Response of Otolith Sr:Ca to a Manipulated Environment in Young American Eels

RICHARD T. KRAUS AND DAVID H. SECOR
University of Maryland Center for Environmental Science, Chesapeake Biological Laboratory, One Williams Street, Solomons, Maryland 20688, USA

Abstract.—There has been increased use of otolith composition data to track eel Anguilla spp. migrations in coastal and estuarine environments. Numerous studies have used strontium (measured as Sr:Ca) to infer salinity related habitat use, yet the method remains largely unverified. It is not known whether otolith Sr:Ca is primarily related to ambient salinity, or whether this relationship is confounded by temperature or growth. We manipulated experimental rearing environments of young American Eels Anguilla rostrata to determine the amount of variability in otolith Sr:Ca related to salinity, temperature, and growth; and estimate the lag time in response of otolith chemistry to changes in salinity. Our results suggest that otolith Sr:Ca in eels can be used to discriminate broad scale life history periods of fresh, brackish, and ocean habitat occupation, but finer scale interpretations cannot be supported. In addition to salinity, temperature, and growth effects, further study is needed concerning the influence of dietary sources of Sr on eel otolith composition.

Introduction

Important anguillid eel fisheries exist worldwide but primarily occur for three species: Anguilla japonica, A. anguilla, and A. rostrata. Each species is comprised of a single panmictic population that has a broad geographical and estuarine range during the juvenile life history stage (i.e., glass eel and elver stages). Eels occupy a variety of habitats in which there is considerable potential for variable reproductive contribution to the population (Tsukamoto et al. 1998). To better understand the population dynamics of different eel species, it is important to identify divergent life cycles and quantify significant differences in vital population rates between major habitat types.

One method that provides retrospective inference of habitat use is microprobe analysis of otolith strontium:calcium ratios (Sr:Ca). Otolith Sr:Ca is expected to be proportional to Sr:Ca in the water (Bath et al. 2000), and in estuaries salinity represents a proxy for Sr:Ca in the water, which typically increases from freshwater to marine habitats. Observed variability in eel otolith Sr:Ca suggests that time spent in fresh, brackish, and ocean waters can be differentiated using microprobe-based chemical analyses in A. rostrata, A. anguilla, and A. japonica (Tsukamoto and Arai 2001; Morrison et al., Limburg, and Tzeng et al., all this volume); however, predictive relationships between salinity and otolith Sr:Ca have not been determined. Although otolith Sr is positively related to ambient salinity for several tested fishes (Secor and Rooker 2000), temperature and growth may also be important in regulating the deposition of Sr and Ca in fish otoliths (Mugiya and Tanaka 1995; Kalish 1989; Radtke 1989; Campana 1999).

The assumption that otolith Sr:Ca is positively correlated with salinity has been examined for A. japonica (Tzeng 1996; Kawakami et al. 1998), but not for other Anguillidae. Further, Casselman (1982) observed differences in otolith Sr:Ca between A. rostrata and A. anguilla, and suggested that uptake of Sr into otoliths may vary between these species. The objective of our study was to experimentally quantify the relationship between otolith Sr:Ca and ambient salinity, temperature and growth in young elverstage (< 7 g) A. rostrata through controlled rearing experiments.

Methods

Young eels were obtained from Anguilla Culture Technology in Hopewell, Virginia. Eels were captured at the glass eel stage, primarily by fishers in Canada and Maine, and cultured in fresh water. Two experiments were conducted
to evaluate effects of environmental salinity and temperature on otolith Sr:Ca in young *A. rostrata*. In the first (short-term) experiment, randomly selected eels were acclimated to five salinities (0.5, 5, 10, 15, 25) and three temperatures (16°C, 20°C, 28°C) and held for 53 days. These treatments were chosen to represent the ranges of temperature and salinity that are potentially encountered by estuarine juveniles during the growing season. Two replicates were run simultaneously. In the second (cycle) experiment, randomly selected tanks of eels were switched from high (15) to low salinity (0.5) or vice versa to compare with control treatments with no salinity change. The cycle experiment was replicated twice at two temperatures (20°C and 28°C) and lasted for 74 days. Salinity was changed on experimental day 53. The durations in both the short-term and the cycle experiments allowed sufficient otolith growth at all treatment levels to conduct the microprobe analyses. De- markation of experimental otolith growth was accomplished with *in vivo* marking with alizarin complexone (50 ppm, by immersion for 24 hours). *Ad libitum* feedings were conducted daily with BioDiet Starter, 1 mm food pellets (from Bio-Oregon, Inc. of Warrenton, Oregon).

