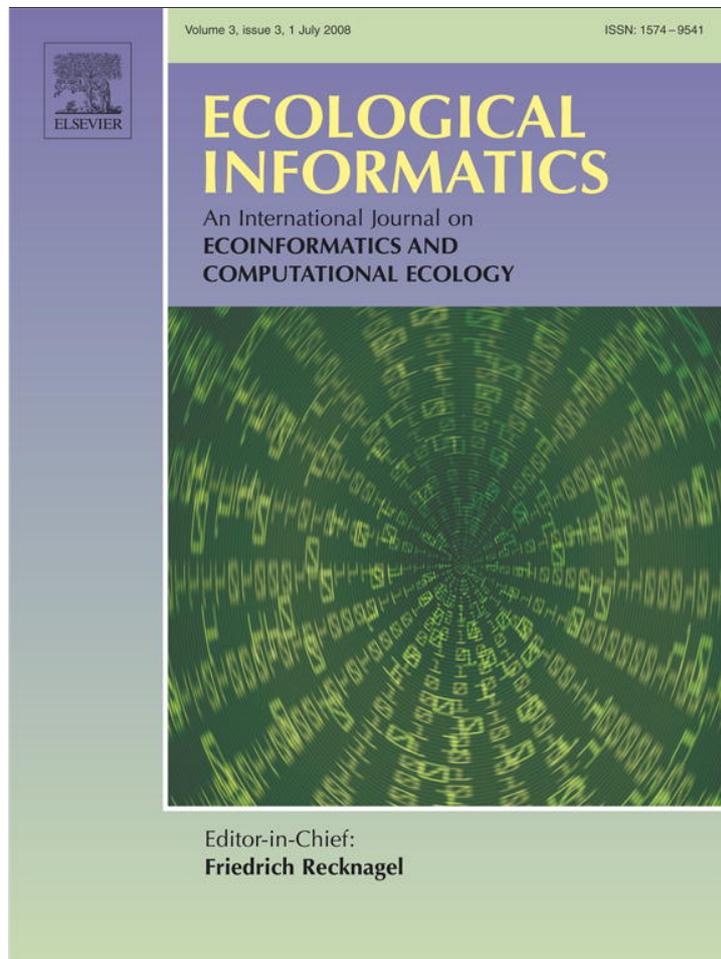


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A quantitative approach for classifying fish otolith strontium: Calcium sequences into environmental histories

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ARTICLE INFO

Article history:

Received 20 September 2007

Received in revised form 9 April 2008

Accepted 12 April 2008

Keywords:

Anguilla rostrata

Otolith

Sr:Ca ratio

Zoning

Environmental history

ABSTRACT

Zoning algorithms were used to quantitatively classify strontium:calcium otolith sequences into fish environmental histories. Otoliths were acquired from 162 American eels (*Anguilla rostrata*) caught in the Gaspé region of Québec, Canada, and Sr:Ca ratios were determined at an interval of 10 μm along a transect from the core to the edge of each otolith (the otolith sequence) using an electron probe microanalyzer. Changes between freshwater and brackish water occupancy were determined with reference to a sample of non-anadromous species including brook char (freshwater) and *Fundulus* sp. (brackish water). Three algorithms were then applied separately to zone the sequences into environmental histories: (i) a local zoning algorithm, which used a split-moving window; (ii) a global zoning algorithm, which used a recursive method; and (iii) an optimization zoning algorithm, which maximized the combined value of selected statistics of the fitted model within a decision-rule framework. Zones were further classified into being of either freshwater or brackish water. All algorithms produced classifications that were not significantly different to those determined using the standard approach of qualitative interpretation, demonstrating the applicability of a quantitative approach. The advantages of the quantitative approach are that (i) the statistics of the model fit provide information on environmental history patterns that is generally not available from qualitative interpretation, and (ii) the parameters of the algorithm can be reported, allowing methodological consistency between different researchers, enabling the potential for more robust meta-analyses.

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1. Introduction

Changes in the Sr:Ca ratio from the core to the edge of a fish otolith (a bone in the inner ear, Campana, 1999) provide information on the fish environmental history (usually resulting from habitat change) because the ratio depends on the salinity of the water body at the time when that part of the otolith was formed (Secor and Rooker, 2000). Analysis of otolith Sr:Ca sequences has been used to determine the environmental history of fish species such as arctic charr (*Salvelinus alpinus*) (Halden et al., 1995), white-spotted charr (*Salvelinus leucomaenis*)

(Arai and Morita, 2005), brown trout (*Salmo trutta*) (Elfman et al., 2000), sockeye salmon (*Oncorhynchus nerka*) (Rieman et al., 1994), American shad (*Alosa sapidissima*) (Limburg, 1995; Elfman et al., 2000; Limburg, 2001), American conger eel (*Conger oceanicus*) (Correia et al., 2004), grey mullet (*Mugil cephalus*) (Chang et al., 2004), white perch (*Morone americana*) (Kraus and Secor, 2004), shirauo (*Salangichthys microdon*) (Arai et al., 2004a), sakhalin taimen (*Hucho perryi*) (Arai et al., 2004b), and American eel (*Anguilla rostrata*) (Thibault et al., 2007).

The interpretation of fish environmental history from the otolith sequence has traditionally been subjective. The conven-

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tional approach is to qualitatively analyze the trend of the Sr:Ca ratio, on a point-by-point basis, and compare this trend with thresholds established for discriminating between fresh, brackish and sea water. In the majority of studies using eels, researchers have interpreted the Sr:Ca sequences by examining individual Sr:Ca observations (Jessop et al., 2002; Morrison et al., 2003; Shiao et al., 2003; Tzeng et al., 2003; Jessop et al., 2004; Daverat et al., 2005). In other studies, researchers have used mean Sr:Ca ratios from the elver mark to the otolith edge to analyze environmental history (Tsukamoto and Arai, 2001; Kotake et al., 2003). Thresholds used to discriminate between water masses of different salinities are also determined differently according to the researcher. Many researchers have compared otolith Sr:Ca ratios of specimens reared or captured in habitats of different salinity (Tzeng et al., 2000; Morrison et al., 2003; Shiao et al., 2003; Daverat et al., 2005), while others have based their thresholds on mean otolith edge Sr:Ca ratios and the distribution of values at the 1st annulus (Jessop et al., 2002). Some researchers have used a Sr:Ca threshold of 4.0×10^{-3} , below which eel growth was classified as occurring in a freshwater habitat (Jessop et al., 2002; Shiao et al., 2003; Tzeng et al., 2003; Jessop et al., 2004), whereas others have used a ratio to 2.5×10^{-3} or less (Tsukamoto and Arai, 2001; Kotake et al., 2003; Morrison et al., 2003; Daverat et al., 2005). Finally, even when categorizations proposed by different researchers have shown similarities, each researcher has adapted the categories used according to the aim of the study. To our knowledge, only one study has used a quantitative approach (Fablet et al., 2007).

