Use of Otolith
Microchemistry to Discriminate <u>O</u>. <u>nerka</u>
of Resident and Anadromous Origin[1]

B. E. Rieman and D. L. Myers

USDA Forest Service
Intermountain Research Station
316 E. Myrtle, Boise, ID 83702

and

R. L. Nielsen
Oregon State University
College of Oceanography
Corvallis, OR 97331-5503

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We used a wavelength dispersive microprobe to describe patterns
in otolith microchemistry of sockeye salmon Oncorhynchus nerka.
Patterns in Sr/Ca ratios in transects across otoliths were
consistent with changes in environmental chemistry associated
with life history. The patterns we observed were consistent with
those described for other salmonids. We found Sr/Ca ratios in
otolith primordia of fish of known anadromous origin were sign-
ificantly higher than those in otolith primordia of fish of known
resident origin. We found also, however, that variation among
samples was associated with differences in chemistry of the
freshwater environments. These differences could confound
attempts to discriminate sympatric resident and anadromous forms
in lakes where Sr/Ca ratios are high. Samples used to
discriminate origin of emigrants from Redfish Lake, ID confirmed
the presence of both resident and anadromous fish, but the two
groups were not clearly resolved. Otolith microchemistry has the
potential to determine the parental origin of O. nerka and to
describe life history patterns, but may not clearly identify all
individuals in all lakes. More work is needed to determine the
inherent variability among individuals and among populations, and
to determine the influence migration, spawning, and incubation
environments have on the chemistry of otoliths.

Otolith chemistry has been used to describe the environmental history of individual fish (Gauldie et al. 1980; Kalish 1989; Radtke 1989; Radtke et al. 1990). Using a wavelength dispersive microprobe, Kalish (1990) found patterns in strontium (Sr) and calcium (Ca) content, expressed as the Sr/Ca ratio, across otoliths of brown trout (Salmo trutta), Atlantic salmon (Salmo salar), and rainbow trout (Oncorhynchus mykiss), that were associated with the varied environments and life histories of anadromous and nonandromous forms. Kalish (1989) showed that sea-farmed rainbow trout passed a chemical signal in the form of elevated Sr to the nucleii of the otoliths of their progeny. He concluded that the Sr/Ca ratio in otolith primordia can be used to distinguish the progeny of sympatric anadromous and nonanadromous salmonids.

Discrimination of resident and anadromous forms is potentially useful to the research and management of sockeye salmon (Oncorhynchus nerka). Anadromous and resident (kokanee) forms of sockeye salmon commonly occur in sympatry throughout much of the species range (Ricker 1940, 1959; Wood and Foote 1990). The two forms give rise to each other (Ricker 1940, 1959, 1972; Foerster 1947; Rounsefell 1958; Bjornn et al. 1968; Nelson 1968; Scott 1984; Graynoth 1987; Foote et al. 1989), but the importance and dynamics of such interaction is unknown. Anadromous sockeye salmon have founded persistent kokanee populations (Ricker 1940; Graynoth 1987) and sustained production of anadromous fish has been established from a kokanee population under artificial

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circumstances (Kaeriyama et al. 1992). Reproductive isolation

and resulting adaptation to the freshwater environment, however,

might limit the success of kokanee or hybrid forms that attempt

anadromy in the wild (Wood and Foote 1990; Taylor and Foote 1991;

Foote et al. 1992).

Anadromous sockeye salmon were once produced in lakes

throughout the Snake River Basin in Idaho and Oregon. Although

kokanee are still found in a number of these lakes, dams and

other barriers have eliminated access for anadromous fish to

most. Survival of salmonid smolts in the Snake River Basin has

declined substantially with hydropower development (Ebel 1977).

Smolt-to-adult survival is probably a limitation for those $\underline{O}$.

$\underline{nerka}$ populations that still have access to the ocean. An

anadromous form of sockeye salmon has persisted only in Redfish

Lake in Idaho, and that run is on the verge of extinction (Waples

et al. 1991). Kokanee persist in most of these lakes and at

least some fish emigrate at the same time and size as typical

sockeye smolts (Bjornn et al. 1968; Idaho Department of Fish and

Game, Boise, ID, unpubl. data). Kokanee might maintain or

refound the anadromous form in Redfish Lake during or following

periods of severe restriction in migrant survival (Bjornn et al.

1968; Waples et al. 1991). Discrimination of origin in $\underline{O}$. $\underline{nerka}$

emigrating from and returning as anadromous adults to Redfish and

other lakes would be informative. The role that resident fish

play in the maintenance or refounding of an anadromous form of

sockeye salmon is clearly an important question to future

management and the potential recovery of anadromous stocks throughout the basin.

This study was conducted to determine whether patterns in otolith microchemistry of sockeye salmon are consistent with life histories and with patterns described for other species. Our approach was two-fold. First, we obtained otoliths from fish of known or assumed origin (anadromous or resident female parent) from several stocks of O. nerka. We used a wavelength dispersive microprobe to determine whether the Sr/Ca patterns in our otoliths were consistent with the varied environments and life histories of resident and anadromous fish. Second, we attempted to discriminate the resident versus anadromous origin of O. nerka that emigrated from or returned as anadromous adults to Redfish Lake.

Materials and Methods

Rationale

Our analysis was based on the low level substitution of Sr for Ca in the aragonite matrix of an otolith during growth (Kalish 1989; 1990). Although the Sr and Ca content in the otolith may be variable (Kalish 1989), Sr/Ca ratios in otoliths and ova reflect the relative amounts of Sr and Ca in the environment (Kalish 1990). In anadromous salmonids vitellogenesis is initiated while the female is still in the ocean. The Sr/Ca ratio of seawater can be much higher than that typically found in

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freshwaters. Kalish (1990) assumed Sr/Ca ratios of 0.0087 for seawater vs. 0.0019 for freshwater on average. He found a corresponding difference in the Sr and Ca content of the ova from nonanadromous and anadromous forms of rainbow trout. The multiple primordia of salmonid otoliths are the first calcified structures to form in the developing embryo and are present at least several weeks prior to hatch. The elemental composition of the primordia that develop directly from the yolk should reflect the elemental composition of that yolk, and ultimately the life history of the female parent. Kalish (1990) found that the chemical composition of sagittal otolith primordia could be used to clearly discriminate individual rainbow trout from female parents held in freshwater or seawater.

Otolith Samples

We obtained otoliths from the progeny of resident and anadromous female parents at Redfish Lake, in Stanley Basin, ID (Table 1). A resident stock of kokanee spawns in Fishhook Creek, a tributary to the lake. The Fishhook Creek stock can be distinguished from anadromous fish on the basis of size, and the time and location of spawning. In addition, all anadromous adults were trapped as they attempted to enter the lake in 1991. Therefore, the fish spawning in the lake or in Fishhook Creek had to be fish that matured in the lake. Eggs were collected and fertilized at Fishhook Creek on two occasions in September 1991

Table 1
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as part of an independent research project. Embryos were transported directly to the Idaho Department of Fish and Game Eagle Island Hatchery where they were incubated, hatched, and reared. We sacrificed fish for otolith samples at several times between hatch and yolk absorption. Otolith samples from the Fishhook Creek kokanee population represented six separate female parents. We also collected otoliths from adult $\underline{O}$. $\underline{nerka}$ spawning in Fishhook Creek. Although the Fishhook Creek adults could have been residual progeny of anadromous adults, they are reproductively isolated from the anadromous stock. Kokanee spawning in Fishhook Creek spawn at least one month earlier than the known anadromous fish which have only been observed spawning along the lake shore. Because of the isolation from the known anadromous spawning site and time we assumed that the Fishhook Creek samples represented fish of resident-parent origin.