**Microchemical Analysis**

Sagittal otoliths were embedded in epoxy resin (Spurr 1969). Longitudinal thin sections were mounted on petrographic slides, ground to the widest plane, and polished to a smooth appearance at 50× magnification. Chemical analysis was accomplished with Wavelength Dispersive X-ray Spectrometry (WDS) using a JEOL JXA-8900 electron probe microanalyzer for the elements, Sr and Ca. Calcite and strontianite standards were used. A nominal beam diameter was used to scan an area 25 μm² with an accelerating voltage of 25 kV and current of 20 na. Peak Sr count time was 30 seconds, and peak count time for Ca was 10 seconds. For the short-term experiment, points were oriented along the periphery of otolith sections. For otolith sections from the cycle experiment, transects with evenly spaced points at 10 μm intervals were analyzed along a major growth axis from the core region to either the rostral or postrostral margin, whichever direction appeared to have the most uniform surface at 600× magnification. Subsequent examination of slides with epifluorescent microscopy was used to view the alizarin complexone mark(s) and assign probe points to the preexperimental or experimental regions of otolith.

Additional chemical analyses were performed on the experimental eel diet and on water samples from the various salinities using a quadrapole inductively coupled plasma mass spectrometer (ICP-MS). Approximately 0.5 g of food was placed into a 20 mL Teflon vial along with 3 mL of concentrated HNO₃ (J.T. Baker, Optima Grade) and 1 mL of concentrated HCl (J.T. Baker, Optima Grade). The vials were tightly capped and placed in a drying oven at 60°C overnight. The samples were diluted with Q-water prior to analysis by ICP-MS. The final dilution was dependent on the sample concentration of Ca and Sr.

**Statistical Analyses**

In the short-term experiment, the mean of Sr:Ca values for multiple points (*n* = 2–10) between the alizarin mark and the edge were used to represent the Sr:Ca response by each eel to experimental treatments. A regression approach was used to model the effects of salinity, temperature and growth (as measured by an index; see below) on otolith Sr:Ca. Individual eels were considered as experimental units, and within-tank covariances between individuals were modeled using compound symmetry structure in the residual error matrix (Littell et al. 1996). Individual eel growth was indexed as the ratio of the increment of otolith growth during the experimental period to the entire otolith diameter (measured along ventral-dorsal axis). The index required the assumption that otolith and somatic growth were proportional, which we tested by comparing otolith weight and wet body weight for a subsample of eel otoliths. The relationship was significant: otolith weight in mg = 0.091 + 0.069 × wet body weight in g (*r*² = 0.77, *p* < 0.001). The Sr:Ca response was log transformed to satisfy homogeneous variance assumptions, while temperature, salinity and growth variables were centered to reduce multicollinearity. Treatment effects on arcsine transformed survival data were analyzed using mixed model analysis. In the cycle experiment, nested analysis of variance (ANOVA) was used to compare the mean Sr:Ca response from the area between the first and second marks (corresponding to the initial salinity) with the area between the second mark and the edge
(corresponding to the final salinity). The four cycle treatments (0–0, 15–15, 0–15, and 15–0) were nested within two temperatures (20°C and 28°C).

