9534, 0.0552; BW-EF: 0.9914, 0.0139; EW-BF: 1.4298, −0.0126; EW-EF: 1.3182, 0.0616. Slopes for treatments EW-BF and EW-EF are significantly different (p < 0.05) from those of treatments BW-BF and BW-EF.

to be strictly regulated by O. *niloticus*, and this regulation applies to calcium from both aquatic and dietary sources.

Strontium deposition on the otolith was affected by strontium concentration in the water, but not by that in food (Fig. 2). For a given otolith weight, otoliths from fish reared in treatments with enhanced water strontium (EW-BF and EW-EF) contained significantly more strontium than did those from fish reared in treatments with background water strontium (BW-BF and BW-EF). Therefore, water strontium concentrations appear to play a significant role in determining otolith strontium content, whereas food strontium concentrations do not.

DISCUSSION

A number of studies have examined calcium deposition on otoliths from water (5,15,23,24). In general, fish obtain calcium primarily through the gills (34), and there does not appear to be a different uptake mechanism for otolith calcium. Our results show that aquatic sources contributed approximately 75% of otolith calcium in O. *niloticus*. This is similar to Berg's (3) estimate for goldfish (*Carassius auratus*), where water contributed 80% of total calcium under background food and freshwater conditions.

Physiological regulation of otolith calcium seems intuitive. Given the importance of the otolith in gravity and sound perception (36), otolith size must be regulated by fish. One way to achieve this is to control the deposition rate of the major component of otolith mass, calcium carbonate,
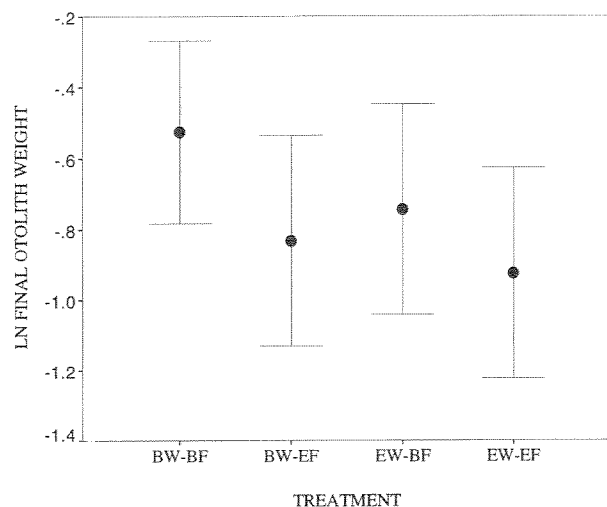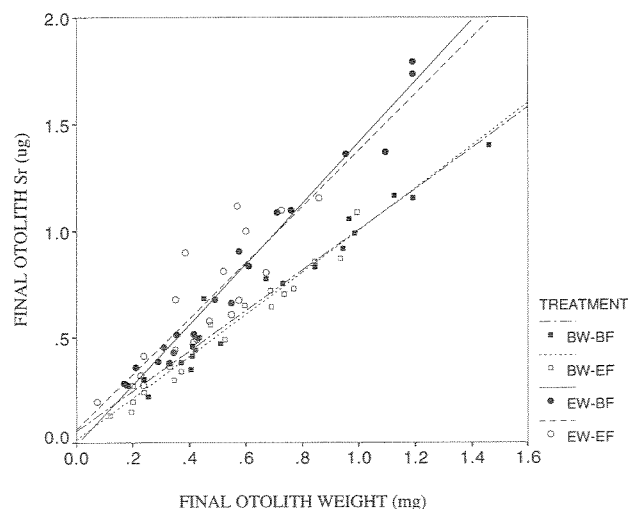

FIG. 1. Final otolith weight (mg) for juvenile tilapia reared under background (B) and enhanced (E) concentrations of calcium and strontium in two sources: water (W) and food (F). Means with 95% CI are shown for ln-transformed data: differences between means are not significant. Otolith weight was used as a proxy for otolith calcium content.

via the regulation of calcium incorporation. Control of calcium levels in both teleost blood plasma (34) and endolymph (16) does occur. Mugiya (23) has shown that calcium is transported by the plasma to the endolymphatic sacs and secreted by the macular cells into the fluid surrounding the otolith. Calcium carbonate then precipitates onto the otolith protein matrix. He also suggested that control of endolymph calcium concentrations may occur through a combination of regulatory processes that affect both plasma calcium levels and secretion of calcium by cells in the inner ear, including those of the maculae. Although the precise nature of regulation remains to be clarified, the absence of a significant effect of calcium availability on otolith size supports the concept that the latter may be carefully controlled through the physiological regulation of calcium incorporation.

Strontium substitutes for calcium in the otolith much more easily than do many other trace elements. This is due to similarities in ionic radius (0.113 Å for Sr^{2+} and 0.099 Å for Ca^{2+}), valence (2^+) and in chemical properties between the two elements (4). Strontium, however, is much less abundant in freshwater than is calcium (in this experiment, water strontium was 0.02 ppm versus 7.25 ppm for calcium) and is not an essential element for fish. It has been suggested by Thresher et al. (37) that strontium uptake is less likely to be physiologically regulated than an essential element but may be affected by a number of constraints, including factors controlling calcium concentration. At the strontium concentrations in this study, the amount of strontium deposited on the otolith did vary with the environmental concentrations of strontium in water. The significant effect of water strontium concentration on strontium uptake is consistent with our estimate that 88% of otolith strontium is derived from aquatic sources. Our results support the idea, then, that strontium deposition on the otolith is less strictly controlled than is that of an essential element such as calcium. It is not clear, however, if otolith strontium varies directly with strontium availability in water or if it is a function of water Sr/Ca ratios.