One female and three male anadromous adult sockeye salmon returned to Redfish Lake in 1991. In October, as part of the endangered species recovery program, the single female was spawned in captivity. Her eggs were fertilized with sperm from each of the three males. The embryos were incubated, hatched, and reared in the same facility, in identical incubation chambers, and with the same water supply as the resident fish. Because of the later spawning time the anadromous origin embryos were brought into the hatchery about one month after those of resident origin. However, because the water source is a well, incubation temperatures did not vary significantly between the

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two groups. We obtained otoliths only from mortalities that occurred during incubation and rearing. Our samples represented all of the three matings.

We obtained additional samples from O. nerka in other lakes (Table 1). We collected otoliths from kokanee in Alturas Lake, about 25 km from Redfish Lake. Although Alturas Lake once supported an anadromous run, anadromous fish no longer have access to the lake and none have returned to the lake in more than 20 years. The Alturas Lake samples represent samples of resident-parent origin, but from a distinct watershed in the Stanley Basin. The National Marine Fisheries Service provided samples of anadromous-origin adults and outmigrating smolts from Wenatchee Lake, tributary to the Columbia River in Washington. Resident-origin fish were not available from Wenatchee Lake. The Canadian Department of Fisheries and Oceans provided samples from anadromous adults and resident adults in Takla Lake in the headwaters of the Fraser River. The parental origin of the Takla Lake fish is not certain, but phenotypic and genetic information were used to characterize the fish as resident, residual (remaining in the lake but originating from an anadromous female parent), and anadromous origin (C. Foote and C. C. Wood, Canadian Department of Fisheries and Oceans, Nanaimo, B.C., pers. comm. of unpubl. data).

Otolith samples of unknown parental origin were available from juvenile fish that emigrated from Redfish Lake in 1991. In May, 861 of an estimated 4,500 emigrants were trapped about 0.5 km

below the outlet of the lake. Trapping was conducted through the full duration of the migration by the Idaho Department of Fish and Game as part of a captive broodstock development program. The emigrants in our sample ranged from 84 to 115 mm fork length. Because no more than four anadromous adults were believed to have spawned in the lake in 1988 or 1989 (Idaho Department of Fish and Game, Boise, ID, unpubl. data) we anticipated that resident origin fish contributed to the emigration. Our otolith samples came from fish that died at the trap site, in transportation, or at the Eagle Island Hatchery captive broodstock site. We also obtained otoliths from the four anadromous adults that returned to the Redfish Lake and were spawned in October 1991, and a single anadromous male that returned to the lake in 1992.

All otoliths were stored dry in plastic micro-centrifuge vials until preparation for analysis.

Analysis

Otoliths were cleaned in deionized water and dried before mounting. Our initial preparation of otoliths from juvenile fish followed Kalish (1990). The otoliths were mounted sulcus side up on glass slides with Crystal Bond 509 (Aremco Products Inc.). We ground the otoliths in the sagittal plane to the approximate level of the primordia with 1200 grit wet-dry sand paper. The much larger otoliths from adult fish were initially mounted sulcus side down and ground in the sagittal plane to a level near

the primordia with 600 grit, and then with 1200 grit sand paper to provide a thinner sample with better optical properties. The glue was then heated and the otoliths were turned sulcus side up and ground to near the primordia as with the juvenile preparations. We ground all (adult and juvenile) otoliths to the precise level of the central primordia with 5.0 μ paper (Buehler Inc.). We finished samples by hand polishing on 1.0 μ and then 0.05 μ alumina paste (Buehler Inc.). All samples were washed with deionized water in an ultrasonic cleaner and then photographed at magnifications of 140X and 280X. Photographs were used to "map" the sample and guide the selection of microprobe sites. Immediately before final analysis, samples were again washed with deionized water, air dried, and coated with a 200 A carbon layer for surface conductivity.

Elemental analyses were done with a Cameca SX-50 wavelength dispersive microprobe at Oregon State University. Our analyses followed the general procedures outlined by Toole and Nielsen (1992). We used a 15 KV, 50 nA, and 5 μ diameter, electron beam for all of our analyses. Standards for Sr and Ca were strontiantite ($SrCO_3$-USNM R10065) and calcite ($CaCO_3$-USNM 136321). For maximum precision, we used a long counting time (40 s) and analyzed each element simultaneously. We report Sr/Ca ratios as normalized mole fractions (Toole and Nielsen 1992) that are equivalent to the atomic ratios reported by Kalish (1990). One potential problem with otolith analysis is the effect of beam damage (Kalish 1990). Under exposure to the electron beam,

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carbonate and organic material will break down, changing the composition of the area under the beam. In the case of otoliths, the concentration of Ca and Sr will increase because of the loss of CO_2 and H_2O by volatilization. Our use of long counting-times and high beam-currents thus precluded analysis for absolute abundances of the individual elements (Kalish 1990; Toole and Nielsen 1992). The technique developed by Toole and Nielsen (1992) and used in this study is based on the assumption that Sr and Ca are equally refractory under exposure to the electron beam. Given that assumption, the ratio of Sr to Ca at the sample site would remain constant throughout the period of beam exposure. To test this assumption we analyzed a single site on a single otolith repeatedly 10 times. The results (Table 2) show no systematic change in Sr/Ca ratio. The coefficient of variation (CV), calculated as the $SD_{Sr/Ca} \cdot$ Mean Sr/Ca^{-1}, for the 10 samples was about 0.11.

We classified sampling regions on individual otoliths as: (1) Primordia-sites directly in the primordia or in nuclear material immediately adjacent to the primordia; (2) Nucleus-the area of growth between the central and distal primordia and between the primordia and point of hatch; (3) Freshwater growth-the region between the point of hatch and the point of saltwater entry for anadromous fish or the last annulus for resident fish, in most samples these sites were in the summer growth region preceding the first or second annulus; and, (4) Saltwater growth-the summer

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growth region preceding the last annulus on otoliths of anadromous adults.

All otoliths were sampled in the primordia. Sample sites in other otolith regions were included on initial analyses where we ran a transect of probe sites from the primordia out toward the edge of the otolith. Transects and samples in freshwater-growth and saltwater-growth regions on the otolith were used only to determine whether patterns in Sr/Ca ratio were consistent with those anticipated based on anadromy and resulting change in chemistry of the environment. We hypothesized that an elevated Sr/Ca signal should be evident in samples from the primordia of fish of anadromous origin relative to those of resident origin. After Kalish (1990) we expected samples from fish of anadromous origin to show a pattern of elevated Sr in the primordia, reduced Sr in the freshwater-growth region and, if an anadromous adult, elevated Sr in the saltwater-growth region.

