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What did you say about my mother? The complexities of maternally derived chemical signatures in otoliths.

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Abstract

Connecting maternal migratory behavior with the behavior and ecology of their progeny can reveal important details in the ecology of a population. One method for linking maternal migration to early juvenile life-history is through maternal chemistry recorded in otoliths. Despite the wide use of maternal signatures to infer anadromy, the duration and dynamics of maternal otolith signatures are not well understood. Shifts in the elemental ratios and strontium isotope ($^{87}Sr/^{86}Sr$) chemistry in otoliths from juvenile Chinook salmon (*Oncorhynchus tshawytscha*) correlate with the timing of hatch and emergence respectively, indicating a chemical marker of these ontological stages. Additionally, analysis of maternal signatures show that maternally derived $^{87}Sr/^{86}Sr$ may be influenced by equilibration of the mother to freshwater, and in some cases the $^{87}Sr/^{86}Sr$ signatures of the eggs can shift significantly after being laid. These results provide guidance in separating maternal and juvenile signatures as researchers increasingly target early juvenile otolith chemistry. These results also caution against the use of $^{87}Sr/^{86}Sr$ alone as a marker of anadromy in populations with significant inland migrations.

Introduction

In many fish species migration is a facultative strategy, often encompassing various degrees of partial migration (Tsukamoto and Arai 2001, Secor and Kerr 2009, Chapman et al. 2011, Brodersen et al. 2014). Even within species and populations that are generally considered to be obligately migratory, the timing of migratory movements can be quite diverse (Isaak et al. 2003, Burke 2004, Hegg et al. 2015a). In many cases life-history characteristics such as growth rate and propensity to migrate are heritable, and understanding their expression in the context of fitness necessitates linking parent and progeny (Stewart et al. 2006, Thériault et al. 2007, Liberoff et al. 2014, 2015, Waples et al. 2017). Further, maternal effects and conditions early in life can have large effects on adoption of particular strategies by individual fish (Taylor 1990, Morinville and Rasmussen 2003). However, it can be difficult without genetic parentage information to link the life-history of the mother to her progeny. Further, understanding behavior and the effects of environment on juvenile fish can be difficult in fish too small to apply electronic tags.

Otoliths provide a window into the link between mothers and progeny, as well as detailed juvenile information. An inner ear structure of bony fish, otoliths grow in size with the addition of daily layers of calcium carbonate (Campana and Thorrold 2001). These layers incorporate elements and isotopes that occasionally substitute into the calcium carbonate structure. Many of these elements and isotopes are recorded in proportion to the water the fish inhabits, creating a temporal and spatial record throughout its life (Kennedy et al. 1997, Thorrold et al. 1998, Campana 2005). Numerous studies have reported the presence of a maternal chemical signature

in the core of otoliths using various chemical proxies, allowing researchers to infer some aspects of maternal location, spawning migration, and anadromy from their progeny (Kalish 1990, Volk et al. 2000, Miller and Kent 2009, Shippentower et al. 2011, Courter et al. 2013, Liberoff et al. 2015). At the same time, otoliths provide information on juvenile natal location, movement, growth and condition in the layers just outside the zone of maternal influence near the otolith core (Thorrold et al. 1998, Walther and Thorrold 2010, Hamann and Kennedy 2012, Hegg et al. 2013a, Schaffler et al. 2014, Shrimpton et al. 2014, Turner et al. 2015).

Despite the amount of available research using these adjacent areas of the otolith, Veinott et al. (2014) highlight variability in some core signatures and a lack of conclusive agreement on the markers of maternal signature and its duration. Further, some assumptions of maternal chemical incorporation and duration in the otolith core are untested (Elsdon et al. 2008). Limburg et al. (2001) note that a lack of understanding of the duration of maternal signatures may affect their interpretation of Baltic sea trout that lack a freshwater phase. It is also unknown whether the maternal transition is related to underlying ontological changes in maturing fish which might provide further information on juvenile development. As the number of studies utilizing otolith chemistry to infer detailed juvenile movement patterns increase in frequency it is important to determine how to distinguish maternally-derived, versus environmentally-derived, chemical signatures of juvenile fish.

The maternal signature is retained in the otolith because the egg is provisioned using nutrients derived from the mother. The larva obtains all of its nutrients from the yolk sac until it commences feeding, which occurs shortly after hatching, at which point the larva begins

significant chemical exchange with the surrounding water (Hayes et al. 1946). Therefore, as the otoliths develop prior to hatching, its isotopic signature should reflect that of the mother (Kalish 1990, Waite et al. 2008). If the mother has migrated from a location with a different chemical signature her eggs will retain a chemical signature related to her past location, attenuated by the degree to which her body chemistry has equilibrated with the chemistry of the spawning stream (Volk et al. 2000, Bacon et al. 2004). This has also been demonstrated as a marking technique, by exposing mothers to known isotopic signatures prior to spawning (Thorrold et al. 2006, Woodcock et al. 2013, De Braux et al. 2014). This maternally derived chemical information recorded in the otolith can then be used to infer information about the maternal provenance or behavior.

The use of maternally-derived chemical signatures for the identification of marine influence in fish has been repeatedly demonstrated (Kalish 1990, Miller and Kent 2009, Liberoff et al. 2015). In most freshwater systems the concentration of strontium (Sr) is much lower than that of the ocean, providing a large and predictable shift in Sr/Ca in dissolved ions that is conserved between anadromous and resident fish (Kraus and Secor 2004, Brown and Severin 2009). However, examples exist showing that in some systems Sr/Ca is a poor indicator of ocean residence (Kraus and Secor 2004), that expected patterns of chemical signatures of ocean residence can be population specific (Hamer et al. 2015), and that Sr/Ca ratios change in relation to the time females spend in freshwater and the difficulty of the migration (Donohoe et al. 2008).

It has also been demonstrated that maternal signatures are conserved in otolith $^{87}Sr/^{86}Sr$ signatures (Barnett-Johnson et al. 2008, Miller and Kent 2009, Hegg et al. 2013a). In contrast to

Sr/Ca, however, the relationship between maternal $^{87}Sr/^{86}Sr$ values and juveniles is less well quantified. The assumption in anadromous species has been that $^{87}Sr/^{86}Sr$ maternal signatures follow the same mechanism as Sr/Ca, reflecting the global marine value of 0.70918 due to maternal investment in the yolk material (Courter et al. 2013). Several studies, however, have shown that migratory distance and migratory difficulty may influence the maternal $^{87}Sr/^{86}Sr$ signature (Volk et al. 2000, Bacon et al. 2004, Miller and Kent 2009). As more studies use $^{87}Sr/^{86}Sr$ as a marker of maternal anadromy (Courter et al. 2013, Hodge et al. 2016), it is necessary to understand the dynamics of maternal chemistry in the otolith core, as well as the migratory conditions under which the assumptions of maternal chemical influence hold.

Based upon a review of the otolith literature, the factors controlling the duration of the maternal signature have been largely unexplored. Many studies mention the need to avoid mistaking the period of maternal influence with the period of natal origin, or vice-versa (Barnett-Johnson et al. 2008, 2010, Donohoe et al. 2008, Hegg et al. 2013a). However, determining the end of maternal chemical influence on the otolith has often been determined subjectively, often based on associations with microstructural checks, without a clear understanding of the link between microstructure and chemistry (Barnett-Johnson et al. 2008, Hegg et al. 2013a). Most studies of the microchemistry of the otolith core have focused on markers of the otolith primordium, rather than the extent of maternally derived influence, using various chemical ratios to calcium including barium (Ba/Ca), magnesium (Mg/Ca), and manganese (Mn/Ca) (Ruttenberg 2005, Macdonald et al. 2008).

Late season spawning Chinook salmon (*Oncorhynchus tshawytscha*) in the Snake River of Idaho provide an ideal population for examining the presence and duration of the maternal otolith signature. Females in this population are entirely anadromous and individuals migrate long distances (500-1000 kms) inland to spawn over a narrowly defined period in October and November (Garcia et al. 2005). Prior work has characterized the isotopic variation across spawning areas in the basin, indicating that juvenile signatures are significantly different between spawning areas and that $^{87}Sr/^{86}Sr$ and Sr concentration in freshwater habitats are significantly different from the marine signature (Hegg et al. 2013a). These factors provide both a range of freshwater strontium concentrations to interpret otolith signatures, as well as the migration distance required to explore the degree that maternal marine signature varies from the global marine signature with inland migration.

This study used individual otolith transects from a collection of known-origin, juvenile Fall Chinook salmon from both wild and hatchery sources to quantify the duration and stability of maternal signatures in otoliths. This work also investigates the variability in these signatures that may be due to the equilibration of mothers to river-specific chemical signatures prior to spawning, as well as changes in the microchemistry of the egg after deposition in the redd. Given the inland location of the population we hypothesized that maternal $^{87}Sr/^{86}Sr$ signatures would reflect some degree of freshwater influence. We used a multi-tracer approach, including ratios of Sr/Ca, Ba/Ca, Mn/Ca, and Mg/Ca as well as $^{87}Sr/^{86}Sr$ to examine the presence and duration of the maternal signature on the otolith. Further, we explored whether changes in each tracer were simultaneous, and thus indicative of a single event, or if individual tracers may signal different ontological time-points in the early life of fish. First, using the suite of chemical signatures we

148 determine the presence and duration of a maternal marine signature in individual juvenile
149 otoliths. Given the variability we quantify in maternal signatures, we then measure the spatial
150 and temporal variability of maternal signatures across the study area.
151

152 Methods

153

154 Study System

155

156 Fall Chinook salmon in the Snake River are listed as threatened under the Endangered Species
157 Act (Good et al. 2005). The population inhabits low-elevation, mainstem habitats of the Snake
158 River and its tributaries. The majority of spawning occurs in the free flowing Snake River
159 between Asotin, Washington and Hells Canyon dam and in the Clearwater River (Garcia et al.
160 2008). The main spawning areas in the Snake River are classified as "Upper", above the
161 confluence with the Salmon River, and "Lower", in the free-flowing section below the Salmon
162 River confluence to Asotin, WA. The watersheds of the basin are geologically heterogeneous
163 with enough distinction to provide significant differences in water $^{87}Sr/^{86}Sr$ ratios between the
164 major spawning reaches that can be used to classify fish to their natal location (Hegg et al.
165 2013a, 2013b). Adult salmon migrate a minimum of 750 km to the main spawning areas in the
166 Snake and Clearwater Rivers above the town of Lewiston, ID. The watershed is heavily
167 influenced by hydropower with eight downstream dams, four on the Columbia River and 4 on
168 the Snake River, creating slow moving reservoir habitat downstream of Lewiston, ID.
169

The population is notable for a recent shift from a historically ubiquitous sub-yearling strategy, whereby individuals migrated shortly after emergence, toward a later, yearling strategy in response to anthropogenic changes to the river system (Connor et al. 2005, Williams et al. 2008). Studies have indicated that this change in juvenile life-history is likely heritable and under active selection in response to reservoirs and hydropower regulation that provide cool water opportunities during summer that did not exist historically (Williams et al. 2008, Waples et al. 2017). A separate study seeks to quantify the spatially explicit outmigration behaviors (Hegg et al. In prep). However, to accurately assess early life-history behaviors using chemical proxies in otoliths, it is essential to determine the degree of maternal influences on the early chemistry of juvenile fish.

Background Water Sample Collection

Water samples were collected from 2008 through 2016 throughout the spawning range of Snake River Fall Chinook salmon to characterize the spatial and temporal variation in strontium isotopic chemistry within the basin (Figure 1). Samples were collected during baseflow periods in late summer and fall in all years to capture the most representative signature of water and rock interactions within each river. Starting in 2009, as resources permitted, samples were taken seasonally. Sampling began in the spring as soon as flows were safe to sample and included summer, and fall seasons to characterize the stability of the signatures. Additionally, in 2010, samples were taken in the Clearwater and Salmon Rivers at three periods encompassing the peak of the hydrograph to characterize seasonal variation observed in prior studies (Hegg et al. 2015b)

193 Samples were collected in acid cleaned 125ml HDPE bottles according to established protocol
194 (Kennedy et al. 2000). Samples were analyzed for $^{87}Sr/^{86}Sr$ isotope ratios on a Finnigan MAT
195 262 Multi-Collector thermal ionization mass spectrometer (TIMS) as well as a Isotopix Phoenix
196 TIMS. Throughout the research period, replicate analysis of the National Institute of Standards
197 and Technology standard reference material (SRM-987) was used to determine the analytical
198 error. The Finnegan MAT 262 yielded mean $^{87}Sr/^{86}Sr$ of 0.710231 (2SD = 0.000032, n=16), the
199 Isotopix Phoenix yielded a mean $^{87}Sr/^{86}Sr$ of 0.710244 (2SD = 0.000009, n=89).
200

201 ## Fish and Otolith Collection

202

203 Wild juvenile fish (n=430) were captured using beach seines from spawning areas between the
204 Snake and Clearwater Rivers as part of seasonal population surveys between April and August in
205 years spanning 2007 to 2014 (Connor et al. 1998). Some of these fish (n=111) were PIT tagged,
206 released, and recaptured at fish passage facilities at Lower Granite Dam on the Snake River
207 during their downstream out-migration, providing two known locations for these fish (tagging
208 site and recapture site). Juvenile fish were also collected from the two hatcheries that produce
209 Fall Chinook in the Snake River basin. Hatchery fish were collected from Lyons Ferry Hatchery
210 in 2009 and 2011 (n=28), and from Nez Perce Tribal Hatchery in 2011 and 2012 (n=35).
211

212 Fish samples were kept frozen until otoliths could be removed through dissection. Otoliths were
213 stored dry in polypropylene microcentrifuge tubes. Otoliths were then mounted on the sagittal
214 plane on petrographic microscope slides using Crystalbond adhesive and ground by hand on fine

grit Micromesh aluminum oxide sandpaper to reveal the otolith primordium and daily otolith increments. (Secor et al. 1992, Hegg et al. 2013a).