**Results**

Calcium and strontium in the tank water were positively correlated with experimental salinity levels (Figure 1; Ca: \( p < 0.0001 \); Sr: \( p < 0.0001 \)). Calcium varied from 1.4 to 206.5 ppm and strontium varied from 0.53 to 6.11 ppm. Slopes of the regressions for calcium and strontium were linear, indicative of conservative mixing across the salinity gradient. There was little variation in the Sr:Ca of the water among the tanks with 5 ppt salinity or greater (mean = 0.031, CV = 8%), and higher and more variable Sr:Ca was observed at the 0.5 ppt treatment (mean = 0.091, CV = 44%). The concentrations of strontium and calcium in the diet used in these experiments were orders of magnitude higher than in the tank water concentrations: 140 and 33,590 ppm, respectively. Dietary concentrations of Sr and Ca are consistent with levels measured by Limburg (1995) with ICP-MS in an artificial diet for American Shad (Sr = 158 and Ca = 19,180 ppm). However, another study that measured Sr and Ca in several artificial diets with atomic absorption spectrophotometry found much lower values: Sr ranged from 1.8 to 7.8 ppm, and Ca ranged from 658 to 5040 ppm (Hoff and Fuiman 1995).

In the short-term experiment (Table 1; Figure 2), Sr:Ca increased significantly with temperature (DDF = 16, \( F = 4.73, p = 0.04 \)) and salinity (DDF = 14.7, \( F = 18.43, p = 0.0007 \)). No significant temperature-salinity interaction was detected. Otolith Ca (measured as calcium oxide, CaO) remained relatively constant across transect points within otoliths and across treatments (mean = 53.9%, s.e. = 0.09); observed otolith Sr:Ca ranged from 0.0019 to 0.0082. No significant effect on otolith Sr:Ca due to growth was detected, and no treatment effects on survival were detected.

In the cycle experiment, nested comparisons were made between the two marked regions, but no significant differences were found at any of the four treatment levels within either temperature treatment (20°C or 28°C). Mean otolith Sr:Ca was significantly different between the temperature treatments (DDF = 44, \( F = 5.0, p = 0.03 \)), and within each temperature level, the mean otolith Sr:Ca was significantly different between the four possible cycle treatments (DDF = 44, \( F = 6.4, p < 0.001 \)). At 20°C, mean otolith Sr:Ca ratio in the 0–0 salinity cycle was significantly lower than both the 15–15 salinity cycle (df = 44, \( t = -3.62, p = 0.02 \)) and the 0–15 salinity cycle (df = 44, \( t = -3.45, p = 0.04 \)), using Bonferroni adjusted probability values. At 28°C, mean otolith Sr:Ca in the 15–15 salinity cycle was significantly higher than the 0–15 salinity cycle (df = 44, \( t = 4.19, p = 0.004 \)). Where significant differences were observed, the sign was consistent with our expectations; however, some of the comparisons in which significant differences were not observed led to contradictory results. For example, at the 20°C/15–0 cycle, observed Sr:Ca was variable and not significantly different from any of the other cycle treatments.

**Discussion**

The observed magnitude and variability of otolith Sr:Ca at the different salinity treatments in this study were consistent with otolith Sr:Ca observed in *A. japonica* reared in a similar range of salinities (Tzeng and Tsai 1994; Kawakami et al. 1998). Contrary to our expectations, a direct relationship between Sr:Ca in the tank water and that in the otolith was not indicated by these results, an outcome which warrants further investigation. Based on the observed variability, inferences from otolith Sr:Ca in wild eels appears to be limited to discriminating periods of freshwater from saltwater habitat occupation. However, a linear extrapolation (to salinities from 25 to 30 ppt) of the trend that we observed suggests that additional information might support discrimination between periods of brackish and ocean habitat occupation. There are three possible confounding influences on this relationship due to temperature and growth effects, diet composition, and the temporal resolution of the electron microprobe.

In our experiments, lower temperatures were associated with decreased otolith Sr:Ca. This result contrasts to the expected inverse relation between ambient temperature and otolith Sr:Ca attributed to molecular kinetics (Radkte 1989). Temperature effects observed in this study may be confounded due to a small, yet significant correlation between temperature and the growth index (\( r = 0.46, p = 0.002 \)). However, the correlation is driven by a low growth index at the 16°C treatment; the growth index did not increase between 20°C and 28°C. Slow growth due to
Figure 1. Strontium (lower panel) and calcium (upper panel) concentrations in water versus salinity level in rearing tanks for juvenile American Eel holding experiments.
Table 1. Effects of temperature and salinity on American Eel otolith Sr:Ca (by weight percent). Model coefficients and tests for significance were determined using a mixed model analysis to account for within tank correlation among individual fish responses. Degrees of freedom were determined using a Satterthwaite approximation. s.e. = standard error.

