



Detecting and tracing farmed salmon with natural otolith 'fingerprint' tags



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OTOLITHS AS DATA LOGGERS

Formed during embryogenesis



Grow continuously



Metabolically inert



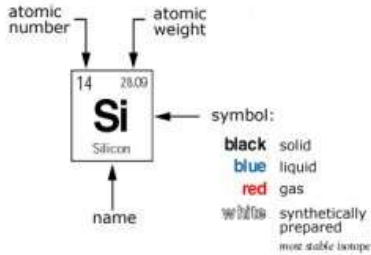
Incorporate impurities into the matrix

COMMON ENVIRONMENTAL MARKERS



Periodic Table of the Elements

1 1.01 H Hydrogen																	2 4.003 He Helium	
3 6.94 Li Lithium	4 9.01 Be Beryllium																	10 20.18 Ne Neon
11 22.99 Na Sodium	12 24.31 Mg Magnesium																	18 39.96 Ar Argon
19 39.10 K Potassium	20 40.08 Ca Calcium	21 44.96 Sc Scandium	22 47.88 Ti Titanium	23 50.94 V Vanadium	24 51.996 Cr Chromium	25 54.94 Mn Manganese	26 55.85 Fe Iron	27 58.93 Co Cobalt	28 58.70 Ni Nickel	29 63.55 Cu Copper	30 65.37 Zn Zinc	31 69.72 Ga Gallium	32 72.59 Ge Germanium	33 74.92 As Arsenic	34 78.96 Se Selenium	35 79.90 Br Bromine	36 83.80 Kr Krypton	
37 85.47 Rb Rubidium	38 87.62 Sr Strontium	39 88.91 Y Yttrium	40 91.22 Zr Zirconium	41 92.91 Nb Niobium	42 95.94 Mo Molybdenum	43 (98) Tc Technetium	44 101.07 Ru Ruthenium	45 102.91 Rh Rhodium	46 106.40 Pd Palladium	47 107.87 Ag Silver	48 112.41 Cd Cadmium	49 114.82 In Indium	50 118.69 Sn Tin	51 121.73 Sb Antimony	52 127.60 Te Tellurium	53 126.90 I Iodine	54 131.30 Xe Xenon	
55 132.91 Cs Cesium	56 137.33 Ba Barium	57 138.91 La Lanthanum	72 178.49 Hf Hafnium	73 180.95 Ta Tantalum	74 183.85 W Tungsten	75 186.21 Re Rhenium	76 186.21 Os Osmium	77 192.22 Ir Iridium	78 196.09 Pt Platinum	79 196.97 Au Gold	80 200.59 Hg Mercury	81 204.37 Tl Thallium	82 207.19 Pb Lead	83 208.98 Bi Bismuth	84 (209) Po Polonium	85 (210) At Astatine	86 (222) Rn Radon	
87 (223) Fr Francium	88 226.03 Ra Radium	89 227.03 Ac Actinium	104 (261) Rf Rutherfordium	105 (262) Ha Hahnium	106 (266) Sg Seaborgium	107 (262) Bh Bohrium	108 (265) Hs Hassium	109 (266) Mt Meitnerium	110 (271) Ds Darmstadtium	111 (272) Rg Roentgenium	112 (277) Cn Copernicium	(113) Nh Nihonium	(114) Fl Flerovium	(115) Mc Moscovium	(116) Lv Livermorium	(117) Ts Tennessine	118 (294) Og Oganesson	
58 140.12 Ce Cerium	59 140.91 Pr Praseodymium	60 144.24 Nd Neodymium	61 (143) Pm Promethium	62 150.40 Sm Samarium	63 151.96 Eu Europium	64 157.25 Gd Gadolinium	65 158.93 Tb Terbium	66 162.90 Dy Dysprosium	67 164.93 Ho Holmium	68 167.26 Er Erbium	69 168.93 Tm Thulium	70 173.04 Yb Ytterbium	71 174.97 Lu Lutetium					
90 232.04 Th Thorium	91 231.04 Pa Protactinium	92 238.03 U Uranium	93 237.05 Np Neptunium	94 (244) Pu Plutonium	95 (243) Am Americium	96 (247) Cm Curium	97 (247) Bk Berkelium	98 (251) Cf Californium	99 (252) Es Einsteinium	100 (257) Fm Fermium	101 (260) Md Mendelevium	102 (259) No Nobelium	103 (262) Lr Lawrencium					



