Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using solution-based ICPMS

Anthony J. Fowler, Steven E. Campana, Cynthia M. Jones, and Simon R. Thorrold

Abstract: Retrospective determination of the early life history of fish using the microelemental analysis of their otoliths is dependent upon understanding the factors that affect this elemental composition. Here, juvenile Atlantic croaker (*Micropogonias undulatus*) were reared under different treatments of temperature and salinity to determine their impacts on elemental inclusion rates in otoliths. Solution-based inductively coupled plasma mass spectrometry (ICPMS) was used to measure 21 isotopes in each otolith: isotopic concentrations ranged over seven orders of magnitude, and differed significantly amongst the temperature–salinity regimes. Univariate analyses identified 13 isotopes that contributed to these multivariate differences; the influence of temperature was stronger than that of salinity. Within each treatment there was a significant relationship between otolith microchemistry and otolith size. To some extent this confounded the interpretation of the between-treatment effect of temperature. In contrast, both the otolith and somatic growth rates were similar between the two salinity treatments, indicating that differences in elemental fingerprints were unambiguously related to the salinity difference, probably a response to the elemental concentrations in the tank water. Overall the study highlighted the current poor understanding of the mechanism of contamination of otoliths by trace elements and their incorporation into the otolith microstructure.

Résumé : La reconstitution du début du cycle biologique d'un poisson à l'aie de l'analyse des micro-éléments présents dans ses otolithes est tributaire de la compréhension des facteurs qui agissent sur la composition élémentaire de ces organes. Nous avons élevé dans diverses conditions de température et de salinité des juvéniles de tambour brésilien (*Micropogonias undulatus*) pour mesurer les effets de ces traitements sur le taux d'absorption des éléments dans les otolithes. La spectrométrie de masse avec plasma inductif en solution a permis de mesurer la concentration de 21 isotopes dans chaque otolithe; ces concentrations couvraient sept ordres de grandeur et différaient de façon significative selon les régimes de température et de salinité. Des analyses univariées ont permis de repérer 13 isotopes contribuant à ces différences dans l'analyse à plusieurs variables; l'influence de la température était plus forte que celle de la salinité. Pour chaque traitement, on observait une relation significative entre la microchimie de l'otolithe et sa taille, ce qui créait une certaine confusion dans l'interprétation de l'effet des divers régimes de température. Par contre, le taux de croissance des otolithes et celui de l'ensemble du corps étaient à peu près les mêmes pour les deux conditions de salinité, ce qui indique que les différences dans les empreintes des éléments liées, sans ambiguïté possible, à la différence de salinité; il s'agit probablement d'une réaction aux concentrations des éléments dans l'eau de l'aquarium. Dans l'ensemble, l'étude a mis en relief les carences qui subsistent dans la compréhension du mécanisme de contamination des otolithes par les éléments traces et de leur absorption par la microstructure des otolithes.

[Traduit par la Rédaction]

Received August 8, 1994. Accepted March 2, 1995.
J12524

A.J. Fowler,[1] S.E. Campana, and S.R. Thorrold. Marine Fish Division, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS B2Y 4A2, Canada.
C.M. Jones. Applied Marine Research Laboratory, Old Dominion University, Norfolk, VA 23529, USA.

[1] Present address: South Australian Research and Development Institute, PO Box 120, Henley Beach, Adelaide, South Australia 5001.

Introduction

Otoliths of teleost fishes are small, calcareous structures located within the inner ear, whose primary function appears to be the detection of sound. These structures have also become valuable research tools for fish biologists, now being the preferred structure for use in large-scale direct ageing studies based on the enumeration of annuli (Pentilla and Dery 1988; Beamish 1992). Furthermore, their microstructure provides an accurate chronology of growth and life-history transitions, which has revolutionized studies of early life history (Campana and Neilson 1985; Jones 1986; Jones 1992). Recently, a significant new research priority has developed based on otoliths. Their elemental composition ("microchemistry") is being used increasingly to retrospectively describe the life history of fish, in particular to identify the environments they have experienced (reviewed Gunn et al. 1992; Radtke and Shafer 1992; Secor et al. 1994).

The use of otoliths as environmental recorders is based on the premise that the differences in water chemistry amongst different aquatic environments are manifested in the elemental composition of otoliths (Radtke and Shafer 1992; Campana and Gagne 1994). It is also dependent on the observation that otoliths are acellular and physiologically static; once accreted, material is not resorbed or metabolically reworked (Campana and Neilson 1985). Already otolith microchemistry has proved to be a useful tool, having been used to determine the age of fish (Bennett et al. 1982; Campana et al. 1990), to distinguish amongst their stocks (Edmonds et al. 1989, 1991, 1992; Campana and Gagne 1994; Campana et al. 1994), and to describe the environmental conditions experienced at various life-history stages (e.g. Radtke 1984; Kalish 1990, 1991*b*; Secor 1992; Radtke and Shafer 1992).

Interpretation of the life-history information that has been chemically encoded within the otolith can only be achieved after the elemental composition has been determined. One of the most powerful procedures now available to achieve this is inductively coupled plasma mass spectrometry (ICPMS). Since its first commercial application in 1983, its multielement capabilities, broad linear range, and its ability to discriminate amongst isotopes made ICPMS a significant tool for use in the earth sciences (Date 1991; Jarvis and Jarvis 1992). ICPMS can be used to analyse otoliths in two ways: solution-based ICPMS for the analysis of whole otoliths, and laser ablation ICPMS (LA-ICPMS) for sampling specific loci within otoliths. To date, the former has been applied to the elemental analysis of whole otoliths from adult fish (Edmonds et al. 1992; Campana and Gagne 1994), in each case successfully discriminating amongst fish collected from different places. Laser ablation ICPMS has recently been successfully applied to the assay of the elemental composition of nuclei in otoliths of adult cod (Campana et al. 1994), also successfully discriminating amongst fish collected from different places.

Application of microelemental analysis to field studies depends on the ability to accurately interpret such analyses. The aim of this project was to improve our understanding of the factors that affect the microchemistry of the otoliths of juvenile Atlantic croaker (*Micropogonias undulatus*), to facilitate its use for understanding the life history of wild fish. Consequently, fish were reared under constant conditions at different temperatures and salinities to assess their effect on otolith microchemistry. The present paper is concerned with the analysis of otoliths by solution-based ICPMS, whilst the following paper reports the results of analyses from the same fish using LA-ICPMS (Fowler et al. 1995). The specific aims here were to (1) identify the elements that occur in the otoliths of Atlantic croaker; (2) determine their absolute concentrations; and (3) determine whether the rate of incorporation varies under different environmental conditions.