<table>
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<th>Effect</th>
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<th>p-value</th>
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<td>14.7</td>
<td>4.3</td>
<td>0.0007</td>
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</table>

Reduced metabolism at 16°C may have resulted in a decreased uptake of strontium that would account for the lower otolith Sr:Ca.

Pre-experimental otolith Sr:Ca averaged 0.002, which is consistent with otolith Sr:Ca in eels from freshwater regions of Hudson River (Morrison et al. these proceedings), and slightly less than, but within the range observed in freshwater eels from Maine (Arai et al. 2000). The higher mean Sr:Ca levels we found in the freshwater treatments (0.0037) may be an artifact of the experimental conditions related to stress or diet, but may also result from high Sr:Ca in water observed at this treatment level. By

![Graph showing the effects of temperature and salinity on juvenile American Eel otolith Sr:Ca.](image)

Figure 2. Effects of temperature and salinity on juvenile American Eel otolith Sr:Ca. Points represent the mean otolith Sr:Ca for a given eel plotted against the mean salinity recorded in the tank. Different symbols represent the three temperature treatments indicated in the key. Approximately 30% of the variability in Sr:Ca was accounted for by salinity (see Table 1 for regression results). For comparison, symbols with 95% confidence bars represent measurements on the marginal increment of wild Hudson River eels captured at fresh and brackish water sites (Morrison et al., this volume).
comparison, the mean otolith Sr:Ca observed in the freshwater experimental treatment is also greater than the means observed in wild individuals from three other species in freshwater: 0.0034 in *A. celebesensis* and 0.0028 in *A. marmorata* (Arai et al. 1999a), and 0.0034 in *A. australis* (Arai et al. 1999b).

Diet could have affected otolith Sr:Ca through increased uptake of strontium relative to calcium. We expect that dietary and water borne sources of calcium did not affect otolith Sr:Ca because calcium is a highly regulated ion. Calcium concentrations are similar throughout the otolith’s microstructure, and past experimental studies have documented that otolith calcium is unresponsive to variation in direct or intestinal exposure of calcium (Berg 1968; Farrell and Campana 1996). In contrast, high levels of strontium in the diet relative to the ambient tank water could have elevated the intercept of the regression relationship (Table 1; Figure 2), explaining the higher otolith Sr:Ca observed for the 0.5 salinity treatment. Because discrimination of strontium by direct absorption is higher than by intestinal absorption (Berg 1968; Farrell and Campana 1996), it is not clear how dietary Sr would affect the slopes in the regression. The linear increase in otolith Sr:Ca with experimental salinity and the conservative mixing behavior of ambient strontium lends support to the assumption that changes in otolith Sr:Ca are in part associated with salinity changes. Our results suggest further study of diet related factors is needed.

Electron microprobe point size (25 μm²) for the Sr:Ca analysis in these experiments roughly integrated growth over 5–25 days on the otolith, depending on the plane in the otolith section and the size and growth history of the individual. Thus, otolith precipitation rates ranged from 0.2 to 1 μm/d along the axes that we analyzed. High otolith Sr:Ca variability coupled with coarse temporal resolution in the microprobe analysis precluded evaluation of a fine scale lag response to ambient salinity changes. However, we believe that the lack of significant change in otolith Sr:Ca between the cycle phases was more likely a function of the high variability of otolith Sr:Ca among individual eels rather than improper temporal scaling. There were some significant differences in otolith Sr:Ca observed among the four different cycle treatments; however, some of the multiple comparisons gave contradictory results, and further study into the sources of variability in otolith Sr:Ca among individuals is warranted.

In summary, the results of the short-term experiment indicated a linear increase in Sr:Ca with salinity, although the absolute relationship should not be applied directly to field observations until further study has determined the contribution of dietary strontium to otolith strontium in eels. In addition, the variability in otolith Sr:Ca suggests that while broad scale inference of fresh, brackish, and ocean habitat use appears valid, finer scale interpretation of estuarine life history movements may not be supported by this method.

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References


