Abstract.—Otolith microchemistry of anadromous and non-anadromous salmonids was investigated to determine if there were differences among migratory and non-migratory individuals and if the habitat where vitellogenesis took place would affect the composition of the otolith primordia of the progeny. Electron microprobe transects across salmonid otoliths showed that there were large differences in otolith Sr/Ca ratios among adult anadromous and non-anadromous individuals, but there were no detectable differences in Na/Ca, K/Ca, and S/Ca ratios. The hypothesis that Sr/Ca ratios in the primordia of the progeny of anadromous salmonids would be greater than those in the primordia of the progeny of non-anadromous individuals because of differences in the composition of ova was tested and confirmed by the results of a controlled experiment. Also, the ova of anadromous *Oncorhynchus mykiss* were found to contain 5 times more Sr than their non-anadromous conspecifics. On the basis of these data, it was concluded that otolith nucleus Sr/Ca ratios can be used to distinguish the progeny of sympatric anadromous and non-anadromous salmonids.

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Use of Otolith Microchemistry to Distinguish the Progeny of Sympatric Anadromous and Non-anadromous Salmonids

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The ability to differentiate between juvenile anadromous salmonids and their sympatric non-anadromous conspecifics is essential for management of these species. However, stock discrimination between sea-run and resident freshwater salmonids has been limited to the adults upon their return from the sea to spawn. Furthermore, for those species where anadromy is a facultative, and not an obligate behavior, such as brown trout *Salmo trutta*, rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar*, cutthroat trout *Salmo clarkii*, and Arctic char *Salvelinus alpinus*, it has been impossible to distinguish between the co-occurring forms on the basis of meristic or morphometric characters (Nordeng 1983, Jonsson 1985, Neilson et al. 1985). McKern et al. (1974) were able to distinguish between winter and summer races of steelhead trout (anadromous rainbow trout) from rivers in British Columbia, Washington, and Oregon on the basis of sagittal otolith nuclear dimensions that they believed to be affected by both qualitative and quantative differences in yolk. Rybock et al. (1975) concluded that differences in the size of female resident non-anadromous rainbow trout and steelhead trout resulted in differences in egg size and, subsequently, the size of the otolith nucleus in the progeny. However, both Neilson et al. (1985) and Currens et al. (1988) found that measurements of otolith nuclear dimensions were of questionable value in distinguishing juvenile non-anadromous and anadromous rainbow trout.

Development of ova in anadromous salmonids is virtually complete through vitellogenesis or yolk formation before the fish enter freshwater. On the basis of this information, I hypothesized that egg composition would, in some way, reflect the chemical composition of the seawater environment and that this would ultimately affect the composition of the otoliths of the progeny, particularly the otolith nuclei that are formed in the early stages of development and well before yolk utilization is complete. Yolk is formed through the deposition of a phospholipoprotein-calcium complex yolk precursor, vitellogenin, in the developing oocyte (Mommsen and Walsh 1988). Solubility and transport of vitellogenin through the circulatory system of the female and to the developing ovaries may be dependent on the presence of calcium which has been shown to increase markedly in female salmonids (Bailey 1957, Booke 1964, Elliot et al. 1979) and other fishes (Oguri and Takada 1967, Woodhead 1968) during gonad development. Vitellogenin has a high affinity for calcium due to the significant negatively-charged phosphate component of the molecule (Hara and Hirai 1978, Hara et al. 1980). The calcium binds to the vitellogenin molecule and, in this complexed form, the vitellogenin and calcium are deposited in

the oocyte. Clearly, yolk deposition is a conservative process in that the overall composition of the yolk is more dependent on the genetic programming of the female parent than the environment. However, in many calcium-binding proteins, such as vitellogenin, the calcium moiety of the complexed molecule can be substituted by strontium due to the similar structural features of Ca^{++} and Sr^{++} (Skoryna 1981). The relative degree of this substitution would be largely dependent on the relative concentrations of calcium and strontium in the ambient environment. Typical Sr/Ca ratios in marine waters (salinity 35‰) are 0.0087 (0.09 mM/kg Sr:10.3 mM/kg Ca) (Bruland 1983) and average 0.0019 (0.00068 mM/kg Sr:0.35 mM/kg Ca) in freshwater (Rosenthal et al. 1970). These differences would be expected to affect the Sr/Ca ratio of the yolk and, ultimately, the composition of the developing embryo and its otoliths.