Materials and methods

Juvenile Atlantic croaker used in this experiment were reared at the National Marine Fisheries Service (NMFS) Laboratory, Beaufort, N.C. Adult fish were induced to spawn through temperature manipulation and injection. Eggs were reared for the first 24 h in a common tank (22°C and 33‰ salinity) and then transferred to eight separate 100-L rearing tanks, with an initial stocking density of 500 eggs per tank. Each tank was supplied with water from the same initial source, with treatments established by diluting with deionized water and (or) heating. The four treatments assigned randomly to two tanks each were as follows treatment 1, 20°C and 26‰ salinity; treatment 2, 20°C and 35‰ salinity; treatment 3, 25°C and 26‰ salinity; treatment 4, 25°C and 35‰ salinity.

After hatching, the larvae were fed ad libitum with rotifers, whose concentration in the tanks was always kept in excess of consumption. A 12 h dark : 12 h light diurnal cycle was maintained. Oxygen was always kept at saturation in each tank using aerators. All tanks were maintained for 71 days from 28 October 1992 (day 1) until 6 January 1993 (day 71), after which the surviving fish were killed, measured (standard and total length), and frozen in individual acid-washed glass vials.

A number of fish were removed from each tank at different times throughout the rearing period for a separate study to determine the periodicity of increment formation in otoliths. Furthermore, there was considerable variation in the survivorship amongst the treatments and tanks. Unfortunately, the total loss of fish from both tanks of treatment 4 eliminated the high temperature – high salinity treatment (25°C and 35‰ salinity), whilst one tank was lost from treatment 2 (20°C and 35‰ salinity). Fish that died prior to the termination date of 6 January were not considered in any subsequent analyses.

General laboratory procedures

To avoid airborne contamination most procedures involving the fish or otoliths were done in a positive flow fume hood, which established a Class 100 clean room. To prevent surface contamination all equipment and plasticware involved in the ICPMS work or otolith storage were acid-washed prior to use. At no stage were fish or otoliths exposed to bare metal.

After the fish were killed, 10 from each tank (except treatment 2 tank 1, $n = 20$), were randomly selected from

Table 1. Details of otolith dissolution prior to solution-based ICPMS.

Treatment	Tank	Fish remaining	Sample size	Mean otolith weight (mg)	Volume of acid (mL)	Volume of H_2O (mL)	Total volume (mL)
1	1	46	10	0.404	0.3	2.7	3.0
(20°C, 26‰)	2	41	10	0.426	0.3	2.9	3.2
2	1	29	20	0.545	0.4	3.7	4.1
(20°C, 35‰)	2	0	—				
3	1	32	10	3.739	2.8	25.0	27.8
(25°C, 26‰)	2	24	10	4.195	3.1	28.1	31.2
4	1	0	—				
(25°C, 35‰)	2	0	—				

Note: Included are the number of fish remaining in each tank at the end of the rearing period, the number of otoliths from each tank analysed by ICPMS, their mean weight, and the volumes of Seastar nitric acid and Super Q water used to dissolve each otolith. All solutions were subsequently diluted twofold.

those available using random number tables. Each fish was dissected using glass probes on a microscope slide using a binocular microscope (ensuring that the otolith or dissection equipment never touched any metal part of the microscope). Both sagittae, one destined for solution-based ICPMS and the other for LA–ICPMS were placed on a clean glass slide, rinsed several times in Super Q water (deionized, purified through reverse osmosis, and millipore filtered), and adhering tissue was removed with the glass probes. These otoliths were then air-dried in the positive flow fume hood. Both air-dried sagittae were weighed (to 0.01mg), and the one destined for solution-based ICPMS was sealed in an acid-washed polyethylene conical vial for acid dissolution.

Solution-based ICPMS

The sagittae were prepared for analysis by dissolution in Seastar brand redistilled nitric acid followed by dilution with Super Q water. It is important to standardize the concentrations of the sample solutions before analysing them with ICPMS (Jarvis and Jarvis 1992), and since the otoliths from fish in the different treatments varied in size, the amounts of acid and water used for otolith dissolution were proportional to the mean weight of the otoliths being dissolved (Table 1). The acid was added to the conical vials first, followed half an hour later by the Super Q water, maintaining a ratio of nine parts water to one part acid (Table 1).

The solution-based ICPMS analyses were done by an external laboratory. The solutions, one from each otolith, were provided to the laboratory in the conical vials in which the otoliths had been dissolved. Each solution was then further diluted twofold prior to assay. Our samples were all analysed on a single day in five blocks. The samples from the five tanks were divided evenly amongst the five blocks, and their within-block order was random. Prior to analyses the ICPMS was calibrated with standards and thereafter let to drift. In the subsequent data analyses, sequence number was used as a covariate to statistically remove this instrument-drift influence.

Data analysis

Results from solution-based ICPMS were expressed in terms of solution concentration (micrograms per litre) for each isotope within each otolith. Isotopic concentrations were converted to elemental concentrations based on percent natural abundances, and then converted to parts per million, based on otolith weight and the total volumes used for dissolution (Table 1). Elemental concentrations were analysed using a combination of multivariate and univariate techniques. Prior to analysis all variables were transformed ($y = \log_e x$), to stabilize variance–covariance matrices in the multivariate tests, and to ensure homogeneity of variances and normality of residuals in univariate analyses.

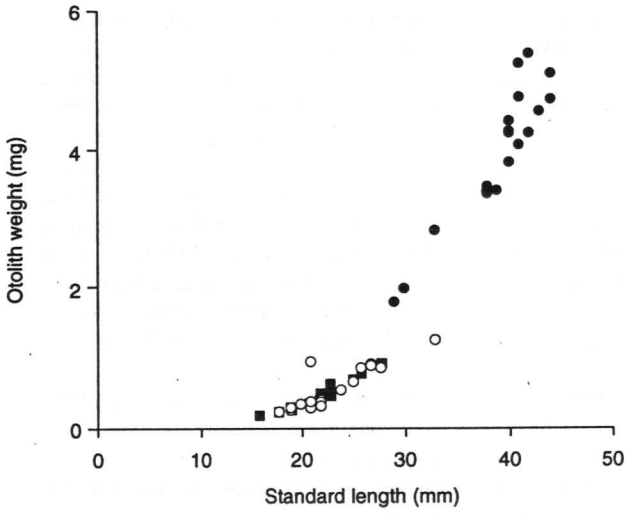
Elemental compositions of otoliths were analysed by multivariate analysis of covariance. Treatment was considered a fixed effect in the design, and sequence number was introduced to the model as a covariate to account for instrument drift. The multivariate analysis of covariance (MANCOVA) was followed by a canonical discriminant analysis (CDA) to identify where differences among treatments occurred. Before the CDA analysis, the effect of sequence was removed from each of the isotopes using the pooled within-groups slope. Finally, a factor analysis was done to identify the isotopes that were loading together in the multivariate analyses.