The development of a quantitative analytical approach for classifying otolith sequences provides (i) information on the accuracy of the classification and (ii) a degree of standardization which would aid in meta-studies. The application of a quantitative approach is potentially difficult because otolith sequences are often characterized by a large amount of variation at short scales. Zoning—the segmentation of a data-series into zones on the basis of similarity or dissimilarity metrics—may have

potential for application to otoliths. The principal method used for zoning data in the environmental and ecological sciences has involved the application of (i) local zoning (Webster, 1973; Cornelius and Reynolds, 1991; Int Panis and Verheyen, 1995) and (ii) global zoning (Gill, 1990). Local zoning involves passing a window across the sequence to obtain information on the local pattern, allowing the identification of discontinuities, with these discontinuities being the breakpoints between zones. Global zoning divides the sequence into a series of zones, usually through a recursive procedure, which are internally homogeneous and different from adjacent zones. Other procedures such as optimization of user-defined functions expressing key statistics of the otolith sequences may be a viable alternative.

The aim of this study was to determine the applicability of zoning algorithms to the classification of otolith Sr:Ca sequences into environmental histories consisting of zones of distinct occupancy of different habitats. This was done using otolith sequences acquired from 162 American eels (*A. rostrata*) which showed evidence of switching between freshwater and brackish habitats. Two standard (local and global) zoning algorithms were applied. An additional algorithm was developed (an optimization zoning algorithm) that maximized selected statistics of the otolith sequences which had been predetermined according to a given rationale. To show the relative classification accuracy of the algorithms, a comparison was made with an analysis conducted using the commonly-used qualitative approach.

2. Materials and methods

2.1. Water salinity and concentrations of Sr and Ca within the freshwater and brackish habitats

Water salinity (psu) and concentrations of Sr and Ca were determined from measurements made at the end of June 2005

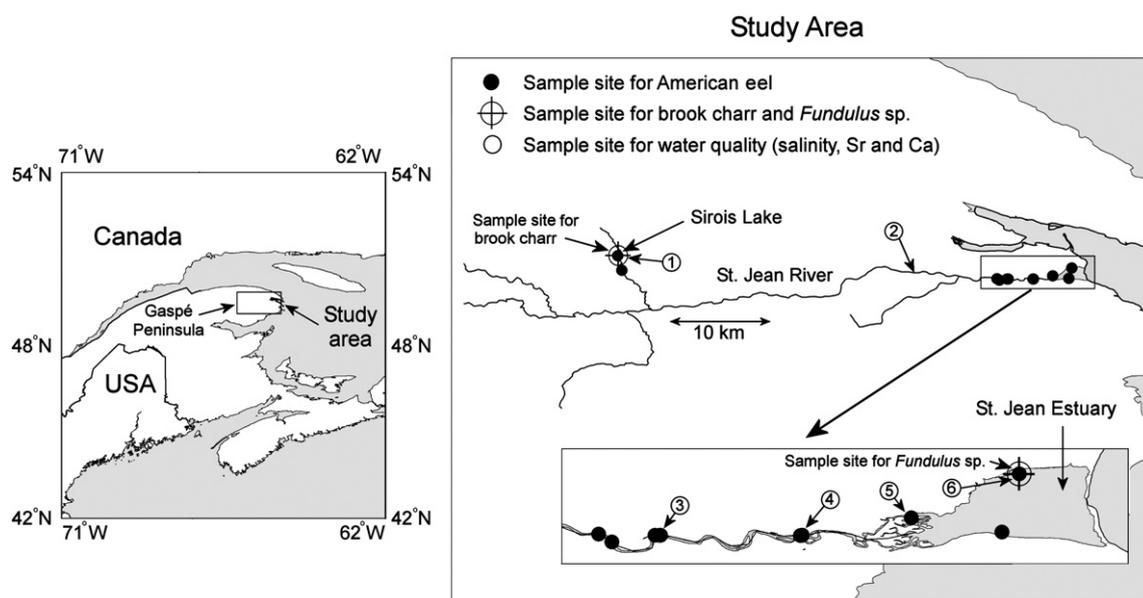


Fig. 1—Map of St. Jean River and St. Jean Estuary (Gaspé Peninsula) showing sample sites for American eel, brook trout and *Fundulus* sp., and water quality.

at six sites: one in Sirois Lake, three in the river, and two in the estuary (Fig. 1). Salinity was determined *in situ* using a YSI Model-33 Salinity–Conductivity–Temperature meter (YSI Inc.). Water samples were then acquired, filtered (45 μm), acidified (HCl) and Sr and Ca concentrations were determined in laboratory with a Perkin Elmer Analyst Atomic Absorption Spectrometer (PerkinElmer Life And Analytical Sciences, Inc.).

The purpose of measuring water salinity and concentrations of Sr and Ca within the freshwater and brackish habitats was (i) to examine the consistency of relationships between water salinity and water Sr:Ca ratios, and (ii) to demonstrate how water Sr:Ca ratios differed to those within otoliths of any species present.

