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Prevalence of stocked whitefish in River Kemijoki, Finland, inferred by micro X-ray fluorescence analysis of otoliths

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Abstract

Micro X-ray fluorescence (µ-XRF) analysis of otoliths was evaluated as a method to estimate the proportion of stocked one-summer-old whitefish *Coregonus lavaretus* L. in catches of adult fish (n = 20) ascending the River Kemijoki to spawn. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis was applied as control. Polished otoliths were scanned with µ-XRF to obtain strontium maps that were used to infer visually the provenance of the whitefish. Thirteen of the fish showed signs of being stocked as one-summer-old fingerlings. LA-ICP-MS was applied to determine the elemental composition in a spot outside the core of the otolith. The results were largely consistent with the visual inspection of the µ-XRF mapped otoliths. In conclusion, µ-XRF mapping successfully identified whitefish stocked as one-summer-old fingerlings. The vast majority of whitefish returning to the River Kemijoki to spawn were stocked fish.

KEYWORDS

µ-XRF, Baltic Sea, elemental analysis, LA-ICP-MS, otolith, whitefish

1 | INTRODUCTION

The whitefish *Coregonus lavaretus* L. stock in the Gulf of Bothnia (Figure 1) is composed of two different ecotypes: sea-spawning whitefish and river-spawning whitefish. Spawning occurs in autumn in shallows in the sea or in rivers. After hatching in spring, fry of the river-spawning ecotype migrate from the river to the sea in mid-June after reaching a length of ≈30 mm (Jokikokko, Huhmarniemi, Leskelä, & Vähä, 2008). Besides their choice of spawning grounds, the ecotypes differ in migratory behaviour and

Short informative: Mapping strontium in whitefish otoliths with µ-XRF helped identify stocked one-summer-old fingerlings in river Kemijoki, Finland, Baltic Sea. The µ-XRF method also has potential to identify wild river-origin whitefish, and as such, it provides useful means for assessing the efficiency of whitefish stocking.

number of gill rakers. The river-spawning ecotype can undertake long feeding migrations all the way from the northernmost part of the Gulf of Bothnia to the Archipelago Sea (≈700 km), whereas the sea-spawning ecotype is relatively stationary (Lehtonen, 1981; Wikgren, 1962). Traditionally, the ecotypes have been differentiated by counting of the number of gill rakers on the outermost gill arch, for which mean gill raker numbers are ≈30 and ≈27 for the river-spawning and sea-spawning ecotypes, respectively (Himberg et al., 2015; Svärdson, 1957). The numbers of gill rakers, however, overlap for the ecotypes rendering this characteristic somewhat problematic to use for differentiation, particularly at the individual level. Size of adult fish has also been used for differentiation, since river-spawning whitefish generally grow larger than the stationary sea-spawning whitefish, due to their feeding migration to the south (Kallio-Nyberg, Veneranta, Salonen, Jokikokko, & Leskelä, 2019; Lehtonen, 1981; Linden et al., 2019). Notably, there are also small short-migrating fish among river spawners (Jokikokko, Hägerstrand, & Lill, 2018).

Owing to loss of spawning grounds caused by damming of rivers for hydropower production, eutrophication of spawning grounds and overfishing, the stock of river-spawning whitefish along the Finnish coast has steadily declined since the 1990s (HELCOM, 2013; Urho, 2011). To mitigate for these losses, a massive stocking programme of whitefish has been undertaken in Finland. Several million one-summer-old whitefish are stocked each year along the Finnish west coast, a major part for compensatory purposes into rivers high up in the northern part of the Bothnian Bay (Jokikokko & Huhmarniemi, 1998).

The River Kemijoki is the longest river in Finland, flowing 550 km from the Russian border and Finnish Lapland before entering the Gulf of Bothnia at the city of Kemi (Figure 1). Before the first power plant was built in 1949, the River Kemijoki was one of most important spawning rivers in Europe for salmon, brown trout and whitefish.

