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# Effects of inter-habitat migration on the evaluation of growth rate and habitat residence of American eels *Anguilla rostrata*

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ABSTRACT: The use of Sr:Ca ratios in fish otoliths to reconstruct historical patterns of fish movement between aquatic habitats of different salinity ranges (fresh, estuarine, marine) can be extended to evaluate the frequency and duration of inter-habitat movements. However, the proportion of otolith growth in a habitat does not necessarily equal the proportion of time spent in that habitat and depends on the difference between otolith growth rates within each habitat. For inter-habitat migrant yellow American eels *Anguilla rostrata* from the East River, Nova Scotia, the mean proportion of residence time in freshwater slightly (2.9%), but significantly, exceeded the proportion of otolith growth in freshwater, but the magnitude of the effect was small and perhaps of little practical consequence. Although the observed effect magnitude was small, where large differences in otolith growth rates occur among habitats or with ontogeny, habitat-specific growth rates should be considered for detailed examinations of inter-habitat migration and residency. A simple habitat residence model provides results consistent with the observed data. For inter-habitat migrants, the mean difference between 2 possible methods of estimating the proportion of fish growth in freshwater from microprobe otolith Sr:Ca ratio transects was small (2.2%). Singleton outliers of eel otolith Sr:Ca ratios may create difficulties in evaluating the frequency and duration of inter-habitat movements that remain to be resolved.

KEY WORDS: *Anguilla rostrata* · American eel · Strontium:calcium ratios · Otolith growth period · Habitat residency period

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## INTRODUCTION

Examination of the ratios of strontium (Sr) to calcium (Ca) along otolith transects has revealed much complexity in the history of anguillid eel migration between marine/estuarine and freshwaters (reviewed by Daverat et al. 2006). Anguillid eel growth rates are typically higher in marine habitats than in freshwater habitats, and for inter-habitat migrants the proportion of time spent in marine/estuarine waters is reflected in their average growth rate (Morrison et al. 2003, Tzeng et al. 2003, Jessop et al. 2004, 2006). More generally, diadromous species of a given genus tend to have higher growth rates than do freshwater species of the genus (Gross 1987), with anadromous species generally more prevalent at temperate latitudes and catadromous species at tropical latitudes (McDowall 1987).

Few studies of *Anguilla* have estimated the proportion of time juvenile inter-habitat migrants are resident in freshwater or estuarine/marine habitats (Jessop et al. 2002, 2004) or examined the frequency or duration of migratory episodes (Daverat et al. 2006, Jessop et al. 2006, Lamson et al. 2006). By definition, otolith growth

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period and habitat residency period are equivalent for fishes that reside entirely in either a marine/estuarine or freshwater habitat. For inter-habitat migrants of diadromous and amphidromous species, the otolith growth and habitat residence periods might be estimated as the proportions on the growth and time scales of a transect from otolith core to edge with Sr:Ca values greater than or less than the criterion value for separating marine/estuarine habitat from freshwater habitat (e.g. Jessop et al. 2004). However, if otolith growth rate varies among habitats, then a given transect proportion will not reflect the time spent in each habitat (Jessop et al. 2006). The degree of difference between the proportions of time and growth in a habitat depends on the relative difference in otolith growth rates between habitats. To extend the interpretation of element ratio or isotope patterns along an otolith transect to more detailed examination of temporal patterns of residence and movement, the issue of relative otolith growth rate among habitats must be considered.

This study evaluates whether, for American eels *Anguilla rostrata* migrating between habitats, measurements of the proportion of otolith growth along a transect with Sr:Ca values above or below a defined habitat transition value can be considered functionally equivalent to the proportion of residence time in a given habitat. We examine: (1) the inter-habitat migratory activity of juvenile (yellow-phase) eels with respect to time and otolith growth, (2) evaluate the difference between the proportion of otolith growth and proportion of time spent (residency period) in marine/estuarine and freshwater habitats, and (3) compare the observed habitat residence/growth period data with the results of a simple habitat residency model.

## MATERIALS AND METHODS

**Study area.** The East River (latitude 44° 35′ 16″ N) along the Atlantic coast of Nova Scotia drains a watershed of 134 km$^2$. A small falls (0.6 m) at the river mouth creates a sharp transition between river and estuary. The Sr:Ca ratio of the river water was measured as $5.6 \times 10^{-3}$ (wt%, equivalent to $2.5 \times 10^{-3}$ molar ratio) at a site 1.3 km upstream of the river mouth, and $4.1 \times 10^{-3}$ (wt%, equivalent to $1.9 \times 10^{-3}$ molar ratio) at a site further upstream (Jessop et al. 2002). Additional habitat details and maps of the watershed can be found in Jessop (2000) and Jessop et al. (2006).