In a study of otolith and endolymph composition, Kalish (1989) showed that the quantity of strontium incorporated into the otolith was directly related to the quantity of strontium present in the endolymph, and that anadromous brown trout collected in an estuary had higher levels of strontium in both the endolymph and otolith than non-anadromous brown trout. Therefore, it seems likely that differences in the elemental constituents of the yolk and embryos of anadromous and non-anadromous salmonids would result in differences in otolith composition. This hypothesis is supported by research that shows little or no exchange of calcium between prehatch salmonid embryos and the environment (Hayes et al. 1946, Zeitoun et al. 1976). This would be expected since calcium present in the yolk of the developing embryo is probably present in a protein-bound form and is destined for the tissues of the fry. Craik and Harvey (1984) found that the calcium composition measured in whole rainbow trout eggs was indistinguishable from the calcium measured in protein precipitate obtained from the egg. This, of course, excludes calcium that would be present in the fluid of the perivitelline space following fertilization (Laale 1980, Alderdice 1988). Strontium would probably behave similarly and there would be minimal loss of any seawater-derived protein-bound ions in the yolk to the freshwater environment where the development of salmonid embryos takes place.

In this paper, I discuss variations in the elemental composition of the otoliths of non-anadromous and anadromous salmonids with emphasis on the composition of the sagittal otolith nucleus. Several "life history" transects (scans of elemental composition across an axis of an otolith made with a wavelength-dispersive electron microprobe) are presented to indicate the variety of forms that these data may take in salmonids with differing life histories. I also present the results of an experiment designed to test the hypothesis that otolith primordia of the progeny of anadromous rainbow trout contain higher levels of strontium than the otolith primordia of non-anadromous rainbow trout. These data are examined in view of their usefulness in distinguishing the progeny of sympatric non-anadromous and anadromous salmonids and in investigations of diadromous behavior.

Methods

Brown trout (non-anadromous *Salmo trutta*) and sea trout (anadromous *Salmo trutta*) were collected by gillnet from the Derwent River and estuary, southeast Tasmania, Australia. Juvenile and adult rainbow trout were obtained from both wild and hatchery stock. Atlantic salmon were obtained from hatcheries. Otoliths were removed from fresh fish, cleaned, and stored in glass vials. Details of otolith preparation and microprobe analyses are described below.

Ripe ova were obtained from ovulating freshwater rainbow trout and sea-farmed rainbow trout and frozen in plastic bags for later analyses of calcium and strontium. Whole rainbow trout eggs were used for the determinations. Groups of 10 eggs from 4 freshwater and 4 sea-farmed trout were used. Preparation of eggs was modified from the methods employed by Craik and Harvey (1984). All solutions were made up with Milli-Q water (Millipore Corp.). Groups of eggs were weighed wet and then oven-dried at 50°C for 24 hours to estimate dry weight. The dried whole eggs were ashed at 500°C for 24 hours and then digested in 2.0 mL Aristar ultrapure hydrochloric acid at 100°C for 2 hours. Samples were separated into two equal aliquots for separate calcium and strontium determinations. Sample digests for estimation of calcium were diluted in 10 mM lanthanum chloride to suppress interference due to phosphate binding. Calcium concentrations were determined by flame atomic absorption spectrophotometry using an air-acetylene flame.

Strontium concentrations in eggs were determined by the method of standard additions using graphite furnace atomic absorption spectrophotometry on a Varian AA-1475 spectrophotometer equipped with a GTA-95 graphite furnace and an autosampler. Argon was used as the purging gas in the graphite furnace. Samples were diluted with a 0.25% solution of an ionic detergent, Triton X-100, and 20 μL of diluted sample were injected by autosampler into a walled, pyrolytically coated graphite tube. Furnace conditions were: drying

Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1

Electron microprobe analytical data for salmonid otolith analyses. Details of counting times, counting precision, and materials used as standards. Calculation of the counting precision is discussed in Goldstein et al. (1981). Precision values are for the 95% confidence level.

Spectrometer/ crystal	Element	Peak counting time (sec)	Counting precision	Standard material	Source
1/PET	Ca-K_α	10	~28,000 counts collected 1.0%	Calcite	USNM* 136321 Jarosewich and MacIntyre (1983)
1/PET	K-K_α	30	15% when $K/Ca = 2 \times 10^{-3}$	Anorthite	USNM 137041 Jarosewich et al. (1980)
2/PET	S-K_α	40	36% when $S/Ca = 1 \times 10^{-3}$	Troilite	BMR** Museum Ramdohr and Goresy (1971)
3/TAP	Na-K_α	20	8.1% when $Na/Ca = 2 \times 10^{-2}$	Anorthite	Same as for K above
3/TAP	Sr-L_α	20	9.9% when $Sr/Ca = 3 \times 10^{-3}$	Strontianite	USNM 10065 Jarosewich and White (1987)

*Natl. Mus. Nat. Hist., Smithsonian Inst., Wash., DC
**Bur. Miner. Resour., Canberra, Australia

at 90°C for 60 seconds; ramp ashing from 90 to 700°C for 20 seconds; ashing at 2600°C for 1 second; and atomization, with no gas flow, at 2600°C for 3 seconds.