- alkali metals
 - alkaline earth metals
 - transitional metals
 - other metals
 - nonmetals
 - noble gases
- black solid
blue liquid
red gas
white synthetically prepared
more stable isotope

Stable isotopes of Barium

Isotope	Frequency	Mass
¹³⁰ Ba	0,106%	129,906320811
¹³² Ba	0,101%	131,905061288
¹³⁴ Ba	2,417%	133,904508383
¹³⁵ Ba	6,592%	134,905688591
¹³⁶ Ba	7,854%	135,904575945
¹³⁷ Ba	11,232%	136,905827384
¹³⁸ Ba	71,698%	137,905247237

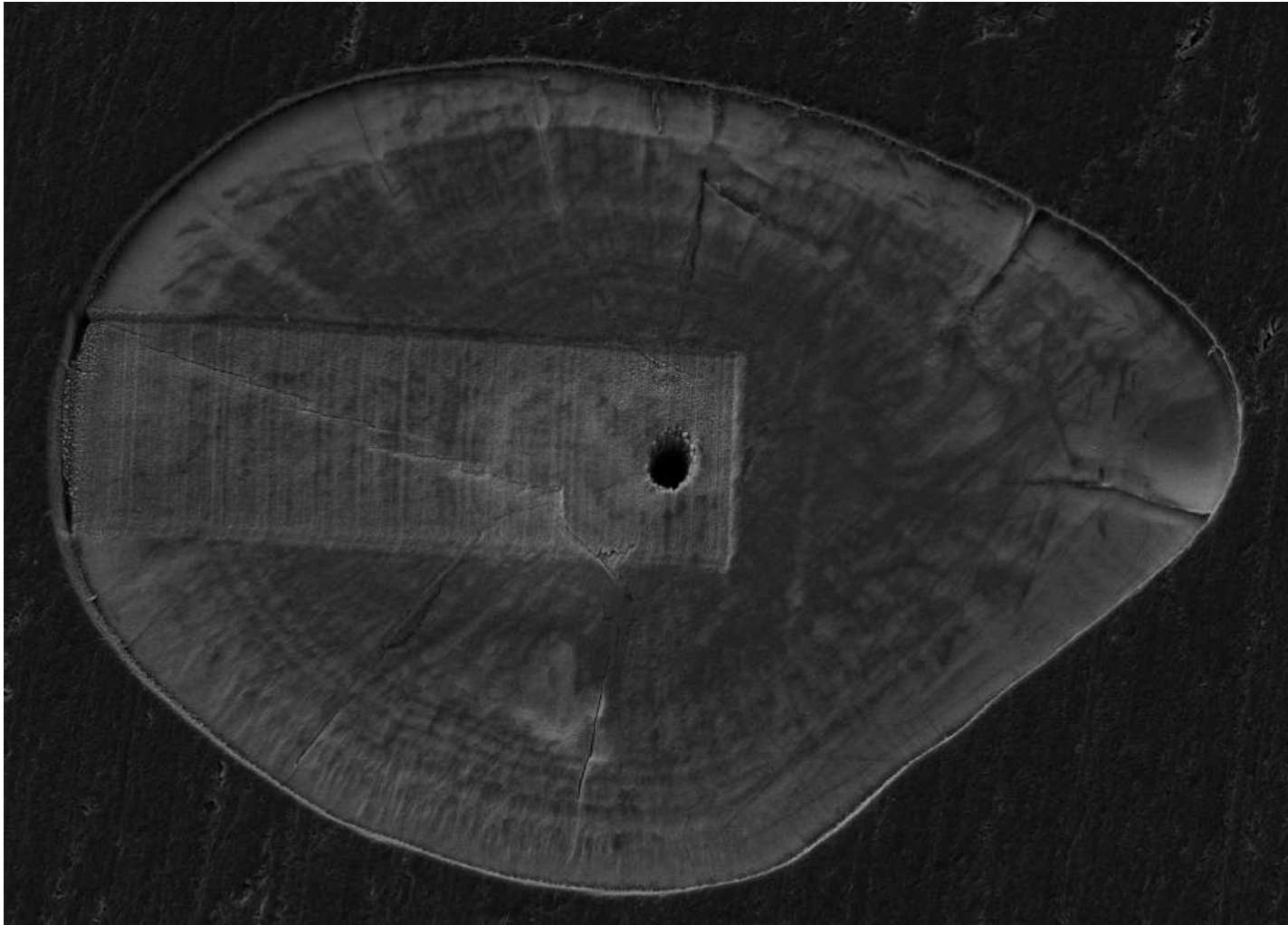
BENEFITS OF ENRICHED STABLE ISOTOPES

- Naturally occurring
- Ratios are largely invariant in nature
- Low concentrations required to change the ratio
- Reliability requires a good baseline