Univariate analyses were used to identify individual isotopes contributing to the differences detected in the MANCOVA and CDA analyses. The data for each isotope were analysed using ANOVA, with tanks nested within treatments. To allow a more powerful test of the treatment effect, data were pooled across tanks when the tank effect was not significant at $p > 0.2$. An alpha level of 0.05 was used here despite the number of comparisons being made, as we were using such analyses as indicators of those

Table 2. Details of the size of fish and otoliths that were considered in the microelemental analyses by ICPMS. In each case the mean (±SD) is given.

Treatment	Tank	Mean standard length (mm)	Mean otolith weight (mg)	Mean otolith length length (mm)
1	1	21.1±3.5	0.40±0.24	1.22±0.27
(20°C, 26‰)	2	22.3±3.5	0.43±0.17	1.29±0.23
2	1	23.1±3.8	0.55±0.31	1.34±0.24
(20°C, 35‰)	2	—	—	—
3	1	36.3±4.3	3.74±1.17	2.48±0.31
(25°C, 26‰)	2	38.7±2.4	4.20±0.73	2.63±0.16
4	1	—	—	—
(25°C, 35‰)	2	—	—	—

Fig. 1. Relationship between otolith weight and standard length for fish from the three surviving treatments. Open circles relate to treatment 1, 20°C and 26‰ salinity; solid squares to treatment 2, 20°C and 35‰ salinity; and solid circles to treatment 3, 25°C and 26‰ salinity.



isotopes likely to have contributed to the multivariate differences identified by the MANCOVA. When an ANOVA was significant, means were compared using Tukey's honestly significant difference, applying Kramer's correction for unequal sample sizes (Quinn and Day 1989).

Results

Fish and otolith growth

The fish that had been maintained at low temperatures were much smaller than those from the higher temperature regime, a difference that was also reflected in the otolith weights and lengths (Table 2). Fish from the low-temperature tanks had otoliths approximately half the length and one tenth the weight of those from fish reared at higher temperatures (Table 2). Consideration of the otolith weight and fish size relationships (Fig. 1) suggested that the high-temperature treatment caused a higher rate of increase in otolith weight relative to standard length, than the low-temperature treatments. There was no apparent effect of salinity here.

Solution-based ICPMS

A total of 21 isotopes representing 16 elements was detected in the otolith solutions. Initial plots for some isotopes against sequence number displayed significant trends related to instrument drift, particularly evident for some major elements such as Ca. Furthermore, in some cases, different isotopes from the same element drifted in different directions. Consequently, sequence number was treated as a covariate and removed from the analyses. The concentrations of the 16 elements calculated from the isotopic concentrations of the 21 isotopes ranged over seven orders of magnitude (Table 3). Calcium dominated the results followed by chlorine and strontium (Table 3). Iron and phosphorus were present in hundreds of parts per million, whilst the remaining elements were at less than 100 ppm. Differences in elemental concentrations from isotopes of the same element are related to different rates of interference from other ions and variation in counting.

The multivariate analysis of variance indicated a significant difference in the elemental fingerprints of otoliths from the three treatments (Pillai's Trace = 1.446, $F = 4.83$, df = 40, 74, $p < 0.0001$), along with a significant effect of the sequence covariate. The canonical discriminant analysis that relates the results from the three treatments to each other indicates a clear separation amongst them (Fig. 2). The isolation of treatment 3 from the other two along canonical variate 1 is coincident with the difference in temperature regimes. The separation between treatments 1 and 2 along canonical variate 2 reflects the effect of salinity on otolith microchemistry. Factor analysis was then used to look for affinities amongst the 21 isotopes. The calcium and strontium isotopes, along with ^{57}Fe, separated from all others along factor 1 (Fig. 3). Each of these had higher concentrations within treatment 3 than treatments 1 and 2, suggesting that their separation could be related to temperature (Table 3). Calcium and strontium isotopes separated from ^{26}Mg, ^{55}Mn, ^{31}P, and ^{85}Rb along factor 2, probably due to salinity.

There was a significant difference in the elemental composition of otoliths from the three treatments (Fig. 2). However, the cause of this is ambiguous as the treatment effect also resulted in differences in growth rate; treatment 3 (high temperature) produced large otoliths and treatments 1 and 2 (low temperature) small otoliths (Table 2). We used principal components analysis (PCA) to assess the influence of growth on otolith composition. Linear combinations of the data for all 21 isotopes were constructed using PCA. The first principal component, accounting for 37.2% of the variability within the matrix, was graphed against otolith weight (Fig. 4). For each treatment there was a monotonic decreasing function between the first principal component and otolith weight, indicating

Table 3. Summary of concentrations of the 21 isotopes that were significantly above background levels in the otoliths of fish from each tank.