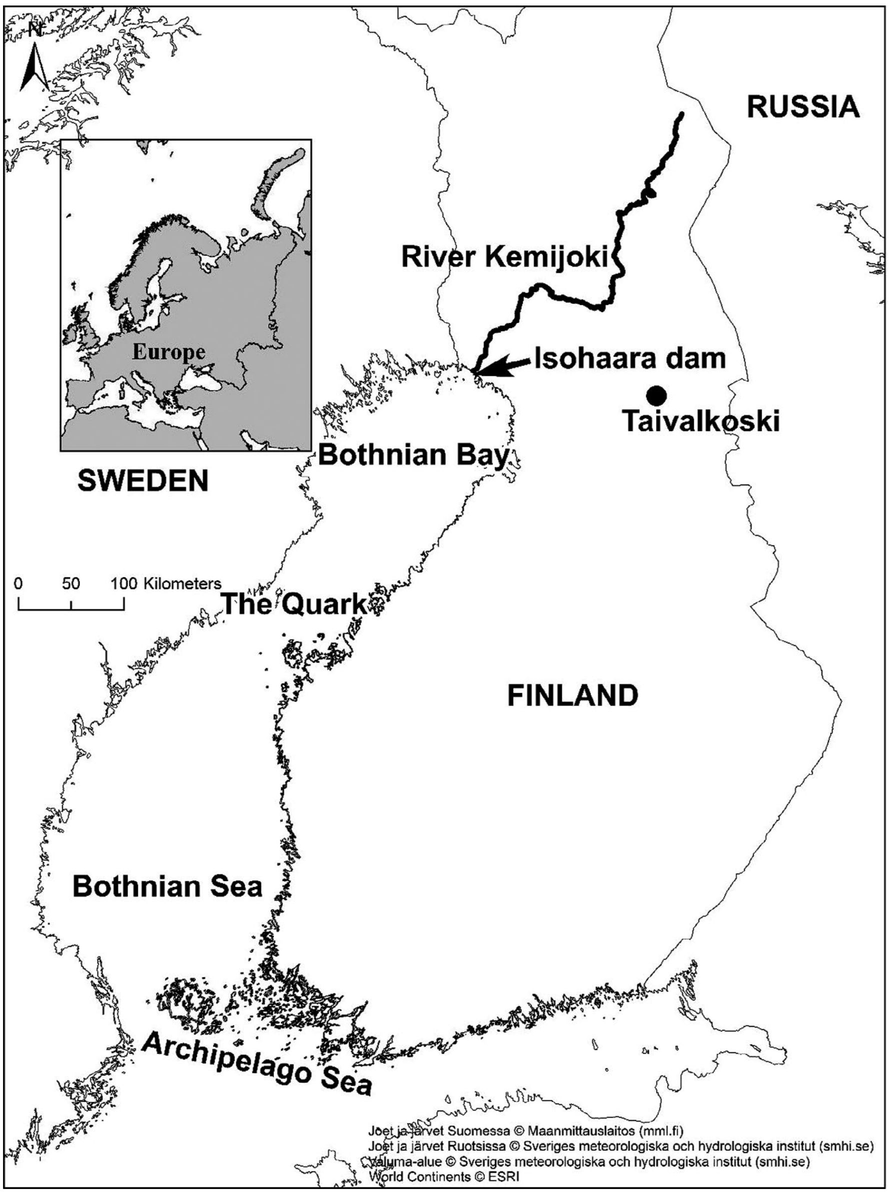

FIGURE 1 Map of the River Kemijoki (Finland) and Bothnian Sea via Bothnian Bay. The Isohaara power plant is located about 5 km upstream of the river

Presently, about 3.1 million one-summer-old whitefish fingerlings are stocked every year into the river below the lowest dam, Isohaara (Figure 1), located a few kilometres from the sea. This constitutes the single largest compensation stocking of whitefish undertaken in Finland.

The stocked fingerlings originate from spawning whitefish caught every autumn in the River Kemijoki, and from a broodstock of whitefish from the nearby River Tornionjoki, kept in freshwater ponds in a hatchery facility in Taivalkoski (Figure 1). After hatching in May, the larvae are transported to several natural food freshwater ponds where they are kept until late September to early November when the ponds are emptied and the fingerlings are stocked into the River Kemijoki. The average length of the fingerlings at stocking is 10 cm, and the weight is 6–7 g. In addition to the stocking of one-summer-old fingerlings, newly hatched larvae of the same origin are occasionally stocked in the coastal areas around the mouth of the River Kemijoki. According to the Natural Resources Institute Finland, stocked numbers of these surplus larvae have varied between 0.3 and 2.5 million larvae/year.