2.2. Otolith data

162 American eels (*A. rostrata*) were captured in freshwater and brackish habitats of the Saint Jean River watershed, located in the Gaspé Peninsular of Quebec (48°46' N, 64°26' W), Canada, from May 17th to October 10th, 2004. The American eel was chosen because recent studies have demonstrated its amphidromous behaviour, using both freshwater and brackish habitat for growth (Daverat et al., 2006), and because it has been demonstrated that otolith Sr:Ca ratios in eels significantly increase with salinity and water Sr:Ca concentrations, but are not affected by temperature, ontogenetic development and otolith dimensions (Tzeng, 1996; Daverat et al., 2005; Elsdon and Gillanders, 2005). For each of the 162 eels, a sagittal otolith was extracted and cleaned with distilled water to minimize chemical contamination, then dried in air and conserved in a plastic ependorf until it was subjected to Sr:Ca analysis. In laboratory, each otolith was embedded in resin, and ground and polished on the sagittal plane until the core was exposed. An electron probe microanalyzer (EPMA; JXA-8900R, JEOL Ltd.) with a wavelength dispersive X-ray spectrometer (WDS) was used to obtain a transect of Sr and Ca concentrations measured along an axis from the core of the otolith to the edge at 10 μm intervals. Beam conditions were 15 keV for the acceleration voltage, 3 nA for the current and a 5 \times 4 μm rectangular scanning beam. Results obtained by this analysis showed a sequence of Sr:Ca variation for each otolith (in wt.%, see Daverat et al., 2006; Jessop et al., 2008).

To interpret the pattern of Sr:Ca variation in each otolith, it was necessary to determine which otolith Sr:Ca values were associated with which type of water mass (freshwater or brackish). It was not possible to determine this by direct measurement of water Sr:Ca ratios because otolith Sr:Ca ratios tend to be less than those of the surrounding water mass (see Dorval et al., 2007) due to physiological barriers to the absorption of Sr. Instead, the association between otolith Sr:Ca values and water mass type was deduced by a comparison with Sr:Ca values in resident species. For the freshwater signal, otolith Sr:Ca values recorded in eels captured in a freshwater lake of the St. Jean River watershed were compared to the otolith Sr:Ca ratios of 5 brook charr (*Salvelinus fontinalis*) from a non-anadromous population captured in the same lake. The same exercise was conducted for the brackish signal, but with eels and 3 *Fundulus* sp. captured in the brackish St. Jean River estuary. The otolith preparation and X-ray analyses were identical to those used for eels. The mean Sr:Ca ratio of the brook charr in freshwater was approximately 2×10^{-3} , and rarely exceeded 4×10^{-3} (Fig. 2a). The mean *Fundulus* Sr:Ca ratio was approximately 5×10^{-3} and was rarely less than 3×10^{-3}

(Fig. 2b). The threshold Sr:Ca ratio in which the proportion of the salt water *Fundulus* population was greater than the freshwater brook charr population occurred at 3.5×10^{-3} (Fig. 2c). As the Sr:Ca ratios of the brook charr and the *Fundulus* were similar to the eel's values, the following thresholds were established:

$$\text{freshwater growth} = \text{Sr:Ca} < 4.0 \times 10^{-3}$$

$$\text{brackish water growth} = 3.0 \times 10^{-3} < \text{Sr:Ca} \leq 8.0 \times 10^{-3}$$

In cases where some values of the overall Sr:Ca pattern were between 3×10^{-3} and 4×10^{-3} , growth was considered to have occurred in freshwater when the fish exhibited an evident freshwater residence growth pattern (i.e. with Sr:Ca values always lower than 4×10^{-3}). Otherwise, Sr:Ca values within this ambiguous range were considered to be evidence of growth during brackish residence. As has been proposed by some authors, when only one point was located above or below the freshwater–brackish Sr:Ca threshold, it was not taken into account.

Otolith sequences were classified into 7 patterns of distinct habitat use: (i) freshwater residence; (ii) brackish residence; (iii) amphidromous freshwater-to-brackish residence; (iv) amphidromous brackish-to-freshwater residence; (v) amphidromous freshwater-to-brackish-to-freshwater residence; (vi) amphidromous brackish-to-freshwater-to-brackish residence; (vii) amphidromous multiple (>2) changes in freshwater and brackish residence. Classification was conducted from the 1st annulus to the otolith edge.

2.3. Quantitative determination of environmental histories: (i) local (ii) global and (iii) optimization zoning algorithms

Otolith sequences were smoothed with a running-mean filter (window size of 30 μm). This removed variation at very short scales which could not be related to a systematic change in habitat use. Sequences were then classified from the local minimum between the 1st annulus (the position at which the elver metamorphosis had occurred) and the 2nd annulus. Classification from all algorithms (local, global or optimization zoning algorithms) were defined as being freshwater or brackish according to where the predicted Sr:Ca ratio was less than or greater than 3.5×10^{-3} (the delineation between freshwater and brackish habitat for brook charr and *Fundulus* sp as shown in Fig. 2c). For all classification algorithms, classification accuracy was determined by (i) the proportion of otolith sequences with the same environmental history pattern as that determined by eye (e.g. freshwater–brackish–freshwater) and (ii) the proportion of observations within each otolith sequence having the same habitat type as that determined by eye.

2.3.1. Local zoning algorithm

The local zoning algorithm used the generalized distance method (Webster, 1973). This consisted of passing a split moving window, comprised of 2 compartments of equal length, over each sequence. The generalized distance (GD) was determined as a ratio of the squared difference between the means of each compartment and the sum of the variances of each compartment:

$$\text{GD} = \frac{(\bar{Y}_1 - \bar{Y}_2)^2}{s_1^2 + s_2^2} \quad (1)$$

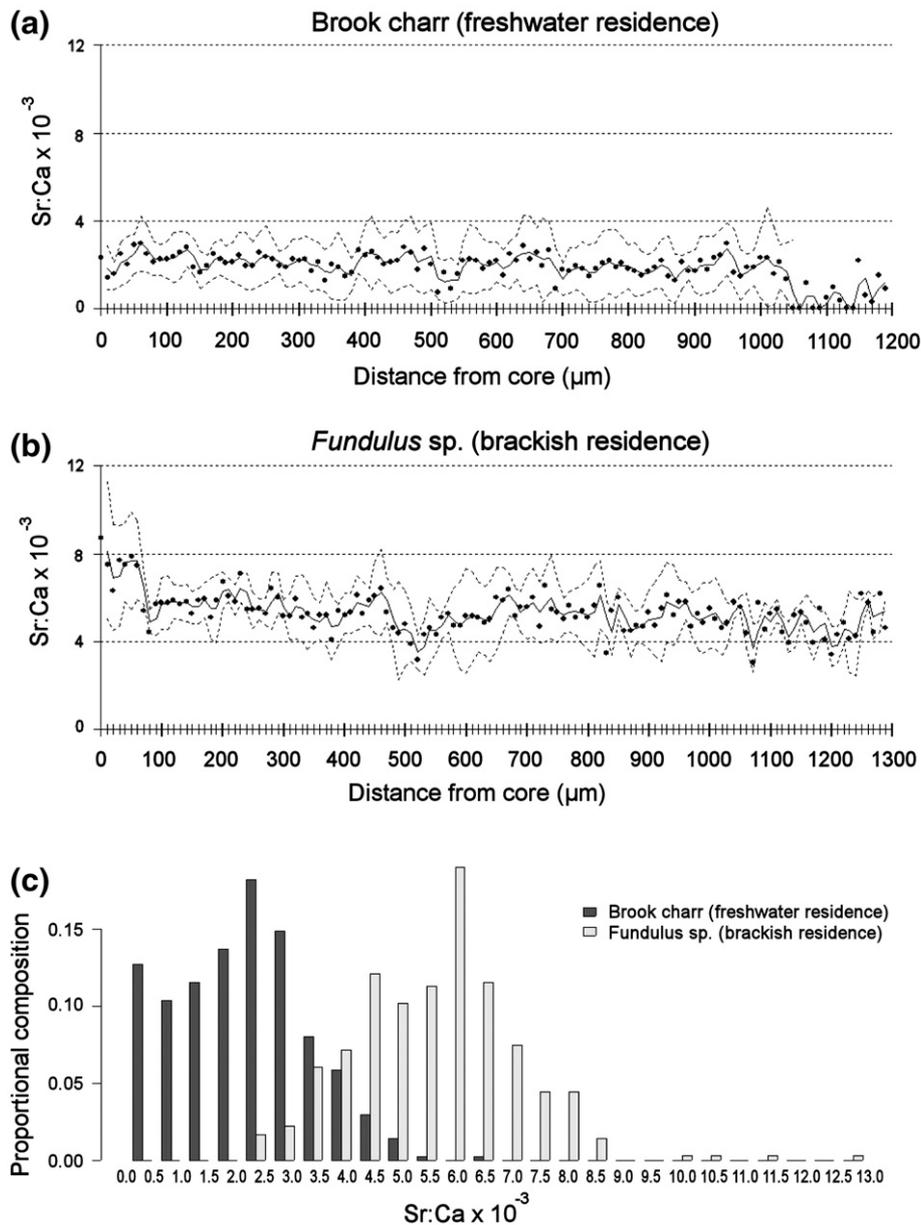


Fig. 2 – Sr:Ca ratios for brook charr (freshwater residence) and *Fundulus* sp (brackish residence): (a) otolith Sr:Ca environmental history sequence of brook charr; (b) otolith Sr:Ca environmental history sequence of *Fundulus* sp; (c) histograms of Sr:Ca ratios for both brook charr and *Fundulus* sp (proportion of total Sr:Ca ratio measurements for brook charr or *Fundulus* sp falling within given Sr:Ca bins). For Fig. 1a and b, filled circles show the mean of the observed values for each distance (from 5 brook charr, or 3 *Fundulus* sp), the solid lines show a running-mean with a window size of 20 μm, and dashed lines show the running mean ± 1 SD.

where \bar{Y}_1 and \bar{Y}_2 are the means and s_1^2 and s_2^2 are the variances of compartments 1 and 2. The window size was determined individually for each otolith as the maximum lag before the auto-correlation of the respective sequence became negative.

Transitions between zones for each otolith sequence were determined by the positions where the generalized distance was greater than the mean generalized difference plus 2 SDs. Contiguous transition zones were merged into individual transition zones according to which transition had the greatest generalized difference.

2.3.2. Global zoning algorithm

The global zoning algorithm divided each sequence into increasingly homogeneous zones, the position of each zone break being determined iteratively such that the sum of the deviance (D) of each of the two zones on either side of the break was the minimum possible. Deviance was defined as:

$$D = \sum_{j=1}^N (y_j - \mu_{[j]})^2 \quad (2)$$

where y_j is the Sr:Ca ratio for position j within the zone and $\mu_{[j]}$ is the mean Sr:Ca ratio of the zone. Each zone was then sub-zoned until specific thresholds were reached: zoning stopped when the number of observations in the zone being partitioned was 10 or less and/or the number of observations in each of its sub-zones was 5 or less.

A potential problem of this type of approach is that it may 'over-fit' a model, particularly if the data are characterized by large amounts of variation over short scales. Zone breaks were therefore pruned using a cross-validation technique. This consisted of (1) splitting the data into 10 equally sized parts, (2) using 9 of these to create a partition model (3) testing this model with the remaining part, and (4) repeating steps 1:3 9999 times (i.e. a Monte-Carlo approach). The optimal number of zoning breaks was determined as being the largest number of partition breaks possible where the residual error (the average error plus 1 SD) was at a minimum. The explanatory power of each fitted model (r^2) was determined as $1 - (\text{residual deviance} / \text{null deviance})$ as follows:

$$r^2 = 1 - \left(\frac{\sum_{j=1}^N (y_j - \hat{y}_j)^2}{\sum_{j=1}^N (\bar{y}_j - \gamma_j)^2} \right) \quad (3)$$

where γ is the observed value, \hat{y} is the estimated value for observation j , and \bar{y} is the mean of the observed values.

2.3.3. Optimization zoning algorithm

The optimization algorithm differed to the local and global zoning algorithms in that a model was fitted to all (n) sequences simultaneously, meaning that the identification of habitats within any one sequence was dependent on those identified within other sequences. The algorithm maximized the combined value of selected statistics of the otolith sequences. Parameters were determined according to a rationale based on the following:

- (1) A maximum of 2 habitats could exist (allowing for the distinction between freshwater and brackish habitats). It was not deemed necessary to have an additional class allowing the option of seawater because the eels had migrated from the marine environment by the time of the elver check (the minimum position from which the otolith sequences were analyzed).
- (2) Class values should remain constant between different otolith sequences.
- (3) Class values along an otolith sequence should be spatially dependent. That is, the probability of 2 positions along the sequence having the same class should be inversely proportional to the distance separating them.