Except for the transects, we sampled six sites within the primordia on each otolith. Analyses of individual sites with high organic content or with surface flaws were eliminated from the data set. Such results were identified by their low total ion concentration (<80% carbonate by weight). Nine otoliths out of the entire sample were limited to five sites in the primordia.

We analyzed water chemistry to describe the relationship between the chemistry of otolith region and the chemistry of the environment in which the otolith, or yolk precursor in the case of primordia, developed. Water samples were collected from each

of the freshwater sources where fish reared except for Takla Lake. Water samples were analyzed by inductively coupled plasma atomic emission spectrometry, according to U.S. Environmental Protection Agency method 215.1 (U.S. Environmental Protection Agency 1983) for Ca and Standard Methods method 303 (American Public Health Association 1989) for Sr. Detection limits for both elements were considered to be 10 μg·l^{-1}. We assumed a Sr/Ca ratio for seawater of 0.00874 (Bruland 1983), for all the otolith sample sites of anadromous origin.

Results

Samples of Known or Assumed Origin

Microprobe transects across otoliths of known or assumed origin showed patterns consistent with life history. Transects across otoliths from sockeye salmon smolts and adults from Wenatchee Lake showed intermediate Sr/Ca ratios in the primordia and nucleus, lower Sr/Ca ratios in the freshwater-growth region, and higher ratios in the saltwater-growth region (Fig. 1). Transects run on otoliths from fish of resident origin in Redfish Lake were low at all sites (Fig.1).

Fig. 1.
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The Sr/Ca ratios in the primordia of otoliths of known anadromous origin fish in Redfish Lake were clearly elevated relative to ratios from otoliths of resident-origin fish (Fig. 2A). The distribution of all sample sites in the two groups

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overlapped, but the distribution of means for individual otoliths did not (Fig. 2B). None of the samples from fish of resident

origin produced a mean Sr/Ca ratio higher than 0.00080 (range 0.00042 to 0.00080); and only one sample from fish of anadromous origin produced a ratio lower than 0.00140 (range 0.00114 to 0.00201). Student's T-tests of log (x+1) transformed ratios between anadromous and resident means of individual otoliths or between all sample sites pooled by origin, were significant ($p < 0.001$). The CV among sites within individual otoliths ranged from about 0.04 to 0.20 in both groups.

The sample means of otolith primordia from fish of anadromous origin in Wenatchee and Takla lakes were within the range of the sample means of anadromous-origin fish in Redfish Lake (Fig. 3). Of all 28 anadromous samples (all lakes) only one mean Sr/Ca ratio was below 0.00140. Samples in the primordia of otoliths from resident kokanee in Takla Lake were lower than those in the primordia of anadromous fish and were similar to samples in the

primordia of resident fish in Redfish Lake. The samples in primordia of kokanee from Alturas Lake were higher (range 0.00155 to 0.00204) than those in kokanee from either Redfish or Takla Lake (Fig. 3), and were similar to those observed in samples in the primordia of anadromous-origin fish.

The Sr/Ca ratios in freshwater-growth regions on otoliths were similar to those we observed in the primordia of resident-origin fish from the same lake. The mean Sr/Ca ratios in freshwater-growth regions were lower than those in the primordia of

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anadromous origin fish for samples from fish in Redfish, Takla and Wenatchee lakes. Differences between anadromous primordia and freshwater-growth sites were significant ($p < 0.001$) whether for the means of individual otoliths or for all sites in common regions pooled among fish.

Concentrations of Sr and Ca varied substantially among the waters we sampled (Table 3). The resulting Sr/Ca ratios ranged from < 0.00120 to 0.00610. We found a direct relationship between the Sr/Ca ratio in primordia, freshwater-growth, or saltwater-growth regions and the Sr/Ca ratio of the water associated with the development of the otolith sites or their yolk precursors (Fig. 4).

Table 3
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Fig. 4.
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Samples of Unknown Emigrants from Redfish Lake

We completed microprobe analyses on otoliths from 94 individuals that emigrated from Redfish Lake in May 1991. The range of mean Sr/Ca ratios in primordia of individual fish was similar to that observed among pooled samples of resident and anadromous origin (Fig. 5). Two modes were evident in samples from freshwater-growth regions. However, all freshwater samples higher than 0.00080 were taken from fish that were held at the Eagle Island Hatchery. The sample sites fell in the region of growth that occurred while the fish were in the hatchery, and thus represent incorporation of Sr that occurred in the hatchery and not in the lake. The range of means from freshwater-growth

Fig. 5.
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regions that represented growth in the lake was much narrower and consistent with the range of means from other freshwater samples, except those from Alturas Lake. Two modes were evident in the primordia means, suggesting the sample included fish of both resident and anadromous origin. We did not find two clearly discrete groups that would allow us to classify the origin of all fish. About one-third (35%) of the samples fell into the range between 0.00080 and 0.00140, while 29% and 36% of the samples fell above and below that range respectively.

The CV among sites within the primordia of individual otoliths ranged from about 0.10 to 0.30. The variation among sample sites in individual otoliths tended to be higher than we observed in the sequential samples at a single site on a single otolith (CV about 0.11), or among sites in the primordia of individuals of known parental origin (0.04 to 0.20). The variation among sites within the primordia also tended to be higher in samples that fell in the midrange between the two modes (Fig. 6).

The Sr/Ca ratios in primordia of the five anadromous fish that returned to Redfish Lake in 1991 and 1992 fell in the range observed for samples in the primordia of fish of known anadromous origin (Table 4).

Discussion

The patterns we observed in Sr/Ca content of sockeye salmon otoliths were consistent with changes in environment related to

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life history and with the patterns Kalish (1990) observed for other salmonids. Differences in our known samples of resident and anadromous origin fish from Redfish Lake were clear, and the expected patterns were evident in other populations as well. The association of otolith and environmental chemistry demonstrates that analysis of Sr content in otoliths of migratory salmonids, including sockeye salmon, can provide information on life history.

There were differences in our results and those of Kalish (1990). The absolute magnitudes of our Sr/Ca ratios in primordia and freshwater-growth sites were lower than the observations reported by Kalish (1990) for brown and rainbow trout and Atlantic salmon. Our sample means in otolith primordia of resident-origin fish ranged from about 0.00040 to 0.00080, and in primordia of anadromous-origin fish from about 0.00110 to 0.00230. Kalish (1990) observed ranges in Sr/Ca ratios of about 0.00070 to 0.00170, and 0.00220 to 0.00520 for the same two groups, respectively. Our observations in saltwater-growth regions, however, were in the same range as those reported by Kalish (1990) (about 0.0025 to 0.0040, his Fig. 1).