In Mahone Bay, about 2 km from the river mouth, the salinity varies seasonally and with depth from ~27 to 31.5 (M. Dadswell, Acadia University, Wolfville, Nova Scotia, pers. comm.). Water temperatures in the East River estuary are about –1 to 2°C during winter, rising to about 18 to 20°C during the summer, but are often in the range of 12 to 17°C. The estuary is well mixed, with a maximum tidal range of ~2 m and an average range of 1.5 m. The freshwater–saltwater mixing pattern in the estuary is unknown, but its extent is governed by seasonal freshwater discharges that range, for example, between about 0.5 and 4.8 m$^3$ s$^{-1}$ between late May and late September (Jessop 2000).

**Data collection and treatment.** Yellow (juvenile) eels *Anguilla rostrata* (N = 107) were collected by electrofishing on 17 July 2001 from 5 sites at various distances upstream from the river mouth. Details of the sample collection and treatment, otolith preparation, Sr and Ca measurement by electron microprobe, and otolith ageing and measurement procedures are described in Jessop et al. (2006). Based on the pattern of Sr:Ca values between the elver check and final annulus, 45 yellow eels remained in freshwater after initial freshwater entrance and were not used in further analysis. Of the 62 yellow eels that exhibited varying degrees of inter-habitat movement, 5 were unusable due to the significant non-coincidence of the age measurement and Sr:Ca transects, leaving 57 eels for analysis (mean length = 277.7 mm, SD = 59.30, range = 179 to 443 mm; mean weight = 42.1 g, SD = 30.62, range = 9.9 to 168.5 g; mean age = 10.4 yr, SD = 3.65, range = 5 to 20 yr).

Small measurement differences, usually <3% of the otolith radius (µm), between the Sr:Ca transect length and age transect length were common, perhaps due to the Sr:Ca transect ending just short of the otolith edge or to imprecision in matching Sr:Ca and age transects. When the age transect length exceeded the Sr:Ca transect length and the Sr:Ca values were all $\leq 4.0 \times 10^{-3}$ for several years, any unmeasured Sr:Ca values were assumed also to have been $\leq 4.0 \times 10^{-3}$. When the Sr:Ca transect length exceeded the age transect length (3 cases), the Sr:Ca transect length was re-scaled to the age radius.

The temporal pattern of otolith Sr:Ca values along a transect was used to evaluate the environmental history of each eel, under the assumption that the temporal pattern of Sr:Ca reflects the habitat salinity (Kraus & Secor 2004, Daverat et al. 2005). Ratios (wt%) of Sr:Ca $\leq 4.0 \times 10^{-3}$ were considered to indicate freshwater residence, and values $> 4.0 \times 10^{-3}$, to indicate estuarine or marine residence (Jessop et al. 2004, 2006).

**Freshwater otolith growth estimation.** Two methods were used to estimate the proportion (converted to percentage, as necessary) of freshwater otolith growth (%fwg), the remainder being estuarine/marine growth. Method 1, used to estimate the %fwg for this analysis differs from that used by Jessop et al. (2004, 2006), which is termed Method 2. Method 1 estimated %fwg for eels that first entered freshwater as either elvers or juveniles as the percentage of Sr:Ca values

$\leq 4.0 \times 10^{-3}$ between the elver check and the last annulus, but not including singletons (Table 1, Fig. 1). Method 2 estimated %fwg for eels that first entered freshwater as elvers as the percentage of Sr:Ca values $\leq 4.0 \times 10^{-3}$ between the first pair of Sr:Ca values $\leq 4.0 \times 10^{-3}$ and the otolith edge, while for eels that entered freshwater as juveniles, the start position was the elver check. Basically, the migration groups, as defined in Jessop et al. (2004, 2006) consisted of eels that entered freshwater as elvers and those that entered as juveniles (≥1 yr old) and subsequently engaged in inter-habitat migration or remained totally freshwater resident.

A singleton is a single Sr:Ca value $>4.0 \times 10^{-3}$ departing from a freshwater norm ($<4.0 \times 10^{-3}$) or a value $<4.0 \times 10^{-3}$ departing from a saline water norm ($>4.0 \times 10^{-3}$) (Fig. 1). If singleton otolith Sr:Ca values are assumed to represent a habitat transition, their prevalence and magnitude affect the interpretation of the frequency and duration of inter-habitat movements and the cumulative growth and residence in a given habitat. The residence period represented by singleton Sr:Ca values was estimated for a representative sample of 10 singletons from 4 eels with values more than $\pm 1 \times 10^{-3}$ from the habitat transition constant.