	Treatment 1 (ppm)				Treatment 2 (ppm)		Treatment 3 (ppm)			
	Tank 1		Tank 2		Tank 1		Tank 1		Tank 2	
Isotope	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
^{7}Li	1.2	0.8	1.2	0.5	1.7	1.4	0.9	0.3	1.3	0.4
^{26}Mg	144.3	50.2	135.5	41.2	122.6	81.8	69.2	26.3	72.3	23.4
^{27}Al	44.1	47.6	50.3	35.7	24.6	22.2	28.3	27.5	26.8	12.2
^{31}P	790.4	545.5	853.6	141.8	874.5	589.5	256.5	113.2	274.3	58.5
^{35}Cl	10 479.6	11 624.2	10 788.6	2 590.5	11 181.8	11 999.7	12 277.6	3 916.2	11 675.6	3 269.8
^{44}Ca	467 974.0	41 855.0	454 170.0	47 907.0	478 058.0	59 060.0	502 815.0	81 915.0	485 423.0	65 100.0
^{48}Ca	338 832.0	24 905.0	330 000.0	37 992.0	335 016.0	14 310.0	363 474.0	12 482.0	365 705.0	8 427.0
^{55}Mn	7.2	1.3	7.2	1.5	7.3	2.7	4.6	0.8	5.8	1.2
^{57}Fe	358.0	128.1	344.9	64.2	362.4	49.7	354.8	38.9	362.4	49.7
^{60}Ni	11.0	12.5	5.0	1.6	5.8	5.3	5.3	3.8	4.9	2.5
^{65}Cu	4.4	1.6	4.7	2.1	3.3	3.2	2.4	1.9	2.7	1.1
^{66}Zn	82.9	50.0	59.7	29.0	54.6	92.1	25.3	54.3	37.2	20.4
^{68}Zn	78.5	51.8	58.6	23.2	54.0	86.9	23.6	46.5	37.9	13.3
^{75}As	0.4	0.2	0.7	0.4	0.6	0.5	0.6	0.3	0.5	0.5
^{85}Rb	0.6	0.7	0.8	0.5	0.6	0.4	0.4	0.2	0.5	0.2
^{86}Sr	1 421.8	229.0	1 350.4	130.9	1 378.4	206.4	1 660.4	216.0	1 804.0	190.5
^{87}Sr	1 378.9	232.8	1 248.8	132.6	1 364.6	226.2	1 640.7	227.8	1 767.0	221.0
^{88}Sr	2 013.1	213.5	1 899.6	114.2	1 929.0	174.1	2 330.1	213.6	2 528.1	376.5
^{137}Ba	5.8	2.4	4.3	5.8	5.3	4.2	5.9	2.9	6.0	9.8
^{138}Ba	5.0	2.7	4.1	6.3	4.5	3.8	5.3	2.6	5.6	10.6
^{208}Pb	2.1	2.6	1.6	1.4	1.3	2.0	0.7	1.7	1.7	2.5

Note: Sample size is 10 except treatment 2, tank 1, where $n = 20$. (Treatment 1, 20°C and 26‰; treatment 2, 20°C and 35‰; treatment 3, 25°C and 35‰.)

that within a given treatment, elemental composition is a function of otolith size and growth rate.

Individual isotopes were analysed independently to identify those contributing to the separation among treatments. No isotope demonstrated a significant tank effect; indeed, p was less than 0.2 for only two isotopes, ^{7}Li and ^{60}Ni (Table 4). Thirteen isotopes demonstrated a significant difference amongst treatments (Table 4, Fig. 5). In most cases the mean for treatment 3 was significantly different from those for treatments 1 and 2, reflecting an effect of temperature. For ^{26}Mg, ^{31}P, ^{55}Mn, ^{65}Cu, ^{66}Zn, and ^{68}Zn this mean was significantly lower whilst for ^{44}Ca, ^{48}Ca, ^{57}Fe, and the three Sr isotopes the mean for treatment 3 was higher. For ^{44}Ca a significant difference between treatments 1 and 2 indicated an effect of salinity.

Discussion

The use of microelemental analysis of otoliths to address questions about the early life history of fish depends on interpreting the elemental fingerprints to identify residence times within water masses of different chemistries. Yet, elemental fingerprints from otoliths may not be a simple reflection of the elemental composition of the seawater, since numerous other factors may influence the rates at which ions are deposited in otoliths (Kalish 1989, 1991*a*; Radtke and Shafer 1992). By rearing juvenile Atlantic croaker under controlled environmental conditions we were able to assess the effect of water temperature and salinity on otolith microchemistry, whilst simultaneously minimizing the effect of other potentially confounding influences.

Factors affecting otolith microchemistry

Our experiment was compromised somewhat by the complete mortality of fish from one treatment. Nevertheless, we were still able to identify significant differences in the elemental composition of otoliths from fish reared under the three remaining regimes of temperature and salinity. The effects were recognizable even though the same water source was used for all treatments, with the different conditions being determined only by dilution with deionized water and (or) heating.

Comparison of the elemental composition of otoliths from treatments 1 (20°C, 26‰) and 2 (20°C, 35‰), allowed us to assess the influence of salinity. The univariate analyses of single isotopes suggested that salinity caused only subtle differences amongst different isotopes, most evident for ^{31}P, ^{55}Mn, and ^{65}Cu. Nevertheless, when the numerous differences amongst different isotopes were combined into multivariate descriptions, the extent of the difference between treatments became apparent. There was no evidence from any of our analyses that the fish or otoliths differed in growth rates between these salinity treatments. Therefore, the most likely explanation for the elemental

Fig. 2. Plot of the first two canonical variates from a CDA comparing the elemental composition of otoliths amongst the three experimental treatments. Open circles relate to treatment 1, 20°C and 26‰ salinity; solid squares to treatment 2, 20°C and 35‰ salinity; and solid circles to treatment 3, 25°C and 26‰ salinity.

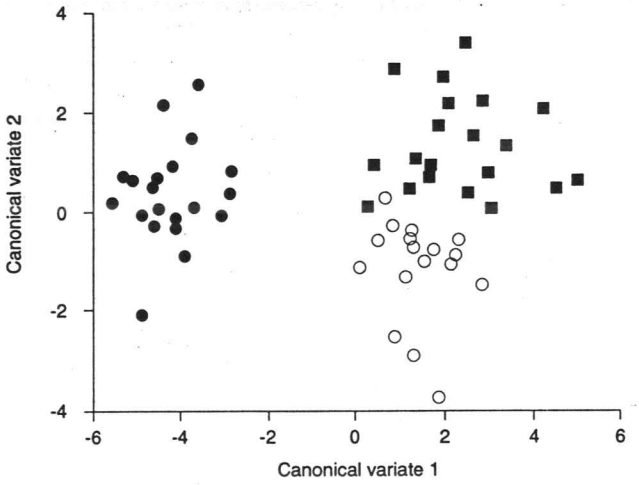
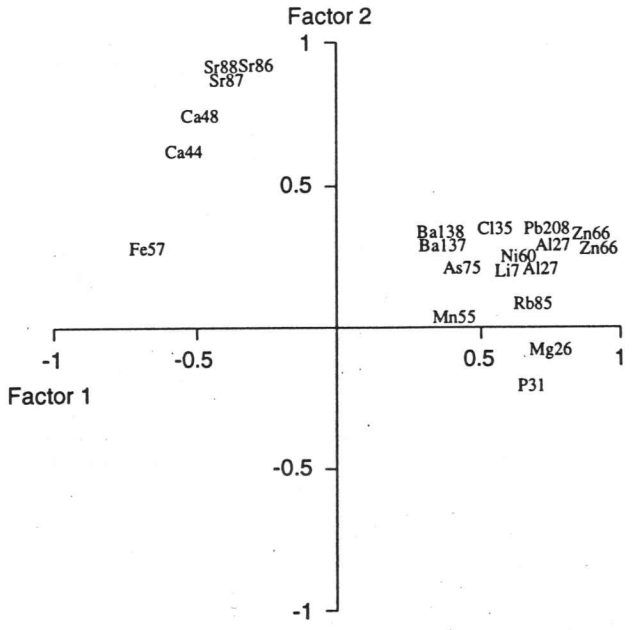


