Subject: **Mary River Project: Steensby Port Arctic Char Otolith Analysis**

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1.0 INTRODUCTION

Freshwater baseline field programs were undertaken in 2021 along the future south rail route and the in Steensby Port Area to support licencing for Phase 3 of the Mary River Project. One component of the field studies entailed examination of potential anadromy of Arctic Char (*Salvelinus alpinus*) at selected waterbodies and drainages in the Steensby Port Area to inform the potential effects relating to Phase 3 Project infrastructure on char. This Technical Memorandum (TM) presents a description of the field and laboratory methods and results relating to this component of the 2021 field program.

2.0 STUDY APPROACH

The general approach of the study entailed collection of Arctic Char from two waterbodies where potential for anadromy was identified within drainages that would be affected by road or rail crossings. Potential for anadromy was identified based on surveys of connectivity of drainages to the marine environment and the results of Inuit Qaujimajatuqangit (IQ) studies summarized in the FEIS (Baffinland 2012). Potential interactions with Project infrastructure were identified based on the Project design information provided to North/South Consultants Inc. (NSC) in spring 2021.

Otoliths were extracted from Arctic Char captured from two lakes for analysis using laser ablation microprobe technology (LA-ICP-MS analysis). LA-ICP-MS analysis is used to determine elemental concentrations in the bony structures of fish. Differences in elemental concentration at different locations on the structure can be used to make inferences about fish movements between fresh and marine water and biological cycles such as reproduction.

3.0 METHODS

3.1 FIELD PROGRAM

All catchments at the Steensby Port Site that could potentially be affected by road or rail crossings or other infrastructure were surveyed in spring 2021 (1-2 July) for downstream connectivity to the marine environment (i.e., Steensby Inlet). Surveys included backpack electrofishing of streams and lake shorelines to confirm presence of char and seasonal and permanent barrier searches in all streams connecting any potential overwintering lakes with Steensby Inlet.

This preliminary survey identified a total of nine lakes from six catchments with potential connectivity to Steensby Inlet (Figure 1). Three of the lakes were upstream of Ikpikitturjuaq Lake, also known as Lake ST-347, which IQ studies identified as a lake that supports an anadromous population of char (Baffinland 2012). In addition, the streams connecting these lakes to Ikpikitturjuaq Lake were large enough to support adult char use and had no seasonal or permanent barriers to fish movements when surveyed in spring and late summer/fall 2021a.

The remaining six lakes were identified as lower priority for further investigations based on the presence of seasonal barriers to fish movements in some streams, presence of smaller streams with lower water levels that could only support juvenile char (< 200 mm fork length), and/or the smaller size of the lakes which may be inadequate to support spawning populations.

A fall 2021 sampling program was undertaken with the objective of capturing 8-10 adult char from selected lakes identified as potentially supporting anadromous populations. Due to logistical constraints posed by persistent inclement weather during the sampling period (6-9 September) only two lakes could be sampled (lakes ST-352 and ST-176). These were the largest of the three lakes that connect to Ikpikitturjuaq Lake and thought to be the most likely to support anadromous char.

Standard index gill nets (1.5, 2, 3, 3.75, 4.25, and 5-inch mesh panels) were deployed in each lake for approximately 3.5-4.5 hours. Depths and universal transverse mercator (UTM) coordinates were recorded at each end of the gillnet sets. A total of six gillnet gangs were set in ST-352 and three in ST-176 (Table 1; Figures 2 and 3). All captured fish were measured for fork length (± 1 mm) and weight (± 10 g) and identified for sex and maturity using gamete extrusion. Otoliths for laser ablation analysis were removed from all char mortalities. Live fish in good condition were released at the site of capture.

3.2 LABORATORY ANALYSES

Otoliths from selected fish were embedded in epoxy resin, and a transverse cut was made to create a dorso-ventral cross section through the nucleus of the otolith, exposing all annuli. The posterior half of each cut otolith was re-embedded in a 25 mm diameter acrylic microprobe mount (up to 10 otoliths per mount). The exposed otolith surfaces were sequentially ground (with 30 and 9 µm wetted aluminum oxide lapping film), polished (0.3 µm dry aluminum oxide lapping film), and finally ultrasonically cleaned in ultrapure water.

To describe the LA-ICP- MS process simply, a laser beam follows a transect across the polished face of the otolith. As the laser moves along, the surface of the otolith is “burned” and elements that compose the otolith are released. Element concentration (strontium and others) is determined using a mass spectrometer. A line scan is then produced by plotting strontium concentration with distance (µm) from the origin of the transect. To produce a line scan that encompasses the duration of a fish’s life, the transect starts at the nucleus of the otolith (formed during egg incubation) and extends to the periphery of the otolith (see Figure 4).