To confirm the hypothesis regarding otolith nucleus composition, eggs were obtained from sea-farmed and freshwater broodstock rainbow trout which originated from a similar hatchery stock (Cressy, Tasmania). These two groups of hatchery fish derive from similar stocks of rainbow trout imported into Tasmania; because of minimal gene flow, inbreeding is relatively great and the diversity of the gene pool is low. Both freshwater and sea-farmed adults had been maintained on jack mackerel *Trachurus declivis* based pellets throughout the period of egg development. Sea-farmed broodstock were kept at the same hatchery as freshwater broodstock for 3 weeks before stripping, and all eggs were fertilized with sperm from a male of freshwater stock. The developing embryos from sea-farmed and freshwater broodstock were maintained in separate baskets in the same channel of a recirculating water system where temperature was maintained at 10°C. A natural photoperiod was maintained throughout the experiment. A random sample of 20 freshwater progeny and 20 sea-farmed progeny was taken 20 days after hatching, and these fish were frozen for later removal of otoliths. Sagittae and lapilli were removed from fry, the adhering otolith capsule was removed, and otoliths were rinsed in deionized water. Otoliths were oven-dried at 40°C. The length of sagittae and lapilli was measured with an ocular micrometer.

Otoliths from adults were embedded in Araldite D epoxy resin (Ciba-Geigy) and polymerized in an oven at 40°C for 48 hours. Several transverse sections of approximately 200 μm thickness were obtained from otoliths, including one section containing the primordium, with a low-speed saw (Struers Accutom) equipped with a diamond cut-off wheel. Sections were affixed to glass slides with epoxy. After drying, sections were ground with a graded series of carborundum paper (wet/dry paper 600–1200 grade) until the primordia were reached. The much smaller otoliths from fry were mounted, sulcus-side-up, in Crystalbond 509 (Aremco Products, Inc.), a heat-labile thermoplastic polymer (Neilson and Geen 1981), and ground in the sagittal plane to the level of the primordia with 1200-grade wet-dry paper. All otoliths were then ground to the precise level of the primordia on a lapping wheel with 0.25 μm aluminium paste (Linde A) and then finished with 0.25 μm diamond paste. Polished specimens were ultrasonically cleaned in reagent-grade mineral spirits followed by ultrasonic cleaning in deionized water and oven drying at 60°C. Samples were then coated in a high-vacuum evaporator with a 25-nm carbon layer and stored in a dessicator.

Otolith elemental analyses were carried out with a Cameca SX-50 wavelength-dispersive electron microprobe. Analyses were made with a square raster of 10×10 μm. Probe current and accelerating voltage were 10 nA (measured on Cu) and 15 kV, respectively. Elements analyzed included Ca, Sr, Na, K, and S. Salmonid otoliths are very susceptible to damage from the electron beam, and it was necessary to utilize a low-beam current and reduced counting times for all analyses. In conjunction with these conditions that reduce the total number of X-ray counts from a specimen, it is important to be aware of detection limits and counting statistics. A treatment of these subjects can be found in Goldstein et al. (1981) and references therein.

Details of the microprobe counting procedures, precision, and standards employed in this study are outlined in Table 1. Background was measured at offsets both above and below peak position for 50% of the peak counting time. The average counts from these two background measurements were subtracted from the peak. Background measurements were made with each analysis. Corrected X-ray intensity ratios were calculated using the ZAF method (Reed 1975), and the final elemental ratios are normalized atom ratios based on concentrations derived from the standards.

Elemental data for otolith life-history transects from adult salmonids were collected with approximately 40 μm separating each 10 × 10 μm sample region (50 μm separating the center point of each sample). This sampling spatial frequency was judged to be adequate because it was desired to collect data relating to major life-history changes only, and not recurring or seasonal events.

For a comparison of otolith composition among the progeny of sea-farmed fish and freshwater fish, microprobe data were collected from five individual primordia within each sagitta or lapillus. In some lapilli it was necessary to make multiple measurements on individual primordia because five primordia were not always exposed or visible. In addition, five microprobe measurements were made on each otolith at points near the otolith edge that were indicative of the otolith composition at a time after the completion of yolk absorption.

Results

Otolith life-history transects representative of several types of adult salmonids are presented in Figure 1. Differences in otolith transects among anadromous or sea-farmed fish and non-anadromous individuals are only evident in the Sr/Ca data and, therefore, only Sr/Ca life-history transects are presented. Sea trout, sea-farmed Atlantic salmon, and sea-farmed rainbow trout exposed to the marine environment display a clear increase in otolith Sr/Ca that is coincident with entry into seawater.