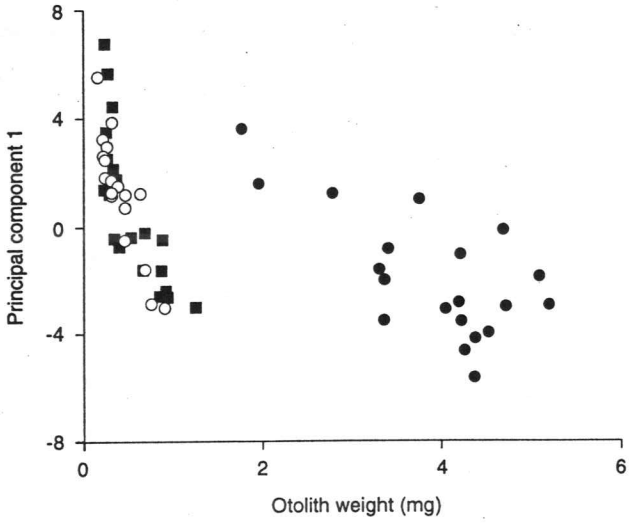
Fig. 3. Plot of first and second factors from the factor analysis indicating affinities amongst the 21 different isotopes measured in juvenile Atlantic croaker otoliths.



differences relates to the different ionic concentrations in the tank water determined by the level of dilution with deionized water. ^{31}P, ^{44}Ca, ^{48}Ca, ^{55}Mn, and ^{65}Cu and the three Sr isotopes all gave higher values in higher salinity. In contrast, ^{26}Mg and ^{85}Rb were recorded at higher levels in the low-salinity otoliths, indicating the influence of some other mechanism, perhaps physiological regulation.

Previous studies have related Sr levels (Coutant and Chen 1993) and Sr/Ca ratios (Radtke et al. 1988; Kalish 1990; Secor 1992) to salinity levels, in each case recording

Fig. 4. Relationship between the first principal component and otolith weight for the three treatments. Open circles relate to treatment 1, 20°C and 26‰ salinity; solid squares to treatment 2, 20°C and 35‰ salinity; and solid circles to treatment 3, 25°C and 26‰ salinity.



higher values from fish collected from higher salinity regimes. Such results have been used to discriminate between anadromous and nonanadromous fish (Kalish 1990) and to identify the timing of migrations between water masses with different salinity regimes (Radtke et al. 1988; Secor 1992; Coutant and Chen 1993). We also recorded higher levels of Sr in the high-salinity treatment but this effect was small compared with that of temperature. Also, since our Sr/Ca ratios were lower from the high salinity treatment, the relative effect of salinity on Ca was higher than on Sr.

The effect of a difference in temperature of 5°C on elemental composition was profound and clearly stronger than that relatable to a 9‰ difference in salinity. The temperature effect was particularly evident for ^{31}P, ^{65}Cu, ^{66}Zn, ^{68}Zn, and the three Sr isotopes. Some of these were at higher concentrations and others at lower concentrations in the higher temperature regime. How temperature might cause such differences is difficult to suggest because of the large number of isotopes involved and their different responses. Furthermore, the effect is confounded with otolith size and growth rate, since fish grew faster and produced larger otoliths at the higher temperature. Numerous authors have previously related Sr/Ca ratios to water temperature suggesting that Sr ions replace Ca ions in the aragonite crystal matrix at a rate controlled by water temperature (Radtke 1984, 1987, 1989; Townsend et al. 1989; Radtke and Shafer 1992). In our study, water temperature clearly affected Sr and Ca levels in the otoliths, but their ratios varied little, suggesting that the deposition rate of both elements responded similarly to the temperature difference, in turn suggesting that in this case Sr/Ca ratios would be poor indicators of water temperature.

The final factor that we identified as influencing the elemental composition of otoliths was otolith size. Within each treatment, and therefore independent of any variation