Otolith Chemical Analysis

Otoliths were analyzed for $^{87}Sr/^{86}Sr$ using a Finnigan Neptune (ThermoScientific) multi-collector inductively coupled plasma mass spectrometer coupled with a New Wave UP-213 laser ablation sampling system (LA-MC-ICP-MS). Analysis for trace element composition was conducted using an Element 2 (ThermoScientific) ICP-MS attached to the same laser ablation system. For each analysis method otoliths were analyzed using a transect moving from the edge of the otolith to the core. This transect was positioned approximately 90° from the sulcus on the dorsal side of the otolith to capture the area containing the clearest succession of rings from the edge to the core. The laser was set to ablate at a constant speed. For $^{87}Sr/^{86}Sr$ ratios the LA-MC-ICP-MS system was set to 10μm/second scan speed, 40μm laser spot size, and 0.262 second integration time. For trace element analysis, the laser was set to scan at 10μ/second, 30μm laser spot size, and a 1 second sampling time in the ICP-MS method. Samples were run using a dry flow of helium (He) through the sample chamber was approximately 0.8L/minute, which combined with .6L/min argon (Ar) before entering the plasma. Oxide formation rates were <1% Th/ThO_2. The trace element analysis included the elements calcium (^{43}Ca), strontium (^{86}Sr), barium (^{138}Ba), Magnesium (^{25}Mg), and Manganese (^{55}Mn).

Strontium ratio data was corrected based on the global marine signature for each analysis day using a marine shell standard (mean $^{87}Sr/^{86}Sr = 0.709186$, SD = 0.000077, n=535). Error across

238 individual otoliths varies based on fish location and is best assessed on stable regions. The
239 maternal period between the otolith core and 150μm is relatively stable across all otoliths. In our
240 study the standard deviation of the $^{87}Sr/^{86}Sr$ signature in the maternal region averaged across all
241 otoliths was 0.00095 (SD = 0.00063, n = 279).
242
243 Elemental counts were corrected to the SRM 610 standard (Jochum et al. 2011). Correction was
244 done using a ten second, within-run blank during which gas was flowing over the sample but the
245 laser was not ablating. Blank counts were then subtracted from the measured concentration of
246 SRM 610 for each element. All measurements were normalized to known CaO in SRM-10 and
247 aragonite, to account for variations in laser ablation efficiency. Three standards were run for
248 every 10-20 samples, and an average of the correction factor for these standards was used to
249 correct those samples. Limits of detection (LOD) for each element were calculated as 3 X SD
250 from the mean of all sample blanks during otolith analysis runs in which our samples were a part
251 (n = 859). Detector problems affected approximately 15% of the samples, causing high blank
252 values and increasing LOD for those samples. The LOD for all samples are included in
253 parentheses. Expressed as a ratio of elements to calcium, detection limits were; Sr/Ca 0.0059
254 (0.029) $mm•mol^{-1}$, Ba/Ca 0.00036 (0.023) $mm•mol^{-1}$, Mn/Ca 0.011 (0.031) $mm•mol^{-1}$, and
255 Mg/Ca 0.003 (0.022) $mm•mol^{-1}$. A suitable solid standard with a similar matrix was not available
256 to report accuracy and precision of elemental analyses.
257
258 The edges of the otolith were identified within the data using a CUSUM algorithm on Sr and Ca
259 counts, then confirmed visually. Extraneous data were trimmed beyond the edge of the otolith
260 before reversing the sequence to form a data transect from the core to the edge. The distance of

261 each data point from the core, in microns, was calculated using the scan speed and
262 integration/sampling time of the laser and ICP-MS software.
263
264 ## Statistical Analysis
265
266 Water chemistry and otolith data were aggregated into chemically distinguishable reaches as
267 detailed by Hegg et al. (2013a) with the inclusion of two groups for hatchery signatures. One
268 chemically distinguishable river group is made up of the Clearwater and Salmon Rivers (CWS).
269 A second group is the Lower Snake River (LSK), which extends downstream from the
270 confluence of the Snake and Salmon Rivers downstream to the town of Asotin, WA. A third
271 group is the Upper Snake River (USK) consisting of the Snake River upstream of the confluence
272 with the Salmon River to Hells Canyon Dam. Finally, the Grande Ronde, Imnaha, and Tucannon
273 Rivers, comprise the fourth river group. Each of these rivers, flowing from the south, runs over
274 the Columbia River basalts, a geological formation of flood basalts (referred to collectively as
275 the CRB river group). Finally, Lyons Ferry Hatchery (LFH) and Nez Perce Tribal Hatchery
276 (NPTH) make up two separate groupings. Hatchery fish were analyzed separately despite the
277 inability to distinguish these groups using $^{87}Sr/^{86}Sr$ in the past (Hegg et al. 2013a). This was done
278 with the hope that multi-tracer data would help to distinguish these groups, and because we
279 expected early juvenile or maternal microchemistry might differ from wild fish.
280
281 ### *Duration of Maternal Signature*
282 Otolith transects were analyzed graphically to determine the location of a change between
283 maternally derived and post-hatch chemistry. Large, rapid, changes in element/calcium ratio in

284 the early otolith allowed for statistical confirmation of the location of chemical change. To
285 confirm the identified location on the otolith did, in fact, represent a significant change in
286 elemental signature, the mean signature from 100μm of otolith growth before the identified
287 change was compared to the mean signature 100μm after, for all fish within each river group.
288 This comparison was done using a two-sided, paired t-test assuming unequal variance ($\alpha = 0.05$)
289 with Bonferroni correction for multiple comparisons. The chemical tracers that showed the most
290 significant change across river groups were then used to develop a multivariate change-point
291 algorithm to determine the otolith location of the maternal/natal change for individual fish.
292
293 The maternal/natal transition in $^{87}Sr/^{86}Sr$ was less distinct and involved a gradual slope during
294 the maternal period (See Variation in Maternal Signature section below), transitioning to a
295 second slope during an extended equilibration period. We used a segmented regression approach
296 to test for the presence of a transition in slope near 150μm, the point that we determined a
297 transition graphically. We used the {segmented} package for R which uses a likelihood
298 maximization approach to determine the optimal breakpoints in a linear model (Muggeo 2003,
299 2008). We applied this algorithm to the segment of each otolith $^{87}Sr/^{86}Sr$ transect between 50μm
300 and 250μm from the otolith core, encompass a range around the expected transition. The model
301 fit was limited to a single breakpoint and each sample was limited to a maximum of 200
302 iterations to converge on a breakpoint. If the model failed to converge it was assumed that the
303 data did not contain a breakpoint and was instead best described by a simple linear model.
304 Breakpoint analysis was only conducted for those river groups whose river signature was
305 different from the expected maternal signature (somewhere between the global marine average
306 and the Lower Snake River), since these were the only signatures likely to exhibit a sharp

enough shift to test. Therefore, fish from Lyons Ferry Hatchery and the Lower Snake River were not tested. Breakpoint analysis was run on data smoothed with a centered, 20-point moving average. To ensure that smoothing did not affect results, breakpoint analysis was run across a range of smoothness from the raw data to 20-point smoothing at increments of 5-points.

We also tested two change-point methods for their ability to identify the location of the maternal/natal chemical transition for individual otolith elemental ratios. Both were applied to the data between 150μm and 350μm from the core of each otolith. The first 150μm was excluded so as to avoid the known peak in Mn/Ca near the core (Brophy et al. 2004, Ruttenberg 2005), while covering the location on the otolith where the presumed maternal/natal change occurs. The first change-point method tested was the multivariate {ecp} package for R, that used the three most significant elemental tracers from the paired t-test above (James and Matteson 2014). Additionally, we applied a univariate change-point algorithm from the {changepoint} package for R (Killick and Eckley 2013). This univariate approach used only the most significant chemical tracer from the prior paired t-test. The univariate change-point algorithm was applied using the "AMOC" (At Most One Change-point) procedure and an asymptotic penalty, for changes in both mean and variance.

Variation in Maternal Signature

To determine the degree that maternal signatures vary we fit a linear model to the maternal $^{87}Sr/^{86}Sr$ signatures of all known juvenile fish and the known average $^{87}Sr/^{86}Sr$ of the river in which they were captured. Under the null hypothesis that all fish maintain an ocean signature we would expect the slope of this regression to be zero, with an intercept of 0.70918, the global

ocean signature. A significant slope other than zero, with an intercept other than 0.70918 would indicate that maternal signatures vary with maternal equilibration to the spawning stream.

Stability of Maternal Signature

During the course of analysis, we noted that the mean $^{87}Sr/^{86}Sr$ in the early otolith (<150μm from the otolith core) of the CWS and NPTH groups appeared to differ. This was striking because contributing mothers of both groups inhabit similar water chemistries from the Clearwater River prior to spawning. Further, we noted that otolith chemistry during this early period appeared to change during development in both groups, with opposite slopes. Chemistry this early in the otolith is likely to reflect chemistry in the egg (Boyd et al. 2010), a period whose rate of chemical change has not been well studied and that some authors claim to be a closed system chemically and isotopically (Volk et al. 2000, Elsdon et al. 2008). To test for differences between these groups we tested the mean $^{87}Sr/^{86}Sr$ ratio between the CWS and NPTH groups using a two-sample t-test. To test for isotopic change within the egg during this isotopically "closed" period we also fit a linear model to the aggregate maternal data in each group to determine the presence and magnitude of change in the maternal signal.

To support our findings, we calculated expected changes in the $^{87}Sr/^{86}Sr$ ratios of the egg under seven scenarios of maternal equilibration and spawning water chemistry (Table 1). In these scenarios, we tested four different maternal equilibration chemistries; mothers equilibrated to the ocean, the Lower Snake River, the Clearwater River (as measured during Oct. & Nov.), and the observed signature at the otolith core (0μm) for known NPTH juveniles taken from the linear model above. For each of these maternal equilibration scenarios the change in $^{87}Sr/^{86}Sr$ was

353 calculated assuming eggs were laid into Clearwater River water, or into water similar to the well
354 water used at NPTH. As a proxy for NPTH well water we used the signature for the Potlatch
355 River, a nearby river that is representative of the low $^{87}Sr/^{86}Sr$ basalt signature of the area.
356
357 The signature observed in the core of NPTH otoliths represents an "intermediate" signature
358 between the Lower Snake and the Clearwater Rivers, an indication that mothers may not be fully
359 equilibrated to the Clearwater River signature at spawning. This "intermediate" signature
360 provided a direct test of whether the changes we observed in the otoliths were supported by our
361 calculations.
362
363 Calculations were based on two-component isotope mixing models including both concentration
364 and isotope ratio differences (Faure and Mensing 2004 p. 350, equation 16.11). We calculated
365 the expected change in $^{87}Sr/^{86}Sr$ during the first 80 hours after fertilization, when the egg takes
366 on the majority of its external water (Loeffler and Løvtrup 1970). We then calculated the further
367 change in $^{87}Sr/^{86}Sr$ due to the small amount of water exchange during the period from
368 fertilization to hatch (0.33% of egg volume per day) as estimated by Loeffler and Løvtrup
369 (1970). Calculations were based on values available from the literature. We used egg Sr
370 concentration data for wild and hatchery steelhead from Kalish (1990), average egg volume in
371 Atlantic salmon from Rombaugh and Garside (1982), and changes in Atlantic salmon egg
372 volume over time from Leoffler and Løvtrup (1970). We assumed the number of days to hatch to
373 be 73, the average for the Clearwater River in 2013 (Bill Arnsberg, Nez Perce Tribal Fisheries,
374 pers. comm.).
375

Results

Duration of Maternal Signature

Graphical analysis of otoliths by known natal location indicated that changes occurred at consistent locations on the otolith for elemental ratios and $^{87}Sr/^{86}Sr$, regardless of river group. However, elemental ratios and $^{87}Sr/^{86}Sr$ did not change simultaneously. Instead $^{87}Sr/^{86}Sr$ exhibited changes at different locations on the otolith than elemental ratios.

Strontium ratio appeared to exhibit an inflection point ~150µm from the otoliths core. This was particularly noticeable in the signatures from the Clearwater, Grand Ronde, and NPTH river groups. Breakpoints within each river group were approximately normally distributed, with no group violating the assumption of normality ($p > 0.4$ for all groups, $\alpha = 0.05$) using the Shapiro-Wilkes normality test (Razali and Wah 2011). The mean breakpoint within the Clearwater River was located at 151µm (SD = 22µm). Mean breakpoints for the Grande Ronde were 150µm (SD = 20µm). For NPTH the mean breakpoint was 148µm (SD=24µm). The median breakpoint for the Upper Snake river group was 145µm (SD=24.9). Both the NPTH and USK groups exhibited a second, smaller, group of breakpoints near 200µm as data smoothness decreased. The mean breakpoint for all groups remained within 15µm across all smoothing profiles, though the results became increasingly abnormally distributed as data smoothness decreased. The median breakpoint remained relatively stable across groups regardless of smoothing, with the exception

of NPTH and the Upper Snake river groups whose median increased to 180μm and 185μm respectively on the raw data.

Elemental ratios, particularly Mn/Ca and Ba/Ca, showed a marked change beginning at 225μm from the otolith core (Figure 2). These changes were less pronounced in fish from the hatchery. Comparison of the 100μm segments on either side of 225μm using two-sided, paired t-tests assuming unequal variance ($\alpha = 0.05$) with Bonferroni correction showed that Mn/Ca was highly significant for all groups, while Ba/Ca was significant for all river groups except hatchery fish from LFH (Figure 3). Sr/Ca ratios were significantly different only for the USK and LSK groups and LFH. Mg/Ca ratios showed no significant differences. Mn/Ca ratios were at or below LOD in many samples during this period, however, the consistent pattern of increasing Mn/Ca after 225μm indicates this increase is likely biological despite low concentrations of Mn.