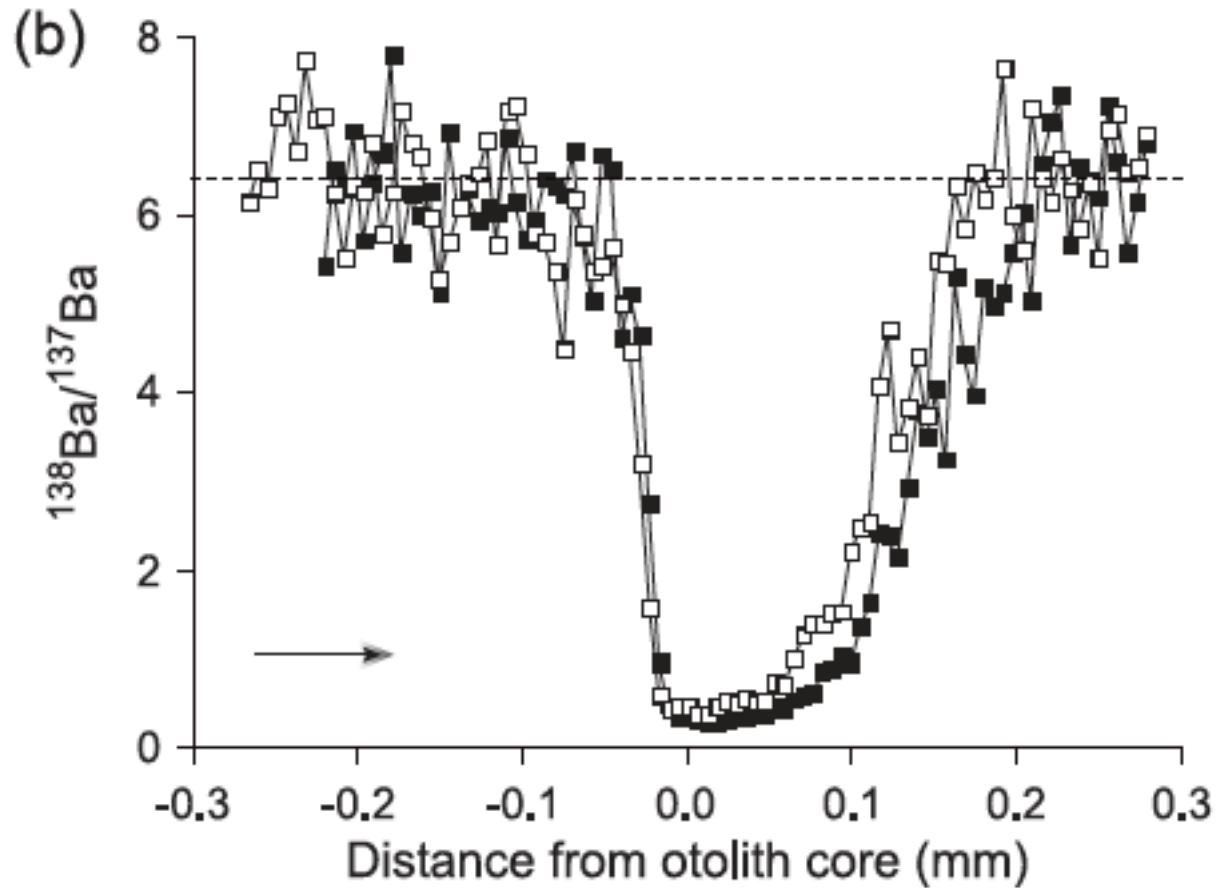
LA-ICPMS ANALYSIS OF FISH OTOLITHS



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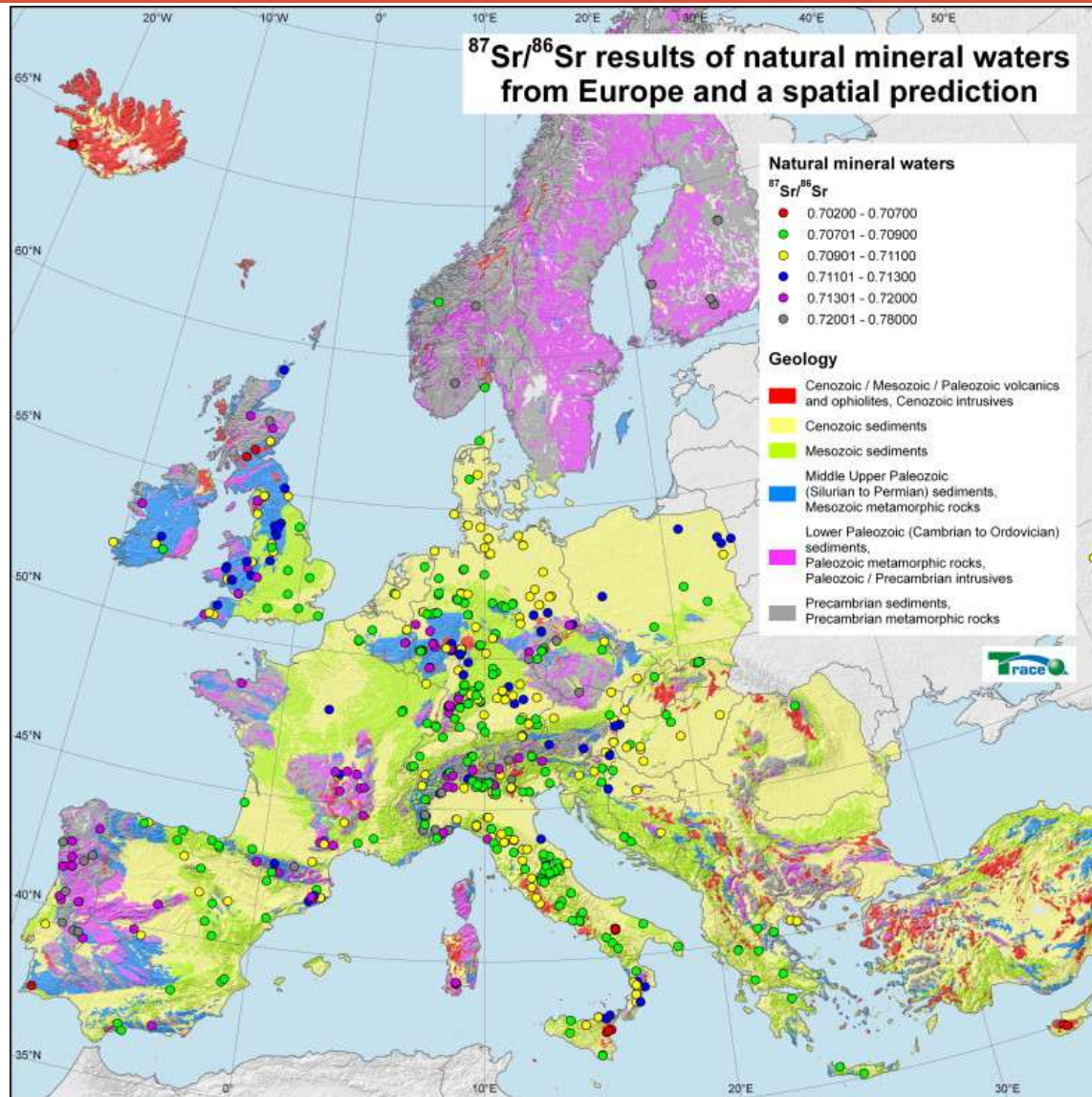
DETECTING ISOTOPIC TAGS



BUILDING A BASELINE

- Natural levels of isotopes vary due to underlying geology

BUILDING A BASELINE



BUILDING A BASELINE

- Establish natural ratios of Ba, Sr & Mg isotopes throughout Norwegian salmon populations
- To ensure our marker concentrations are correct
- Spatially: 23 rivers from north to south
- Temporally: 2 rivers with long-term collections to assess isotope variations through time
- Collections from IMR & NINA

Three Techniques

- **Vaccination:** Injection of stable isotope via a vaccine into parr
- **Maternal Transfer:** Injecting stable isotope solution into female brood stock
- **Egg Induction:** Immersion of freshly fertilised eggs in a stable isotope solution

Maternal transfer – Experimental design

4 Treatments plus a control using a septuple isotope combination. (^{86}Sr , ^{87}Sr , ^{134}Ba , ^{135}Ba , ^{136}Ba , ^{137}Ba & ^{26}Mg)

- T1: 2 μg per g female weight (n=6)
- T2: 0.2 μg per g female weight (n=6)
- T3: 0.02 μg per g female weight (n=6)
- T4: 0.002 μg per g female weight (n=6)
- C1: Control – saline solution (n=6)

Otoliths for analysing isotope fingerprint signatures will be collected from offspring at hatching, and at maturity.