Given the above, the function (O) to be maximized by the optimization algorithm was written as a function of two statistics: (i) the explanatory power of the model; and (ii) the auto-correlation among the model predictions as a function of lag:

$$O = |k_R R + k_C C|_{\max} \quad (4)$$

where k_R and k_C are weighting coefficients. R represents the explanatory power of the model prediction ($1 - (\text{residual deviance} /$

null deviance)) and C represents the sum of the auto-correlation between the model predictions \hat{y}_i separated by a series of lags of $h=1,2,\dots,H$ in the x -axis space for otolith sequences i to n :

$$C = \sum_{h=1}^H \sum_{j=1}^m \sum_{i=1}^n \left(\frac{\hat{y}_i \times \hat{y}_{i(j+h)}}{\sqrt{\sum \hat{y}_i^2 \sum \hat{y}_{i(j+h)}^2}} \right) \quad (5)$$

The objective of the optimization algorithm was to maximize the explanatory power (R) while maximizing the auto-correlation (C). The procedure for optimizing O was as follows:

- (1) An initial O was estimated.
- (2) One otolith sequence was then chosen randomly.
- (3) Three contiguous points on the chosen sequence were then chosen randomly and their class values (and thus \hat{y}_{ij}) were switched to the alternative class value to that held by the majority of the 3 contiguous points. O was re-estimated and the switch was retained if this resulted in a decrease in O .
- (4) Steps 2–3 were re-initiated a further 3239 times. That is, otolith sequences were chosen randomly and points were switched 3240 times, corresponding to a mean of 20 switches per otolith (20×162 otoliths = 3240 switched).
- (5) The \hat{y}_{ic_1} value (freshwater) was changed, O was re-estimated, and this change was retained if there was a reduction in O .
- (6) The \hat{y}_{ic_2} value (brackish) was changed, O was re-estimated, and the change was again retained if there was a reduction in O .
- (7) Steps 2–7 were repeated until the maximum number of iterations (40) had been reached.

For the initial O , the 2 class means (freshwater and brackish) were determined respectively using the 1st and 3rd quartile of the Sr:Ca ratio values of all the otolith sequences, and each point was assigned to either of the classes on a random basis with equal probability. No bias was involved in selecting otolith sequences or points to be switched. When changing class values, the new value was the sum of the preceding values and an additional value determined randomly from a normal distribution with a mean of 0 and a SD of 0.1. The maximum lag, H , was set at a separation distance of 5 observations (i.e. 50 μm). Weights were assigned as $k_R=1$ and $k_C=0.5$. The maximum number of iterations was set at 40.

3. Results

3.1. Water salinity and concentrations of Sr and Ca within the freshwater and brackish habitats

A strong positive correlation existed between water salinity and water Sr:Ca ratio (Pearson's correlation, $t_6=5.74$, $p=0.001$, $r=0.91$) (Table 1). This strong correlation was largely a result of the difference between the freshwater and brackish habitats, and there was a large amount of variation in the relationship between salinity and Sr:Ca ratio within an individual habitat type. For a constant salinity in the freshwater habitat (salinity=0 psu), the Sr:Ca ratio varied between 3.66 and

Table 1 – Water quality (salinity, Sr and Ca) of the freshwater and brackish habitats

Habitat type	Sample site	Site	Salinity (psu)	Sr:Ca (mmol/mol)	Sr:Ca $\times 10^{-3}$ (wt.%)	Mean Sr:Ca (wt.%)	Mean salinity (psu)
Freshwater	1	Sirois Lake	0.0	1.67	3.66	6.78	0.00
	2	River	0.0	4.00	8.75		
	3	River	0.0	3.40	7.45		
	4	River	0.0	3.31	7.24		
Brackish	5	Upper estuary (ebb tide)	6.0	1.08	2.36	47.28	14.38
	5	Upper estuary (high tide)	3.0	17.98	39.32		
	6	Lower estuary (ebb tide)	25.0	33.67	73.62		
	6	Lower estuary (high tide)	23.5	33.75	73.80		

8.75×10^{-3} ; and in the brackish habitat, one measurement had a low salinity associated with a relatively large Sr:Ca ratio (salinity=3 psu, Sr:Ca ratio= 39.32×10^{-3}) whereas another measurement had a greater salinity associated with a smaller ratio (salinity=6 psu, Sr:Ca ratio= 2.36×10^{-3}).

Water Sr:Ca ratios (mean Sr:Ca ratio= 6.78×10^{-3} in the freshwater habitat, mean Sr:Ca ratio= 47.28×10^{-3} in the brackish habitat) were much greater than those of the otoliths of the resident species (mean Sr:Ca ratio= 2×10^{-3} of brook charr in the freshwater habitat, mean Sr:Ca ratio= 5×10^{-3} of *Fundulus* sp. in the brackish habitat).

3.2. Otolith patterns

Transition from a marine habitat to a non-marine habitat always occurred before the 2nd annulus. After smoothing with a running-mean filter (window size=30 μm), 7 patterns of habitat use were evident in the otolith sequences (Fig. 3): freshwater ($n=35$); brackish ($n=60$); freshwater–brackish ($n=15$); brackish–freshwater ($n=29$); freshwater–brackish–freshwater ($n=2$); brackish–freshwater–brackish ($n=13$); and multiple ($n=8$).

Otolith sequences were characterized by a large amount of variability over short scales. Auto-correlation varied according to otolith sequence, with sequences remaining positively correlated up to ranges of between 30 and 570 μm (mean=153 μm , SD=95 μm). This range varied according to pattern of habitat use: freshwater (mean range=172 μm); brackish (mean range=114 μm); freshwater–brackish (mean range=184 μm); brackish–freshwater (mean range=191 μm); freshwater–brackish–freshwater (mean range=330 μm); brackish–freshwater–brackish (mean range=160 μm); multiple (mean range=91 μm).

3.3. Quantitative determination of environmental histories

Examples of the environmental history classification from the local zoning, global zoning and optimization zoning algorithms are shown in Fig. 4. Predicted environmental history sequences were not statistically significantly different from those observed: local zoning algorithm (Chi-square test: $\chi^2_{df=6}=8.64$, $p=0.19$); global zoning algorithm (Chi-square test: $\chi^2_{df=6}=3.38$, $p=0.75$); optimization zoning algorithm (Chi-square test: $\chi^2_{df=6}=2.52$, $p=0.86$). Overall, the optimization zoning algorithm achieved the greatest classification accuracy (85% of the sequences classified correctly), followed by the global zoning algorithm (78% classified correctly) and then the local zoning algorithm (76% classified correctly) (Table 2).