Although high numbers of anadromous whitefish ascend the River Kemijoki to spawn in October, natural reproduction is assumed to be low due to the variable water current below the dam. However, some natural reproduction has been observed (E. Jokikokko, unpublished results). In 1996–1998, a large-scale spray-marking study of stocked whitefish fingerlings was carried out in the River Kemijoki (Leskelä, Jokikokko, Huhmarniemi, Siira, & Savolainen, 2004). When the mature fish returning for spawning were sampled in later years, it was noticed that not all of them had been stocked as fingerlings but a portion was unmarked fish originating from either natural spawning, stocking of larvae or straying from other stocks. However, the proportions of wild and stocked fish were not evaluated. Furthermore, based on dipnetting studies of newly hatched larvae carried out by the Natural Resources Institute Finland in recent years, natural reproduction was also observed in the River Kemijoki below the Isohaara dam. Since no larvae were stocked into the river before dipnetting, these larvae must have originated from natural spawning. Therefore, it follows that there are both naturally reproduced and stocked fish among the whitefish ascending the River Kemijoki to spawn. To summarise, whitefish ascending the River Kemijoki to spawn can have several different provenances/origins. They can be: (a) stocked into the river as one-summer-old fingerlings; (b) stocked around the river mouth as newly hatched larvae; (c) hatched in the river; or (d) strayers from other rivers; and although not previously observed (e) straying sea spawners may occur.

The main aim of this study was to evaluate μ-XRF analysis of otoliths as a method to estimate the proportion of stocked one-summer-old whitefish in catches of adult fish ascending the River Kemijoki to spawn. The concentration of strontium is known to be very low in freshwater ponds where whitefish are held to grow prior to stocking (Lill et al., 2018), compared with the seawater content where the salinity of the surface water increases from ≈1 to ≈7 PSU in the north–south direction of the Gulf of Bothnia. Therefore, μ-XRF analysis, a method well-suited for semi-quantitative mapping of strontium (Holbach, Cowley, Kramar, & Neumann, 2012;Lill et al., 2019), and LA-ICP-MS, a sensitive method suitable for the quantitative spot analysis of a wide range of trace elements, including strontium (Campana, 1999;Elsdon et al., 2008;Lill et al., 2019;Rohtla et al., 2017), were used. The LA-ICP-MS analysis functioned as a control for the μ-XRF mapping. The assumption was that μ-XRF mapping of otolith strontium can be used to visually detect stocked one-summer-old whitefish, thereby providing a easy to use method for wider application in fisheries management. Knowledge gained by this study is useful when the efficiency of stocking of whitefish in general and the importance of the River Kemijoki as a spawning ground in particular are evaluated.

2 | METHODS

Whitefish ascending the lowest reach of the River Kemijoki (Figure 1) were caught with gillnets during the spawning season in October 2015. Twenty individuals, 10 males and 10 females, were sampled. These individuals were used as broodstock to produce whitefish for future stocking. Following euthanisation, the length, weight and sex of each sampled fish were recorded, the gill rakers were counted and the otoliths were removed for elemental analysis and age reading by counting the annuli (Table 1).

Before the otoliths could be analysed, it was necessary to grind them down to the core, exposing the annual increments. The otoliths were placed in silicone moulds, sulcus side facing downward and were then fixed in epoxy (Struers, EpoFix 8.33 g epoxy/1 g hardener). After being left overnight for the epoxy to cure, the fixed otoliths were removed from the mould. The fixed otoliths were first wet ground on the anti-sulcus side using 800 p sandpaper (Buehler, Carbimet) until the edges of the otoliths were exposed. Thereafter, the grinding was done in stages with regular visual inspections under a microscope until the first annuli was exposed. At this stage, the fixed otoliths were alternately ground by hand using a wet 2,500 p sandpaper (Buehler, Carbimet) and polished using a wet 3-micron polishing disc until the core was visible under the microscope. The final polishing was done using a soft polishing disc and a 1-micron polish suspension (Kemet, Liquid Diamond Type WXXStr) until the surface of the otolith was completely smooth and the core clearly visible. A Buehler Beta Grinder-Polisher was used for the grinding and polishing. The polished otoliths were finally rinsed in ethanol and cleaned with a lens cleaning tissue before they were mounted on a glass slide. The polished otoliths (20 pcs) were used for age determination and elemental analyses.