Mortality, growth and condition will be monitored over the time frame of the experiment. X-rays to be taken at maturity to check for skeletal deformities

Egg induction

Egg induction has not been successfully tested before, however immersion of larvae, and juveniles has been shown to be successful in some cases.

Immersion of Murray cod larvae (*Maccullochella peelii*) in lab experiments using ^{137}Ba , ^{138}Ba & ^{88}Sr (Woodcock *et al.* 2011)

- Important things to consider
- 1) Immersion time
- 2) Concentration
- 3) Side-effects



Egg induction – Experimental design

4 Treatments plus a control using a septuple isotope combination (^{86}Sr , ^{87}Sr , ^{134}Ba , ^{135}Ba , ^{136}Ba , ^{137}Ba & ^{26}Mg)

T1: Ba at 1000 μg per litre, Sr & Mg at 2500 μg per litre

T2: Ba at 100 μg per litre, Sr & Mg at 250 μg per litre

T3: Ba at 10 μg per litre, Sr & Mg at 25 μg per litre

T4: Ba at 1 μg per litre, Sr & Mg at 2.5 μg per litre

T5: 10 Rare earth elements at 1000 μg per litre each

3 batches of eggs per treatment (1000 eggs per batch)

Otoliths for analysing isotope fingerprint signatures will be collected from offspring at hatching, and at maturity.

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The cost to mark one years production supply

Costs comparisons of the different methods to mark one year's salmon production (500 million fish) using a **septuplet** isotope marker

Concentration gradient	2 µg	0.2 µg	0.02 µg	0.002 µg
Vaccination - µg per gram Parr	kr 18,330,900,000	kr 1,833,090,000	kr 183,309,000	kr 18,330,900
Maternal Transfer- µg per gram broodfish	kr 523,740,000	kr 52,374,000	kr 5,237,400	kr 523,740
Egg Imersion - ug per litre of immersion solution	kr 1,371,240,000	kr 137,124,000	kr 13,712,400	kr 1,371,240

Costs comparisons of the different methods to mark one year's salmon production (500 million fish) using a **quadruple** isotope marker

Concentration gradient	2 µg	0.2 µg	0.02 µg	0.002 µg
Vaccination - µg per gram Parr	kr 10,474,800,000	kr 1,047,480,000	kr 104,748,000	kr 10,474,800
Maternal Transfer- µg per gram broodfish	kr 299,280,000	kr 29,928,000	kr 2,992,800	kr 299,280
Egg Imersion - ug per litre of immersion solution	kr 783,565,714	kr 78,356,571	kr 7,835,657	kr 783,566

Costs comparisons of the different methods to mark one year's salmon production (500 million fish) using a **single** isotope marker

Concentration gradient	2 µg	0.2 µg	0.02 µg	0.002 µg
Vaccination - µg per gram Parr	kr 2,618,700,000	kr 261,870,000	kr 26,187,000	kr 2,618,700
Maternal Transfer- µg per gram broodfish	kr 74,820,000	kr 7,482,000	kr 748,200	kr 74,820
Egg Imersion - ug per litre of immersion solution	kr 195,891,429	kr 19,589,143	kr 1,958,914	kr 195,891