**Freshwater residency period estimation.** Freshwater habitat residency period was calculated as the sum of segments of freshwater otolith growth (µm) as applied to the time scale (yr). Growth segments within each habitat were defined by the points where the line (slope) connecting adjacent Sr:Ca values crossed the $4.0 \times 10^{-3}$ *x*-axis habitat transition value, as estimated by linear regression between the adjacent Sr:Ca values. Segments of freshwater Sr:Ca values were summed over the growth and time scales (time was estimated annually to account for differences in annual growth rate) and converted to proportions of the total transect for growth and time. The last annulus was chosen as the end point for Method 1, because the Sr:Ca spot transect did not always extend precisely to the otolith edge and the distance to the edge represents an uncertain time period. Measurements taken only to the last annulus represent full years, assuming

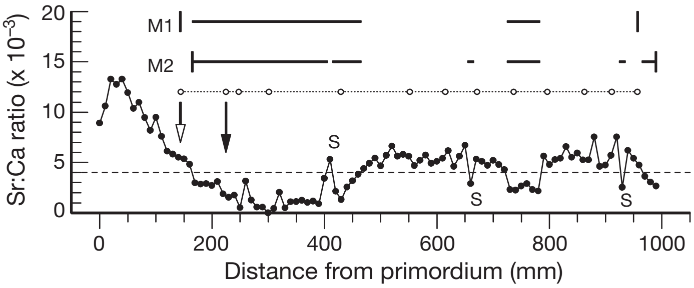


Fig. 1. *Anguilla rostrata*. Comparison between Method 1 (M1, %fwg = 43.3) and Method 2 (M2, %fwg = 48.2) of the proportion of otolith growth in freshwater for an eel that entered freshwater as an elver. For Method 2, if the eel had entered freshwater as a juvenile (>1 yr old), measurement would begin at the elver check. The elver check is indicated by the open-head arrow, the first annulus by the filled-head arrow, Sr:Ca ratio values by filled circles, annuli by open circles and singleton Sr:Ca values by S. Horizontal dashed line: $4.0 \times 10^{-3}$ habitat transition criterion

that the time between the elver check and first annulus represents approximately 1 yr and that, over the life of the eel, the distance between annuli represents 1 yr of growth on average. Formation of the elver check may shortly precede the entrance to freshwater (Michaud et al. 1988, Jessop et al. 2002), but is not dependent upon freshwater entrance (Jessop et al. 2006).

For comparison, data from 9 female silver eels from the East River (Jessop et al. 2004) were also examined so as to extend the freshwater residency range to lower values. Method 1 was used to evaluate freshwater otolith growth, and residency times were evaluated.

The significance of the mean difference between percent freshwater growth methods and between otolith growth and residence time was estimated by paired permutation test with 10 000 repetitions (Manley 1997), because the sample distributions of percent freshwater growth (converted to proportions) and proportions of otolith growth and residence time were negatively skewed and kurtotic, but sample variances were homogeneous (Levene's test $p \geq 0.41$). Confidence intervals (95% CI) for each mean difference were estimated by bootstrapping. The mean number of singleton Sr:Ca values less than (negative) or greater than (positive) $4 \times 10^{-3}$ along a transect was examined for significant difference by paired *t*-test. The mean numbers of both positive and negative singletons for eels grouped in 10% fwg increments were examined by analysis of variance (ANOVA) followed by the Tukey multiple-comparison test. Statistical significance was accepted at $\alpha \leq 0.05$. Statistical significance does not necessarily imply biological importance (Johnson 1999), so Hedge's *g*, a

Table 1. *Anguilla rostrata.* Differences between Methods 1 and 2 for estimating the proportion of otolith growth in freshwater for yellow American eels

| Method | Freshwater entry group | Start | Singletons | End |
|---|---|---|---|---|
| 1 | Elver + juvenile | Elver check | Not included | Last annulus |
| 2 | Elver | First pair[a] | Included | Otolith edge |
| 2 | Juvenile | Elver check | Included | Otolith edge |

[a]From first value of first pair of Sr:Ca values $\leq 4 \times 10^{-3}$

measure of effect size (standardised mean difference between treatment group), was calculated for the mean difference between percent freshwater growth methods and between otolith growth and residence time (Hedges & Olkin 1985) following arcsine transformation of each variable. The correlation between variable pairs is high, and the sample standard deviations were used to calculate the pooled standard deviation for Hedge's $g$ because correction for the correlation overestimates the true effect size (Dunlop et al. 1996).

The relation between the observed proportion of residence time in freshwater ($y$) and the proportion of otolith growth in freshwater ($x$) was fitted by a second-degree polynomial, and the fit of the model was evaluated by Mallows' $C_p$ (Mallows 1973). Centring the $x$ variable before use by subtracting its mean reduced to non-significance the collinearity between the unsquared and squared terms.