Two-dimensional element distribution maps of the polished otoliths were acquired using a M4 Tornado μ-XRF spectrometer. The spectrometer was equipped with a Rh X-ray tube and two silicon drift detectors manufactured by Bruker Nano. The X-ray beam was focused to 20 μm for Mo(Kα) using polycapillary X-ray optics. Multi-elemental maps were obtained with an X-ray tube voltage of 50 kV and a current of 300 μA. The pixel step size was 18 μm. Acquisition time of each spectrum was set to 10×10^{-3} s per pixel. All measurements were carried out in a 20-mbar vacuum. Prior to XRF element mapping, both

TABLE 1 Individual data for whitefish caught in the River Kemijoki 13/10/2015

ID	Length (mm)	Weight (g)	Sex	Age (year)	GRC	Year of hatch	Fingerlings stocked into R. Kemijoki	Larvae stocked at river mouth	Suggested provenance
F1	395	548	1	3	31	2012	0	295,000	Wild RS
F2	467	1,017	2	5	28	2010	240,000	1,810,000	Stocked 1-S
F3	452	869	1	6	29	2009	385,000	985,000	Stocked 1-S
F4	403	619	2	5	28	2010	240,000	1,810,000	Stocked 1-S
F5	472	916	2	6	29	2009	385,000	985,000	Stocked 1-S
F6	371	456	1	5	29	2010	240,000	1,810,000	Stocked 1-S
F7	413	653	2	6	30	2009	385,000	985,000	Stocked 1-S
F8	425	720	2	7	29	2008	3,200,000	2,200,000	Stocked 1-S
F9	331	290	1	9	27	2006	1,050,000	0	Wild SS
F10	382	574	1	5	29	2010	240,000	1,810,000	Stocked L/wild RS
G1	387	552	1	4	28	2011	0	910,000	Stocked 1-S RS †
G2	423	574	2	5	31	2010	240,000	1,810,000	Stocked 1-S RS
G3	385	480	1	6	26	2009	385,000	985,000	Stocked 1-S RS
G4	403	507	2	7	27	2008	3,200,000	2,200,000	Stocked 1-S RS
G5	395	603	1	7	31	2008	3,200,000	2,200,000	Wild RS
G6	392	527	2	5	31	2010	240,000	1,810,000	Stocked 1-S
G7	461	757	2	6	27	2009	385,000	985,000	Wild RS
G8	427	641	2	6	32	2009	385,000	985,000	Stocked 1-S
G9	378	540	1	8	30	2007	1,454,000	5,125,000	Stocked L/wild RS
G10	419	704	1	4	29	2011	0	910,000	Stocked L/wild RS
Mean	409.1	627.4		5.8	29.1				
SD	35.4	168.7		1.4	1.6				

Note: The data include sex (male = 1 and female = 2), age and gill raker counts (GRC) of each individual. The amount of fish stocked into the River Kemijoki and at the coast near the river mouth in the same year as the fish was hatched are also included. Suggested provenance for the whitefish, based on the μ-XRF maps and the LA-ICP-MS based cluster analysis, are presented in the column to the far right.

Abbreviations: Stocked 1-S RS, stocked as one-summer-old river spawner origin fingerling; Stocked L, stocked river spawner origin larvae; Wild RS, wild river spawner; Wild SS, wild sea spawner; †, strayer.