Multivariate change-point analysis on Mn/Ca and Ba/Ca ratios using the {ecp} package did not provide consistent determination of the location of chemical change in individual otoliths. Similarly, univariate change-point analysis on Mn/Ca using the {changepoint} package resulted in inconsistent determination of the maternal/natal chemical shift at the individual level. Using a value of 225μm appeared to describe the location of the maternal/natal transition as well or better than change-point analysis. Individual variation in the magnitude of the chemical change, as well as data noise, was likely to blame for the difficulty in determining logical change-points at the individual level.

Plots of $^{87}Sr/^{86}Sr$ for each non-hatchery river group showed changes in the signature at approximately 150µm from the otolith core, as much as 100µm earlier than the location of chemical changes in the elemental ratios (Figure 4). Following the initial change in $^{87}Sr/^{86}Sr$ ratio after ~150µm the signature then began moving toward a second stable period near ~250-300µm. The signature for LFH was consistent throughout the life of the fish, with no distinct changes. Fish from NPTH began at a signature near 0.7096, before a sudden transition to a signature near 0.70918 around 150µm, before beginning near 300µm to move toward a signature near 0.7110 toward the end of their life.

Variation in Maternal Signatures

The regression of maternal signatures to the signatures of the rivers in which juveniles were captured resulted in a significant linear model ($p<0.00001$, $\alpha=0.05$) with the form,

$$Maternal\ ^{87}Sr/^{86}Sr\ =\ 0.2673 * Maternal\ ^{87}Sr/^{86}Sr + 0.5197$$

Both the slope and intercept terms were highly significant ($p<0.00001$, $\alpha=0.05$), providing support for the alternate hypothesis that maternal signatures are different from the ocean signature, and significantly influenced by the signature of the natal river. This effect is apparent in the histogram of maternal signatures expressed in our study, with Clearwater and Grande Ronde juveniles exhibiting maternal signatures that trend in the direction of their natal river from the global marine average (Figure 5)

Stability of Maternal Signatures

Despite mothers experiencing similar $^{87}Sr/^{86}Sr$ regimes, the maternal signatures of CWS and NPTH juveniles were significantly different prior to 150μm in a two-sample t-test ($p<0.00001$). CWS juveniles had a mean maternal $^{87}Sr/^{86}Sr$ signature of 0.7104, while NPTH juveniles had a mean maternal $^{87}Sr/^{86}Sr$ signature of 0.7096 (Figure 6A).

Maternal signatures in both groups also showed significant, but opposing, slopes and intercept values when a linear model was applied to the maternal data in each group, indicating changes in chemistry within the egg (Figure 6B). The linear model fit to Clearwater River juveniles returned an intercept of $^{87}Sr/^{86}Sr = 0.7101$ ($p=<0.00001$, $\alpha=0.05$) and a slope of .00000314 microns ($p=0.0016$, $\alpha=0.05$). Variability in the data was high ($R^2 = .0115$). Juveniles from NPTH were fit to a linear model with an intercept of $^{87}Sr/^{86}Sr = 0.7098$ ($p=<0.00001$, $\alpha=0.05$) and a slope of -0.00000236 microns ($p=0.0025$, $\alpha=0.05$). Variability in the NPTH juvenile data was also high ($R^2 = 0.0102$).

Our calculation of the expected change in egg maternal signatures indicated that $^{87}Sr/^{86}Sr$ can exhibit changes nearing 0.0001 between the time eggs are laid and when they hatch in some cases (Table 1). The degree of change was driven largely by concentration differences, with mothers equilibrated to the high concentration of the ocean showing little change. The largest change in $^{87}Sr/^{86}Sr$ signature (0.00094) was seen for mothers equilibrated to the "intermediate" signature observed in NPTH fish, with eggs laid into NPTH well-water. This change, interestingly, is very similar to the significant difference (0.0009) between the mean $^{87}Sr/^{86}Sr$

maternal signatures of the Clearwater and NPTH groups above (Figure 6A). In this case, the egg changed its $^{87}Sr/^{86}Sr$ ratio by 0.00005 in the direction of the ambient water between initial swelling and hatch, supporting our observation of a slope in maternal signatures in Clearwater and NPTH juveniles (Figure 6B). In all cases approximately half of the $^{87}Sr/^{86}Sr$ ratio change occurred in the first 80 hours after hatch, with the subsequent change occurring slowly during the period after hatch, further supporting the observation of a slope in $^{87}Sr/^{86}Sr$ signature prior to 150μm in the otolith.

Discussion

Connecting maternal migratory behavior with the behavior and ecology of their progeny can reveal important details in the ecology of a population. In the case of fish, the maternally derived chemistry stored in the core of otoliths provides important clues about the behavior of mothers. This maternally derived chemistry has been particularly effective as a signature to identify ocean residence for partially migratory salmonid populations (Kalish 1990, Volk et al. 2000, Miller and Kent 2009, Shippentower et al. 2011, Liberoff et al. 2015). Recent work has extended this tool, using $^{87}Sr/^{86}Sr$ ratio to infer the degree of anadromy in multiple inland populations of steelhead (Courter et al. 2013). Additional studies have cited a period of maternal influence near the core of the otolith, however the duration and chemical makeup of this maternal signature is unclear (Barnett-Johnson et al. 2008, Miller et al. 2011, Hegg et al. 2015b).

While the use of maternal Sr/Ca signature as a marker of anadromy has been validated (Kalish 1990, Zimmerman 2005), very little information is available regarding the duration of this

maternal signature. Several elemental tracers have been proposed as markers of the otolith core, including Ba/Ca and Mn/Ca, but the origin of these elevated elemental ratios may have more to do with juvenile ontogeny and formation of the core itself than maternal behavior (Brophy et al. 2004, Ruttenberg 2005). Whether these elemental systems change in concert with a single developmental stage, or whether there are asynchronous patterns of chemical changes during development has not been tested.

The hatching of juvenile fish, or alternatively the moment of first exogenous feeding, is usually cited as the ontological event that precipitates a change between maternal and natal chemical signatures. The assumption made in most studies is articulated by Volk et al. (2000); that the egg makes up a closed system reflecting the chemistry of the mother during the time at which the eggs are sufficiently developed to close to outside chemical influence. Under this assumption, the juvenile otolith only begins to equilibrate to the external chemistry of the river after hatching when it begins to interact directly with the external environment. Some authors have extended the assumption to conclude that the equilibration of $^{87}Sr/^{86}Sr$ must be correlated to the first exogenous feeding (Barnett-Johnson et al. 2008).

Duration of the Maternal Signature

Our data indicate that Mn/Ca and Ba/Ca, and to a lesser degree Sr/Ca, appear to mark a sharp transition in the otolith chemistry of juvenile fish at 225μm from the otolith core (Figure 2). The simultaneous changes in these elements argue for an underlying ontological change in the juvenile, however it is unclear whether this indicates hatch, the onset of exogenous feeding, or

another physiological or external driver. There is considerable individual variation in the magnitude and location of this change, however, making individual determination difficult and indicating that individual conditions may play a large role in this chemical change.

The results of our $^{87}Sr/^{86}Sr$ analysis show that in contrast to elemental signatures, $^{87}Sr/^{86}Sr$ ratios appear to change near 150μm from the otolith core, with a more gradual change in signature than that seen for elemental ratios (Figure 4). At this point the signature moves steadily toward the signature of the natal river, reaching a stable plateau between ~250μm and ~300μm depending on the population. This change in signature is especially visible in juveniles from the CWS and CRB groups, natal locations whose $^{87}Sr/^{86}Sr$ values are farthest from the global marine signature and therefore might be expected to exhibit the fastest change toward equilibrium.

The difference in the timing of change between elemental and $^{87}Sr/^{86}Sr$ isotopic ratios was interesting. Strontium ratios in our study began to change at 150μm, ~75μm earlier than do elemental signatures (225μm). This distance corresponds to roughly 12 - 19 days, based on the range of known Chinook growth rates in the basin (Zabel et al. 2010). Further, experimental results indicate that otolith radius at emergence varies from 173μm to 259μm (Paul Chittaro, unpublished data). It is reasonable, based on this difference in timing, to assume that the shift in elemental ratios and $^{87}Sr/^{86}Sr$ are synchronized with different ontogenetic changes in the juvenile fish.

Since hatching represents the first time the egg is capable of a large degree of ion exchange with the surrounding water, the change in $^{87}Sr/^{86}Sr$ ratio at ~150μm likely represents hatching.

Strontium and calcium uptake in juvenile fish begins to climb steadily after hatching, and experimental results indicate that it is possible to isotopically mark non-feeding salmonid fry using water spiked with ^{84}Sr (Hayes et al. 1946, Yamada and Mulligan 1987, De Braux et al. 2014). Further, between 30 and 83% of strontium is incorporated into the otolith from the surrounding water, enough to begin changing the $^{87}Sr/^{86}Sr$ signature once the fish begins exchanging ions directly with the surrounding water through its gills and endothelium (Hayes et al. 1946, Walther and Thorrold 2006).

Previous research indicates that the onset of exogenous feeding could be accompanied by a change in elemental ratios. Experimental evidence indicates that the rate of strontium intake increases to an even faster rate following first-feeding (Yamada and Mulligan 1987), and that magnesium concentration also increases 12-15 days after juveniles hatch (Hayes et al. 1946). The physiological changes that accompany the onset of exogenous feeding could change the regulation of these elements in relation to calcium within the fish's tissues, as well as changes related to the intake of food sources with differing concentrations of elements as compared to that of the yolk sac. The chemical change also broadly correlates with a first-feeding microstructural check at 235-240μm determined by Barnett-Johnson (2007) in Chinook salmon from California's Central Valley.

Taken together our data suggest that both elemental ratios and $^{87}Sr/^{86}Sr$ ratios provide information on maternally derived chemical influence on the otolith. However, the change from maternal to natal chemistry in $^{87}Sr/^{86}Sr$ and elemental data appear to correspond to different ontological stages. Changes in $^{87}Sr/^{86}Sr$ ratio appear to correspond to the hatching of the larval

556 fish ~150µm, with equilibration continuing until sometime at, or soon after, the onset of
557 exogenous feeding. Elemental ratios of manganese, barium, and strontium appear to reflect the
558 onset of exogenous feeding at ~225µm with a more sudden shift to some equilibrium. While the
559 equilibration of $^{87}Sr/^{86}Sr$ seems to coincide with the change in elemental data, we have no
560 evidence to indicate that this is necessarily causal.
561
562 ## Variation in Maternal Signature
563
564 Although Sr/Ca in the core of the otolith can be used to determine maternal anadromy, long
565 inland migrations may attenuate the ocean-derived maternal signature, resulting in variation in
566 the maternal signature. Rieman et al. (1994) showed that Sr/Ca was an incomplete predictor of
567 resident and anadromous maternal behavior in juveniles from a population of *O. nerka* in Idaho,
568 900km from the ocean. Bacon et al. (2004) found that inland populations in the Pacific
569 Northwest had attenuated or nonexistent Sr/Ca and $^{87}Sr/^{86}Sr$ maternal signatures and Donohoe et
570 al. (2008) showed that a metric of migratory difficulty could explain attenuation of the maternal
571 signature.
572
573 This attenuation of maternal signature indicates that mothers who spend significant time in
574 freshwater equilibrate to some degree to the freshwater chemistry along their migratory path and
575 in the natal stream. This equilibration should also be reflected in maternally derived $^{87}Sr/^{86}Sr$
576 ratios. Our results show that maternal $^{87}Sr/^{86}Sr$ signatures of juvenile Snake River fall Chinook
577 salmon do vary significantly from the global marine value (Figure 5). As might be expected, the
578 maternal signatures vary in the direction of the water chemistry of the natal stream, indicating a

large degree of maternal equilibration, not just to the mainstem river in which they reside for most of their upstream migration but to the spawning tributary itself. This is especially evident in fish captured from the Clearwater and Grande Ronde Rivers, spawning reaches whose $^{87}Sr/^{86}Sr$ signatures deviate considerably from the global marine signature, making these changes more apparent. This variation in maternal $^{87}Sr/^{86}Sr$ signatures indicates that for inland populations, maternal $^{87}Sr/^{86}Sr$ ratio does not correlate perfectly to marine residence of the mother.

This result, it seems, would argue that care should be taken in using $^{87}Sr/^{86}Sr$ as a marker of maternal anadromy. However, Courter et al. (2013) used $^{87}Sr/^{86}Sr$ to infer the production of anadromous juveniles in resident rainbow trout in the Yakima River, another inland system in the Columbia River basin with similar inland migration distances. Their success may indicate some degree of species or life-history specific retention of ocean signatures in spawning female salmon. However, without an understanding of these hypothetical species or life-history specific mechanisms, our results would suggest caution in interpreting maternal $^{87}Sr/^{86}Sr$ signatures from the otoliths of populations with significant migration distances. This is particularly the case given that our results indicate that the $^{87}Sr/^{86}Sr$ signature of the egg can vary from that of the mother, and that the maternal signature may not be stable in all cases.