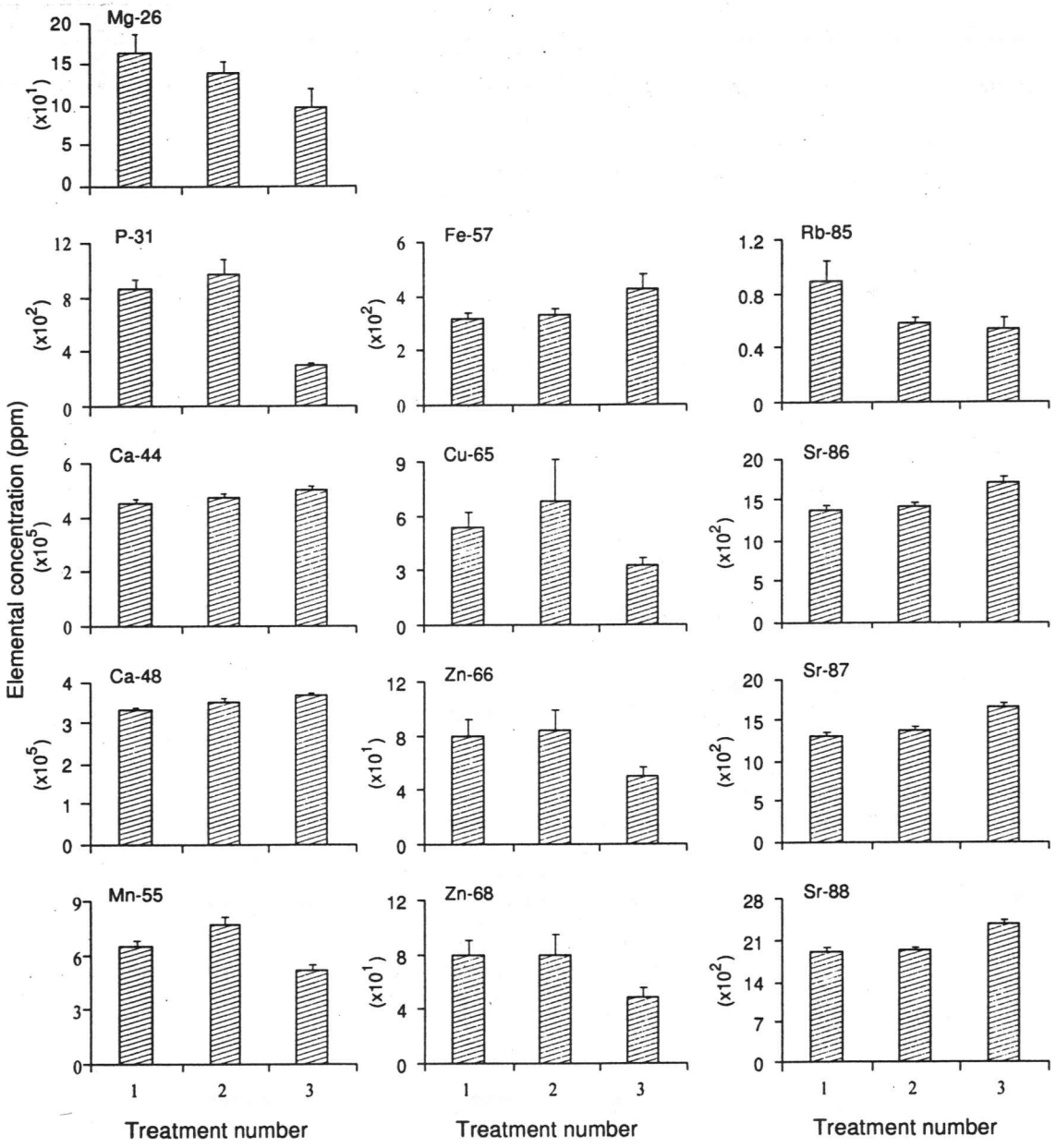
Table 4. ANOVA table examining treatment and tank effects ($\alpha = 0.05$) for each of the 21 isotopes considered in the analysis of otolith microchemistry using solution-based ICPMS.

Isotope	Source	Mean square	F	$p > F$	Pooled
^{7}Li	Treatment	1.1553	2.62	0.2760	
	Tank	0.4403	2.57	0.0855	
	Error	0.1710			
^{26}Mg	Treatment	1.8443	23.28	0.0412	0.0005
	Tank	0.0792	0.37	0.6953	
	Error	0.2143			
^{27}Al	Treatment	1.5493	16.97	0.0557	0.0666
	Tank	0.0913	0.16	0.8517	
	Error	0.5671			
^{31}P	Treatment	8.2579	623.09	0.0016	
	Tank	0.0132	0.11	0.8971	
	Error	0.1219			
^{35}Cl	Treatment	0.1637	3.39	0.2280	0.5015
	Tank	0.0484	0.2	0.8175	
	Error	0.2391			
^{44}Ca	Treatment	0.0494	6.98	0.1253	0.0005
	Tank	0.0071	1.3	0.2817	
	Error	0.0056			
^{48}Ca	Treatment	0.0557	7.53	0.1173	0.0002
	Tank	0.0074	1.37	0.2632	
	Error	0.0054			
^{55}Mn	Treatment	0.6608	12.67	0.0731	0.0001
	Tank	0.0522	0.92	0.4059	
	Error	0.0569			
^{57}Fe	Treatment	0.3533	3.66	0.0323	0.0311
	Tank	0.0724	0.75	0.4771	
	Error	0.0965			
^{60}Ni	Treatment	0.7716	0.79	0.5592	
	Tank	0.9788	2.11	0.1306	
	Error	0.463			
^{65}Cu	Treatment	1.6172	75.5	0.131	0.0399
	Tank	0.0214	0.04	0.9571	
	Error	0.4878			
^{66}Zn	Treatment	1.6506	11.13	0.0825	0.0330
	Tank	0.1484	0.31	0.7325	
	Error	0.4738			
^{68}Zn	Treatment	1.6161	13.17	0.0706	0.0326
	Tank	0.1228	0.27	0.7675	
	Error	0.4617			
^{75}As	Treatment	0.025	0.06	0.9404	0.9170
	Tank	0.3949	1.16	0.3216	
	Error	0.3409			
^{85}Rb	Treatment	1.3156	12.25	0.0755	0.0111
	Tank	0.1074	0.4	0.6731	
	Error	0.2963			
^{86}Sr	Treatment	0.2606	25.55	0.0377	0.0001
	Tank	0.0102	0.51	0.6016	
	Error	0.0199			
^{87}Sr	Treatment	0.2705	15.33	0.0612	0.0001
	Tank	0.0176	0.85	0.4328	
	Error	0.0207			
^{88}Sr	Treatment	0.2461	26.7	0.0361	0.0001
	Tank	0.0092	0.48	0.6234	
	Error	0.0193			
^{137}Ba	Treatment	0.216	0.16	0.8585	0.8011
	Tank	1.3107	1.56	0.2192	
	Error	0.8396			
^{138}Ba	Treatment	0.2557	0.2	0.8354	0.7703
	Tank	1.2975	1.49	0.2334	
	Error	0.8679			
^{208}Pb	Treatment	1.0367	3.3	0.2326	0.3135
	Tank	0.3142	0.35	0.7095	
	Error	0.9097			

Note: The "pooled" column shows the probability for treatment effects when the tank and residual sum of squares were pooled when the probability for a tank effect was >0.2; otherwise it was left blank. Degrees of freedom: treatment, 2; tank, 2; error, 54.

in salinity, temperature, or ionic composition of the water, there was a monotonic relationship between the multielemental composition of the otoliths and their size (and therefore growth rate). This provides strong support for a physiological model that relates otolith chemistry to rates of growth of fish and their otoliths. Previous work based on analyses with the electron microprobe has related Sr/Ca ratios (Gallahar and Kingsford 1992; Sadovy and Severin 1992, 1994), and Na/Ca, K/Ca and S/Ca ratios (Kalish 1989) to fish growth rates. Kalish (1989, 1991*a*) proposed that physiological responses of fish are associated with changes in the chemistry of the blood plasma and endolymph, which are reflected in the rate of ionic deposition in otoliths. This provides a mechanism whereby otolith microchemistry can be correlated with water temperature, because of its influence on fish physiology, but not necessarily directly determined by it (Kalish 1989, 1991*a*; Sadovy and Severin 1992, 1994).