Classification accuracy was dependent on environmental history. The environmental history pattern involving multiple changes had a lower classification accuracy (mean of the three zoning algorithms=25%) than environmental history patterns involving little or no change (for example, mean of the three zoning algorithms=93% for freshwater) (Table 2). The specificity of classification accuracy to environmental history pattern was maintained regardless of zoning algorithm, such that no statistically significant difference existed between the environmental history sequences classified according to the different algorithms: local zoning vs global zoning (Chi-square test: $\chi^2_{df=6}=3.16$, $p=0.78$); local zoning vs optimization zoning (Chi-square test: $\chi^2_{df=6}=5.54$, $p=0.47$); and global zoning vs optimization zoning (Chi-square test: $\chi^2_{df=6}=2.05$, $p=0.91$). The exception to this was the large variation in the classification accuracy for the environmental history pattern showing a freshwater–brackish–freshwater habitat residence which may be attributed to the relatively small sample size of this pattern: a single misclassification would result in a decrease in the classification accuracy by 50%.

The strongest fit, in terms of the RMSE between observed and estimated Sr:Ca ratios, was obtained for the global zoning algorithm (mean RMSE for all habitat types=1.03), followed by the local zoning algorithm (mean RMSE for all habitat types=1.04) (Table 2). The optimization zoning algorithm produced a weaker fit (mean RMSE for all habitat types=1.21), which may be attributed to the fact that the optimization algorithm only permitted 2 habitats for the entire dataset. RMSE was dependent on the habitat, tending to increase when the otolith sequence indicated a high proportion of occupancy in a brackish rather than freshwater habitat (this was expected given that brackish habitats tend to have greater variance in salinity than freshwater habitats).

Of the 162 sequences, the optimization zoning algorithm correctly classified at least 95% of the observations of habitat type in 128 sequences (79% of the total number of sequences) (Fig. 5). The local and global zoning algorithms performed less well but still correctly classified at least 95% of the observations of habitat type in the majority of sequences: 119 sequences (73% of the total) for the local zoning algorithm and 105 sequences (64% of the total) for the global zoning algorithm.

4. Discussion and conclusion

Key to establishing a threshold otolith Sr:Ca ratio for delineating eel otolith formation in freshwater or brackish

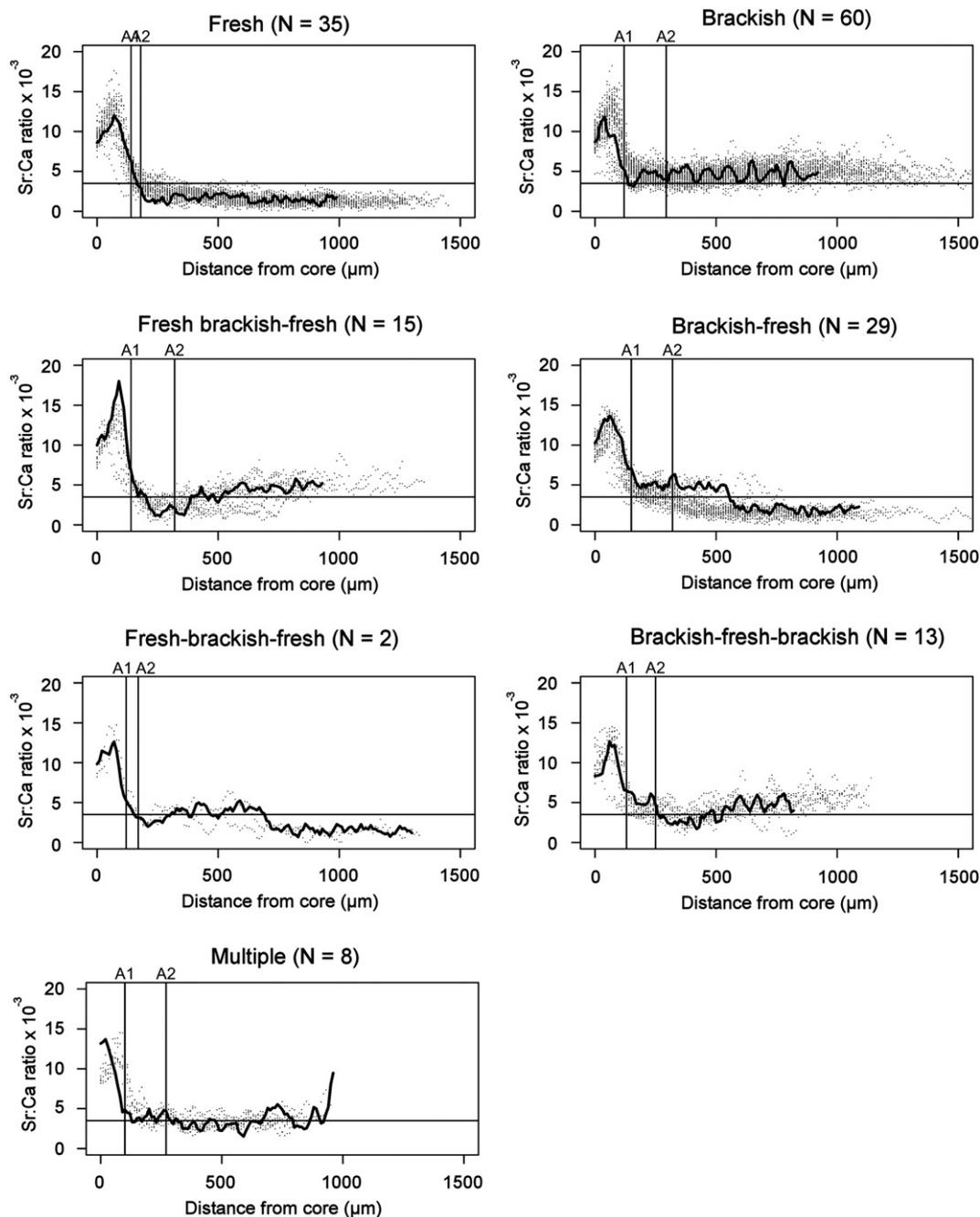


Fig. 3 – Sample Sr:Ca ratio sequences showing the 7 qualitatively derived environmental history patterns. A sequence typical of each environmental history pattern is shown using a continuous line produced from a running-mean with a window size of 30 μm , along with the 1st (A1) and 2nd (A2) annuli. Points show Sr:Ca ratios from all sequences with the same migratory pattern.