A variety of factors including environmental chemistry and stress may influence the mobilization and availability of Ca and Sr in the blood plasma, vitellogenin, the resulting yolk proteins of developing ova, and ultimately in the substitution of Sr for Ca in the matrix of developing otoliths (Kalish 1989). The observed differences of our samples from wild fish relative to

the captive (seafarm or hatchery) stocks represented in Kalish's

(1990) samples could be explained by differences in life history

and environment. The anadromous fish in our samples all moved

into freshwater well before spawning (2 to 4 mo) and undoubtedly

experienced a variety of stresses related to the adaptation from

saltwater to freshwater and the extended migrations (up to 900 km

and several months). It is not clear whether yolk development

was complete when the sockeye salmon represented in our samples

left the ocean. Regardless, some ion exchange can occur between

the female and the egg in the period prior to spawning (Alderdice

1988). Such exchange might continue to influence the yolk

content during migration and might explain the lower ratios we

observed in primordia of anadromous origin relative to those of

Kalish (1990). We observed that the Sr/Ca content in primordia

of anadromous origin fish was typically less than that in

saltwater-growth sites, while the ratios in primordia of

resident-origin fish were similar to those in freshwater-growth

sites (Fig. 4). The difference suggests some attenuation of the

Sr content of the yolk in anadromous females that does not occur

in resident females. The anadromous adults in Kalish's (1990)

study were held in freshwater for only 3 weeks versus several

months in the migration and staging of sockeye salmon.

Substantial differences could result in anadromous origin

primordia through such differences in prespawning environments.

Differences in Sr/Ca ratios among freshwater environments

could also explain differences observed in primordia of resident

origin fish between our study and that of Kalish (1990). The variation in freshwater chemistry can clearly influence the Sr content observed in otoliths derived from resident parents. We observed substantial variation in Sr/Ca ratios for the bodies of water represented in our study, and a strong association between water chemistry and otolith chemistry. Kalish (1989, 1990) did not report water chemistry for his study sites, but the variation we observed among lakes demonstrates that differences could be large.

Our attempt to characterize the origin of the fish leaving Redfish Lake was equivocal. The samples produced a range and two modes in Sr/Ca ratios indicating that migrants of both resident and anadromous origin were present. The sample distributions, however, did not break into two clearly discrete groups. None of our known Redfish primordia samples had mean Sr/Ca ratios above 0.00080 or below 0.00110. Only 1 of the 28 known anadromous samples from all lakes fell below 0.00140. About one third of the 94 unknown samples were between 0.00080 and 0.00140.

There are two possible reasons for the lack of resolution. First, the increased variability in the known samples could have been due to analytical error. The variability among sample sites within an otolith tended to be higher in the samples that were in the range of intermediate Sr/Ca ratios. As a precaution we re-examined the otolith preparations and sample sites for each sample that fell in the intermediate range. We found no evidence of poor sample preparation, site selection outside of a

primordia, or surface flaws that could have contributed to any unusual variation.

Second, our sample size of known-origin fish may not have described the full variability inherent in otolith chemistry of the wild resident-origin fish. Our known sample included only fish from the Fishhook Creek kokanee stock. It is possible that another group of fish may have contributed to the emigration. We recently observed a small group of resident or residual fish spawning along the lake shore. Our known samples were incubated in a relatively stable hatchery environment. Each individual experienced essentially identical conditions of water temperature and chemistry. Embryos from wild fish incubate in a range of conditions that are found in both Fishhook Creek and along the lake shore. Eggs may be ionically permeable and exhibit active uptake of Ca (and presumably Sr) at fertilization (Alderdice 1988; S. Schroder, WA Department of Fisheries, Olympia, WA, USA, pers. comm.) Differences in the chemistry of the incubation microhabitats caused by groundwater or local tributary influences could contribute to additional variability in otolith chemistry (J. Kalish, Ministry of Agriculture and Fisheries, Wellington, NZ. pers. comm.). Some studies have also suggested that Sr/Ca ratio in otoliths can be related to temperature (Radtke et al. 1990; review in Toole and Nielsen 1992) or rate of growth (Kalish 1989; Sadovy and Severin 1992). Temperature during incubation can influence the growth and number of otolith primordia that develop in other salmonids (Nielson et al. 1985) and might well

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influence the physiology of Ca deposition. Temperatures among and within the spawning habitats in Redfish Lake may vary by 10 °C and could well have influenced variation in otolith chemistry.

Our results indicate that the utility of otolith micro-chemistry will be highly dependent on the variation in chemistry of freshwaters and on variation in timing and length of exposure to those waters. The observed variation in chemistry among lakes, for example, could seriously confound attempts to discriminate resident and anadromous origin fish. The Sr/Ca ratios we observed in Alturas Lake kokanee otoliths were similar to ratios observed in primordia from fish of anadromous origin in other lakes. If anadromous fish were present in Alturas lake, it would be impossible to distinguish their progeny from those of resident fish. Clearly, the simple assumption that freshwater Sr/Ca ratios are less than those in seawater is inappropriate. Detailed water chemistry should be part of any further application.

Experimentation with spawning and incubation environments might explain the lack of resolution in our unknown samples from Redfish Lake. Collection of known origin samples in the wild might also be possible by trapping emergent fry following observation of known female spawning. Until that work can be done, however, further studies attempting to discriminate origin of fish in Redfish Lake or any other system will be vulnerable to the same uncertainties.

We conclude that otolith microchemistry has the potential to provide important information on the life history of sockeye salmon. The Sr/Ca ratios in otoliths are strongly associated with the Sr/Ca ratio of the different environments experienced during growth and yolk development. The similarity in pattern among species, the association between otolith chemistry and environmental chemistry, and the similar magnitudes of Sr incorporated in saltwater-growth regions among sockeye salmon and other salmonids, all indicate that different species incorporate Sr into otoliths in similar fashion. There is strong evidence that the Sr content of otolith primordia reflect the environmental history of the female parent. Discrimination of sympatric resident and anadromous forms of O. nerka is possible but problematic. Our results show that discrimination in wild stocks can be confounded by the local environment. Any application to wild stocks requiring discrimination of every individual must first resolve the level of variation inherent among populations, habitats, and individuals of known origin.

We cannot conclude that resident-origin fish are successfully producing anadromous adults in Redfish Lake. The chemistries of all five anadromous adults that returned to Redfish Lake in 1991 were consistent with an anadromous origin. Our data did indicate that both resident and anadromous fish were present among the 1991 emigrants and that resident fish may have represented a large if not dominant proportion of the sample. The potential for anadromous behavior has apparently been retained in fish from

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resident parents but we cannot, as yet, estimate the rate of emigration or its contribution to anadromous returns.

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TABLE 1. Sources, origin, and number of otoliths used for microchemistry analysis.

	Origin	Number Analyzed
Redfish Lake, ID		
1991, 1992 adult returns	Anadromous	5
Progeny of adult returns	Anadromous	15
Fishhook Creek adults	Resident	10
Progeny of Fishhook Creek adults	Resident	11
1991 emigrants	Unknown	94
Alturas Lake, ID		
Juveniles	Resident	8
Wenatchee Lake, WA		
Adults	Anadromous	6
Juveniles	Anadromous	2
Takla Lake, B.C.		
Anadromous adults	Anadromous	4
Residual adults	Anadromous	4
Resident adults	Resident	3

TABLE 2. Elemental Sr/Ca ratios of 10 sequential micro-
probe samples of a single site on a single otolith.