**Habitat residence model.** A simple 'habitat residency' model that considers the effect of differing habitat growth rates on the estimation of habitat residency times was compared with the observed relationship between the proportion of otolith growth in freshwater and residency time in freshwater based on measured values. If we assume linear relations between otolith and fish growth and between otolith (fish) growth rates occurring in fresh- and saltwater, then fish length-at-age would be:

$$L_t = fgt + (1 - f)\mathrm{A}gt \qquad (1)$$

where $L_t$ is fish length at age $t$, $f$ is the proportion of time spent in freshwater, $g$ is the growth rate in freshwater, A is a constant of proportionality between salt- and freshwater growth rates, and A × $g$ is the growth rate in saltwater. The proportion of total fish (otolith) growth in freshwater, measured by the proportion ($P$) of 'freshwater' otolith Sr:Ca values, is the amount of fish (otolith) growth in freshwater ($fgt$) divided by the total amount of fish (otolith) growth [$fgt + (1 - f)\mathrm{A}gt$] or:

$$P = \frac{fgt}{fgt+(1-f)\mathrm{A}gt} = \frac{f}{f+(1-f)\mathrm{A}} \qquad (2)$$

Given estimates of A and $P$, $f$ can be estimated as

$$f = \frac{\mathrm{A}P}{1-P+\mathrm{A}P} \qquad (3)$$

An estimate of A (ratio of salt- to freshwater growth rates) for yellow eels was obtained from the ratio of the mean annual growth ($g$), based on back-calculated lengths-at-age, of estuarine eels (<50% fwg, n = 9, $P$ = 0.35, $g$ = 26.29 mm yr$^{-1}$) and freshwater eels (100% fwg estimated by Method 2, n = 43, $P$ = 1.0, $g$ = 22.03 mm yr$^{-1}$; Jessop et al. 2006); thus, A = 26.29/22.03 = 1.19. Other estimates of A can be derived from the mean annual growth rates estimated from the back-calculated lengths-at-age for male and female silver eels from the East River, Chester. Thus, the mean annual growth rate of 21.8 mm yr$^{-1}$ for male silver eels with largely (<50% fwg) estuarine growth and 19.2 mm yr$^{-1}$ with largely freshwater (>50% fwg) growth gave an estimate of A = (21.8/19.2) = 1.14, while for female silver eels the estimate was A = (27.7/22.7) = 1.22 (Jessop et al. 2004). These are underestimates of A to the extent that the saltwater component is not fully saltwater.

Values of $f$ (proportion of time in freshwater) were calculated with the habitat residency model for values of $P$ (proportion of growth in freshwater) ranging from 0 to 1.0 in increments of 0.05 and with A = 1.19. Model values of $f$ were converted to percentages and compared with values of percent freshwater residency estimated from otolith measurements. The usefulness of the habitat residency model was evaluated by graphic comparison with a polynomial curve fitted to the otolith-based estimates of freshwater habitat residency period for individual eels.

## RESULTS

### Percent freshwater growth

Method 2 produced generally higher estimates than Method 1 of the percentage of otolith growth in *Anguilla rostrata* occurring in freshwater (Fig. 2). The mean difference between methods was statistically significant (n = 57, p = 0.0001) at 2.2% (95% CI = 1.22 to 3.20), with the measure of effect size, Hedge's $g$ = 0.16 (95% CI = 0.10 to 0.22). The greatest differences between methods occurred for eels with intermediate percentages of freshwater growth and large numbers of singleton Sr:Ca values. The variability about the 1:1 reference line (represents equivalence between methods) depended on both the number of singleton Sr:Ca values and, for eels with otolith %fwg greater than about 90%, the degree of estuarine growth occurring between the elver check and freshwater entry (Figs. 1 & 2).

Singleton otolith Sr:Ca values occurred in 81% of inter-habitat migrants, but were most common in eels exhibiting intermediate (30 to 70%) levels of freshwater growth (Fig. 3A). The mean number of singletons per fish was 3.2 (n = 57, SD = 3.22, range = 0 to 8), with no significant difference ($t$ = 0.77, df = 112, p = 0.44) in the mean number of singletons indicating freshwater (<4.0 × 10$^{-3}$) or estuarine (>4.0 × 10$^{-3}$) incursion. However, when pooled in 10% fwg bins (so as to keep n ≥ 3) over the range 20 to 100% fwg, the mean number of positive singletons (>4.0 × 10$^{-3}$) did not vary significantly among bins ($F$ = 1.49, df = 7,49, p = 0.19), but the mean number of negative (<4.0 × 10$^{-3}$) singletons did