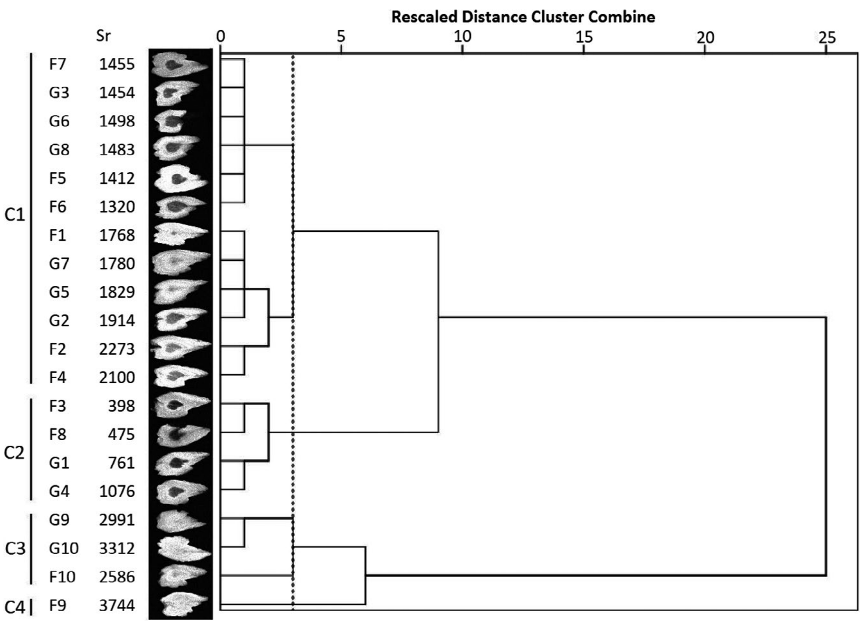

FIGURE 2 Relationships among whitefishes using the between-groups linkage method for strontium (Sr) concentrations (in μg/g) measured with LA-ICP-MS. The dotted vertical line marks the step at which the clusters (C1–C4) were defined for further statistical analyses. The concentrations of Sr are given in the middle column to the left of the graph. The maps display the distribution of Sr in the otoliths from low concentration (dark) to high concentration (bright), measured using μ-XRF

detectors were calibrated by measuring a pure Zr standard and tuning the spectrum according to the Zr(Kα) peak at 15.775 keV. All 20 otoliths on the slide were automatically scanned in the same run. Acquisition time was 12 hr. The size of the scanned area was 61.5 × 20.2 mm, and the total number of data pixels was ≈3.8 million. A map of a single otolith contains between 30,000 and 60,000 pixels. A distinct Kα energy peak of strontium was observed from the sum spectrum. The element map of strontium is based on the $K\alpha_1$ energy peak area and a three spots averaging filter for background removal. The depth range within the otolith from which the detectable X-rays were emitted was calculated to be 50% from 0 to 104 μm and 90% from 0 to 344 μm (J-O. Lill, pers. comm., January 18, 2019; Lill et al., 2019). The colour scale of the μ-XRF otolith maps in Figures 2 and 3 is the same for all otoliths, which means that they are comparable. The lightest and darkest areas correspond to the extreme values in strontium concentrations, representing high and low strontium concentrations, respectively. The sensitivity of μ-XRF was not high enough to map barium.

The LA-ICP-MS analysis was performed at the Geological Survey of Finland (GTK), using a Nu AttoM SC-ICPMS (Nu Instruments Ltd.) and an Analyte 193 ArF laser ablation system (Photon Machines). The laser was run at a pulse frequency of 10 Hz and a pulse energy of 5 mJ at 30% attenuation to produce an energy flux of 2.5 J/cm^2 on the sample surface with an 85 μm spot size (Figure 4). The location of the spot, about 200 μm outside the core, was specifically chosen to identify stocked one-summer-old whitefish by their low strontium concentration at this spot. The depth of the spot was measured to be 40 μm using a scanning white-light interferometer (SWLI). The samples were analysed for 40 s, followed by 20 s of background measurements. Analyses were made using time-resolved analysis (TRA) with continuous acquisition of data for each set of spots (2 standards, 15 unknowns and 1 quality control standard). For the calibration and evaluation of the analysis, FEBS1 (certified otolith standard for trace metals, NRCC, Canada), NIST612 (synthetic silicate standard, NIST, USA), MACS-3 (synthetic carbonate standard, USGS, USA) and CACB-1 (calcium carbonate standard, NRCC, Canada) certified reference materials were used. Based on homogeneity and accuracy results, the standard MACS-3 and its compiled concentrations from GEOREM were used for external standardisation, while the other standards were used for quality control. ^{43}Ca was used as an internal standard for the quantification. The measurements were performed for 12 elements at low resolution ($\Delta M/M$ = 300) using the fast scanning mode (Table 2). Data reduction was handled using the software GLITTER TM (Van Achterbergh, Ryan, Jackson, & Griffin, 2001), which allows for the baseline subtraction, for the integration of the signal over a selected time-resolved region, and the quantification using known concentrations of the external and internal standards.