In more technical terms, LA-ICP-MS analysis is done using a Thermo Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd-YAG laser. Table 2 describes the instrument parameters used during analysis. For fish otoliths, the optimum conditions used a 30 µm diameter beam travelling at 10 µm/s. This provided good sensitivity at a beam size capable of resolving all but the smallest (~20 - 30 µm) annular growth features in the otoliths. Calcium (as 40.04 wt.% CaO) was used as an internal standard. The external calibration standard used is NIST glass 610 using the nominal values reported by Pearce et al. (1997). The measured trace element concentrations, standard deviations and detection limits are processed from intensities using Iolite 3.7 (Paton et al., 2011) and exported to Microsoft® Excel for final presentation.

3.3 DATA ANALYSIS AND INTERPRETATION

Strontium deposition in otoliths roughly occurs in annual cycles due to the cyclic nature of growth in temperate regions, seasonal patterns in feeding rates, migration, and differences in strontium concentration depending upon where a fish was feeding. Inferences regarding fish movement patterns between fresh and marine water can be made by interpreting patterns of strontium deposition in the fish's otolith. In general, fish that remain in freshwater display pulses of strontium increases that correspond to seasonal feeding bouts, but the pulses tend to be consistent in magnitude throughout the line scan.

A distinct and abrupt increase or decrease in strontium concentration indicates the fish may have moved between environments with distinctly different water chemistries such as between marine and freshwater habitats. This can be standardized by calculating the average and standard deviation of strontium concentrations across the entire scan line. Sections of scan line that are greater than two standard deviations from the mean would indicate a potential migration into a marine environment (Swanson et al. 2010). Results are graphed such that strontium concentration is displayed at increasing distance from the origin of the microprobe scan (i.e., the centre of the otolith). Changes to strontium concentration with increasing age are then identifiable providing a chronological record of habitat use over the entire lifetime of the fish.

4.0 RESULTS

A total of eight fish, ranging in fork length from 242 to 582 mm, from Lake ST-352 were retained for strontium analysis (Table 3). Of these, only one fish (a male) was preparing to spawn in fall 2021. The remainder were all immature or resting.

Seven fish, ranging in length from 452-580 mm, were retained from Lake ST-176 (Table 3). All but one of these fish were preparing to spawn in fall 2021 and the majority were captured in one gillnet set, suggesting a potential nearby spawning location.

Results of strontium analysis from otoliths of Arctic char captured in 2021 are presented in Figure 5. In general, patterns of strontium concentration in the otoliths of sampled fish remained relatively stable over the years and do not indicate anadromy. As well, none of the otoliths measured had sections of strontium scan lines that were greater than two standard deviations from the mean. Despite this, without direct comparisons to strontium concentrations from both fish and ambient water chemistry from the nearest downstream marine habitat, anadromy cannot be completely dismissed. Otoliths from three of the 15 Arctic Char sampled (ARCH 3, 5, 15) showed distinct patterns of strontium increasing at the start of their scan lines (i.e., 0-200 µm) corresponding to the core region of the otoliths. Given this increase is within range of subsequent strontium signatures it likely indicates these fish may have moved within freshwater habitats or received some residual contribution from their mothers during gametogenesis and egg incubation.

Habitat-specific otolith strontium concentrations can be observed between fish caught in different waterbodies. Arctic Char 1-8 captured in Lake ST-352 had an average strontium concentration of 1142.3 parts per million (ppm), while fish 9-15 captured in Lake ST-176 had a lower average strontium concentration of 771.4 ppm. This may indicate differences in ambient strontium concentration between the two lakes, although water chemistry information from both lakes would be required for confirmation. It also appears that fish captured in Lake ST-176 had smaller fluctuations in strontium concentration within annuli suggesting these fish likely remained in the same waterbody for the majority of their life cycle. The relatively larger fluctuations in strontium signatures observed in otoliths sampled from fish in Lake ST-352 could indicate greater seasonal differences in ambient strontium concentrations or that fish are making regular periodic movements between different waterbodies or between habitats within the lake.

For context, an example of a strontium pattern from an anadromous char from Swanson et al. (2010) is presented in Figure 6. Strontium concentrations measured in surface water from Lake ST-352 and Steensby Inlet during baseline field studies provide further evidence that fish analysed from Lakes ST-352 and ST-176 were not anadromous. The mean concentration measured in Lake ST-352 in 2008 (mean of 0.000321 mg/L from two samples collected in 2008) was three orders of magnitude lower than the mean concentration measured in Steensby Inlet (means ranged from 6.18-7.57 mg/L over four sampling periods from 2007-2008; Bafflinland unpublished data). Differences in ambient strontium concentrations between the marine and freshwater environment of this magnitude would result is clear patterns of strontium increases in otoliths of anadromous char.