	Sample in order of analysis				
Sample	1	2	3	4	5
Sr/Ca ratio	0.00232	0.00176	0.00214	0.00241	0.00202
Sample	6	7	8	9	10
Sr/Ca ratio	0.00213	0.00194	0.00237	0.00212	0.00254

TABLE 3. Concentration[a] and elemental ratios of strontium and calcium in three lakes and the Eagle Island Hatchery water supply. Sample sizes are shown in parentheses.

Location	Elemental Ca ($mg \cdot l^{-1}$)		Elemental Sr ($mg \cdot l^{-1}$)		Sr/Ca ratio[b]
Redfish Lake	3.88	(6)	< 0.010	(6)	< 0.0012
Alturas Lake	8.29	(6)	0.110	(6)	0.0061
Wenatchee Lake	2.00	(1)	0.015	(1)	0.0034
Eagle Island Hatchery	32.00	(2)	0.340	(2)	0.0049

[a] Detection limits were 0.010 $mg \cdot l^{-1}$

[b] The Sr/Ca ratios were calculated as normalized mole fractions.

TABLE 4. Mean elemental Sr/Ca ratios from microprobe sites in the primordia of otoliths of five anadromous <u>Oncorhynchus</u> <u>nerka</u> that returned to Redfish Lake, ID in 1991 and 1992.

	1991		1992
Female	0.00233	Male	0.00223
Male A	0.00213		
Male B	0.00175		
Male C	0.00131		

FIG. 1. Elemental Sr/Ca ratios from microprobe transects across three general regions on otoliths from adult resident (Redfish Lake) and anadromous (Wenatchee Lake) Oncorhynchus nerka.

FIG. 2. Frequency distribution of elemental Sr/Ca ratios from microprobe sites in primordia of otoliths from Oncorhynchus nerka of known resident or anadromous origin. All fish were the progeny of fish spawned at Redfish Lake, ID. They were incubated at the Eagle Island Hatchery. 'A' represents all sample sites in the primordia for all fish; 'B' represents the means of sample sites in the primordia for individual fish. Numbers on the x axis represent the midpoint of bins used in the frequency distributions.