habitats was the assumption that ratios found in otoliths of resident species in either habitat would approximate those found in parts of the eel otolith formed when it was present in the respective habitat. Inter-specific physiological differences in ion transport and substitution resulting from different environmental tolerances may cause different Sr:Ca ratios for different species under the same set of environmental conditions (Gillanders and Kingsford, 2003; Swearer et al., 2003; Hamer and Jenkins, 2007). However, given that we were delineating between habitats involving large differences in

water quality (freshwater to brackish), we maintain that species-specific effects would have been relatively small. Information on Sr:Ca ratios in resident species suggested a similar range of values to those found in eels. For example, Arai and Morita (2005) found Sr:Ca ratios of approximately 2×10^{-3} in *S. leucomaenis* resident within rivers; Radtke (1989) found Sr:Ca ratios of approximately 11×10^{-3} for *Fundulus heteroclitus* in a marine environment. In both cases, these are similar ratios to those established for eels in river and marine habitats in Eastern Canada (see for example, Jessop et al.,

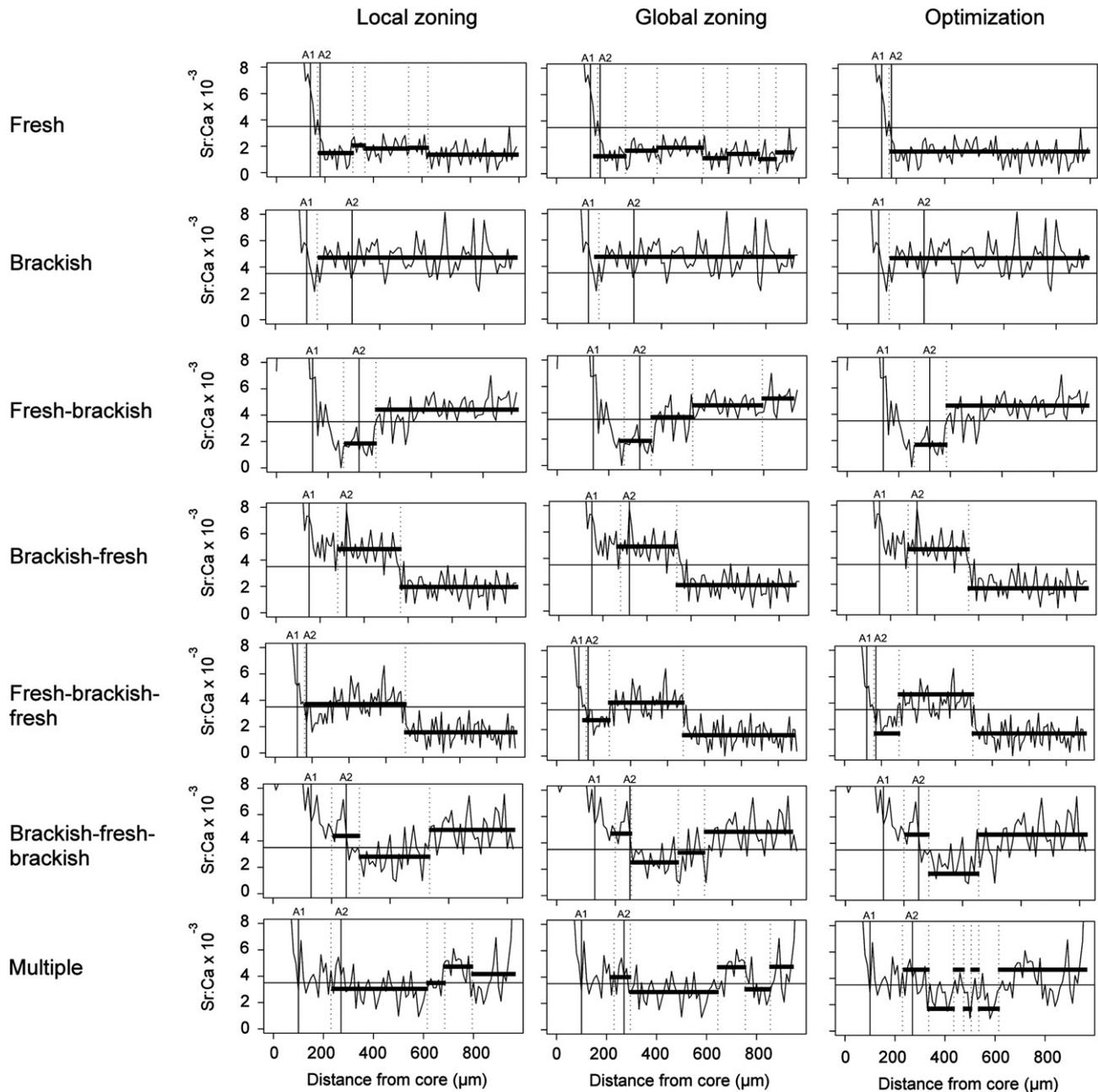


Fig. 4– Sample Sr:Ca ratio sequences showing the 7 qualitatively derived environmental history patterns classified by the local zoning, global zoning and optimization zoning algorithms. The solid vertical lines represent the position of the 1st and 2nd annuli; the horizontal vertical line represents a Sr:Ca ratio of 3.5×10^3 . The dashed vertical lines represent the position of the change from one habitat type to the next.

2004). Given this, we maintain that it was justified to use resident species to establish the threshold otolith Sr:Ca ratio for delineating formation in freshwater and brackish habitats in eels.