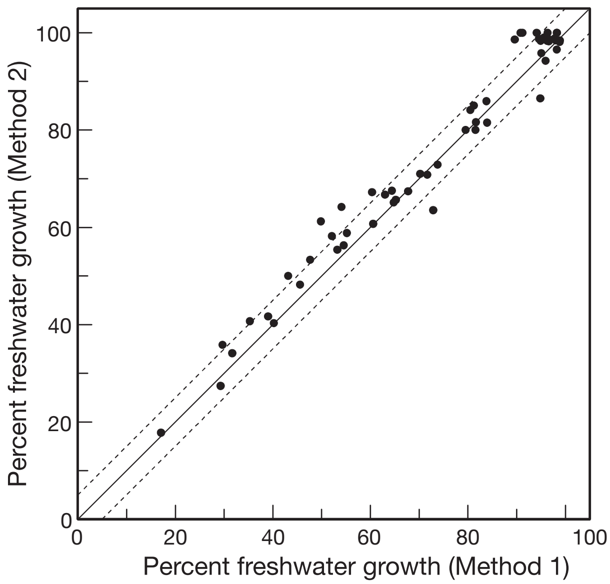


Fig. 2. *Anguilla rostrata*. Relation between the percentage of otolith growth in freshwater estimated by Method 1 and that by Method 2 for yellow American eels from the East River, Nova Scotia. The dashed lines represent proportions ±5% from the central 1:1 ratio solid line

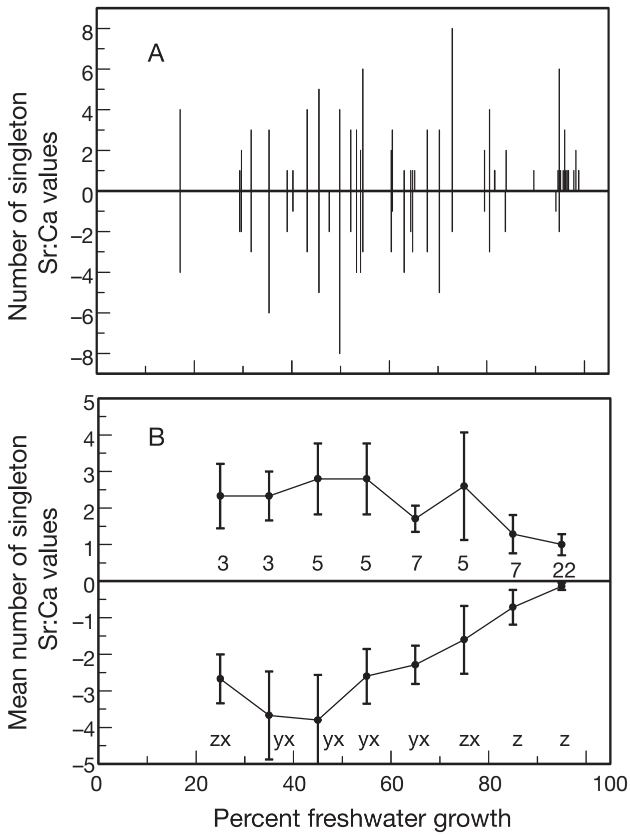


Fig. 3. *Anguilla rostrata*. (A) Number of positive and negative (relative to the habitat transition criterion of $4.0 \times 10^{-3}$) singleton Sr:Ca values along an otolith transect in relation to the percent of growth in freshwater (%fwg; Method 1) for yellow American eels (each vertical line represents 1 eel; 46 of 57 eels had 1 or more singletons) from the East River, Nova Scotia. (B) Mean (±SE) number of positive and negative singletons, centred in 10% fwg bins, in relation to %fwg. The number of eels contributing to each mean is given along the zero axis; negative means without a letter in common are significantly different at $\alpha = 0.05$

vary significantly ($F = 7.31$, df = 7,49, $p < 0.001$), being greatest at 40 to 49.9% fwg and decreasing with increasing %fwg (Fig. 3B). Singleton Sr:Ca values were usually isolated, but small clusters (2 to 3 spots) or closely spaced singletons fluctuating across the habitat transition constant occurred infrequently. The number of Sr:Ca singletons decreased with increasing magnitude of the Sr:Ca value (Table 2).