FIG. 3. Frequency distribution of mean elemental Sr/Ca ratios from microprobe sites in primordia and freshwater-growth regions on otoliths of individual Oncorhynchus nerka from Alturas, Wenatchee, and Takla lakes. The Wenatchee Lake samples are of anadromous origin; the Takla Lake samples are assumed to be of resident, anadromous but residual, and anadromous origin; the Alturas Lake samples are of resident origin. Numbers on the x axis represent the midpoint of bins used in the frequency distributions.
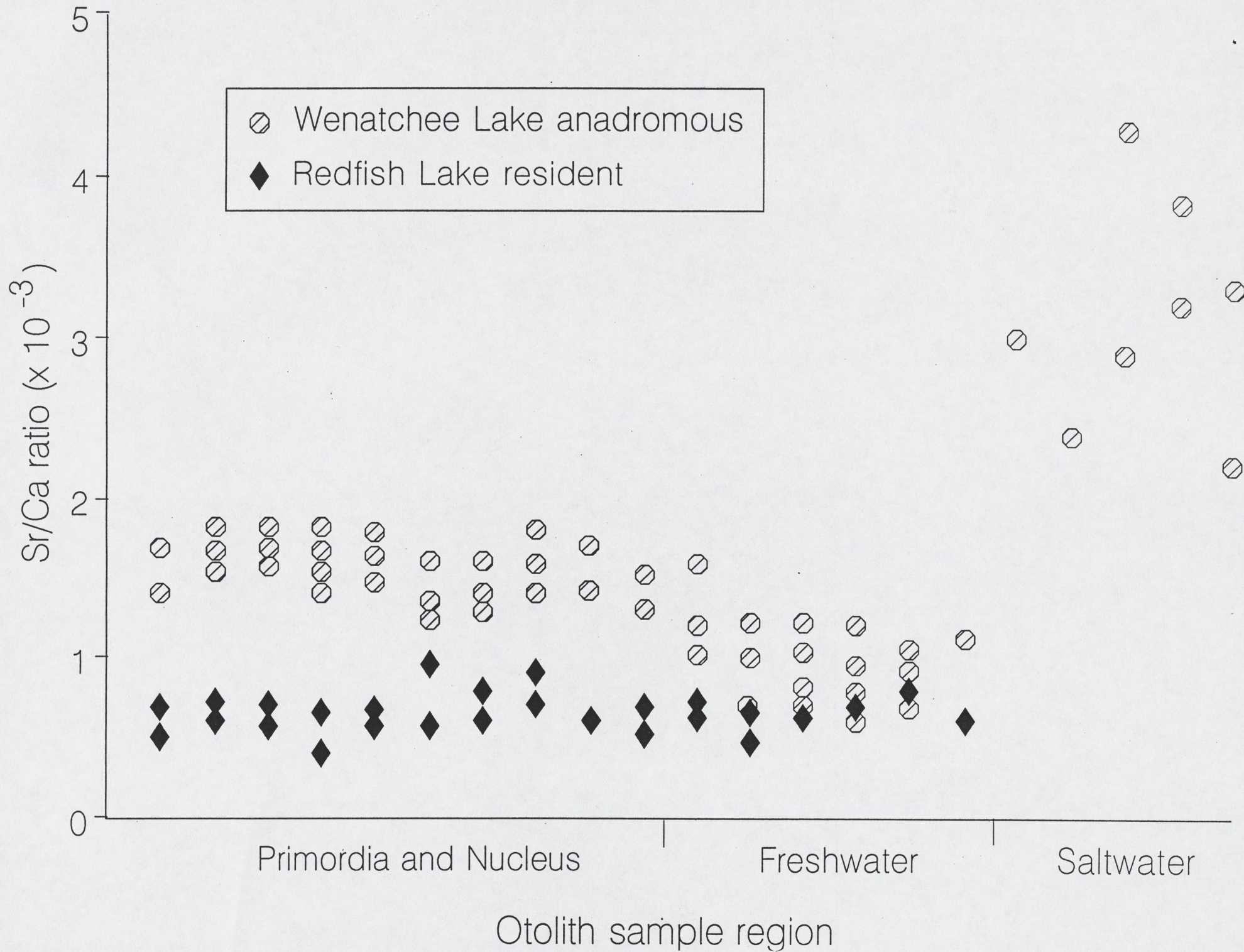
FIG. 4. Relation of mean elemental Sr/Ca ratios in primordia, and freshwater and saltwater-growth regions of otoliths and the water chemistry associated with the growth of that area or development of its yolk precursors. Samples are from three lakes and the Eagle Island fish hatchery. Samples from all anadromous fish or anadromous-origin primordia were pooled from all lakes to relate to the water chemistry for seawater. Vertical bars represent the range of observations.
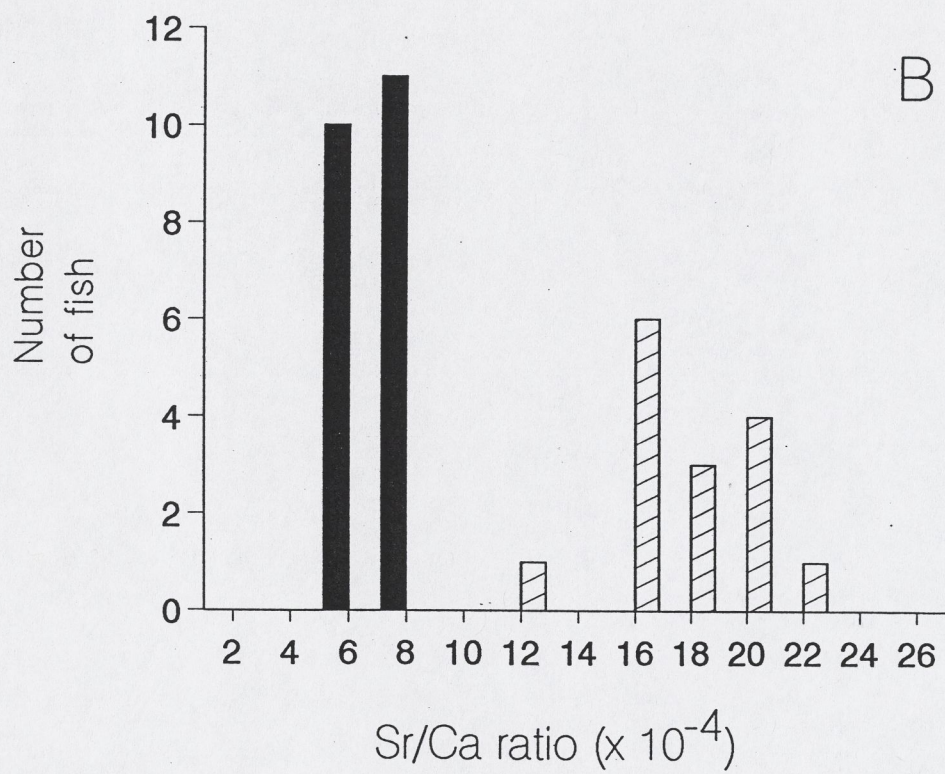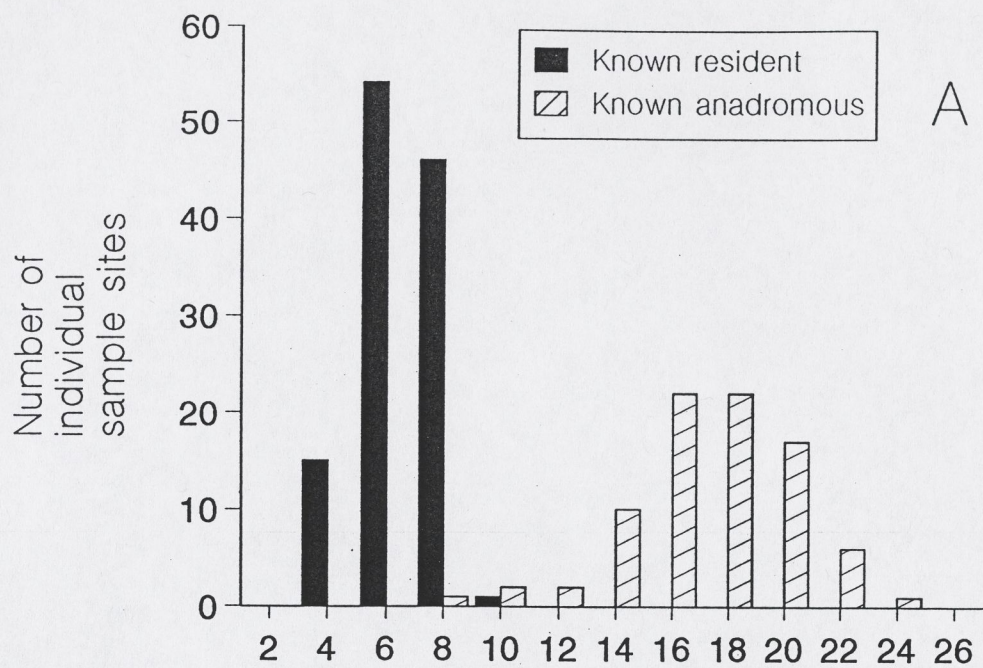
FIG. 5. Frequency distribution of mean elemental Sr/Ca ratios from microprobe sites in primordia and freshwater-growth regions on otoliths of individual Oncorhynchus nerka trapped while emigrating from Redfish Lake, ID in May 1991 (n = 94). Freshwater sample regions are identified as those that were in areas of growth that occurred in the lake and in Eagle Island Hatchery. Numbers on the x axis represent the midpoint of bins used in the frequency distributions.