If the uptake of a calcium analogue such as strontium is indirectly regulated as a consequence of calcium regulation, an apparent effect of absolute strontium availability on otolith uptake may in fact be due to the Sr/Ca ratio of the source. If so, then the assumption that strontium is one of the elements that varies directly with environmental availability may be invalid. In this study, both calcium and strontium concentrations were increased/decreased simultaneously. Our experimental design therefore could not differentiate between the effects of relative versus absolute water strontium availability on otolith strontium incorporation. Treatments with enhanced water strontium contained over one hundred times more absolute strontium (Table 1), as well as seven times more strontium relative to calcium, than treatments with background water strontium (Sr/Ca ratio of 0.0077 for enhanced water strontium vs.

0.0011 for background water strontium, where Sr/Ca ratios were calculated as normalized mole fractions). We note, however, that the rate of enhanced strontium uptake on the otolith was only 30–40% higher than that of background, and this was lower than that expected of either relative or absolute availability effects. Lehtonen et al. (19) suggested that for a given water ^{85}Sr concentration, experimental whitefish (Coregonus lavaretus) accumulated more ^{85}Sr than individuals in preliminary trials because the experimental incubation water contained a lower calcium concentration than that used in preliminary tests. Rieman et al. (29) emphasized the importance of detailed water chemistry when applying otolith microchemistry to discriminate fish of resident and anadromous origin. They found that the Sr/Ca ratios of lakes varied considerably and that this variation was reflected in the otolith strontium content of sockeye salmon (Oncorhynchus nerka). Furthermore, they observed that resident O. nerka from Altura Lake had otolith Sr/Ca ratios that were similar to those of fish of anadromous origin in other lakes. Although the strontium concentration of saltwater was not specified, it was presumably significantly higher than that of the lakewater. This suggests that otolith strontium concentrations may be indirectly regulated in terms of a Sr/Ca ratio rather than absolute strontium availability. Using otolith strontium as one of the trace elements to differentiate between geographically distinct fish stocks would therefore only be applicable if the Sr/Cr ratios differed between stock areas (or if other factors also influenced strontium uptake). Further studies on otolith strontium, in which water calcium is kept constant but absolute strontium concentrations are varied, are needed to distinguish between the two possibilities.

The presence of significant amounts of ^{89}Sr on the otoliths of fish fed radioactive food implies that diet does in fact contribute to otolith strontium. Although few studies have investigated the contribution of diet to strontium deposition on the otolith, food with enhanced strontium levels has been used to mark the scales and vertebrae of fish in freshwater (1,2,26). The lack of a significant effect of strontium availability in the diet on the deposition of otolith strontium in our study may be due to the low contribution (12% of the total) food sources are estimated to make to otolith strontium. Berg (3) found a similar result for C. auratus, where food also contributed less than 20% of total strontium, as did Schiffman (32), who estimated that O. mykiss obtains ten times more strontium from water than from food. It appears that diet is not an important source of otolith strontium in either saltwater (14) or freshwater fish. For a nonessential trace element such as strontium, then, the assumption made in trace element applications that contributions from the diet to otolith composition are minimal appears to be valid. Dietary uptake of nutritionally important trace elements, however, may be considerably more important than that of nonessential elements. Miller et al. (22) found that diet was a more important source than

water for copper incorporated into the gills, digesta, liver and kidneys of O. mykiss. It is not known if copper availability in the diet affects otolith composition in a similar manner. Also, treatments with enhanced food strontium in our study contained more absolute strontium (Table 1) but only twice as much strontium relative to calcium than treatments with background food strontium (Sr/Ca ratio of 0.0016 for enhanced food strontium vs. 0.0008 for background food strontium, where Sr/Ca ratios were calculated as normalized mole fractions). As discussed above, if strontium regulation occurs indirectly through the Sr/Ca ratio of the source, doubling the availability of food strontium relative to that of calcium may not have been sufficient to produce a significant effect on otolith strontium concentrations. This may also help explain why our results contradict those of Limburg's (20) study, in which an enhanced Sr/Ca ratio of 0.00377 in food did produce a significant increase in the Sr/Ca ratio of A. sapidissima otoliths.

Berg (3) observed that for C. auratus, the contribution from water to total otolith calcium and strontium decreased as the calcium concentration in food increased. Ichii and Muiya (15) have suggested that C. auratus balances uptake from aquatic and dietary sources in order to maintain total calcium requirements. It is not known if calcium and/or strontium deposition on the otolith reflects this compensatory process. It is unlikely that such a process exists for nonessential elements, but a calcium analogue such as strontium may be indirectly affected by the need to maintain a total, whole body calcium level. If so, the contribution made by food to otolith strontium may increase in waters where calcium concentrations are low. Further investigation of the physiological regulation of strontium uptake for deposition on the otolith is needed to clarify to what extent the relative contributions from aquatic and dietary sources may vary and the effects such variations may have on the use of strontium in stock discrimination studies based on otolith chemistry.

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