The duration of the residence period in the minority habitat depends upon the growth rate over the given otolith growth increment. Assuming that singleton Sr:Ca values represent habitat transition, a mean residence period of 60.7 d (n = 10, SD = 20.36, range = 24 to 97 d) was estimated for singleton Sr:Ca ratio values, each with a value greater than $\pm 1 \times 10^{-3}$ from the habitat transition constant. The 'time period' of a singleton Sr:Ca value is proportional to its microprobe spot width (approximately 5 µm) relative to the otolith annulus transect length, which varies with growth rate, and the magnitude of the Sr:Ca value. The time period is greater for values further from the $4.0 \times 10^{-3}$ reference line than for closer values because of the greater slope of the line connecting adjacent Sr:Ca values as it crosses the reference line. This method assumes that the otolith spot Sr:Ca ratio value represents a habitat residence transect length greater than just the spot width and includes, on average, half of the distance (10 µm) between adjacent spots. In practise, the spot width on the otolith exceeds 5 µm because of electron beam spreading, and there is little distance between spots.

Table 2. *Anguilla rostrata*. Deviation and frequency of singleton Sr:Ca values (n = 180) from the habitat transition criterion ($4.0 \times 10^{-3}$)

| Deviation ($\pm \times 10^{-3}$) | Frequency (%) |
|---|---|
| 0–0.49 | 39 |
| 0.5–0.99 | 22 |
| 1.0–1.49 | 21 |
| 1.50–1.99 | 11 |
| ≥2.0 | 7 |

## Otolith growth versus residence time

For inter-habitat migrant yellow eels, the proportion of eel residence time in freshwater typically exceeded the proportion of otolith (eel) growth in freshwater. The mean difference was 0.029 (95% CI = 0.015 to

0.044, $p < 0.0001$) and the effect size measure, Hedge's $g$, was $g = 0.13$ (95% CI = 0.08 to 0.18). The observed relation between otolith growth and habitat residence time changed non-linearly from small differences at low and high proportions of freshwater otolith growth to larger differences at intermediate proportions (Fig. 4). However, the quadratic term of a second-degree polynomial fitted to the yellow eel data ($r^2_{adj}$ = 0.94; adjusted for the number of independent variables) was non-significant (n = 57, $t = -1.171$, $p = 0.25$, 95% CI = –0.468 to 0.123) and Mallows' $C_p = 3.4$, indicating that the polynomial model fitted the data insignificantly better than a linear model (Mallows 1973).

### Habitat residence model

As the difference increased between growth rates in fresh- and saltwaters (increased A), so too did the difference between the proportion of otolith growth in freshwater and the proportion of time resident in freshwater, particularly at intermediate proportions of freshwater otolith growth (Fig. 4). The habitat residence model relating $P$ (proportion of otolith growth) and $f$ (proportion of habitat residence) in freshwater for yellow eels with A = 1.19 was almost coincident with the quadratic polynomial curve fitted to the observed estimates of otolith $P$ and $f$ and was well within the 95% CI of the polynomial curve (not shown due to crowding in Fig. 4). The habitat model curves for male and female silver eels (A = 1.14 to 1.22) were also within the upper 95% CI of the polynomial curve for yellow eel observed data, indicating that the variability of the observed data is sufficient to encompass a modest range of A values for the habitat residence model.

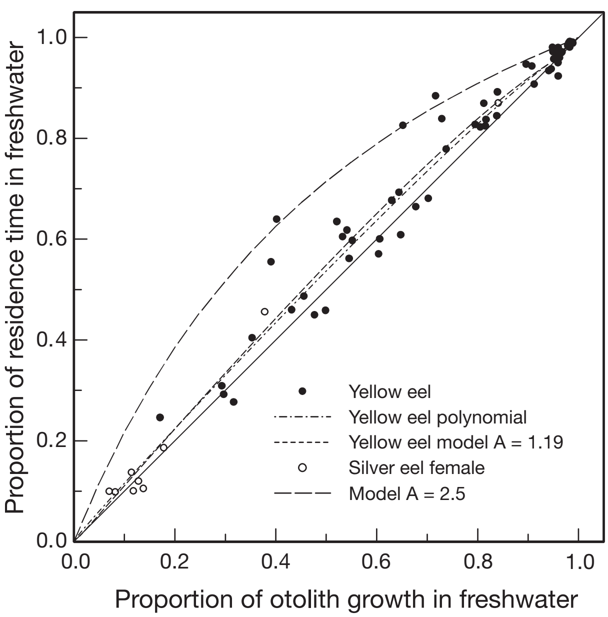


Fig. 4. *Anguilla rostrata*. Relation between the proportions (Method 1) of residence time ($f$) and otolith growth ($P$) in freshwater for yellow American eels from the East River, Nova Scotia. Plots are given for the polynomial regression fitted to the yellow eel data, for female silver eel data from Jessop et al. (2004), for the habitat residence model for the yellow eel data (A = 1.19), and for an illustrative case with A = 2.5. The central solid line represents the 1:1 ratio of A, the ratio of fish annual growth rates in salt- and freshwater