Stability of Maternal $^{87}Sr/^{86}Sr$ Signature

The apparent differences, and significant slope of change, in the maternal $^{87}Sr/^{86}Sr$ ratio of fish from the Clearwater River and NPTH groups challenge the assumption made in many studies that the egg is a closed system, reflecting only the chemical signature of the mother. Spawning

602 females used as broodstock at NPTH are captured at Lower Granite dam and make up a random
603 subsample of the run (Milks and Oakerman 2016). They are then transported to the NPTH
604 complex where they are housed in Clearwater River water until they are spawned (Bill Arnsberg,
605 Nez Perce Tribal Fisheries, pers. comm). Adults who spawn naturally in the Clearwater River
606 move upstream past Lower Granite dam, through the remainder of the Snake River and into the
607 Clearwater River, spawning at a similar time. Thus, adults taken for broodstock at NPTH
608 ultimately spend as much, or perhaps more, time exposed to Clearwater River water as adults
609 who spawn naturally in the river. Despite the similar duration of time mothers are exposed to
610 high $^{87}Sr/^{86}Sr$ Clearwater River water, the mean maternal signature of NPTH juveniles is
611 significantly lower than that of juveniles originating in the Clearwater River (Figure 6A).
612
613 Because adult spawners experience similar water chemistries before spawning in both groups,
614 the discrepancy in their progeny's maternal signature is best explained by changes in the isotopic
615 signature of the egg after spawning. The eggs of the two groups of fish do experience different
616 water chemistries between spawning and hatch, providing a mechanism for the observed
617 difference in maternal $^{87}Sr/^{86}Sr$ if the egg takes up Sr from the surrounding water.
618
619 While NPTH adults are kept in Clearwater River water before spawning, spawned eggs are
620 reared in a different water source. This water is a mix of water from the Clearwater River itself
621 and a well drawing from an aquifer below the river that changes through the year. As the river
622 level rises in the spring, the proportion of Clearwater River water increases (Bill Arnsberg, Nez
623 Perce Tribal Fisheries, pers. comm.). The Potlatch River and Lapwai Creek, both nearby low-
624 elevation streams influenced by the same basalt sources as the aquifer from which well water is

625 taken, exhibit signatures of 0.7089 and 0.7068 respectively (Hegg, unpublished data). The
626 mixing of low $^{87}Sr/^{86}Sr$ ratio water influenced by the Columbia River basalts, and the higher
627 $^{87}Sr/^{86}Sr$ water from the Clearwater River that is influenced by older metamorphic rocks
628 upstream, creates the characteristic movement from low $^{87}Sr/^{86}Sr$ ratio early in NPTH otoliths to
629 higher $^{87}Sr/^{86}Sr$ signatures more reflective of the Clearwater River as fish age (Figure 3).
630 Therefore, it is possible that the differing signatures of the water in which eggs at NPTH and in
631 the Clearwater River incubate is responsible for the difference we observed in their maternal
632 signatures.
633
634 The idea that the maternal signature of eggs might change seems to be in conflict with the idea
635 that the egg is a closed system (Volk et al. 2000). It is also in contrast to several studies showing
636 that the Sr/Ca chemistry of eggs does not change (Waite et al. 2008, Gabrielsson et al. 2012), and
637 that Sr/Ca signatures are inherited from mothers directly (Kalish 1990, Rieman et al. 1994).
638 However, it should be kept in mind that these studies have been conducted in fish with relatively
639 short spawning runs, whose strontium and calcium concentrations are relatively high compared
640 to the freshwater signatures into which their eggs are laid. Isotope ratio mixing is highly
641 dependent on the concentration of the sources being mixed (Faure and Mensing 2004).
642 Therefore, it would require a relatively large amount of fresh water introduction into the egg to
643 change either Sr/Ca or $^{87}Sr/^{86}Sr$ if the ocean-acquired maternal contribution is significantly
644 elevated.
645
646 Salmon eggs do take in as much as 12-15% of their volume in water during the first hours after
647 being laid, and external calcium is required during the process of water hardening, indicating that

strontium would also be absorbed in proportion to its concentration in the water (Potts and Rudy 1969, Finn 2007). Further, the egg does not actively osmoregulate and continues to take on water at an approximate rate of 1/300th of its mass per day (Loeffler and Løvtrup 1970). Recent research has shown that eggs can be successfully tagged using isotopes of strontium and barium during this initial uptake of water (De Braux et al. 2014, Warren-Myers et al. 2015). Thus, changes to the $^{87}Sr/^{86}Sr$ signature is possible in the hours after eggs are laid, and before the otolith is formed, creating a difference between the maternal signature of the juvenile otolith and the true maternal signature of its mother at the time the egg was laid. Further, under certain combinations of water and egg chemistry, the slow water exchange during development could create changes in the $^{87}Sr/^{86}Sr$ signature during the period between water-hardening and hatch, much as we observed in NPTH and Clearwater River juveniles.

Our calculations make clear that spawning females must equilibrate substantially to the concentration of freshwater before the $^{87}Sr/^{86}Sr$ signature of the egg could be changed by influx of freshwater (Table 1). The high concentration of Sr in ocean-equilibrated fish effectively buffers changes in $^{87}Sr/^{86}Sr$. But, for females that have substantially equilibrated to freshwater, our calculations show that the $^{87}Sr/^{86}Sr$ signature of the egg can change up to 0.00086 within the first 80 hours after being laid, and as much as 0.00091 by the time of hatch.

The largest calculated change between the mothers' signature and the signature of the egg at hatch was in the case testing NPTH equilibrated maternal signatures, with eggs laid into NPTH well water. In this case the signature changed significantly, and, it should be noted that this change is very close to the value of 0.0009 that we observed between the CWS and NPTH

groups, an indication that our calculations are accurately representing the observed shift in $^{87}Sr/^{86}Sr$ for these fish.

Further, our data provide evidence that the signature of the egg can change significantly even after the initial hours of water-hardening. This is shown by the significant slopes of $^{87}Sr/^{86}Sr$ ratio during the maternal period in CWS and NPTH juveniles. Despite large amounts of individual variation, CWS and NPTH maternal signatures show highly significant slopes moving in the direction of equilibration to the ambient water, a positive slope in CWS fish and a negative slope in NPTH juveniles (Figure 6B). This is further supported by our calculations showing that a change in $^{87}Sr/^{86}Sr$ ratio in the fourth digit, well within the analytical precision, could be expected in each case.

Conclusions

Maternally derived chemical signatures in fish otoliths, and Sr/Ca in particular, have been instrumental in connecting maternal anadromy to the life-history of their progeny. More recently researchers have begun to infer anadromy from maternally derived $^{87}Sr/^{86}Sr$ signatures as well. At the same time researchers are examining ever more detailed early movement and life-stages of juvenile fish. As more inferences are made from otoliths about the maternal and early juvenile periods it is increasingly important to know when the maternal signature ends and juvenile signature begins to avoid including erroneous chemical data that could bias results. Despite this need, there has been little understanding of when the influence of maternally derived chemistry ends on the otolith. Our study indicates that both elemental and $^{87}Sr/^{86}Sr$ ratios mark ontogenetic

694 changes within larval fish. Further, these signals can be used to determine the end of maternal
695 influence, and the beginning of signatures derived from the water of the natal location. However,
696 our results show that $^{87}Sr/^{86}Sr$ and elemental data are asynchronous, and likely signal two
697 different ontogenetic changes in the developing fish. We believe it is likely that changes in
698 $^{87}Sr/^{86}Sr$ signal hatching, while elemental signatures of Mn/Ca and Ba/Ca likely signal the onset
699 of exogenous feeding. Further, our results indicate that as female spawners equilibrate toward
700 freshwater concentrations, the $^{87}Sr/^{86}Sr$ signature of their eggs may shift after they are laid, and
701 in some cases significant changes can occur. Thus, eggs may not directly reflect the maternal
702 signature, complicating the use of $^{87}Sr/^{86}Sr$ as a method for determining maternal anadromy in
703 inland populations with significant migrations. Further work is needed to verify the duration and
704 stability of maternal signatures under varying elemental concentrations and signatures, and the
705 relationship of elemental signatures to early ontological changes in larval fish.

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961

Tables

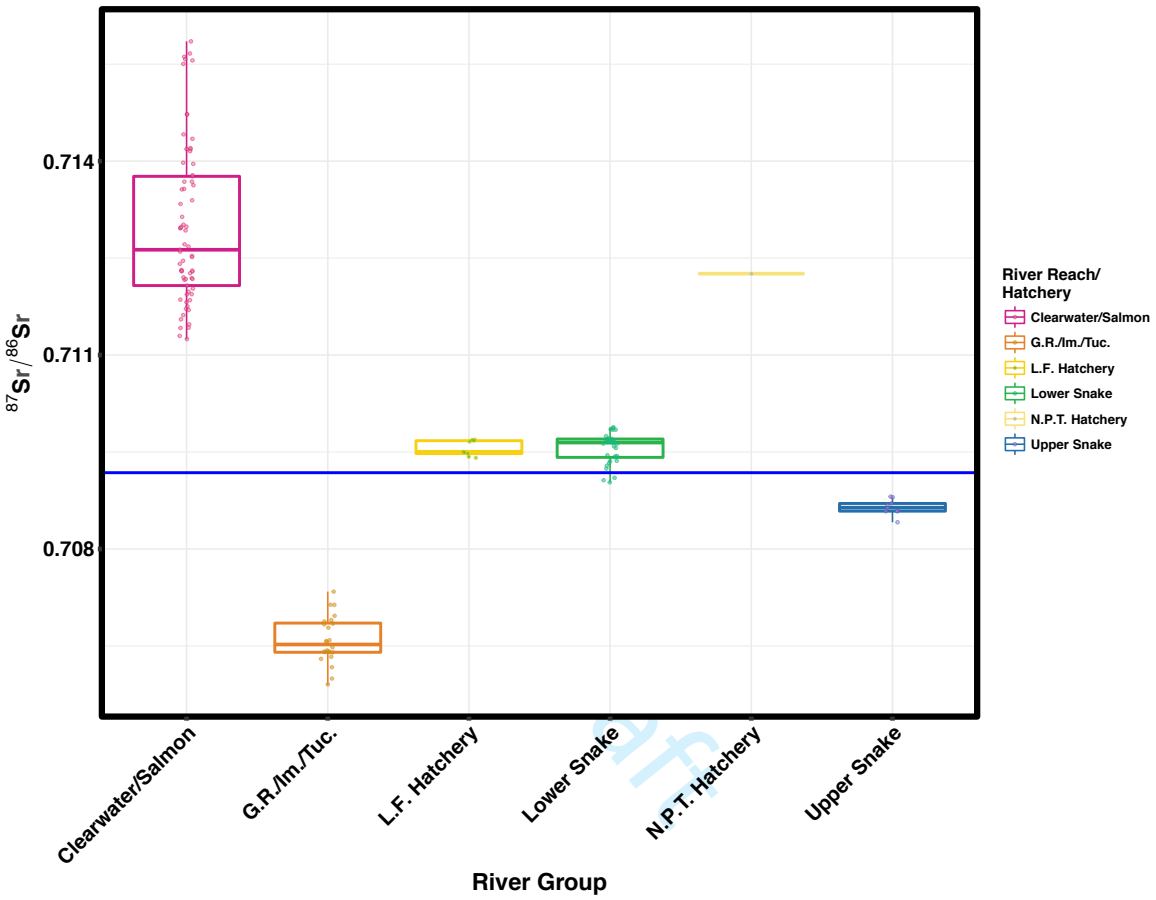
Table 1 - Calculated change in $^{87}Sr/^{86}Sr$ in eggs between laying and hatch

The change in $^{87}Sr/^{86}Sr$ signature was calculated for six different scenarios of maternal equilibration and laying location. In the table strontium concentrations increase from top to bottom, and decrease from left to right. The largest changes within the egg were calculated for scenarios with low maternal concentration and high concentration in the surrounding water (grey outline). This indicates that concentration likely controls the change in strontium ratio of the egg. Calculations were based on sampled water chemistry and values from the literature. Strontium concentration in fish tissue was taken from Kalish et al. (1990), measured in ocean and freshwater reared steelhead. Changes were calculated for the first 80 hours, when the egg takes in the majority of external water, as well as the remaining 73 days of maturation (the average estimated days to hatch for Clearwater River juveniles in 2013). The furthest right-hand column represents the signature observed at the core of known NPTH juveniles, equilibrating to a signature similar to the well-water used to rear NPTH eggs.

978

			Mother's signature (starting signature of egg)			
			High Concentration ⟶ *Low Concentration*			
			Ocean $^{87}Sr/^{86}Sr$ = 0.70918 Sr ppm = 4.73	**Lower Snake** $^{87}Sr/^{86}Sr$ = 0.70956 Sr ppm = 0.88	**Clearwater** $^{87}Sr/^{86}Sr$ = 0.71321 Sr ppm = 0.88	**Observed signature of NPTH fish at 0μm** $^{87}Sr/^{86}Sr$ = 0.70981 Sr ppm = 0.88
Signature of Surrounding Water	***Low Conc. ⟵ High Conc.***	**Potlatch (similar to NPTH well water)** $^{87}Sr/^{86}Sr$ = 0.70891 Sr ppm = 0.15	First 80h = 0.00000 To hatch = 0.00001 **Total Δ = 0.00001**	First 80h = -0.00003 To hatch =-0.00003 **Total Δ = 0.00006**	First 80h = -0.0002 To hatch = -0.0002 **Total Δ = -0.00040**	First 80h = -0.00086 To hatch = -0.00005 **Total Δ = -0.00091**
		Clearwater River $^{87}Sr/^{86}Sr$ = 0.71321 Sr ppm = 0.03	First 80h = 0.00001 To hatch = 0.00001 **Total Δ = 0.00002**	First 80h = 0.00003 To hatch = 0.00004 **Total Δ = 0.00007**	N/A	First 80h = 0.00004 To hatch = 0.00003 **Total Δ = 0.00007**

979 # Figures



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981 Figure 1 - Water $^{87}Sr/^{86}Sr$ Chemistry of the Study Area

982

983 Water samples of $^{87}Sr/^{86}Sr$ within the range of Fall Chinook salmon in the Snake River basin
984 show distinct grouping between major river groups in the basin. Lyons Ferry Hatchery is located
985 on the Lower Snake River. The Nez Perce Tribal Hatchery is located on the Clearwater River.
986 Water within this hatchery is mixed from two sources, low $^{87}Sr/^{86}Sr$ well water and water from
987 the Clearwater River, depending on water conditions. Thus, care should be taken in interpreting a
988 single sample.