There is an initial peak, associated with the otolith nucleus, in the Sr/Ca ratio of the sea trout and Atlantic salmon, but this peak does not appear in the sea-farmed or freshwater rainbow trout. In Tasmania, freshwater broodstock are used for the production of sea-farmed rainbow trout. The peak in otolith Sr/Ca in the otolith nucleus of some individuals is presumably due to the presence of strontium sequestered in the egg yolk proteins during the seawater phase of ovarian development of the fishes' female parent.

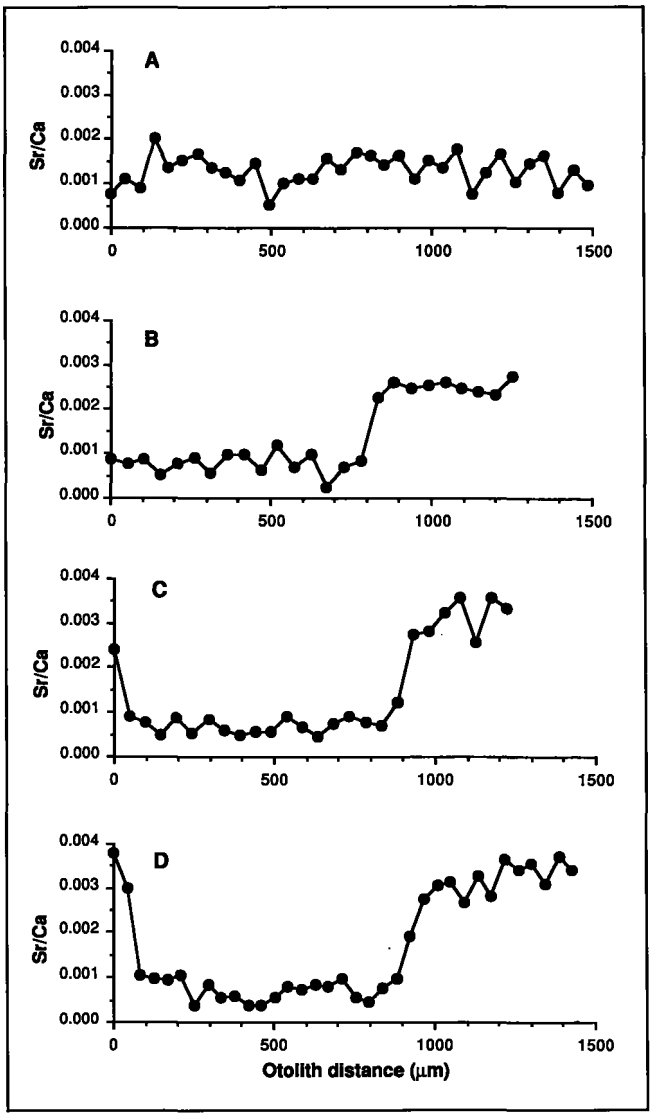

Figure 1

Transects of otolith Sr/Ca ratios measured with a wavelength-dispersive electron microprobe from the primordium (0 μm) to the otolith edge along transverse sections of sagittae from 4 female adult salmonids with differing life histories. Each point represents a single measurement made over an area of 100 μm^2. (A) Wild non-anadromous *Oncorhynchus mykiss* from Great Lake, Tasmania; (B) sea-farmed *Oncorhynchus mykiss*; (C) sea-farmed *Salmo salar*; and (D) wild anadromous *Salmo trutta* from the Derwent River estuary.

The hypothesis that the seawater strontium contribution to the egg would result in increased otolith strontium was confirmed by the results of the rearing experiment. Results of this experiment are presented in Table 2. Mean Sr/Ca ratios based on measurements in five primordia from each of the 20 sea-farmed broodstock progeny was 0.00313 ± 0.00068, significantly greater than the mean value of 0.00114 ± 0.00014 measured in the primordia of the freshwater brood-

Table 2

Mean elemental ratios measured in primordia and post-yolk absorption regions in the sagittae and lapilli of progeny of sea-farmed and freshwater rainbow trout *Oncorhynchus mykiss*. Values are means ±1 standard deviation based on 5 microprobe measurements in the nucleus and post-yolk absorption regions of each otolith. $N = 20$ for sagittae, and $N = 5$ for lapilli. Significance tests alongside sagittae and lapilli primordia data are the results of paired two-tailed t-tests between those otolith primordia and the post-yolk absorption regions of the same otoliths.