FIG. 6. Mean elemental Sr/Ca ratios from microprobe sites in the primordia of otoliths of individual Oncorhynchus nerka trapped while emigrating from Redfish Lake, ID, in May 1991. The vertical bars represent ± 1 SD.
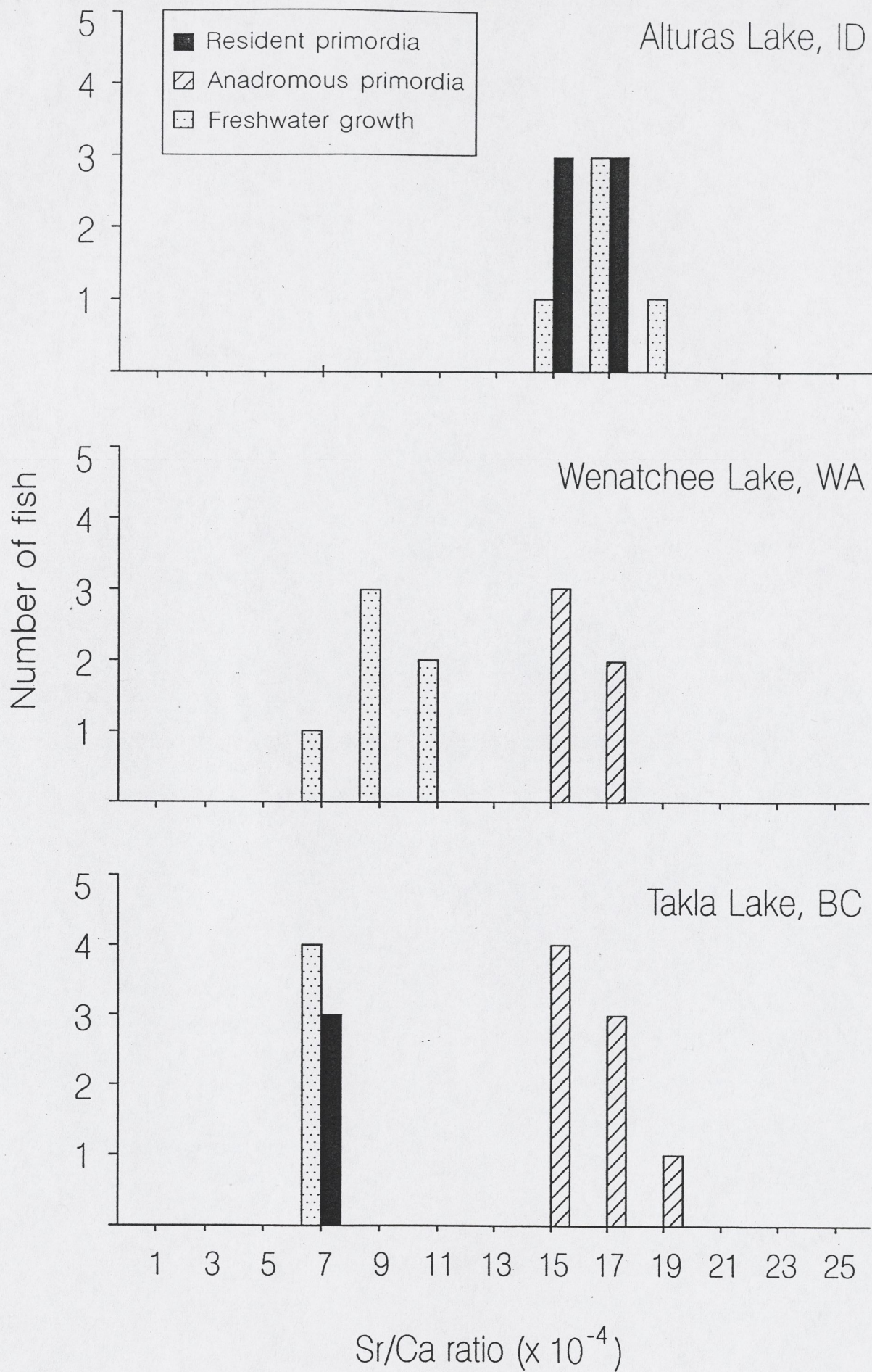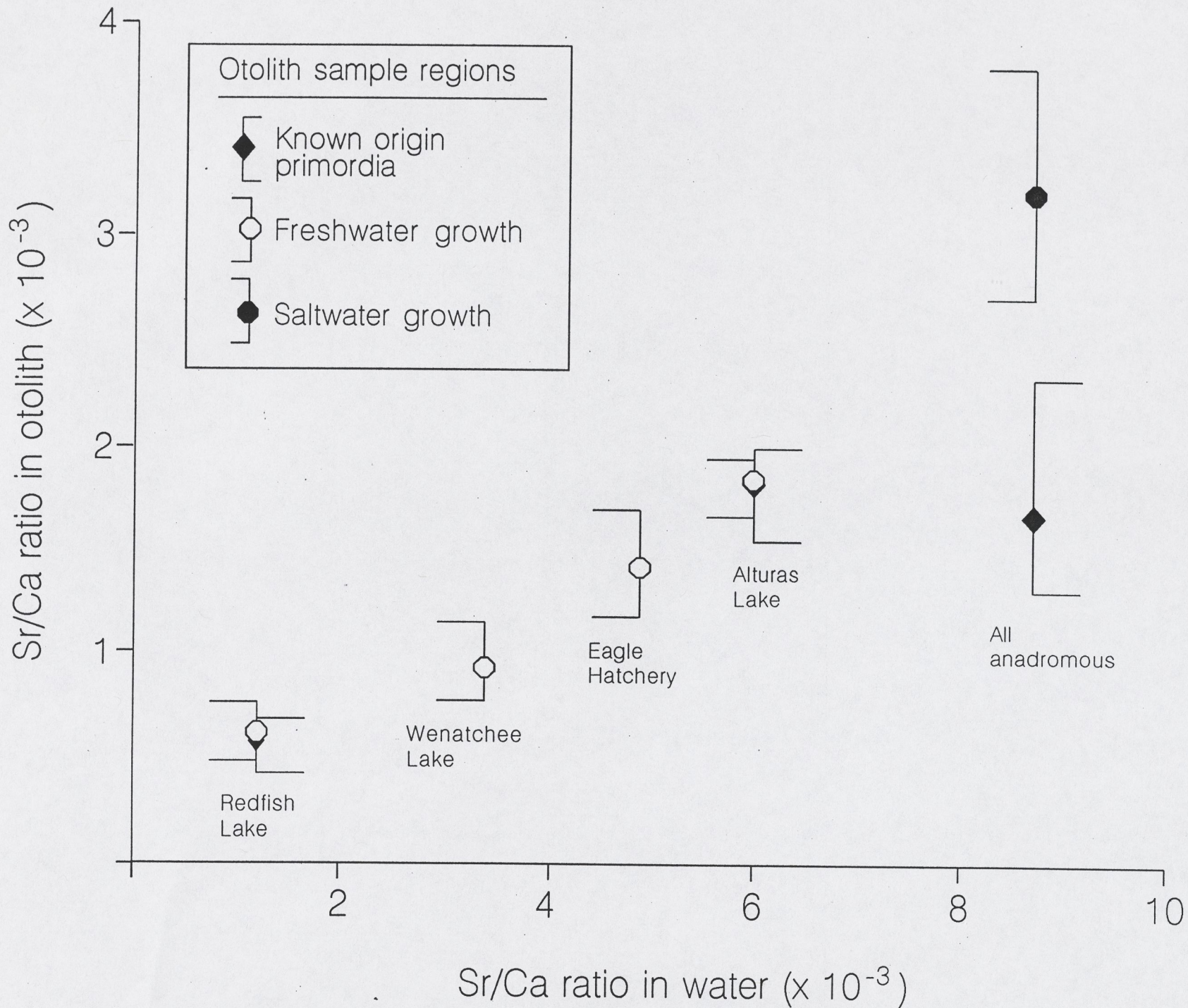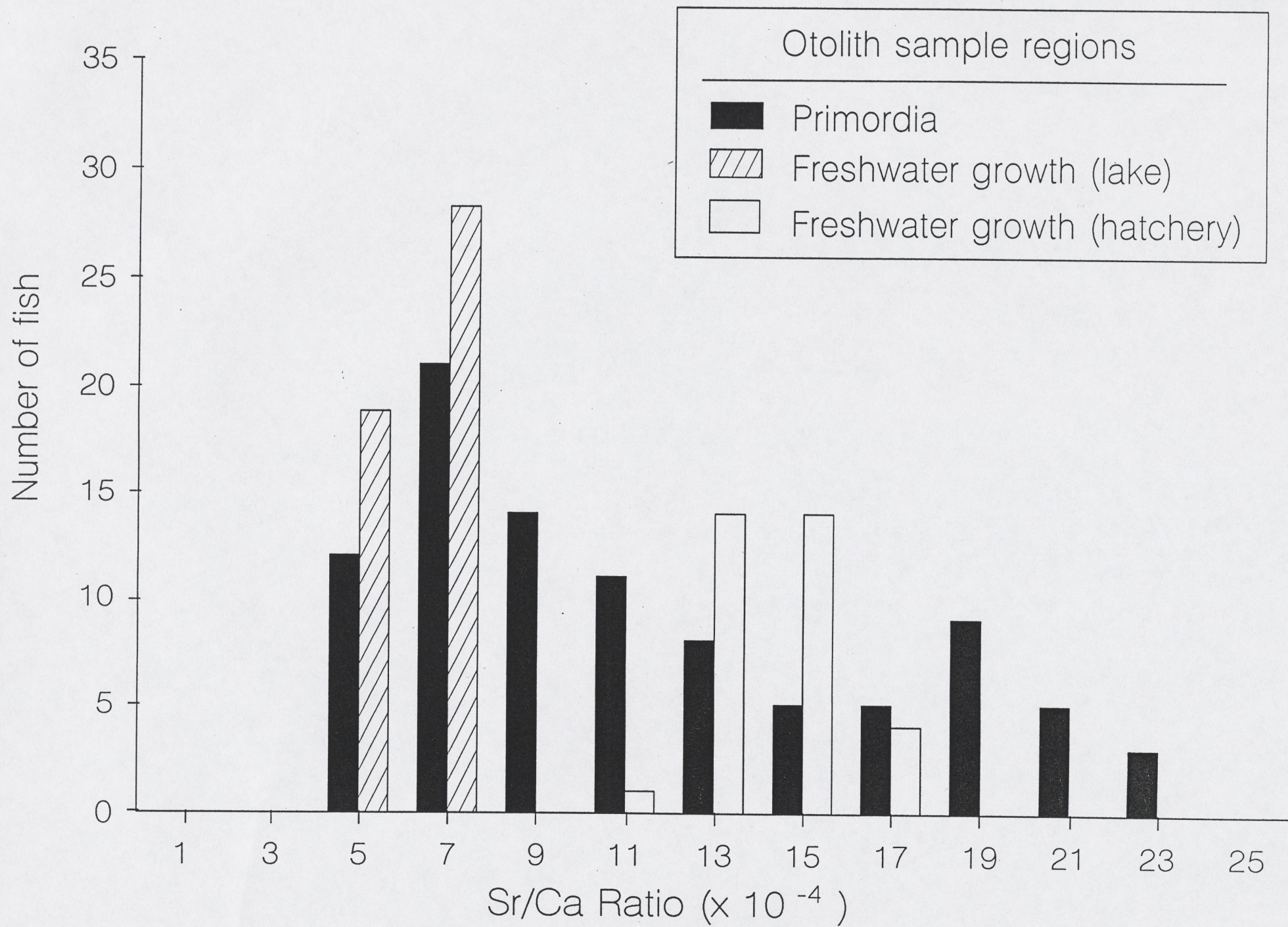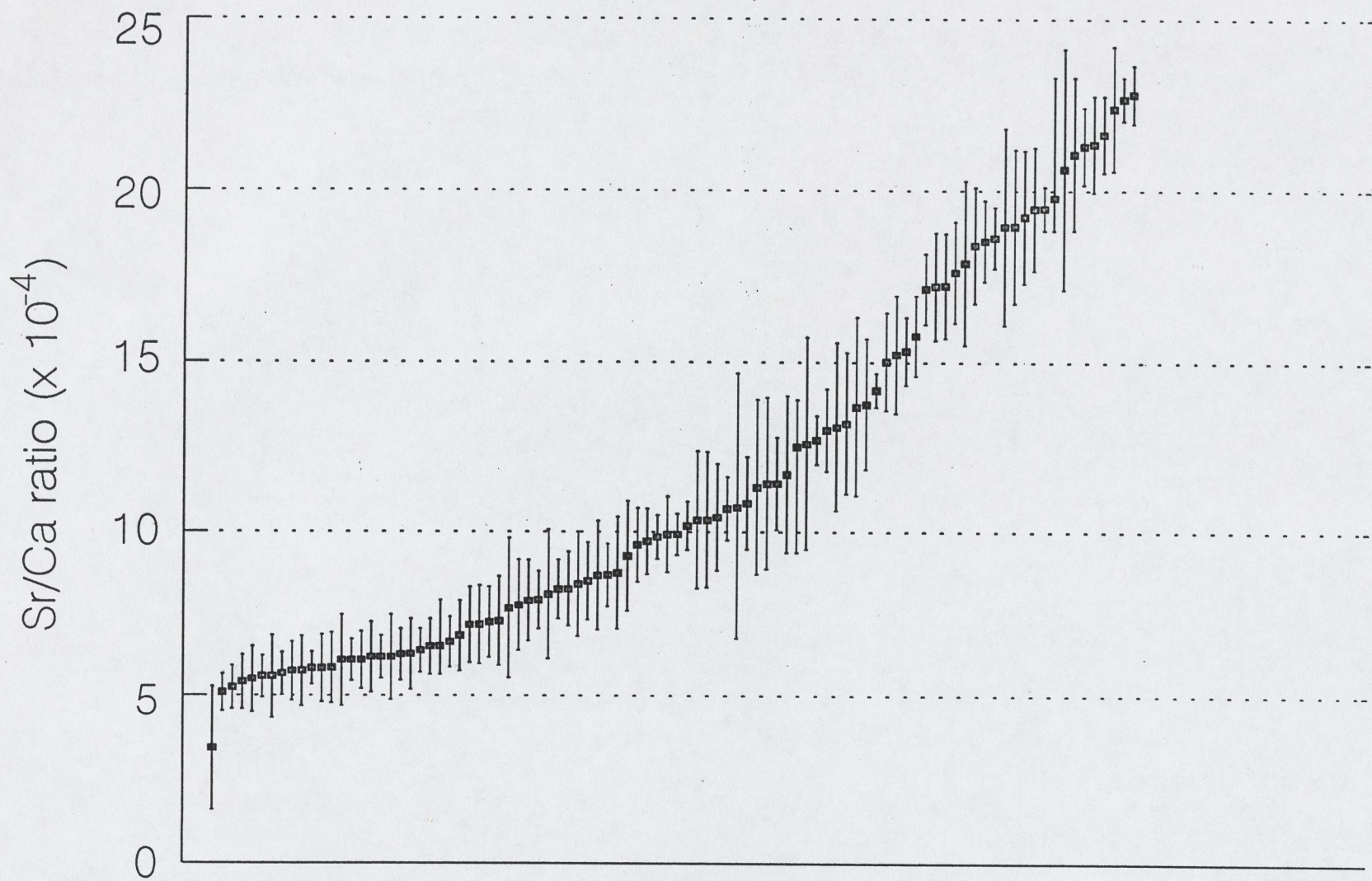
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Legend:
- ■ Known resident
- ▨ Known anadromous

A — Number of individual sample sites vs. Sr/Ca ratio (x 10⁻⁴)

B — Number of fish vs. Sr/Ca ratio (x 10⁻⁴)

Sr/Ca ratio (x 10^{-4})

Legend:
- Resident primordia
- Anadromous primordia
- Freshwater growth

Alturas Lake, ID

Wenatchee Lake, WA

Takla Lake, BC

Number of fish

Sr/Ca ratio (x 10^{-4})