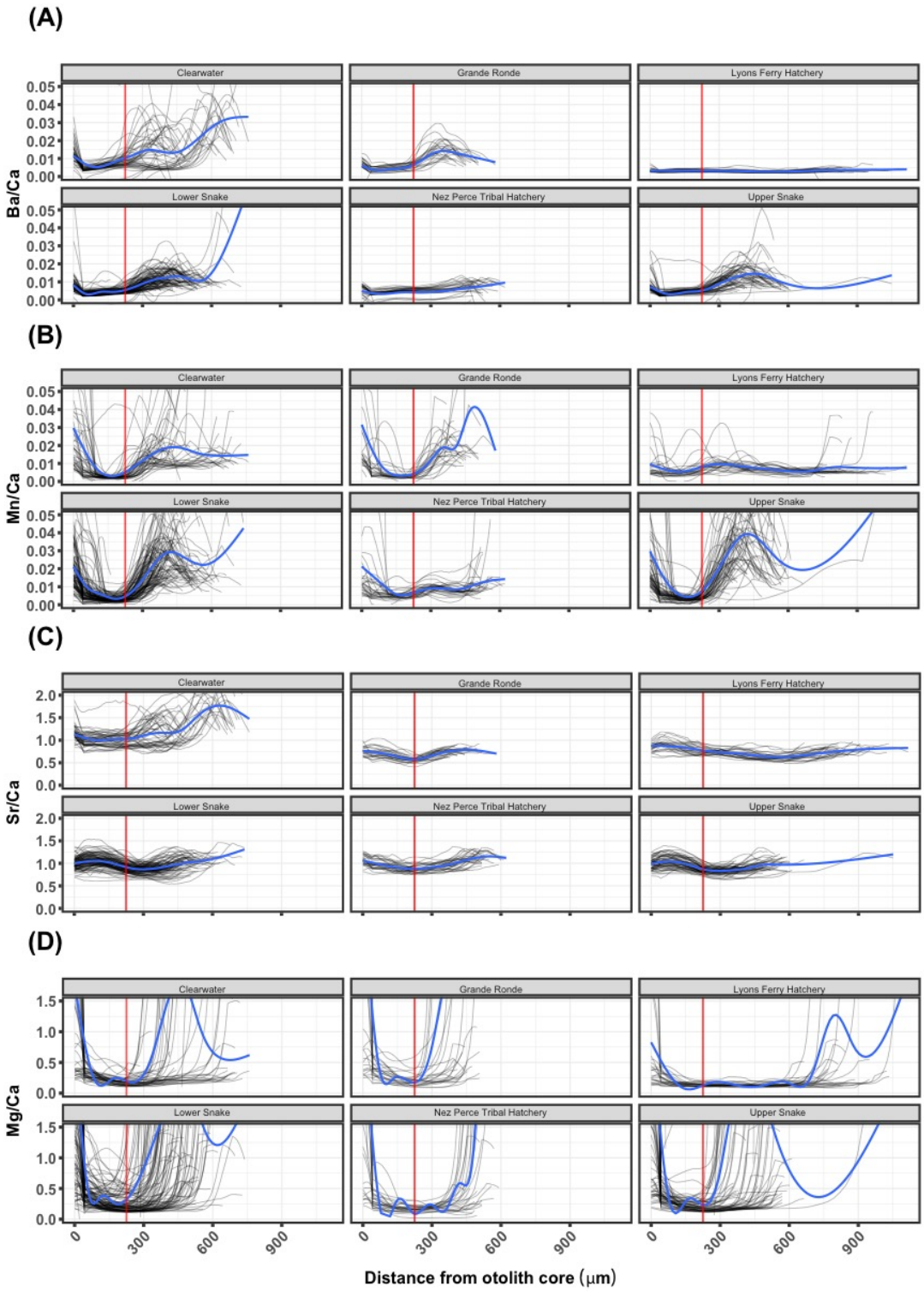


Figure 2 - Element to Calcium Ratios for Juvenile Fish from known locations

992 Plots of individual element to calcium ratios ($mm \bullet mol^{-1}$), plotted by river and hatchery grouping,
993 show a shift in chemistry beginning at ~225μm from the otolith core (red line). This shift is most
994 apparent in Ba/Ca (A) and Mn/Ca (B), and less apparent in Sr/Ca (C) and Mg/Ca (D). Plots are
995 smoothed with a 10-point moving average and exclude high values on the y-axis to maintain
996 detail of the maternal/juvenile transition. Blue lines represent the smoothed average of all
997 individual transects using a generalized additive model.
998

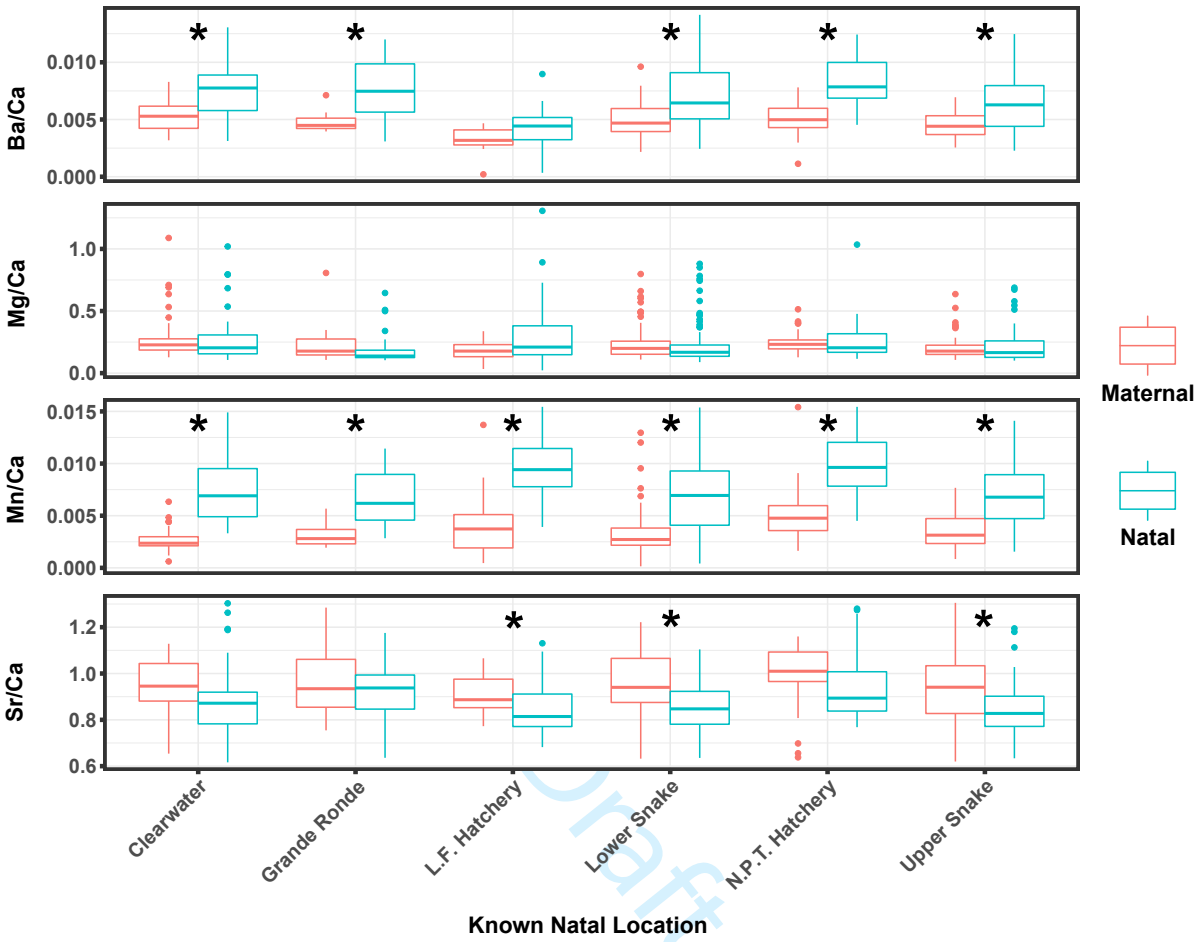


Figure 3 - Differences in elemental ratios of maternal vs. juvenile periods

Boxplots show the difference in elemental ratios 100µm before and after the 225µm otolith radius. Asterisks indicate cases in which maternal signatures (red) were significantly different than natal signatures (blue) based on paired t-tests with Bonferroni correction for multiple comparisons.

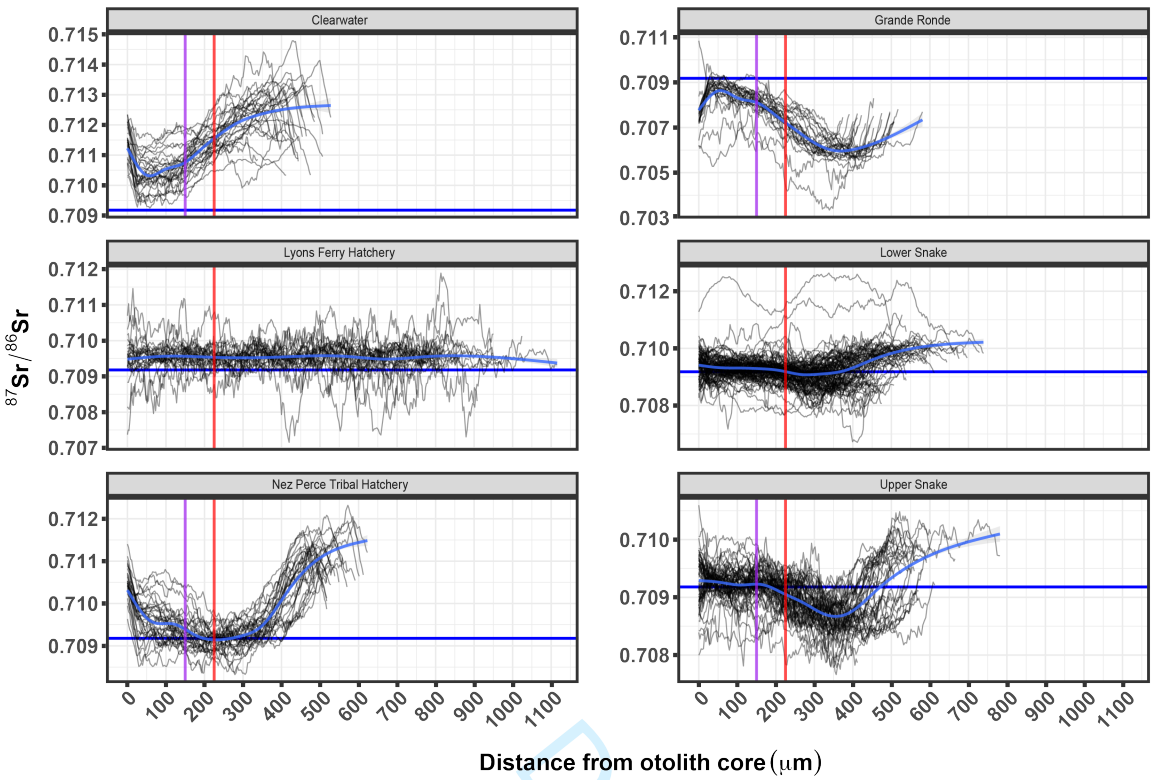


Figure 4 - Strontium isotope ratios ($^{87}Sr/^{86}Sr$) of individual fish from known locations

Plots of individual $^{87}Sr/^{86}Sr$ transects show a relatively stable period from the core of the otolith to ~150μm from the otoliths core (purple vertical line). This region was not apparent in the Lyons Ferry and Lower Snake river groups, presumably due to the similarity in signature between maternal and natal water. After this point transects slowly equilibrate toward the expected $^{87}Sr/^{86}Sr$ value for their river group. Strontium ratio does not seem to change at 225μm (red vertical line) where elemental ratios show a change, indicating that $^{87}Sr/^{86}Sr$ may be recording a different ontological change within the developing fish. Fish from NPTH equilibrate toward an unknown well-water signature before moving upwards to signature reflecting the Clearwater river as river water is mixed with the hatchery well-water late in the season. Some late-season juveniles were removed from the Clearwater River plot for clarity (n=21) because

1022 their transects followed a pattern of movement that suggested hatchery origin and acclimation in
1023 unknown water sources. The global marine signature, 0.70918, is noted for reference (horizontal
1024 blue line).
1025