5.0 LITERATURE CITED

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Table 1. Steensby Inlet Port Site gillnet set information, fall 2021.

Lake ID	Site ID	Date	Gill Net Start				Gill Net End				Set Time	Pull Time	Set Duration
			Easting	Northing	Depth	Mesh	Easting	Northing	Depth	Mesh			
ST-352	GN-01	06-Sep-21	601011	7806602	10	1.5	600981	7806477	8.7	5	10:20	14:10	3:50
ST-352	GN-02	06-Sep-21	599312	7808078	11	5	599445	7807963	9.8	1.5	10:46	14:22	3:36
ST-352	GN-03	06-Sep-21	598977	7808386	8.5	1.5	599018	7808490	19	5	12:10	15:45	3:35
ST-352	GN-04	08-Sep-21	601630	7808072	2.5	1.5	601911	7807959	3.8	5	9:40	13:40	4:00
ST-352	GN-05	08-Sep-21	599783	7808923	3.9	5	599793	7809052	5.2	1.5	10:00	14:10	4:10
ST-352	GN-06	08-Sep-21	599028	7808327	5.7	5	598921	7808381	3.8	1.5	10:15	14:25	4:10
ST-176	GN-07	09-Sep-21	598388	7804341	2.1	5	598455	7804439	4	1.5	9:01	13:10	4:09
ST-176	GN-08	09-Sep-21	598883	7804669	2.9	5	598689	7804644	6	1.5	9:10	13:32	4:22
ST-176	GN-09	09-Sep-21	599252	7803472	4.1	5	599168	7803384	2.7	1.5	9:16	13:51	4:35

Table 2. Instrument parameters used to ablate arctic char otoliths.

Instrument Parameter	Unit	Value/Description
Plasma Power	W	1235
Cool gas	L/min	15.5
Auxiliary gas	L/min	0.82
Sample gas	L/min	0.85
Carrier gas, He	L/min	0.86
Oxide (ThO/Th) formation	%	0.2
Wash time	s	30
Beam size	µm	30
Repetition rate	Hz	5
Fluence	J/cm^2	~4
Standard reference material	Glass	NIST 610
Internal standard	Weight %	Ca (40.04)

Table 3. Fork length (mm), weight (g) and spawning status of Arctic Char from Steensby Inlet lakes sampled for strontium analyses in fall 2021.

Waterbody	Field Site No.	Check Date	Fish Species	Fish No.	Fork Length (mm)	Weight (g)	Sex	Spawning in Fall 2021
ST-352	GN-02	06-Sep-21	ARCH	1	483	930	M	No
ST-352	GN-02	06-Sep-21	ARCH	2	540	1410	F	No
ST-352	GN-03	06-Sep-21	ARCH	3	417	600	F	No
ST-352	GN-03	06-Sep-21	ARCH	4	285	190	M	No
ST-352	GN-03	06-Sep-21	ARCH	5	242	200	F	No
ST-352	GN-05	08-Sep-21	ARCH	6	314	280	F	No
ST-352	GN-06	08-Sep-21	ARCH	7	301	220	M	No
ST-352	GN-06	08-Sep-21	ARCH	8	582	1810	M	Yes
ST-176	GN-07	09-Sep-21	ARCH	9	542	1730	M	Yes
ST-176	GN-07	09-Sep-21	ARCH	10	500	1350	M	Yes
ST-176	GN-07	09-Sep-21	ARCH	11	502	1060	F	Yes
ST-176	GN-07	09-Sep-21	ARCH	12	486	1040	F	Yes
ST-176	GN-07	09-Sep-21	ARCH	13	580	1570	M	No
ST-176	GN-07	09-Sep-21	ARCH	14	514	1190	F	Yes
ST-176	GN-09	09-Sep-21	ARCH	15	452	1030	F	Yes