The three zoning algorithms (local, global and optimization) were based on a similar rationale: that a temporal dependence in the Sr:Ca ratio could be used to zone otolith sequences. This temporal dependence was characterized by comparing the within-zone and between-zone variances for the local and global zoning algorithms, and by the implementation of an auto-correlation function for the optimization

zoning algorithm. Two fundamental differences existed between the local and global zoning algorithms and the optimization zoning algorithm. Firstly, the local and global zoning algorithms classified each sequence independently of all the other sequences whereas the optimization zoning algorithm used all sequences to concurrently classify each individual sequence. Secondly, the local and global zoning algorithms allowed for multiple habitats within each sequence (which were later classified into freshwater and brackish residence according to a predetermined limit), whereas the optimization zoning algorithm only allowed for

Table 2 – Comparison of qualitative versus quantitative classification of otoliths

Qualitative interpretation: environmental history pattern* (and number of cases)	Proportion classified correctly according to zoning algorithm				RMSE according to zoning algorithm			
	Local zoning	Global zoning	Optimization zoning	Mean	Local zoning	Global zoning	Optimization zoning	Mean
F (35)	1.00	0.97	0.83	0.93	0.80	0.80	0.96	0.85
B (60)	0.88	0.77	0.97	0.87	1.16	1.14	1.35	1.22
F–B (15)	0.67	0.73	0.67	0.69	1.09	1.05	1.25	1.13
B–F (29)	0.55	0.79	0.90	0.75	0.96	0.92	1.02	0.97
F–B–F (2)	0.00	0.50	1.00	0.50	0.96	0.89	0.99	0.95
B–F–B (13)	0.61	0.65	0.77	0.69	1.23	1.24	1.44	1.30
Multiple (8)	0.12	0.25	0.38	0.25	1.05	1.02	1.35	1.14
All sequences (165)	0.76	0.78	0.85	0.80	1.04	1.03	1.21	1.09

* F (freshwater), B (brackish), F–B (freshwater-to-brackish), B–F (brackish-to-freshwater), F–B–F (freshwater-to-brackish-to-freshwater), B–F–B (brackish-to-freshwater-to brackish).

a maximum of two separate habitat types. The choice of algorithm selection should depend on the characteristics of the fish population. In situations where the bulk of a population migrates in a punctuated fashion between two distinct habitats, the optimization zoning method might be more appropriate; in situations where there is more within-population heterogeneity in environmental histories, local or global zoning methods may be more appropriate (and a further classification into either freshwater or brackish habitat residence according to a predetermined limit may not even be required). Of the three algorithms, the optimization zoning algorithm performed better than the other two when the life-history sequence involved multiple habitat changes, so may be more suitable for this environmental history pattern, but a larger sample size would be required to confirm this.

The optimization zoning algorithm has an additional advantage in that it may be used in an *a posteriori* determination of the Sr:Ca ratios associated with the residence of a given fish population in a given habitat. Typically, a sample population is used to determine the Sr:Ca ratios associated with a given habitat residence. However, if this is not known, the optimization algorithm may be used to estimate class means approximating those associated with residency in different habitats using initial class values that have been defined arbitrarily. For example, if it known that an amphidromous fish population remains in freshwater and brackish habitat for the vast majority of its life-cycle, and migrates between the two habitats in a punctuated fashion, the class values determined by the optimization algorithm may approximate those associated with otolith formation in the freshwater and brackish habitats.

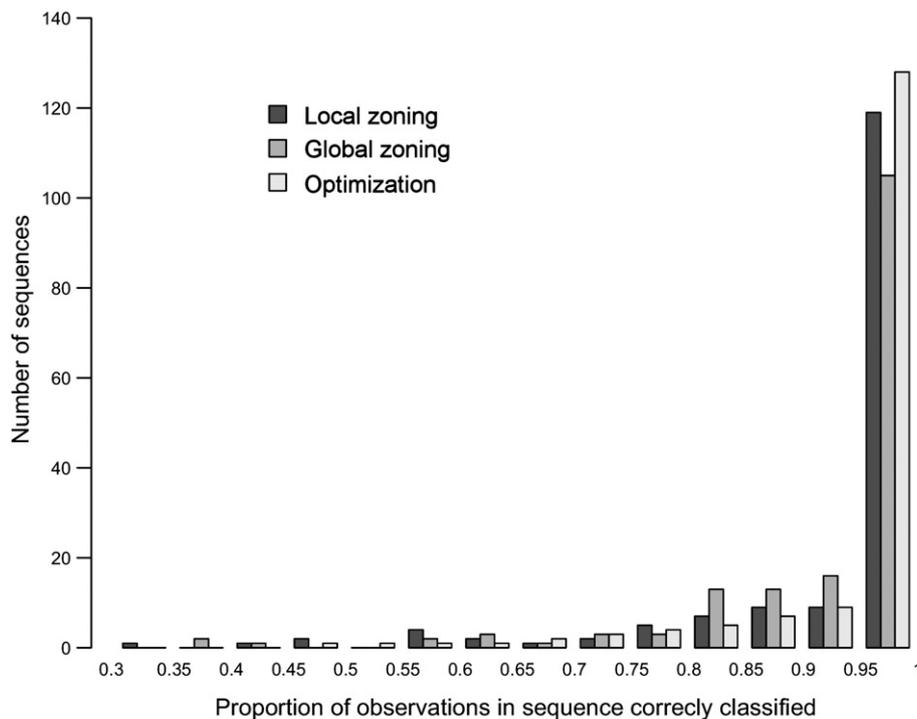


Fig. 5 – Proportion of observations correctly classified by the local zoning, global zoning and optimization zoning algorithms.

The zoning algorithms classified otolith sequences into environmental histories that were not significantly different to those subjectively deduced by eye. Indeed, despite the variation in algorithm characteristics, the algorithms gave remarkably similar results to one another. Given this, it can be concluded that a quantitative algorithmic approach is suitable for determining environmental histories from otolith sequences. The advantages of the quantitative approach are that it is possible to (i) infer attributes of the environmental history from the statistics of the model fit and (ii) report the parameters of the algorithm used to obtain this fit. Firstly, the statistics of the model fit may provide information on the environmental history of the fish population that may be difficult to determine from qualitative interpretation. For example, the residual variance of the model, compartmentalized according to habitat type, may show a habitat-specific relationship. Secondly, reporting of the parameters of the algorithm (such as the smoothing window length) is important because it allows for methodological consistency in future studies. A researcher can use exactly the same parameters as those of previous studies, allowing for comparisons of otolith studies in different geographical regions or time periods and therefore enabling more robust meta-analyses.

Acknowledgements

The study was funded by the *Natural Science and Engineering Research Council of Canada*, the *Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec* and the *Ministère des Ressources Naturelles et de la Faune du Québec*. We thank François Caron who was joint supervisor on this project, and thank Denis Fournier, Vanessa Cauchon, Daniel Hatin, Lee-Anne Julyan, Véronique Lavoie, François Dubé and Jean-Nicolas Bujold for their assistance in the field-work. We would particularly like to thank Wann-Nian Tzeng, Yoshiyuki Iizuka and Jen-Chieh Shiao for their invaluable advice and assistance with the otolith Sr:Ca analyses.

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