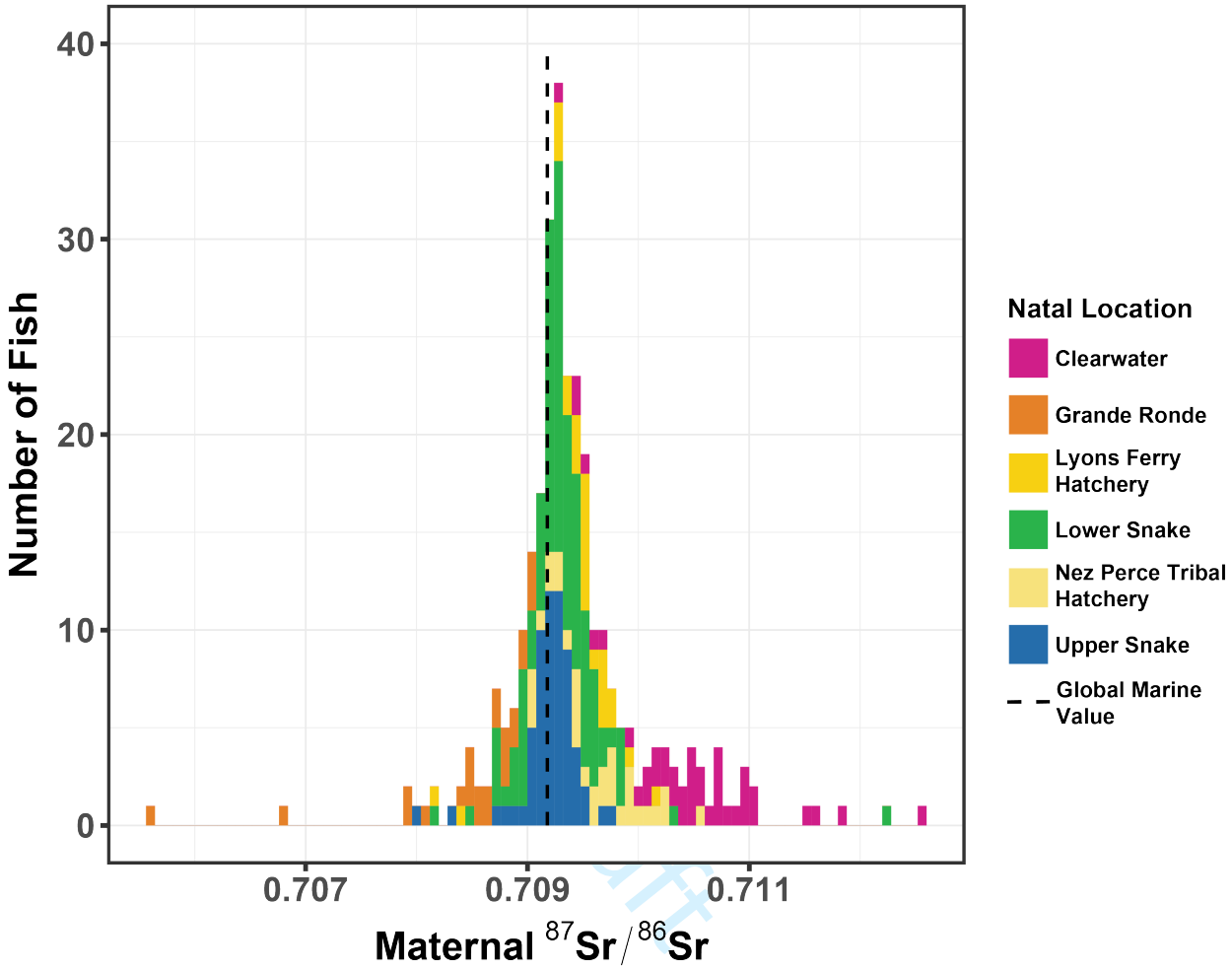
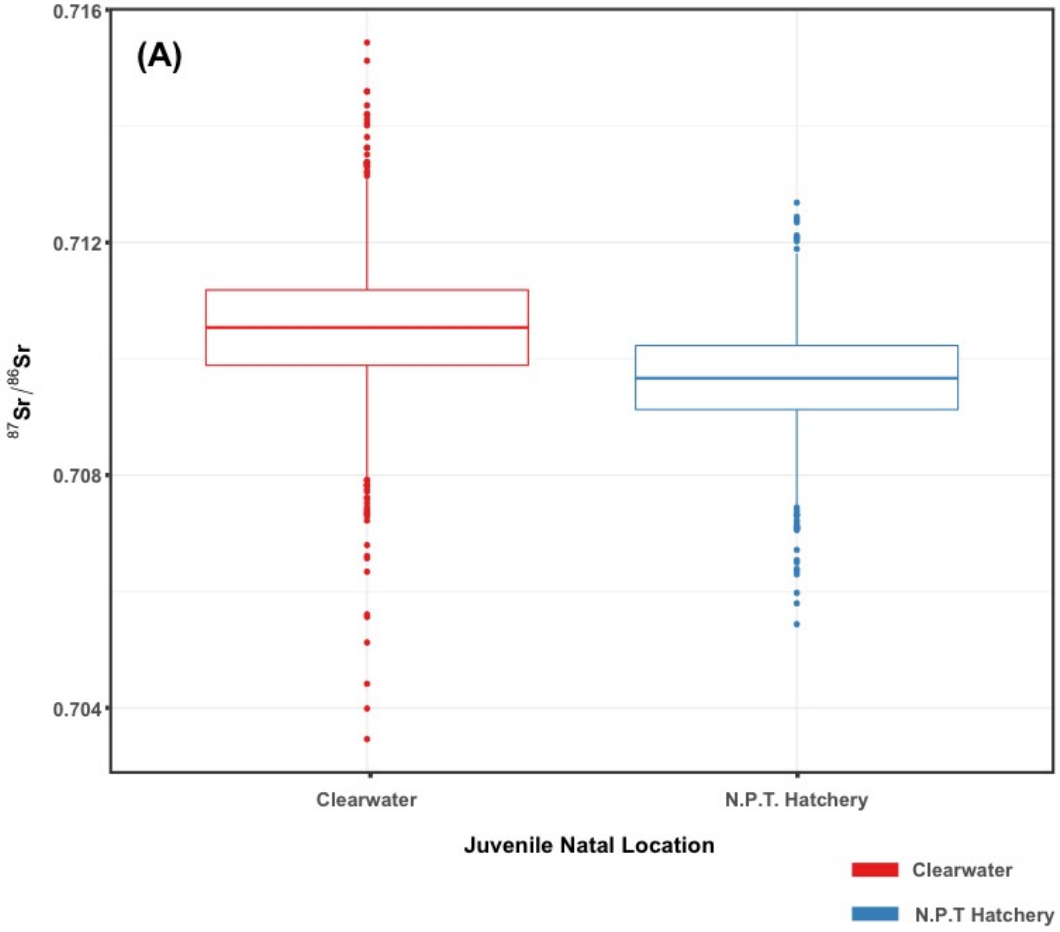
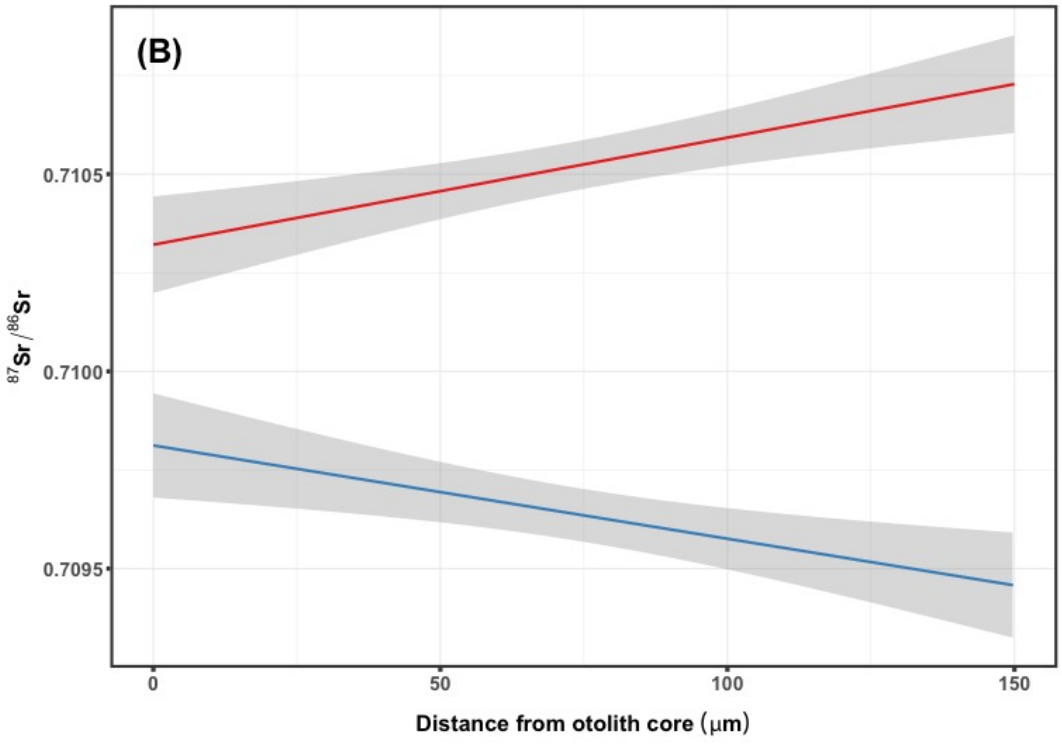


Figure 5 - Variation in Maternal $^{87}Sr/^{86}Sr$ signature

Maternal $^{87}Sr/^{86}Sr$ signatures of known origin juvenile fish vary significantly from the global marine value of 0.70918 (dotted line). Individual fish are colored by their known location, showing that maternal signatures vary in the direction of the water chemistry of the natal stream of the fish (Figure 1). Means for each group are; Clearwater (0.71050), Grande Ronde (0.70845), Lyons Ferry Hatchery (0.70946), Lower Snake (0.70931), Nez Perce Tribal Hatchery (0.70964), Upper Snake (0.70920).


(A)
0.716
0.712
0.708
0.704
$^{87}Sr/^{86}Sr$
Clearwater
N.P.T. Hatchery
Juvenile Natal Location
Clearwater
N.P.T Hatchery


(B)
0.7105
0.7100
0.7095
$^{87}Sr/^{86}Sr$
0
50
100
150
Distance from otolith core (μm)

1036

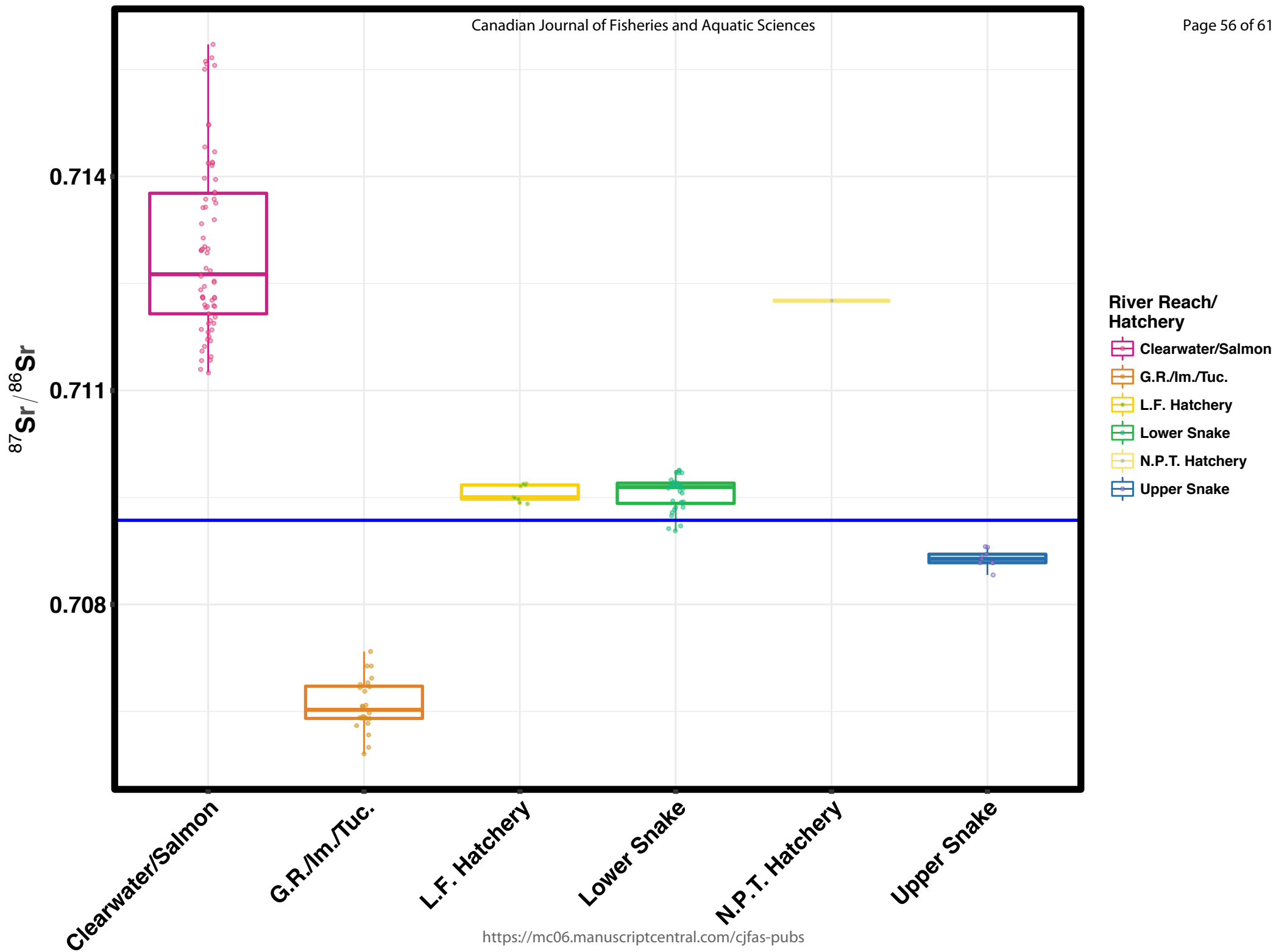
1037 Figure 6 - Mean maternal signatures of hatchery and natural origin Clearwater juveniles