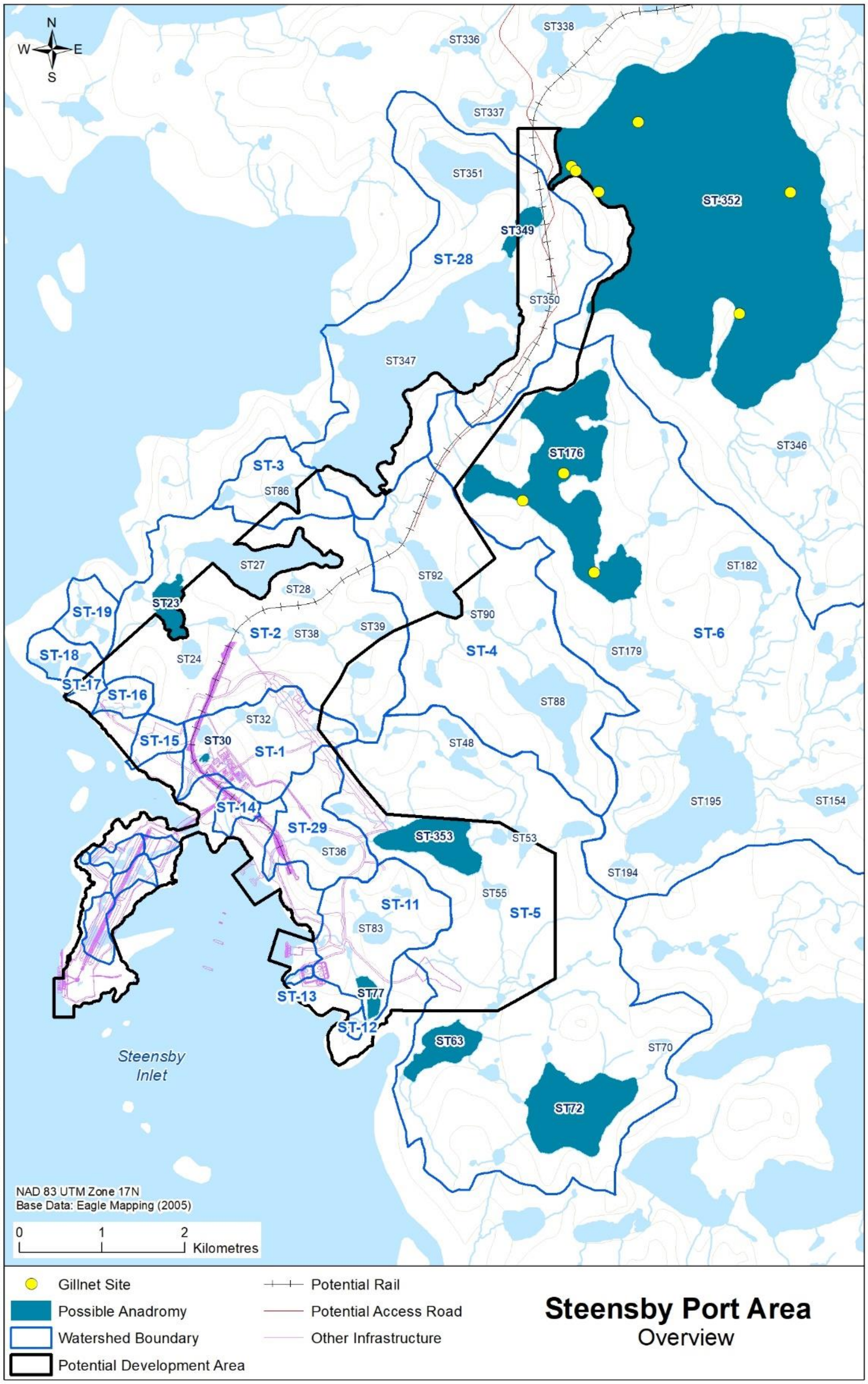

Figure 1. Map of Steensby Inlet Port Site lakes. Lakes in dark blue indicate potential for anadromous populations of Arctic Char.