## DISCUSSION

### Percent freshwater growth estimation

Estimates of the percentage of otolith (fish) growth in freshwater may vary significantly depending upon the method used, yet no standard method exists. Although statistically significant, the mean difference between Methods 1 and 2 was relatively low (2.2%) and may be of little biological or analytical consequence, depending upon the requirements of any further use. One might, *a priori*, conclude that individual and mean differences of less than about 5% are of little consequence given the variability typical of biological systems. Another approach is provided by the estimate of the effect size measure (Hedge's $g = 0.16$), for which values <0.2 are regarded as a small effect (Hedges & Olkin 1985, Cohen 1988) and perhaps of little biological significance. Thus, the differences between Methods 1 and 2 may be minor, for most purposes, despite large differences for some individual fish. Although a single, accepted method for estimating the proportion of otolith growth in freshwater might be preferred, it is not clear which method would be most accurate.

Method 2 produced higher estimates of the percentage of otolith growth in *Anguilla rostrata* in freshwater than did Method 1, because it ignored, for eels that entered freshwater as elvers, those cases where the measurement start occurred after the freshwater check and before the first pair of Sr:Ca values $<4.0 \times 10^{-3}$, thus increasing the relative proportion of freshwater growth. The proportion of freshwater growth was altered in Method 2 by including singleton Sr:Ca values $>4.0 \times 10^{-3}$ or $<4.0 \times 10^{-3}$, with the net effect determined by their relative numbers, while inclusion of the growth zone between the last annulus and the otolith edge increased the proportion of freshwater growth.

The causes and importance of singleton Sr:Ca values (see Fig. 1) are uncertain. If they are technical artefacts (see Kotake et al. 2003, Zimmerman & Nielsen 2003), then singletons are properly ignored when assessing habitat transition activities. In this case, smoothing of the transect data may assist the evaluation of trends. However, if large, off-trend singletons represent inter-habitat shifts, then ignoring them will underestimate the frequency of inter-habitat movement and time spent in another habitat. The degree of underestimation may be large given the substantial (39%) number of singleton Sr:Ca values $\geq 1 \times 10^{-3}$ different from the habitat transition criterion and the moderate frequency of their occurrence in eels with an obvious history of inter-habitat migration. Excursions from freshwater to estuary (positive singletons) were not significantly more frequent for eels with high %fwg than for eels with intermediate %fwg, perhaps due to small sample sizes and high variability, or because, once in freshwater, there is a lower biological motivation to make short-term excursions to the estuary relative to estuarine resident eels returning to freshwater, at least for eels with an intermediate history of %fwg. Inter-cohort competition, and resultant growth rates and survival among fishes are typically asymmetric, being influenced by factors such as fish size, individual fish physiology and behavioural aggressiveness, and local density and habitat productivity (Ward et al. 2006). The significantly decreasing number of excursions from estuarine to freshwaters (negative singletons) with increasing %fwg suggests that eels with a high %fwg history migrate less frequently between habitats than do eels with an intermediate %fwg history. The larger number of singletons at intermediate %fwg values implies that singleton frequency is related to the degree of inter-habitat movement and may be an indicator of brief (up to several months), intra-seasonal, inter-habitat movements. Additional insight into the nature of large singleton Sr:Ca values and high Sr:Ca ratio values might be obtained by the comparison of similar otolith transects by the same or different analytical methods and examination of strontium isotope ratios or other elements such as barium that also reflect environmental salinity (Kennedy et al. 2000, Elsdon & Gillanders 2004, 2005b, Milton & Chenery 2005).

Large-value singleton Sr:Ca microprobe spots representing weeks or months of time during a growing season of about 180 d (Jessop et al. 2006) could represent habitat changes. Environmental variability in Sr:Ca values requires about 20 d to become fully incorporated into fish otoliths at growth-period temperatures, with greater changes in ambient Sr:Ca concentration reaching saturation more rapidly than lesser changes (Elsdon & Gillanders 2005a). Seasonal (spring, autumn) migrations of juvenile eels between river and estuary are well known (Smith & Saunders 1955, Medcof 1969, Jessop et al. 2002). Freshwater Sr:Ca values of $1.9 \times 10^{-3}$ to $2.5 \times 10^{-3}$ (mmol mol$^{-1}$) in the East River are unlikely to have created difficulties in interpreting otolith Sr:Ca ratios because of their typical freshwater values, given partition coefficients <1:1 for Sr:Ca ratio incorporation into the otolith (Campana 1999, Bath et al. 2000, Kraus & Secor 2004, Elsdon & Gillanders 2005b).