1038

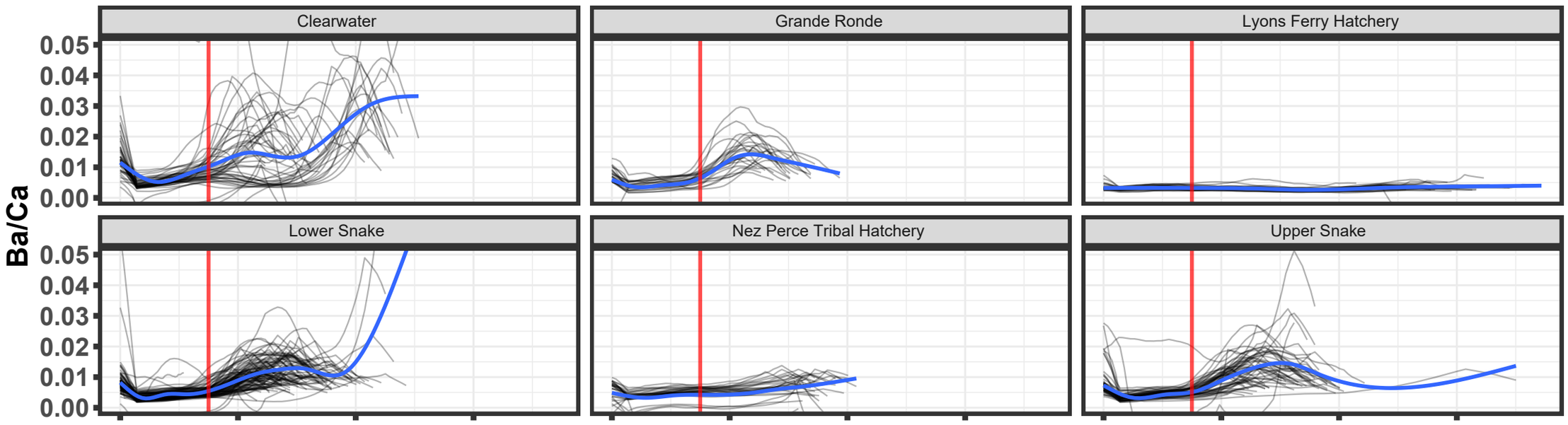
1039 Despite NPTH adults being exposed to the same, or more, time in Clearwater River water, their
1040 progeny exhibit significantly lower $^{87}Sr/^{86}Sr$ maternal signatures (A) than juveniles spawned
1041 naturally in the Clearwater River (T-test, $p<0.0001$). Eggs at NPTH are reared in well-water, not
1042 Clearwater River water. Further, the maternal signatures of juveniles from each location exhibit
1043 significant slopes in the direction of the signature of their natal water sources, indicating
1044 equilibration of the egg signature before hatch (B). Dark blue lines represent the slope of the
1045 aggregate data, while grey shading represents confidence intervals for each regression.

1046

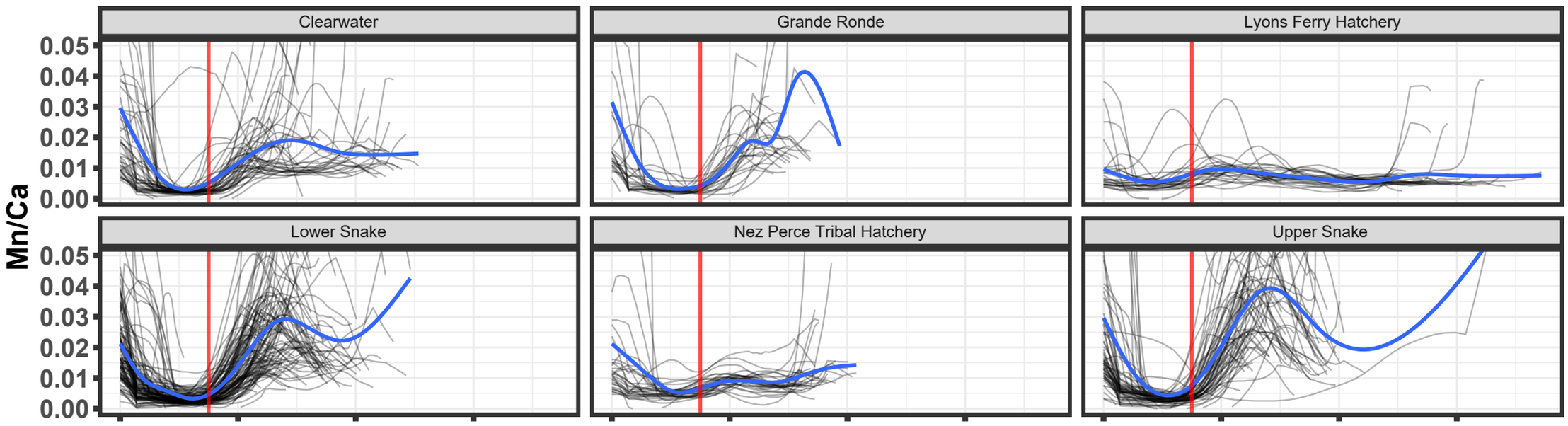
1047


0.714
0.711
0.708
$^{87}Sr/^{86}Sr$
Clearwater/Salmon
G.R./Im./Tuc.
L.F. Hatchery
Lower Snake
N.P.T. Hatchery
Upper Snake
River Group
River Reach/ Hatchery
Clearwater/Salmon
G.R./Im./Tuc.
L.F. Hatchery
Lower Snake
N.P.T. Hatchery
Upper Snake

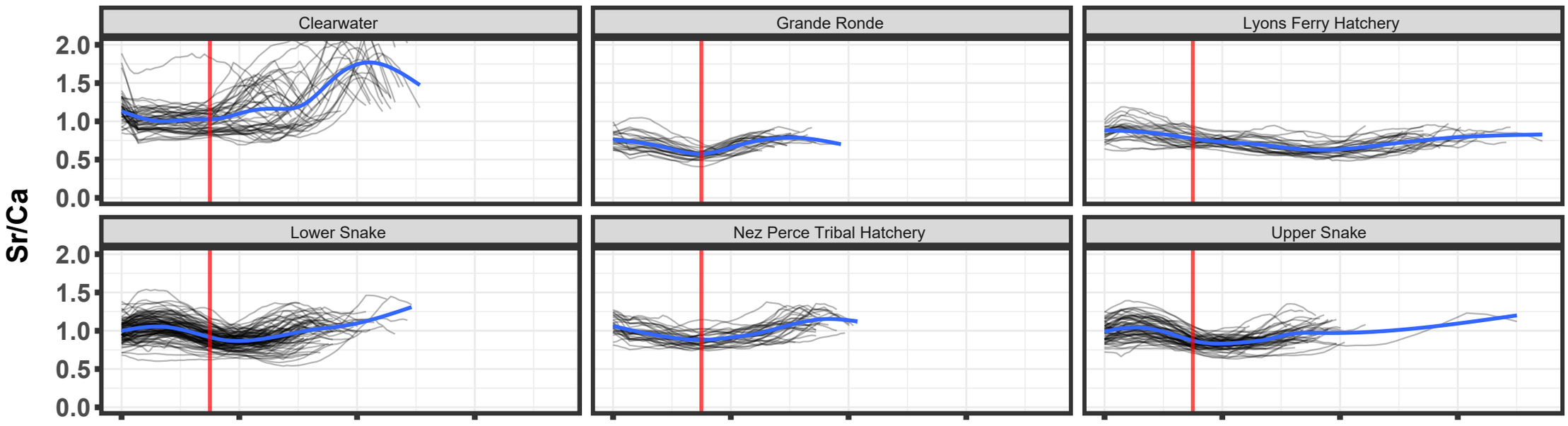
(A)



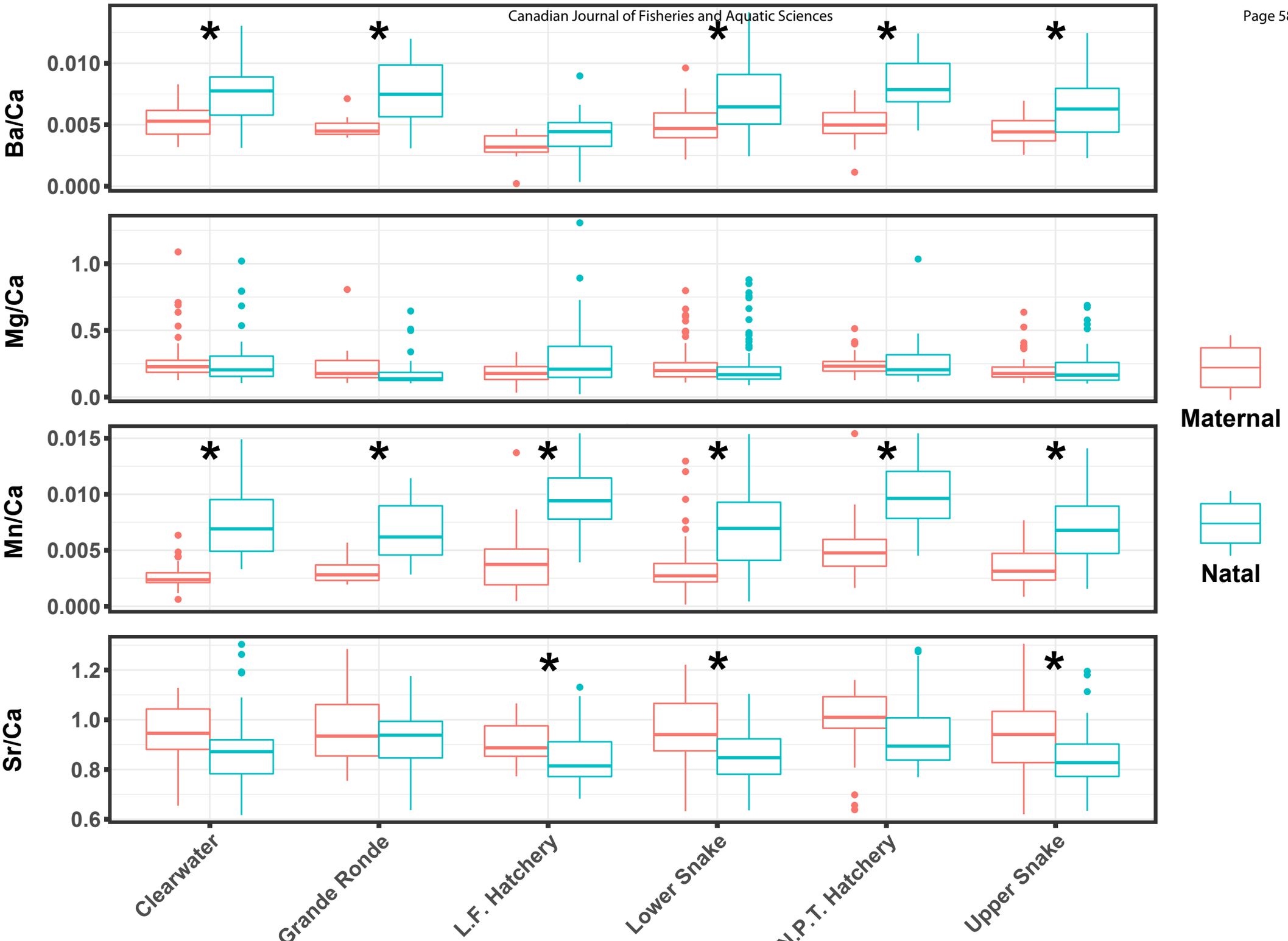
(B)