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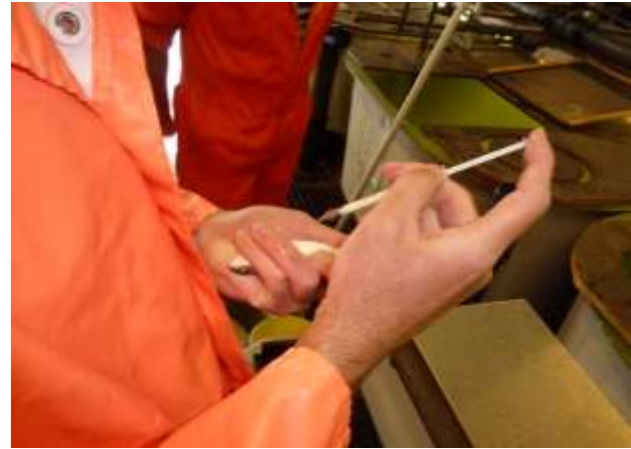
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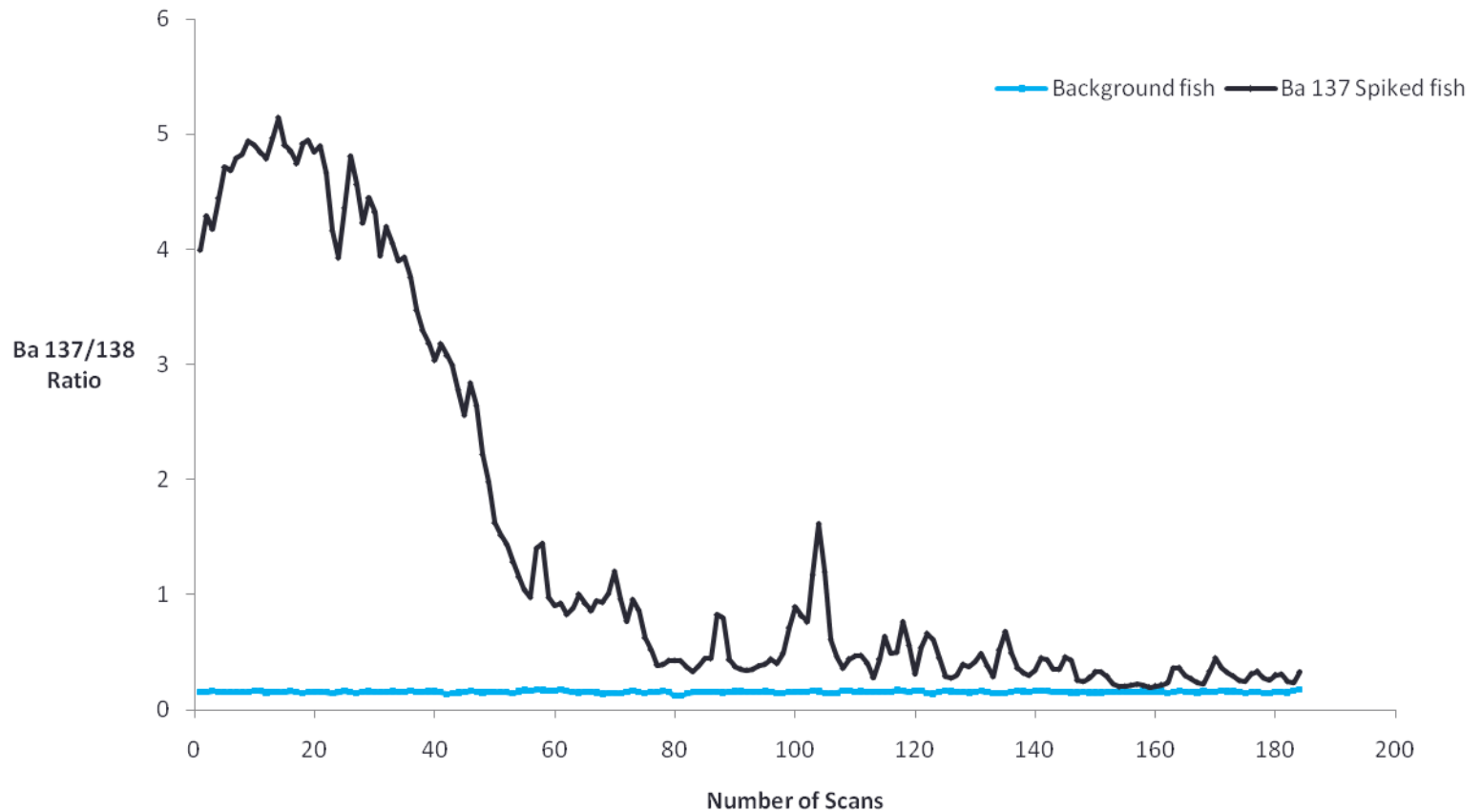
Vaccination Pilot Results