Based on the concentration of strontium in their otoliths in the spot beside the core measured with LA-ICP-MS, the whitefish otolith data were divided into clusters using a hierarchical cluster analysis, using the between-groups linkage method (cluster method of SPSS, IBM) and the distance measure of squared Euclidian distance. Four clusters (C1–C4) were chosen based on inspection of the agglomeration levels. Differences in fish weight, length, age and the number of gill rakers between the clusters were analysed using a one-way ANOVA. The statistical probability of the ratio of stocked one-summer-old versus the other whitefish in the sample (n = 20) to represent the ratio in the entire population was calculated using binominal probability.

3 | RESULTS

The mean total length (40.9 ± 3.54 cm) and mean gill raker number (29.1 ± 1.6) of the fish indicated river-spawning whitefish comprised most of the sample (Table 1). Interpreting the μ-XRF mapped otoliths, 13 out of 20 (65%) whitefish caught in the River Kemijoki were stocked as one-summer-old fingerlings (Figure 2, photographs). These individuals spent the first 4–5 months of their lives in fresh water and therefore show low concentrations of strontium (dark areas) in the central part of the otolith corresponding to this period. This area of the otolith is relatively large and covers about one third of the total width of the otoliths (Figure 2, photographs). The statistical interval estimate (95% CI) for the proportion of one-summer-old whitefish of all whitefish in the River Kemijoki was 41%–85% (0.65%

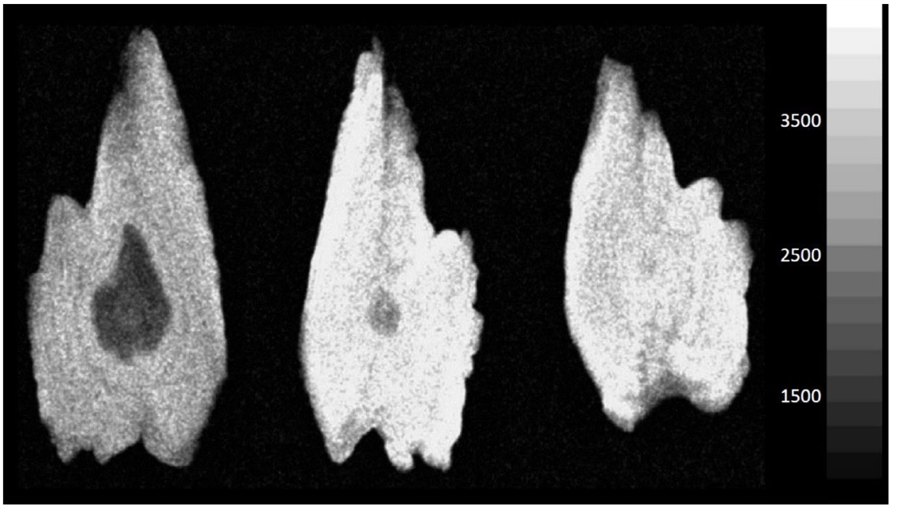

FIGURE 3 Otolith (μ-XRF) images for fish IDs F7, F1 and F9 extracted from whitefishes stocked as one-summer-old fingerlings (left), wild river spawners (middle) and wild sea spawners (right). The grey scale represents the concentration of strontium in μg/g

and 95% CI = {0.408, 0.846}). Furthermore, three whitefish (F1, G5 and G7) show small dark areas around the core of the otoliths and probably are wild river-origin fish that stayed only a short time in the river before entering the sea. The remaining four whitefishes (G9, G10, F9 and F10) did not show dark, strontium-low areas in their otoliths and therefore appear to be of sea origin.

Four clusters (C1–C4) were chosen from the cluster analysis, based on inspection of the agglomeration levels, and there were no significant differences in fish weight, length, age and the number of gill rakers between the clusters (ANOVA, $p \geq 0.05$). Clusters C1 and C2 consisted of 16 otoliths having Sr concentrations ranging from 398 to 2,273 µg/g. The Sr concentrations of these 16 otoliths were indicative of a freshwater provenance, suggesting (but see discussion) that all these fishes were stocked as one-summer-old fingerlings (Lill et al., 2018). Clusters C3 and C4 consisted of four otoliths (G9, G10, F9 and F10) having Sr concentrations ranging from 2,586 to 3,744 µg/g suggesting a provenance at sea (Lill et al., 2018).