Fig. 5. Comparison amongst the three treatments for the 13 isotopes found to differ significantly by ANOVA. Treatment 1, 20°C and 26‰ salinity; treatment 2, 20°C and 35‰ salinity; treatment 3, 25°C and 26‰ salinity. Note the scales on the abscissae differ.



Ionic contamination of otoliths

To understand the method of contamination of the otolith by many trace elements it is necessary to determine where within the bipartite microstructure the contaminant ions are attached, and how they are chemically bound. From the current understanding of the bipartite microstructure of otoliths (Campana and Neilson 1985; Jones 1986; Morales-Nin 1987) and how these form on a diurnal basis (Mugiya 1985, 1986; Mugiya and Tanaka 1992), three positions where contaminant ions can attach are most likely: within the aragonite crystalline structure, adsorbed to the aragonite microcrystals; and within the proteinaceous matrix. Sr is a likely example of the former (Kinsman and Holland 1969; Radtke and Shafer 1992). Na is more likely to occur within the proteinaceous matrix (Radtke and Shafer 1992). S occurs in the two amino acids, cysteine and methionine, and also in sulphated mucopolysaccharides, which are all components of the organic matrix (Degens et al. 1969; Radtke 1984; Kalish 1989).

That different ions may be located within different parts of the diurnally formed, bipartite structure provides a mechanism for differential deposition rates amongst isotopes. When the relative rates of calcification and matrix production vary, the potential deposition rates of the different ions and the multivariate elemental composition will also vary. Changes to the relative rates of deposition of $CaCO_3$ to protein do occur naturally because of seasonal variation in growth rates (Casselman 1980; Radtke et al.

1985) and as a consequence of laboratory rearing procedures (Kalish 1989). Mugiya and Tanaka (1992) proposed that the formation of the incremental and discontinuous zones of the microincrements in otoliths are likely to be independent and subject to different controlling factors. The rate of production of the organic matrix is a biological process controlled by hormonal and biochemical processes, but the physicochemical process of seeding and growth of aragonitic microcrystals, is likely to be influenced by other processes as well as biological events (Mugiya and Tanaka 1992).

The otoliths of Atlantic croaker that we studied contained many elements and isotopes, mostly at trace levels. In this regard they appear similar to otoliths from other fish species from around the world that have been analysed by microelemental techniques (Papadopoulou et al. 1980; Edmonds et al. 1989, 1991, 1992; Campana and Gagne 1994). If concentrations of some isotopes are compared amongst these studies, differences of as much as several orders of magnitude are apparent. Whilst undoubtedly some of this variation is attributable to different analytical procedures, it is reasonable to conclude that many of the differences are real, reflecting the influence of many factors (Kalish 1991*a*; Radtke and Shafer 1992). Such factors are likely to involve biological ones, such as phylogeny, ontogeny, physiology, age, and diets, as well as physical factors, such as water temperature (Radtke 1984; Radtke and Shafer 1992), salinity (Kalish 1990; Secor 1992), and water chemistry (Kalish 1993). Indeed, the results from our analysis by solution-based ICPMS of otoliths from reared Atlantic croaker have confirmed the influence of temperature, salinity, and growth rates on otolith microchemistry. We also had an opportunity to explore the effects of one more factor on the elemental composition of otoliths: ontogeny. This was done using LA-ICPMS and the results are reported in Fowler et al. (1995).

Acknowledgements

We thank Dr. William Hettler and Ms. Valerie Comparetta of the NMFS Laboratory, Beaufort, N.C., who reared the fish and maintained the treatments for the duration of the rearing period. Anita Hamilton and John Dalziel provided technical advice and assistance on the chemical procedures. The Fenwick Laboratories, Halifax, N.S. were responsible for the ICPMS analyses. This research was supported by a National Science Foundation grant (OCE-9202937) to C.M.J. and S.E.C. and is also a contribution of Canada Fisheries and Oceans.

References

Beamish, R. 1992. The importance of accurate ages in fisheries science, p. 8-22. *In* D.A. Hancock [ed]. Proceedings of the Australian Society for Fish Biology Workshop on the Measurement of Age and Growth in Fish and Shellfish. Bureau of Rural Resources, Australian Government Publishing Service, Canberra, Australia. Proc. 12.

Bennett, J.T., G.W. Boehlert, and K.K. Turekian. 1982. Confirmation of longevity in *Sebastes diploproa* (Pisces: Scorpaenidae) from $^{210}Pb/^{226}Ra$ measurements in otoliths. Mar. Biol. (Berlin), 71: 209–215.

Campana, S.E., and J.A. Gagne. 1994. Cod stock discrimination using ICPMS elemental assays of otoliths. *In* D.H. Secor, J.M. Dean and S.E. Campana [ed]. Recent developments in fish otolith research. University of South Carolina Press, Columbia, S.C.

Campana, S.E., and J.D. Neilson. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42: 1014–1032.

Campana, S.E., K.C.T. Zwanenburg, and J.N. Smith. 1990. $^{210}Pb/^{226}Ra$ determination of longevity in redfish. Can. J. Fish. Aquat. Sci. 47: 163–165.

Campana, S.E., A.J. Fowler, and C.M. Jones. 1994. Otolith elemental fingerprinting for stock identification of Atlantic cod (*Gadus morhua*) using laser ablation ICPMS. Can. J. Fish. Aquat. Sci. 51: 1942–1950.

Casselman, J.M. 1980. Chemical analyses of the optically different zones in eel otoliths. Ont. Fish. Tech. Rep. Ser. 4: 74–82.

Coutant, C.C., and C.H. Chen. 1993. Strontium microstructure in scales of freshwater and estuarine striped bass (*Morone saxatilis*) detected by laser ablation mass spectrometry. Can. J. Fish. Aquat. Sci. 50: 1318–1323.

Date, A.R. 1991. Inductively coupled plasma-mass spectrometry. Spectrochim. Acta, Rev. 14(1/2): 3–32.

Degens, E.T., W.G. Deuser, and R. Haedrich. 1969. Molecular structure and composition of fish otoliths. Mar. Biol. (Berlin), 2: 105–113.