- 144 fish Vaccinated in August 2012
- First sample taken 2 weeks after vaccination
- Otoliths from 72 fish analysed using laser ablation (6 fish per treatment)



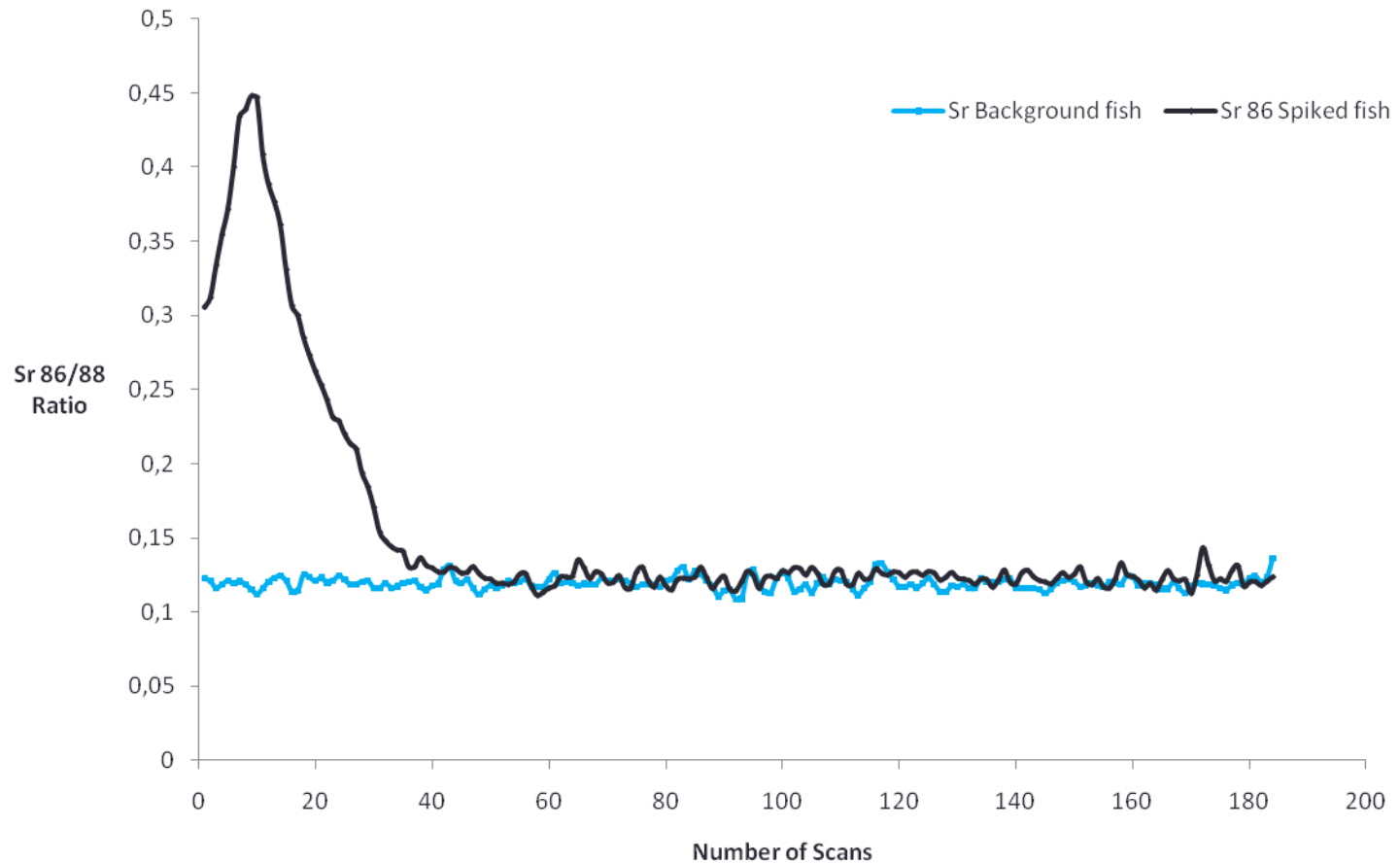
Marking successful with Barium 137

Barium 137/138



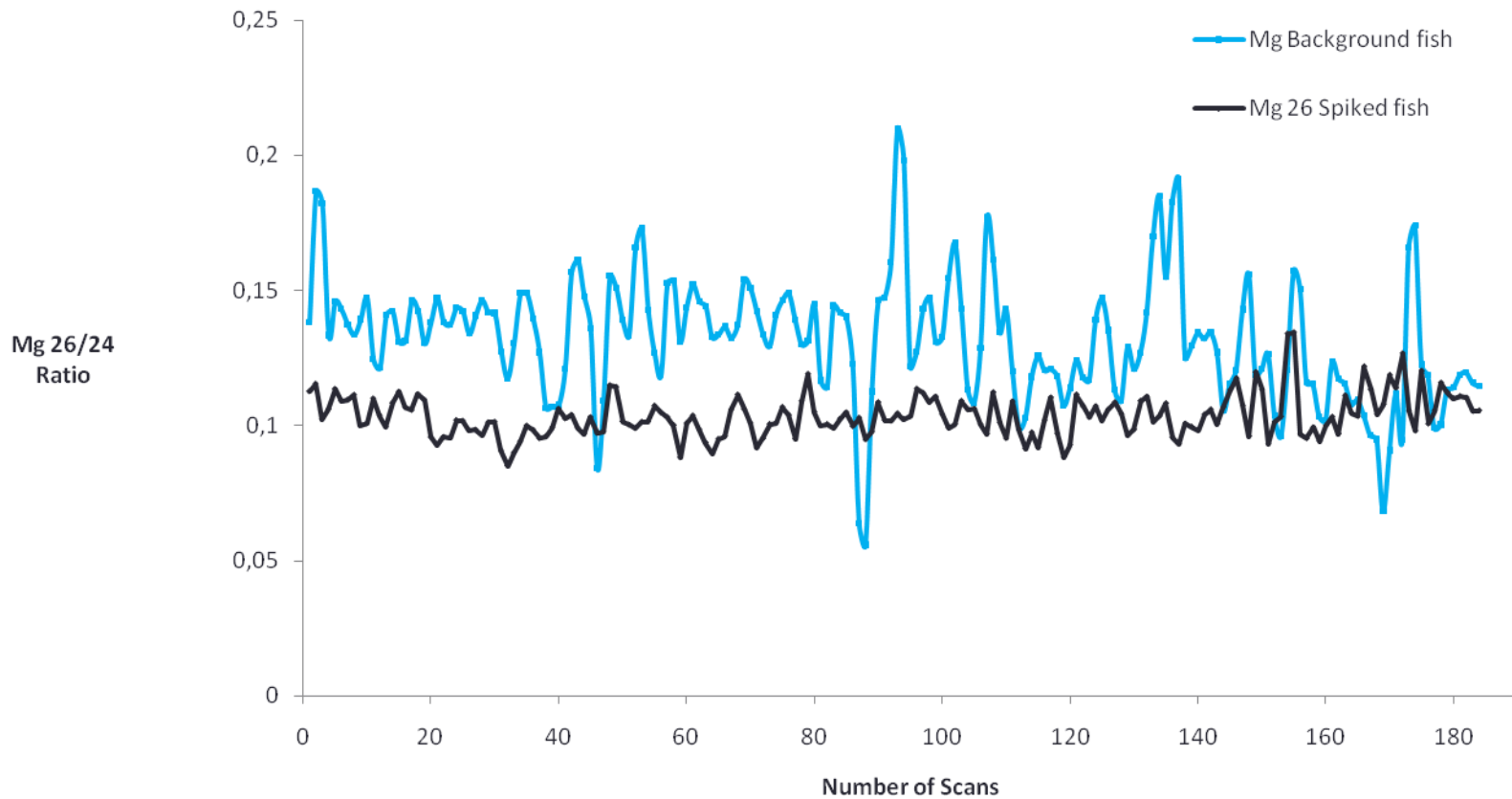
Mark successful with Strontium 86

Strontium 86/88



Mark not successful with Magnesium 26

Magnesium 26/24



Progress - Vaccination

- Full vaccination experiment started 6th October 2012
- 650 fish divided into 12 concentrations/combinations plus a control group
- First otolith samples to be taken February 2013



Progress - Maternal Transfer

- Maternal Transfer experiment started 26th October 2012
- 30 brood stock divided into 4 concentrations plus a control group
- 27 females spawned so far
- First otolith samples to be taken March 2013



Progress - Egg Immersion

- Egg immersion experiment started 1st November 2012
- 5 concentrations plus a control group
(18000 fertilised eggs in total)
- First otolith samples to be taken March 2013