The division of the 16 fishes with an apparent freshwater provenance into two different clusters, C1 and C2, suggested that they were stocked from different freshwater sources (Figure 2). The mean strontium concentration of the C1 cluster was 1,691 ± 301 µg/g, which agrees well with concentrations reported in otoliths from fingerlings farmed at the Taivalkoski hatchery facility (1,664 ± 128 µg/g, Lill et al., 2018). However, among the individuals in this cluster, three individuals (F1, G5 and G7) displayed only small dark areas of low concentration of strontium around the core of the otoliths in the µ-XRF maps (Figure 2). Apparently, the provenance of these individuals lies in fresh water as well, but probably as wild river-origin whitefish flushed into the sea shortly after hatching. However, an origin as larvae stocked in the coastal areas around the river mouth cannot be excluded. The four whitefish individuals in cluster C2 displayed large dark areas of low concentration of strontium around the core in the µ-XRF maps, similar to the majority in cluster C1, but the strontium concentrations measured with LA-ICP-MS were much lower (677 ± 308 µg/g). The precise provenance of these whitefish remains uncertain but may be freshwater pond(s) with a low concentration of strontium (Lill et al., 2018). Notably, whitefish G1 in this cluster hatched in 2011 when no fingerlings were released into the River Kemijoki. It therefore follows that this individual is a strayer from another stock (Table 1).

The remaining four (G9, G10, F9 and F10) of the 20 whitefish, divided in clusters C3 and C4, had high concentrations of otolith strontium ranging from 2,586 to 3,744 µg/g in the LA-ICP-MS measured spots. The strontium was homogenously distributed within each otolith. Furthermore, the barium concentrations in the spots were low, indicating a sea provenance (Table 2). However, three of these individuals (G9, G10 and F10), clustered in C3, resembled typical river-spawning whitefish in number of gill rakers (29.3 ± 0.5 gill raker counts) and were too large to be sea spawners (606 ± 87 g) (Table 1; Himberg et al., 2015;Jokikokko & Huhmarniemi, 1998). Therefore, these fishes are likely river-origin whitefish stocked as larvae in coastal areas outside the river mouth or hatched in the river mouth and quickly flushed into the sea, where they have attained

FIGURE 4 Polished surface of the otolith analysed from whitefish ID F1: (a) core of the otolith, (b) the spot ≈200 µm outside core for which the analytical results are given in Table 2 and used in the cluster analysis (Figure 2). The results from the unmarked ablated craters were not used

high otolith strontium levels. The fourth fish (F9) separated into cluster C4 was the smallest (294 g) and oldest (9 yr) of all the fish in the sample. It had a low gill raker count (n = 27) and the spot analysis with LA-ICP-MS gave the highest strontium and lowest barium concentrations of all the fish otoliths (Table 2), suggesting this fish was a straying sea spawner.

4 | DISCUSSION

The discrepancy of wild river-spawning whitefish (individuals F1, G5 & G7) being clustered together with fish stocked as one-summer-old fingerlings in the cluster C1 is probably caused by a spot analysis using LA-ICP-MS too close to the core (≈200 µm). Due to the proximity of the spot to the core, it is likely that it measured a part of the otolith formed as the wild river-spawning whitefish still resided in the river, giving it a freshwater signature similar to that of fish stocked as one-summer-old fingerlings. Notably, discarding these three specimens from the 16 in the clusters C1 and C2, leaves the 13 fishes that, based on the µ-XRF maps, were concluded to be stocked as one-summer old fingerlings.

Due to the information depth of the µ-XRF and the placement of the spot analysed using LA-ICP-MS, the present study was not well set up to identify short-term residency of larvae in fresh water, that is whitefish hatched in the river and quickly flushed into the sea or whitefish stocked as larvae. Taking into consideration, the information depth of the µ-XRF, which was quite large (90% from 0–344 µm), any small area of low strontium in the core of the otolith, signifying stocking as larvae, may be obscured by X-rays originating from high concentration Sr annuli below. Furthermore, the spot analysed with LA-ICP-MS was placed too far away from the core to be useful in detecting fish stocked as larvae.