Edmonds, J.S., M.J. Moran, N. Caputi, and M. Morita. 1989. Trace element analysis of fish sagittae as an aid to stock identification: pink snapper (*Chrysophrys auratus*) in Western Australian waters. Can. J. Fish. Aquat. Sci. 46: 50–54.

Edmonds, J.S., N. Caputi, and M. Morita. 1991. Stock discrimination by trace-element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. Aust. J. Mar. Freshwater Res. 42: 383–389.

Edmonds, J.S., R.C.J. Lenanton, N. Caputi, and M. Morita. 1992. Trace elements in the otoliths of yellow-eye mullett (*Aldrichetta forsteri*) as an aid to stock identification. Fish. Res. (Amsterdam), 13: 39–51.

Fowler, A.J., S.E. Campana, C.M. Jones, S.R. Thorrold. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser ablation ICPMS. Can. J. Fish. Aquat. Sci. 52: 1431–1441.

Gallahar, N.K., and M.J. Kingsford. 1992. Patterns of increment width and strontium:calcium ratios in otoliths of juvenile rock blackfish, *Girella elevata* (M.). J. Fish. Biol. 41: 749–763.

Gunn, J.S., I.R. Harrowfield, C.H. Proctor, and R.E. Thresher. 1992. Electron microprobe microanalysis of fish otoliths—evaluation of techniques for studying age and stock discrimination. J. Exp. Mar. Biol. Ecol. 158: 1–36.

Jarvis, I., and K.E. Jarvis. 1992. Plasma spectrometry in the earth sciences: techniques, applications and future trends. Chem. Geol. 95: 1–33.

Jones, C.M. 1986. Determining age of larval fish with the otolith increment technique. Fish. Bull. U.S. 84: 91–103.

Jones, C.M. 1992. Development and application of the otolith increment technique, p. 1–11. *In* D.K. Stevenson and S.E. Campana [ed.] Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci. 117.

Kalish, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J. Exp. Mar. Biol. Ecol. 132: 151–178.

Kalish, J.M. 1990. Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fish. Bull. U.S. 88: 657–666.

Kalish, J.M. 1991*a*. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph, and otoliths of bearded rock cod *Pseudophycis barbatus*. Mar. Ecol. Prog. Ser. 74: 137–159.

Kalish, J.M. 1991*b*. Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). Mar. Biol. 110: 37–47.

Kalish, J.M. 1993. Pre- and post-bomb radiocarbon in fish otoliths. Earth Planet. Sci. Lett. 114: 549–554.

Kinsman, D.J.J., and H.D. Holland. 1969. The co-precipitation of cations with $CaCO_3$. IV. The co-precipitation of Sr with aragonite between 16° and 96°C. Geochim. Cosmochim. Acta, 33: 1–17.

Morales-Nin, B. 1987. Ultrastructure of the organic and inorganic constituents of the otoliths of the sea bass, p. 331–343. *In* R.C. Summerfelt and G.E. Hall [ed.] The age and growth of fish. The Iowa State University Press. Ames, Iowa.

Mugiya, Y. 1985. Phase difference between calcification and organic matrix formation in the diurnal growth of otoliths in the rainbow trout, *Salmo gairdnerii*. Fish. Bull. U.S. 85: 395–400.

Mugiya, Y. 1986. Effects of calmodulin inhibitors and other metabolic modulators on in vitro otolith formation in the rainbow trout, *Salmo gairdnerii*. Comp. Biochem. Physiol. 84A: 57–60.

Mugiya, Y., and S. Tanaka. 1992. Otolith development, increment formation, and an uncoupling of otolith to somatic growth rates in larval and juvenile goldfish. Nippon Suisan Gakkaishi, 58: 845–851.

Papadopoulou, C., G.D. Kanias, and E. Kassimati. 1980. Trace element content in fish otoliths in relation to age and size. Mar. Pollut. Bull. 11: 68–72.

Pentilla, J., and L.M. Dery. [Ed]. 1988. Age determination methods for Northwest Atlantic species. NOAA Tech. Rep. NMFS72: 135 p.

Quinn, G.P. and R.W. Day. 1989. Comparisons of treatments after an analysis of variance in ecology. Ecol. Monogr. 59: 433–463.

Radtke, R.L. 1984. Cod fish otoliths: information storage structures. Flodevigen Rapportser. 1: 273–298.

Radtke, R.L. 1987. Age and growth information available from the otoliths of the Hawaiian snapper, *Pristipomoides filamentosus*. Coral Reefs 6: 19–25.

Radtke, R.L. 1989. Strontium–calcium concentration ratios in fish otoliths as environmental indicators. Comp. Biochem. Physiol. 92A: 189–193.

Radtke, R.L., and D.J. Shafer. 1992. Environmental sensitivity of fish otolith microchemistry. Aust. J. Mar. Freshwater Res. 43: 935–951.

Radtke, R.L., M.L. Fine, and J. Bell. 1985. Somatic and otolith growth in the oyster toadfish (*Opsanus tau* L.). J. Exp. Mar. Biol. Ecol. 90: 259–275.

Radtke, R.L., R.A. Kinzie, III, and S.C. Folsom. 1988. Age at recruitment of Hawaiian freshwater gobies. Environ. Biol. Fishes, 23: 205–213.

Sadovy, Y., and K.P. Severin. 1992. Trace elements in biogenic aragonite: correlation of body growth rate and strontium levels in the otoliths of the white grunt, *Haemulon plumieri* (Pisces: Haemulidae). Bull. Mar. Sci. 50: 237–257.

Sadovy, Y., and K.P. Severin. 1994. Elemental patterns in red hind (*Epinephalus guttatus*) otoliths from Bermuda and Puerto Rico reflect growth rate, not temperature. Can. J. Fish. Aquat. Sci. 51: 133–141.

Secor, D.H. 1992. Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay striped bass *Morone saxatilis*. Fish. Bull. U.S. 90: 798–806.

Secor, D.H., J.M. Dean, and S.E. Campana [Ed]. 1994. Recent developments in fish otolith research. University of South Carolina Press, Columbia, S.C. 735 p.

Townsend, D.W., R.L. Radtke, M.A. Morrison, and S.C. Folsom. 1989. Recruitment implications of larval herring overwintering distributions in the Gulf of Maine, inferred using a new otolith technique. Mar. Ecol. Prog. Ser. 55: 1–13.