	Sr/Ca ($\times 10^3$)	Na/Ca ($\times 10^2$)	K/Ca ($\times 10^3$)	S/Ca ($\times 10^3$)
Sea-farmed progeny				
Sagittae ($N = 20$)				
Primordia	3.13 ± 0.68***	2.03 ± 0.16***	2.07 ± 0.47***	1.65 ± 0.63**
Post-yolk absorption	1.07 ± 0.31	1.86 ± 0.16	2.62 ± 0.52	1.08 ± 0.21
Lapilli ($N = 5$)				
Primordia	4.34 ± 0.80**	1.98 ± 0.09*	1.84 ± 0.30*	1.30 ± 0.35 ns
Post-yolk absorption	0.83 ± 0.24	1.74 ± 0.06	2.30 ± 0.07	1.00 ± 0.14
Freshwater progeny				
Sagittae ($N = 20$)				
Primordia	1.14 ± 0.14 ns	2.10 ± 0.08***	2.61 ± 0.41 ns	1.75 ± 0.38***
Post-yolk absorption	0.96 ± 0.19	1.73 ± 0.15	2.46 ± 0.37	1.21 ± 0.21
Lapilli ($N = 5$)				
Primordia	1.20 ± 0.18 ns	1.95 ± 0.04*	2.43 ± 0.25 ns	1.32 ± 0.14 ns
Post-yolk absorption	1.06 ± 0.13	1.72 ± 0.06	2.68 ± 0.26	1.27 ± 0.27

*$P \leq 0.01$; **$P \leq 0.001$; ***$P \leq 0.0001$; ns = not significant ($P > 0.05$).

stock progeny (unpaired one-tailed t-test, $t = 12.69$, $P < 0.0001$). One-way analysis of variance showed that there were no significant differences in mean otolith primordia Sr/Ca ratios among individuals from either seawater ($F = 0.88$, $P = 0.476$) or freshwater broodstock ($F = 1.29$, $P = 0.279$). Differences in otolith primordia composition between sea-farmed progeny and freshwater progeny were not significant for Na/Ca (unpaired two-tailed t-tests, $t = 1.82$, $P = 0.076$) and S/Ca ($t = 0.58$, $P = 0.568$), but were significant for K/Ca ratios ($t = 3.88$, $P = 0.0004$). Similar results were obtained for measurements made in lapilli primordia from five freshwater and five marine progeny. Table 2 also presents the results of comparisons between the composition of the nucleus and post-yolk absorption regions of the otoliths.

A histogram of the frequency distribution of Sr/Ca ratios determined for five primordia in the sagittae of 20 progeny of sea-farmed rainbow trout and 20 progeny of freshwater rainbow trout shows that there is little overlap between individual measurements from the two groups (Fig. 2). Only 5% of the measurements from the sea-farmed progeny overlap with 13% of the freshwater progeny measurements. A frequency histogram of the mean Sr/Ca ratios obtained from each sagitta nucleus shows that the sea-farmed and freshwater progeny are clearly separated on the basis of otolith primordia Sr/Ca ratios (Fig. 2).

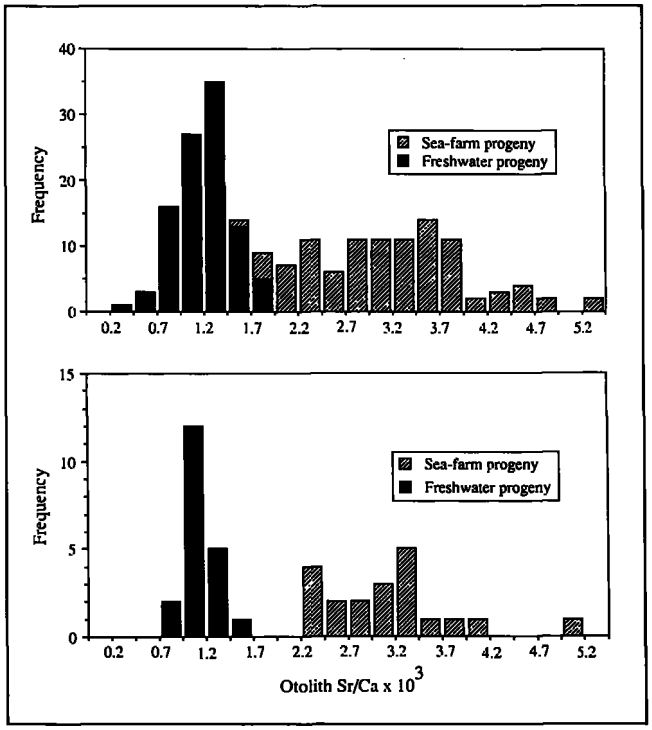

Figure 2

(Above) Frequency distribution of individual otolith Sr/Ca measurements made in the primordia of 20 sagittae from *Oncorhynchus mykiss* fry of sea-farmed broodstock and 20 sagittae from fry of freshwater broodstock ($N = 200$), and (below) frequency distribution of mean otolith Sr/Ca ratios from the same 40 sagittae.