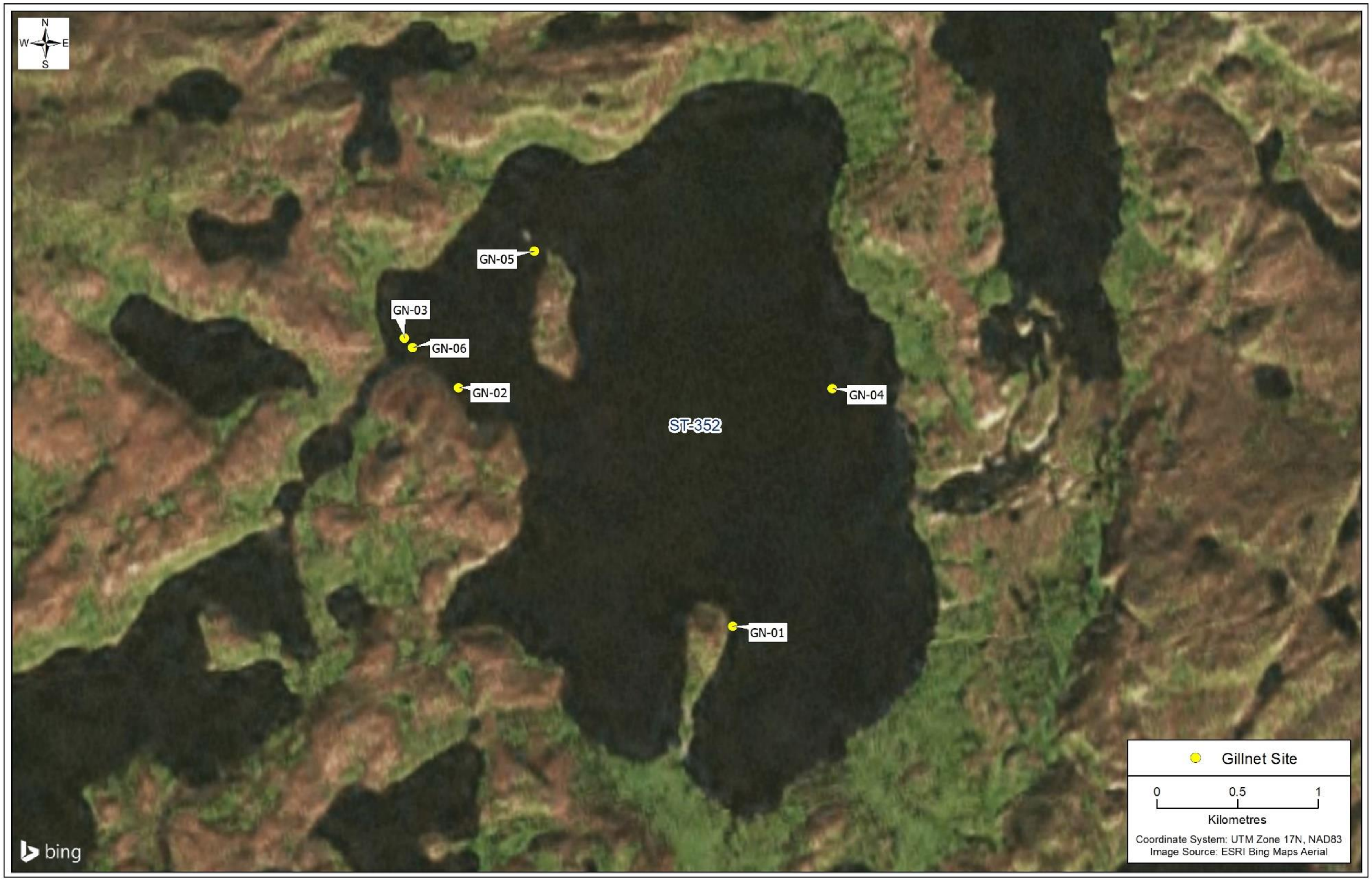

Figure 2. Gillnet set locations in Lake ST-352, fall 2021.

Figure 3. Gillnet set locations in Lake ST-176, fall 2021.