The present study is the first to investigate the provenance of whitefish spawning in the River Kemijoki. Signs of natural reproduction were found in spray-marking studies from 1996 to 1998, but

TABLE 2 Elemental composition of whitefish otoliths measured with LA-ICP-MS. The concentrations represent a spot placed about 200 µm away from the core of the otolith. All concentrations are presented in µg/g of dry weight

	Sr	Ba	Mg	Si	P	Mn	Ni	Cu	Zn
F1	1767.8	18.1	34.6	292.3	74.0	8.7	0.4	0.4	192.0
F2	2,272.8	120.5	31.4	277.3	91.4	14.7	0.5	0.5	194.8
F3	397.9	15.3	29.0	256.6	114.8	5.5	0.6	0.4	195.9
F4	2,100.4	123.5	31.6	257.5	120.8	7.5	0.6	0.6	150.1
F5	1,412.3	18.6	30.3	208.9	92.6	4.1	0.5	0.4	108.3
F6	1,320.3	52.6	36.4	296.0	99.7	10.4	0.3	1.0	151.5
F7	1,455.5	54.3	36.4	249.6	96.5	12.0	0.3	0.3	271.3
F8	474.8	13.3	27.6	244.1	58.6	4.8	0.3	0.1	123.4
F9	3,744.0	4.4	19.8	217.5	170.3	11.4	0.4	0.3	125.4
F10	2,585.9	10.2	35.0	241.2	153.0	16.6	0.4	0.3	142.7
G1	761.0	24.1	51.8	254.4	453.2	17.1	0.4	0.4	187.0
G2	1914.2	97.4	34.6	234.0	101.3	4.6	0.6	0.5	164.3
G3	1,454.4	67.5	26.3	225.2	107.9	6.3	0.3	0.3	146.0
G4	1,075.7	32.9	27.5	249.5	134.8	4.9	0.4	1.4	130.4
G5	1829.0	9.9	35.3	222.2	89.2	11.5	0.5	0.7	175.1
G6	1,497.6	67.6	28.6	269.8	148.2	8.4	0.4	0.2	207.1
G7	1,780.4	14.8	36.8	238.5	73.6	13.1	0.3	0.7	148.8
G8	1,483.2	60.8	29.7	247.5	136.2	8.8	0.4	0.3	187.0
G9	2,991.1	6.9	28.6	236.9	120.4	10.5	0.5	0.3	120.8
G10	3,312.2	6.6	35.5	237.6	139.3	8.7	0.3	0.3	211.0
Mean	1781.5	41.0	32.3	247.8	128.8	9.5	0.4	0.5	166.6
SD	875.4	38.1	6.3	23.0	81.7	3.9	0.1	0.3	39.7

no proportions of wild and stocked fish were estimated (Leskelä et al., 2004). In 2017, natural reproduction was estimated from stocking and dipnetting of alizarin-marked larvae. The study showed that hundreds of thousands of wild larvae were flushed into the sea during May–June (P. Jounela, pers. comm., September 20, 2019). Moreover, newly hatched larvae have been stocked quite regularly into the river below the Isohaara dam (Table 1). Furthermore, wild adult whitefish from the nearby River Tornionjoki tagged with t-anchor tags have been caught in the River Kemijoki below the lowest dam (Natural Resources Institute Finland tagging data).

Taking into account, the previous indications of natural reproduction in the River Kemijoki, stocking of larvae into the river, the possibility of whitefish straying from the River Tornionjoki and the vast stocking of one-summer-old fingerlings into the River Kemijoki (3.1 million every year), the proportions of stocked and wild fish in the present spawning sample seem reasonable.

In conclusion, the µ-XRF strontium maps were useful in identifying whitefish stocked as one-summer-old fingerlings. The method also has the potential to identify wild river-origin whitefish. Quantitative methods, such as LA-ICP-MS, should be applied to obtain detailed knowledge in fish life histories. A major advantage of otolith studies over marking studies is the short experimental time and relative simplicity. Instead of marking fish and then proceed to wait for years for their recapture, the otolith study can be applied to otoliths from recently sampled fish, no previous long-term planning needed. A vast majority of whitefish returning to the River Kemijoki for spawning were stocked. µ-XRF analysis of polished otoliths provides a handy method to determine the efficiency of whitefish stocking.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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