Measurements of otolith composition made in regions of the sagittae formed after the completion of yolksac absorption and corresponding with a period soon after first feeding corroborated data that showed the influence of yolk on otolith composition. There were no significant differences in Sr/Ca (unpaired two-tailed t-tests, $t = 1.30$, $P = 0.201$), S/Ca ($t = 2.02$, $P = 0.051$), or K/Ca ($t = 1.09$, $P = 0.282$) ratios measured in the later-formed regions of the otoliths from the two groups of fry and only slightly significant differences for Na/Ca ratios ($t = 2.70$, $P = 0.010$). Differences between the Sr/Ca ratio of the sagitta edge and primordium were highly significant in the sea-farmed broodstock progeny (paired two-tailed t-tests, $t = 10.38$, $P<0.0001$), and only slightly significant in the progeny of the freshwater broodstock ($t = 3.13$, $P = 0.003$).

Six electron microprobe transects over a sagitta from a single *Oncorhynchus mykiss* fry of sea-farmed broodstock origin were made to show Sr/Ca ratios over the entire otolith including the nucleus and pre- and post-yolk absorption regions (Fig. 3). The outermost points towards the otolith rostrum and posterior show the sagitta Sr/Ca ratios after the completion of yolksac absorption and provide evidence for much reduced otolith Sr/Ca ratios when compared with the otolith material produced during the period of yolksac utilization. The data also indicate that, in general, high Sr/Ca ratios prevail throughout the region of the nucleus and are not confined to the primordia alone.

Measurements of egg calcium and strontium composition show that ova of the sea-farmed rainbow trout contain higher levels of strontium than their freshwater conspecifics. The mean strontium content was 0.054 ± 0.013 μM/g of ova (dry weight) for ova that had developed in seawater and 0.010 ± 0.002 μM/g for ova from freshwater fish, and the difference between these values was significant (unpaired two-tailed t-test, $t = 6.60$, $P = 0.0006$). Mean calcium content of yolk was 0.035 ± 0.005 mM/g from sea-farmed broodstock and 0.026 ± 0.004 mM/g from freshwater fish and these values were also significantly different ($t = 3.06$, $P = 0.022$).