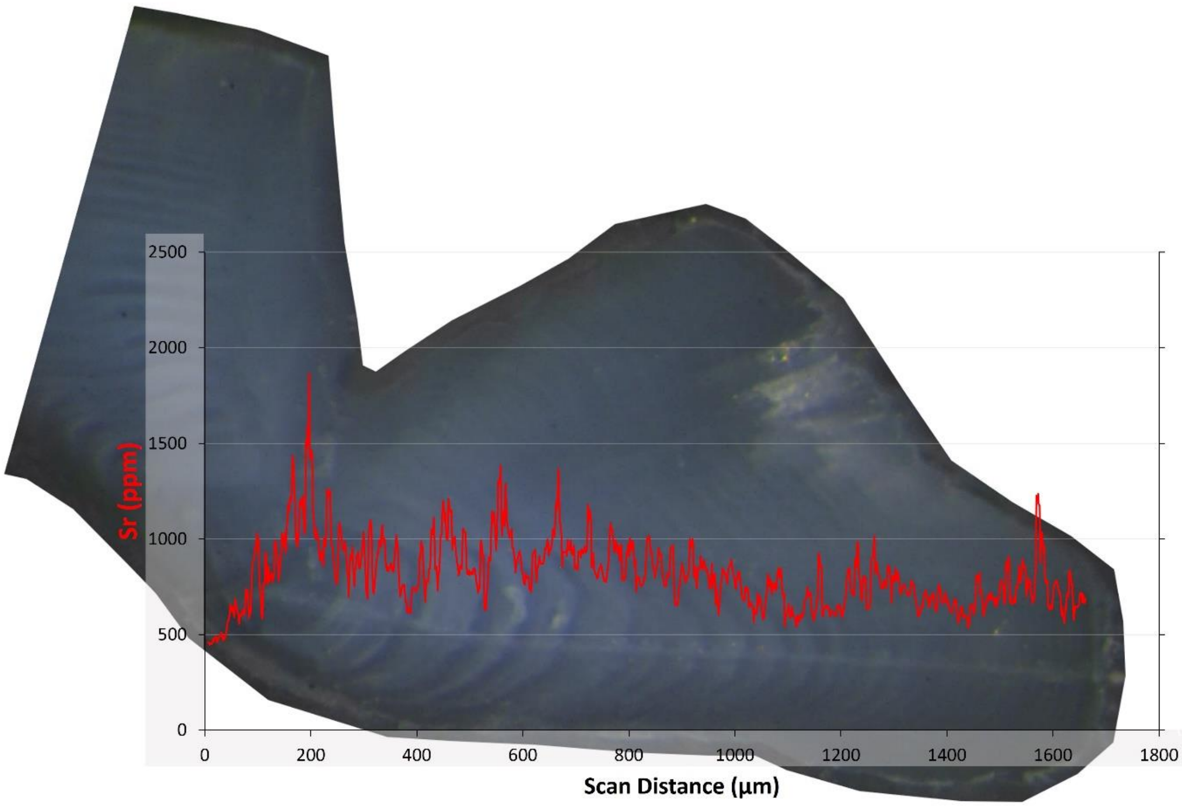

Figure 4. Example of strontium scan line (ppm) over scan distance (µm) overlayed on image of sectioned Arctic Char otolith. Identification of annuli positioning on scan line provides chronological record of changes in strontium concentration over time.

Figure 5 - A. Strontium concentrations (ppm) of Arctic Char captured in Lakes ST-352 (ARCH 1-8) and ST-176 (ARCH 9-15) in 2021. Numbers on graphs correspond to annuli positions estimated during visual examination of scan overlays. Blue horizontal arrows represent sections of otolith where annuli were too compact to distinguish. Shaded red areas should be disregarded as laser ablated over cracked section of otolith.

Figure 5 - B. Strontium concentrations (ppm) of Arctic Char captured in Lakes ST-352 (ARCH 1-8) and ST-176 (ARCH 9-15) in 2021. Numbers on graphs correspond to annuli positions estimated during visual examination of scan overlays. Blue horizontal arrows represent sections of otolith where annuli were too compact to distinguish. Shaded red areas should be disregarded as laser ablated over cracked section of otolith.

Figure 5 - C. Strontium concentrations (ppm) of Arctic Char captured in *Lakes* ST-352 (ARCH 1-8) and ST-176 (ARCH 9-15) in 2021. Numbers on graphs correspond to annuli positions estimated during visual examination of scan overlays. Blue horizontal arrows represent sections of otolith where annuli were too compact to distinguish. Shaded red areas should be disregarded as laser ablated over cracked section of otolith.