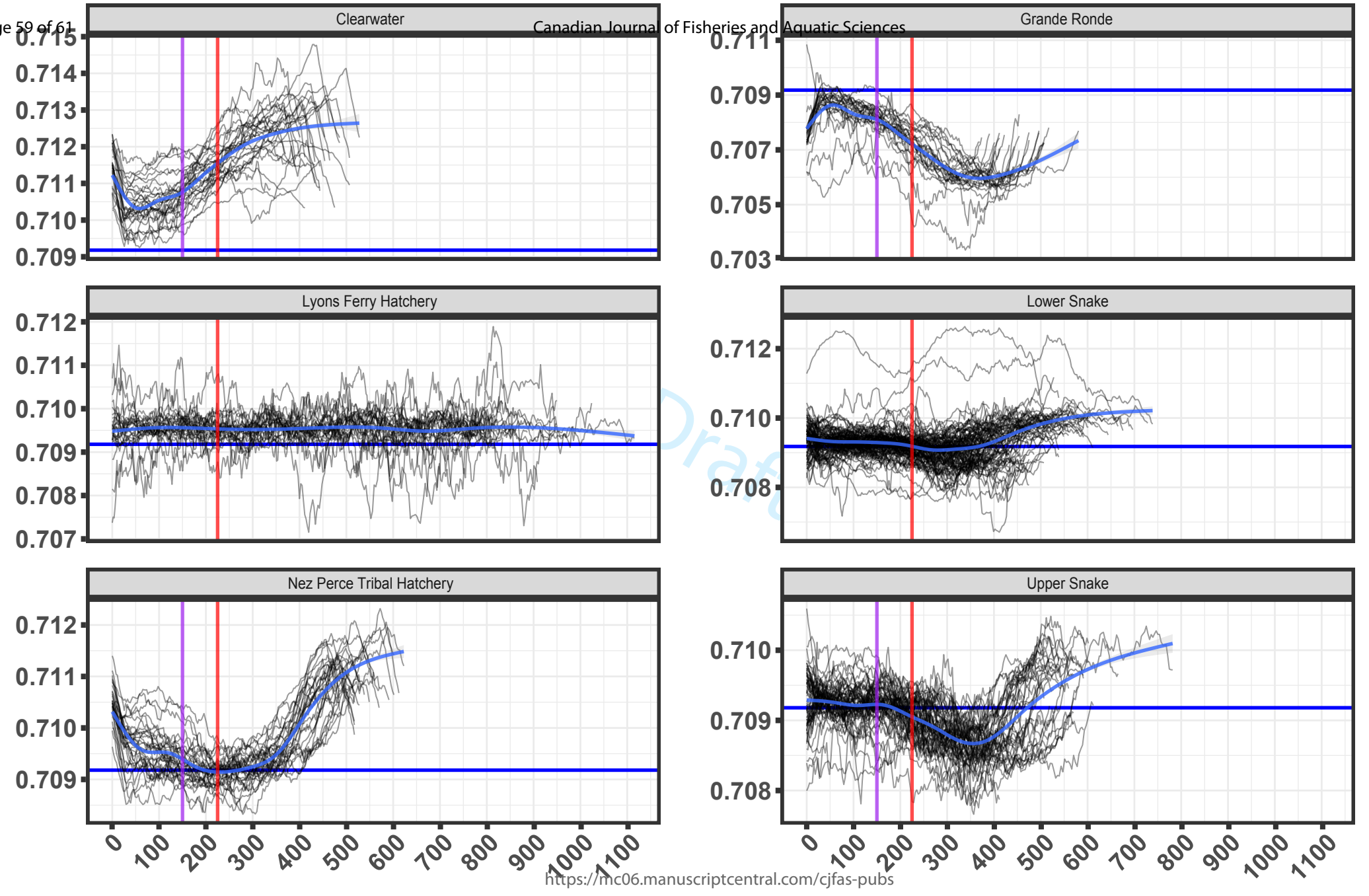


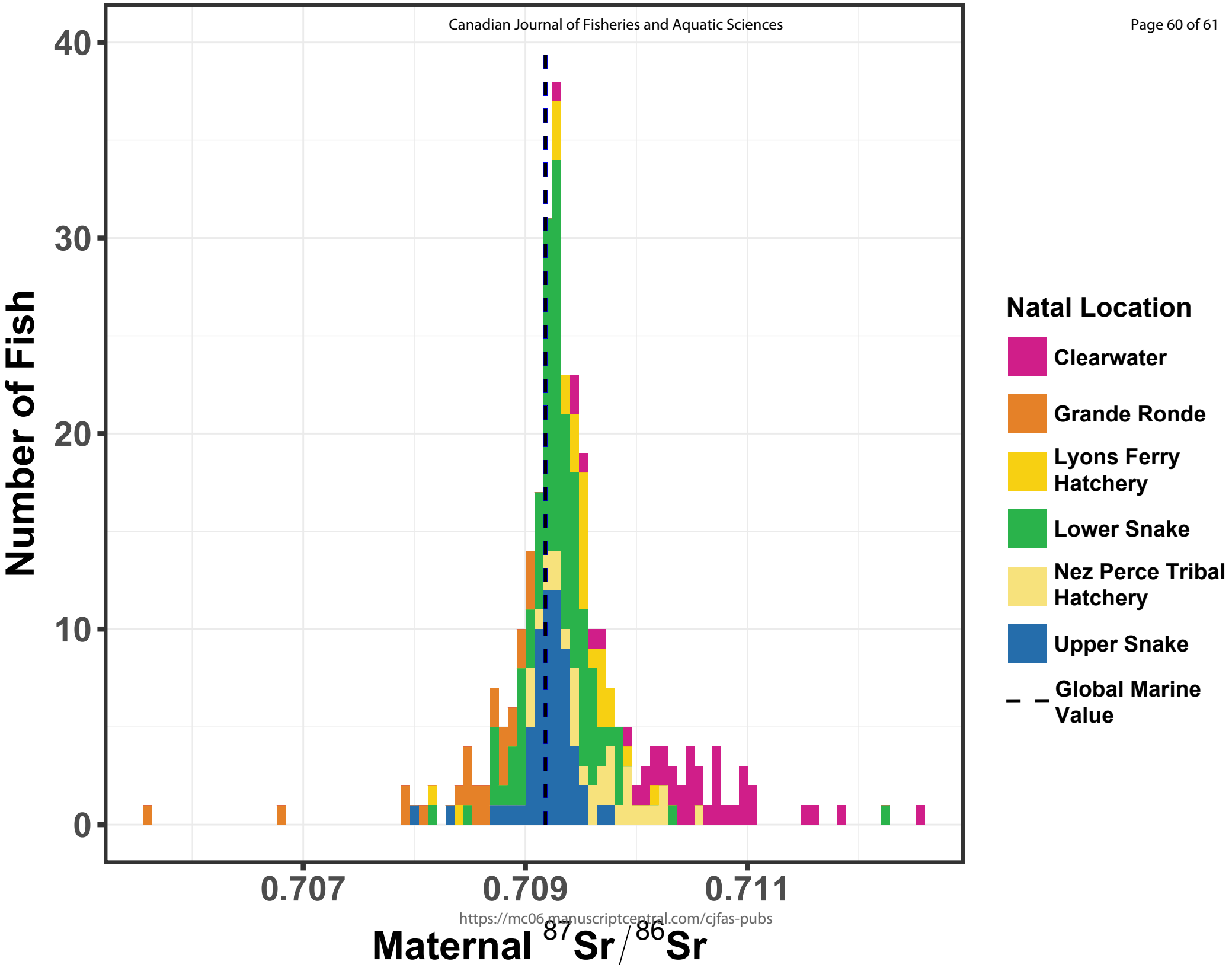
(C)

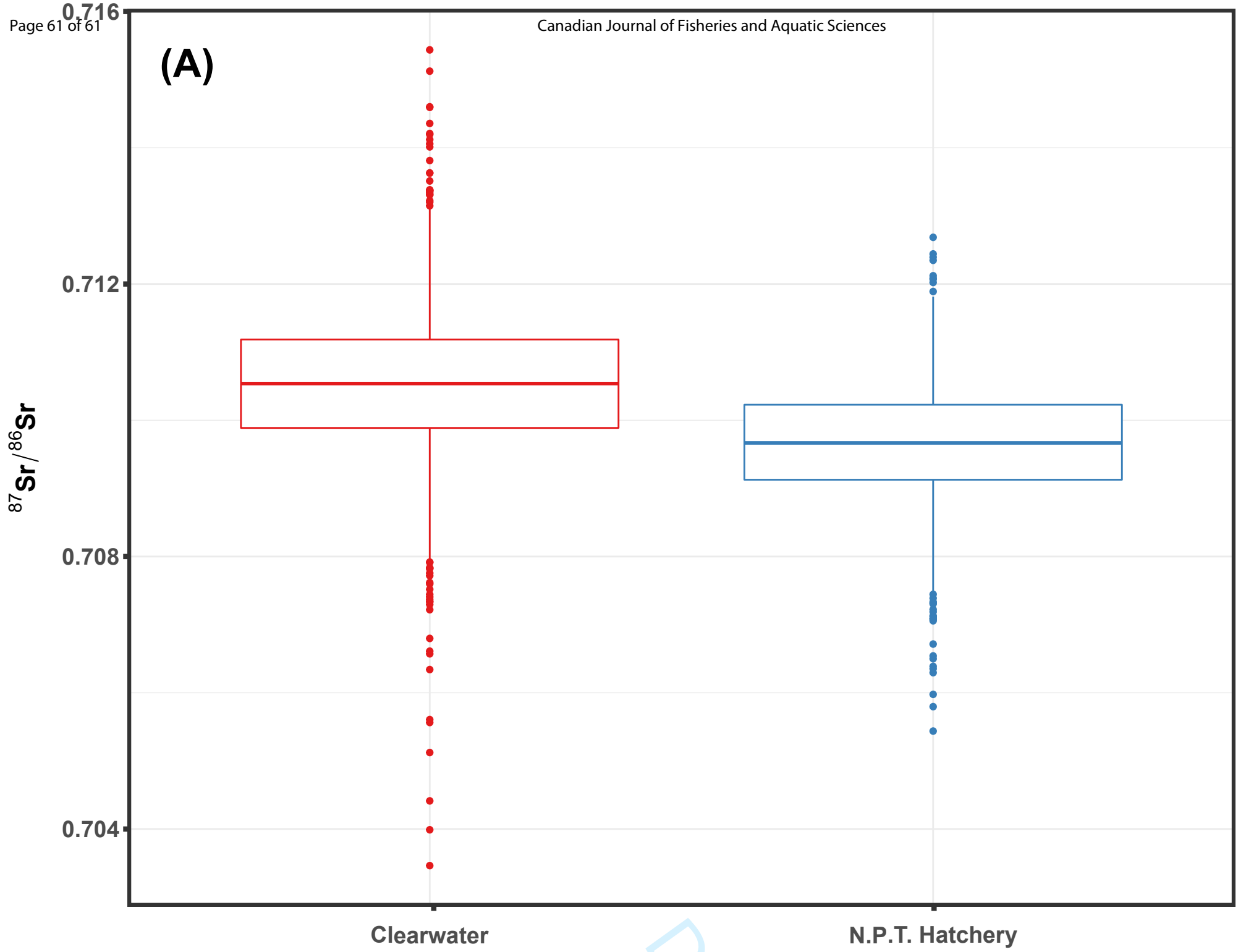


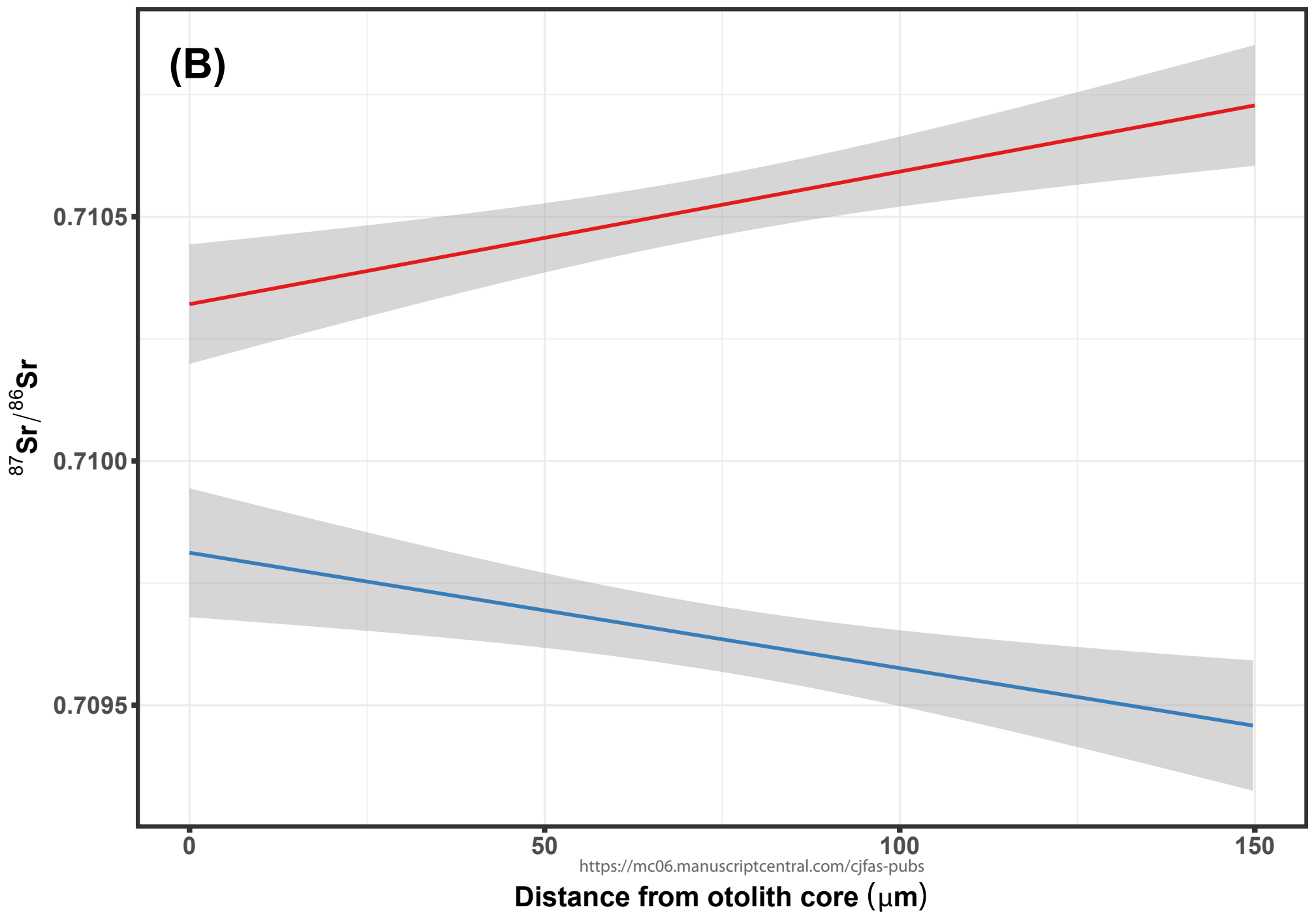
(D)


Ba/Ca
0.010
0.005
0.000
Mg/Ca
1.0
0.5
0.0
Mn/Ca
0.015
0.010
0.005
0.000
Sr/Ca
1.2
1.0
0.8
0.6
*
Maternal
Natal
Clearwater
Grande Ronde
L.F. Hatchery
Lower Snake
N.P.T. Hatchery
Upper Snake
Known Natal Location




Number of Fish
40
30
20
10
0
0.707
0.709
0.711
Maternal $^{87}Sr/^{86}Sr$
Natal Location
Clearwater
Grande Ronde
Lyons Ferry Hatchery
Lower Snake
Nez Perce Tribal Hatchery
Upper Snake
Global Marine Value


(A)
0.716
0.712
0.708
0.704
$^{87}Sr/^{86}Sr$
Clearwater
N.P.T. Hatchery
Juvenile Natal Location
Clearwater
N.P.T Hatchery


(B)
0.7105
0.7100
0.7095
$^{87}Sr/^{86}Sr$
0
50
100
150
Distance from otolith core (μm)