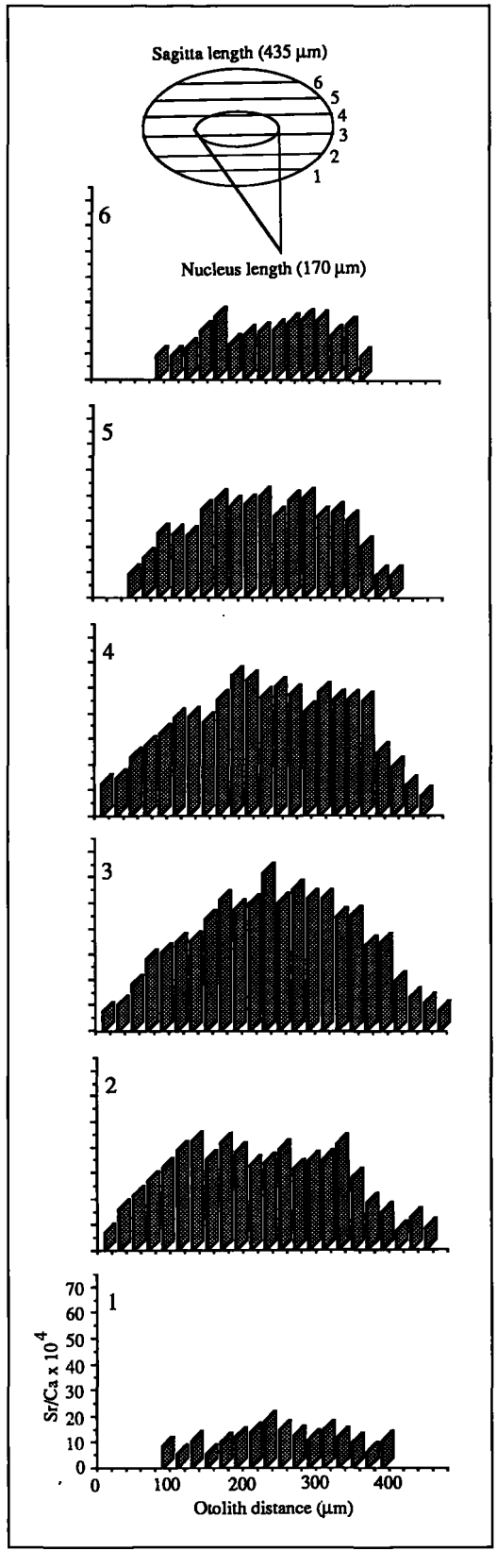

Figure 3

Six transects of otolith Sr/Ca ratios made with a wavelength-dispersive electron microprobe on a single sagitta from an *Oncorhynchus mykiss* fry of sea-farmed broodstock origin. The otolith was ground to the level of the primordia in the sagittal plane. Each column in the histograms represents an individual microprobe measurement ($N = 120$). Outermost points show the sagitta Sr/Ca ratios after the completion of yolksac absorption.

Discussion

The results present evidence that the Sr/Ca ratios of otolith primordia formed during embryonic development are directly influenced by the strontium content of the individual's yolk and that yolk composition is also influenced by the composition of the waters where vitellogenesis took place. These findings are important to the study and management of salmonid species where anadromy is a faculative behavior. They may also be important to studies of other diadromous species, but it remains to be seen whether the relatively small nutritive contribution of the yolk in these fishes is adequate to influence otolith composition. However, since otolith primordia develop very early in the ontogeny of fishes (Brothers 1984), there may be detectable differences in otolith strontium content within other species that display facultative diadromy. In many cases, the analysis of otolith primordium composition should make it possible to investigate the relative importance of parentage and environment in determining diadromous behavior.

The basis for the differences in otolith primordium composition among anadromous and non-anadromous fishes is such that there is probably little, if any, effect of race or population. Both wild and hatchery salmonids involved in this study displayed similar ranges of elemental ratios, depending on habitat (freshwater or marine) indicating that different salmonid species may incorporate into their otoliths a similar proportion of the ions present in the endolymph. Furthermore, this indicates that the relationship between endolymph and blood plasma composition is regulated in a similar manner in each of these species. Kalish (1989) showed that there was a tendency for both endolymph and otolith Sr/Ca ratios to be similar among individuals of a species, whereas differences were greatest among species. On the basis of the data presented here and in Kalish (1989), it seems likely that freshwater and marine salmonid otolith Sr content could be predicted on the basis of endolymph composition.

The results of this study confirm that differences in the composition of freshwater and seawater can be reflected in the composition of fish otoliths. These findings are similar to those obtained by Casselman (1982), Radtke et al. (1988), and Kalish (1989). Wavelength-dispersive electron microprobe analyses of strontium content in diadromous fish otoliths can provide information on the rates of migration to and from the sea, residence times at sea and in freshwater, the age at which migrations take place, and confirmation of data obtained from scales.

Confirmation of data obtained from scale reading is important because of the possibility of scale resorption (Bilton 1974) and the equivocal nature of data obtained from scales in the discrimination of adult anadromous and non-anadromous salmonids. Bagenal et al. (1973) used the strontium content of whole scales to distinguish between brown trout and sea trout, and Moreau and Barbeau (1979) used a similar rationale to discriminate anadromous from non-anadromous whitefish *Coregonus clupeaformis*. However, Castonguay and FitzGerald (1982) found that this method was unreliable for distinguishing between anadromous and non-anadromous brook char *Salvelinus fontinalis*, and Gausen and Berg (1988) had similar results when investigating migratory and non-migratory Atlantic salmon. Measurements of otolith Sr may provide a more reliable means of determining migratory behavior in these species because otoliths are not resorbed during periods of stress (Simkiss 1974, Campana 1983), although Mugiya and Uchimura (1989) present evidence for otolith resorption in goldfish *Carassius auratus* during extreme anaerobic stress. Also, otolith Ca and, most likely, Sr are derived from ions taken up by the gills (Simkiss 1974), and any variations in the Sr content of the diet would not be reflected in the composition of the otoliths. This is not the case for scales, and differences in the Sr content of the diet as well as the effect of different levels of discrimination against Sr would be manifested in scale composition.

It is important to point out the inadequacy of energy-dispersive (ED) electron microprobe analysis for the determination of Sr at the levels typically found in marine fish otoliths and particularly in the otoliths of freshwater species. Under optimal operating conditions, the minimum detectability limit for a wavelength-dispersive (WD) electron microprobe is approximately 10 times lower than that of an ED microprobe for all elements (Geller 1977, Goldstein et al. 1981). Generally, the minimum detection limits attainable with an ED spectrometer are on the order of 0.10% wt (1000 ppm), and these conditions are frequently not realized due to various factors. The range of Sr concentrations measured in this study was approximately 300–5000 ppm, and all marine fish otoliths investigated to date appear to contain Sr in excess of 1000 ppm (Radtke 1987, Edmonds et al. 1989, Kalish 1989). Theoretically the ED spectrometer should be capable of making all otolith Sr measurements in marine fish and some of those from freshwater fish. However, the detection and accurate quantitative estimation of Sr creates special problems for the ED microprobe.