In addition to salinity, water temperature, water chemistry, fish size and exposure time may also influence otolith Sr:Ca ratios (Kraus & Secor 2003, Elsdon & Gillanders 2004, 2005b, Martin et al. 2004). Ambient chemistry may dominate the effects of temperature and salinity (Elsdon & Gillanders 2004, Kraus & Secor 2004). Water chemistry is governed by freshwater element concentrations and the mixing curve with saltwater, producing a direct relation between otolith Sr:Ca and ambient water Sr:Ca (Kraus & Secor 2004, Elsdon & Gillanders 2005b). Although lower estuarine water temperatures might reduce the effects of ambient Sr level (Elsdon & Gillanders 2004), Kraus & Secor (2004) estimated that the effect of freshwater–estuarine water chemistry variability was ~7-fold greater than the effect of a water temperature variation of 2°C. The relation between temperature and otolith Sr:Ca ratio appears negative below 10°C and positive above, with a response magnitude of about 0.1 mmol mol$^{-1}$ in otolith Sr:Ca for each 1°C of temperature change (Kraus & Secor 2004). No interaction has been found between salinity and water temperature for Japanese eels (Tzeng 1996, Kawakami et al. 1998), but it is unknown whether this applies to other anguillid species. Differences among species in the nature of their partition coefficients and fish size, perhaps due to differences in osmoregulatory ability with size, may also contribute to the variability in otolith Sr:Ca values (Zimmerman 2005). However, given a typically low freshwater Sr:Ca ratio, low seasonal discharge, a seasonal mean temperature differential between fresh- and estuarine waters of about 2 to 8°C and a rapid transition between river and estuary (Jessop 2003), it may reasonably be concluded that otolith Sr:Ca patterns of juvenile American eels moving between the East River and its estuary will be more influenced by water chemistry than by temperature.

## Otolith growth versus residence time

For this data set, the proportion of eel otolith growth and proportion of residency in a habitat may be functionally equivalent. Thus, for inter-habitat migrant eels, Sr:Ca data usefully reflect the residence time in a given habitat as well as the growth in that habitat. Statistical significance of the small (<3%) difference between the mean proportion of otolith growth in a habitat and the proportion of residency in a habitat may be of little biological or analytical importance because of the small effect size measure (Hedge's $g$ = 0.13). However, the researcher's judgement and study purpose are also considerations. The position (early, mid, late) in the life history and duration of a habitat residence period may influence the difference between the otolith growth period and the habitat residence period because of the interaction of the decrease in growth rate with age and the variable growth rate in each habitat relative to the periods of residence in each habitat type.

Variability in growth and inter-habitat migratory history may create differences between otolith growth and habitat residence period that are of concern for small numbers of eels, such as those near the dividing line between habitat residence categories. Where sample sizes are moderate or large, shifts of a few fish from one category to another may make little difference to category means. Habitat categories should be based on broad rather than narrow salinity ranges, because of the high variability in otolith Sr:Ca ratios (Kraus & Secor 2003, Zimmerman 2005). The data (N = 107) of Jessop et al. (2006) were re-examined with percent freshwater habitat residence values estimated from the habitat model (A = 1.19), resulting in 1 eel in each of the 2 lower percent freshwater otolith growth period categories being moved into the next higher percent freshwater habitat residence period category, with no statistically significant change to any resultant conclusion. Similarly, of the 57 eels examined in this study, the number assigned to each %fwg habitat group did not differ significantly ($\chi^2 = 0.58$, df = 2, p = 0.75), whether categorised by percent otolith growth or percent residence time, although 4 and 2 eels were moved from the middle (50 to 75% fwg) and lower (<50% fwg) groups, respectively, to their next higher groups when based on residence time. Thus, otolith growth proportion was again found to be basically equivalent to habitat residency proportion. However, where the growth rate differences between habitats are much higher than those observed here, such as for American eels from Prince Edward Island (Lamson 2005), biologically and analytically important differences may occur between otolith growth proportion and habitat residency period. The close match of the habitat residence model with the observed data suggests that the model may be usefully applied to predict the relationship between otolith growth and habitat residence period for yellow and silver eels, with mean growth rates similar to those observed in this study, but it requires further verification for higher mean growth rates.

In summary, the small differences between 2 methods of estimating the proportion of otolith growth in freshwater and estuarine/marine habitats were found to be of little practical effect. Similarly, small differences in the proportion of otolith growth in a habitat and the duration of residence in that habitat permit the terms to be considered functionally equivalent, except perhaps where the difference between freshwater and estuarine/marine growth rate is large. However, the approaches developed here may contribute to more accurate estimates of habitat residence period when otolith (fish) growth rates differ between habitats. Additionally, the importance of singleton Sr:Ca values as indicators of habitat transition remains to be determined.

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