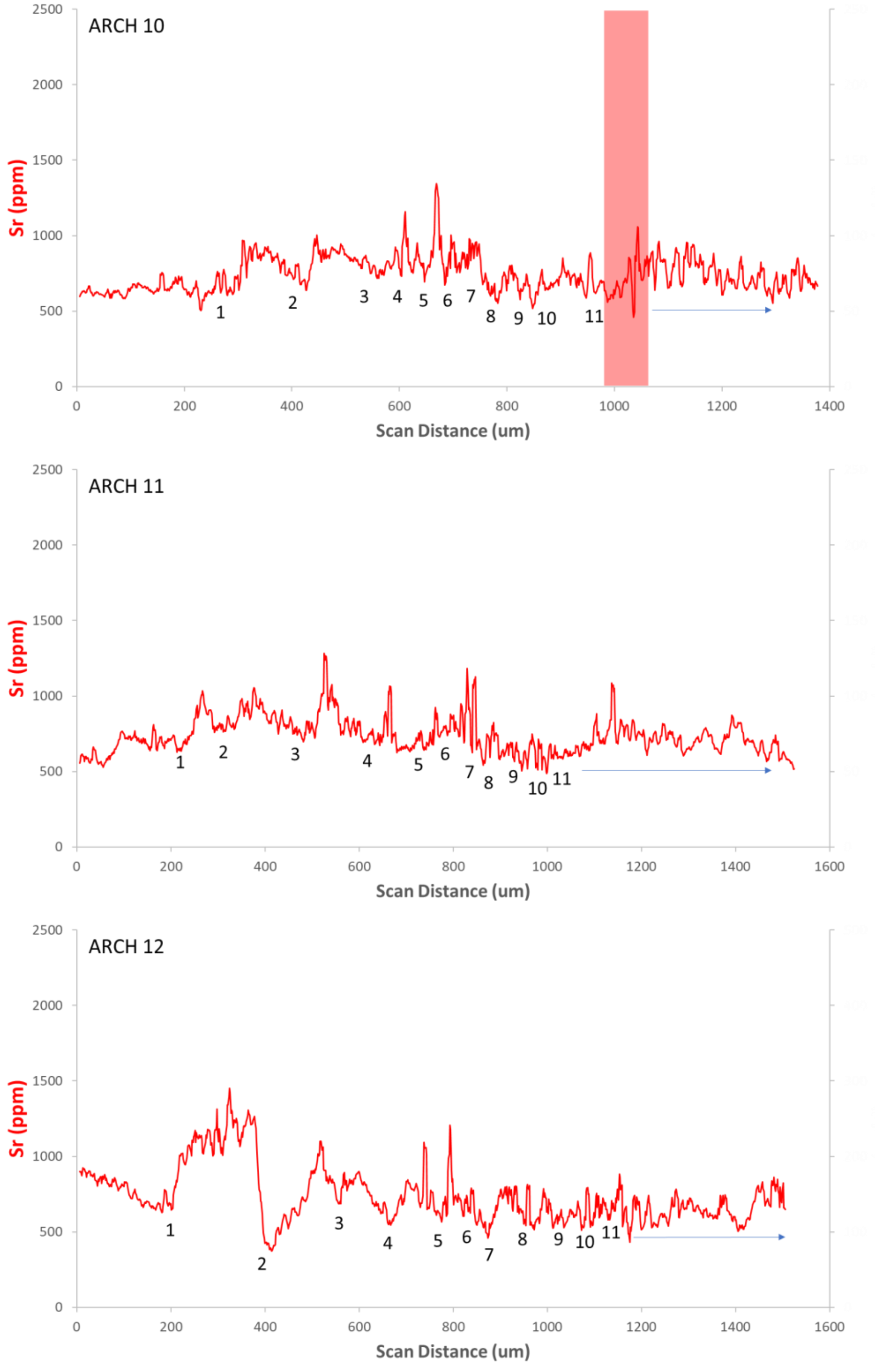

Figure 5 - D. Strontium concentrations (ppm) of Arctic Char captured in Lakes ST-352 (ARCH 1-8) and ST-176 (ARCH 9-15) in 2021. Numbers on graphs correspond to annuli positions estimated during visual examination of scan overlays. Blue horizontal arrows represent sections of otolith where annuli were too compact to distinguish. Shaded red areas should be disregarded as laser ablated over cracked section of otolith.