The X-ray line that is used to detect Sr at 1.806 keV (L_α) is affected by the silicon escape peak associated with the large Ca K_α peak produced when measuring specimens that are largely calcium (aragonite fish otoliths are approximately 38% wt Ca). The silicon escape peak results from the production of Si K_α photons following absorption of relatively high energy (>1.841

keV) photons by the silicon detector of an ED spectrometer (Reed and Ware 1972). The energy of the photon producing the escape peak is $E_x - E_{Si}$, where E_x is the incident photon energy and E_{Si} is the energy of a Si K_α photon (1.739 keV). The silicon escape peak due to Ca K_α would have an energy of 1.951 keV (3.690 keV − 1.739 keV) and an intensity 0.78% that of the Ca K_α peak (Reed 1975). Such a peak, within less than 150 eV of the Sr peak, would create significant errors in the estimation of low levels of Sr. Measurements of trace levels of phosphorus with the K_α line (2.013 keV) would be even more vulnerable to errors because of the closer proximity to the Ca K_α derived silicon escape peak. Other errors can result from a silicon internal fluorescence peak (Reed and Ware 1972) and the silicon absorption edge (Goldstein et al. 1981, Statham 1981), both artifacts of the ED spectrometer and associated silicon detector, and of particular interest to the determination of trace levels of Sr. These sources of error are not present when using a WD electron microprobe.

There are numerous other potential sources of error in otolith trace-element studies carried out with ED electron microprobes. These errors can occur due to the relatively poor energy resolution of the ED spectrometer and the resultant inability to discriminate between X-ray lines separated by less than approximately 150 ev (Statham 1981). Furthermore, the presence of a greater proportion of continuum background radiation or "bremsstrahlung" in the ED spectrometer, due to decreased energy resolution, results in a fivefold increase in the peak to background ratio in a WD spectrometer over an ED spectrometer (Reed 1975). These factors, and others, make it necessary to view with caution studies with ED microprobes that investigate the distribution of large numbers of trace (<0.5% wt) elements, particularly without reference to criteria for determining detection levels or precision. In an investigation of otolith composition using X-ray fluorescence spectroscopy (XRF) Cu, Cd, Cr, and V were below the detection limits of the instrument (<3.0 ppm), Ni was found at levels averaging 2.0 ppm, and Fe, Zn, and Ba levels were below 10 ppm in the four species studied (J.M. Kalish, unpubl. data). Edmonds et al. (1989) used inductively coupled plasma atomic-emission spectroscopy (ICP—AES) to study otolith composition of the pink snapper *Chrysophrys auratus* for stock discrimination, and their data indicate that Mg, Si, and Fe are at levels below the detection limits achievable using either ED or WD microprobe analysis.

With the above results in mind it appears that with a WD electron microprobe the only elements that can be reliably quantified in otoliths are Ca, Na, Sr, K, S and, in some cases, Cl. However, in the case of Cl, the presence of this element in the most frequently used mounting medium, epoxy, makes the quantitative determination of this element difficult. If it is desired to detect Cl, an alternative mounting medium should be considered. The utility of ED microprobe analysis to studies of the quantitative composition of fish otoliths seems to be limited to Ca and, in some rare instances, Na and Sr.

Although WD microprobe analysis is generally capable of detecting the levels of Sr found in freshwater and marine fish otoliths, there are some limitations to the method in studies of anadromous fishes. Most important is the limited spatial resolution of the microprobe. In this study the probe size was maintained at 10×10 μm to minimize beam damage of the specimen. A spot of this size on the otolith of an adult fish would encompass more than a single day of otolith growth and in slow-growing individuals could encompass a month or more. In slow-growing fish, this would limit the ability to determine the temporal scale of migratory events. Determination of the temporal resolution of the microprobe data would be dependent on estimates of fish age based on otolith annuli and microincrement data. In many instances, the utility of otolith Sr data is dependent on the accurate determination of age using the same otolith.

The elemental analysis of otolith primordia should provide an objective criterion for assessing if an individual is the progeny of an anadromous or non-anadromous female. This information alone, or in combination with data from life-history transects, should make it possible to investigate the relationships between genetics and environment on diadromous behavior and aid in the management of species that display faculative diadromy. However, as with any new method, it is important to confirm the validity of these results for the particular species and habitat in question.

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