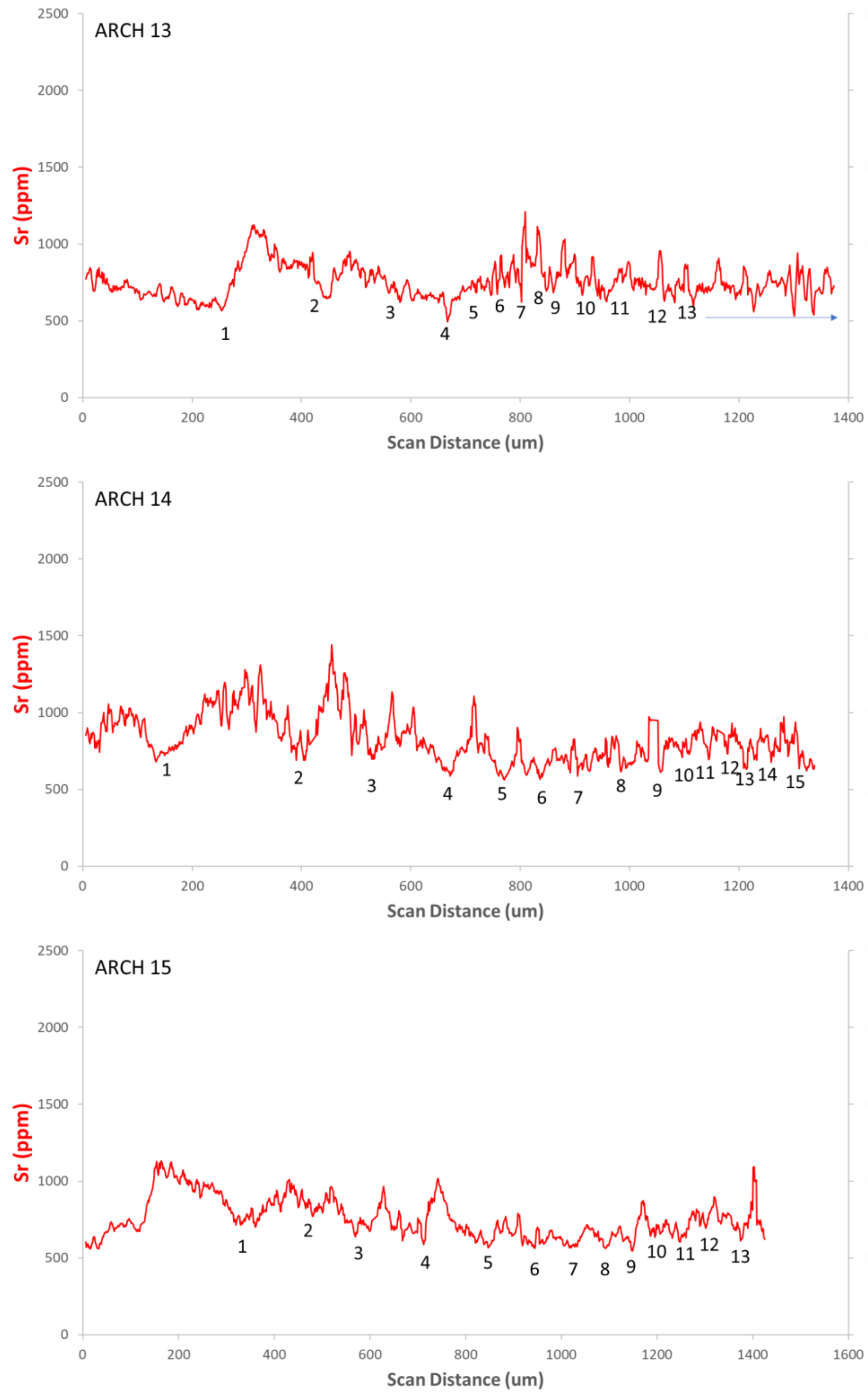

Figure 5 - E. Strontium concentrations (ppm) of Arctic Char captured in Lakes ST-352 (ARCH 1-8) and ST-176 (ARCH 9-15) in 2021. Numbers on graphs correspond to annuli positions estimated during visual examination of scan overlays. Blue horizontal arrows represent sections of otolith where annuli were too compact to distinguish. Shaded red areas should be disregarded as laser ablated over cracked section of otolith.

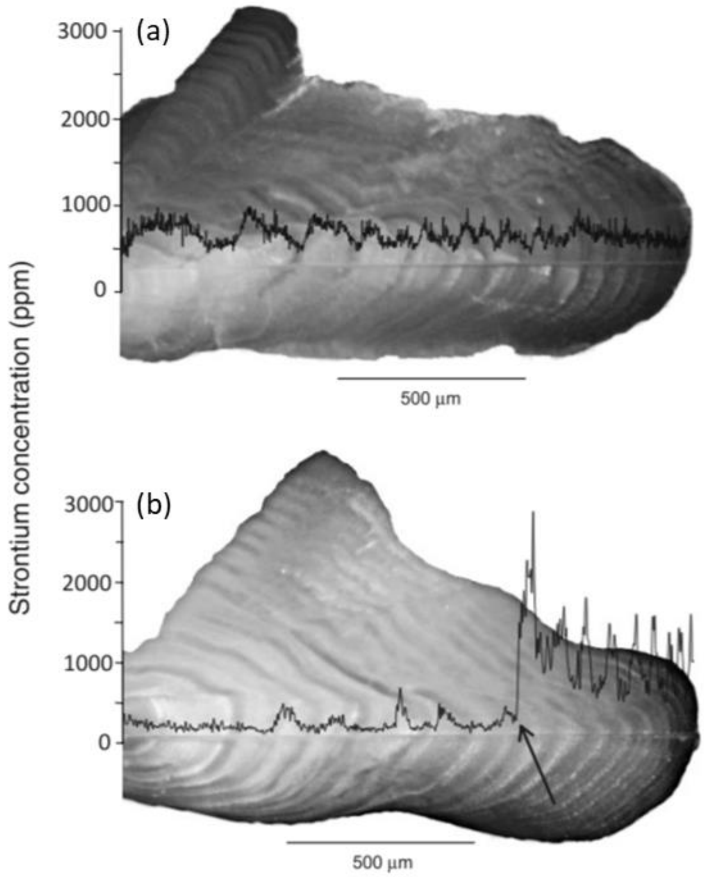

Figure 6. Example of strontium scan lines from (a) a resident Arctic Char and (b) an anadromous Arctic Char otolith from Swanson et al. (2010). Note the strontium profile for the resident fish (a) resembles that of fish from the current study where strontium concentration is relatively flat with low amplitude and annual oscillations across the entire otolith. The black arrow on the anadromous fish (b) indicates first migration into marine waters as indicated by a sharp increase in strontium followed by well-defined, high amplitude oscillations.