



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



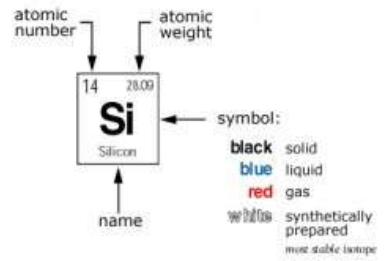
Incorporate impurities into the matrix

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 47.87 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 | 76 190.20 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) (289) Lv Livermorium | (117) Ts Tennessine | 118 (291) Og Oganesson | |



- alkali metals
- alkaline earth metals
- transitional metals
- other metals
- nonmetals
- noble gases

| | | | | | | | | | | | | | |
|--------------------------------------|---|--|--|---------------------------------------|---------------------------------------|---|---------------------------------------|---|---|--------------------------------------|--|--|---|
| 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 |

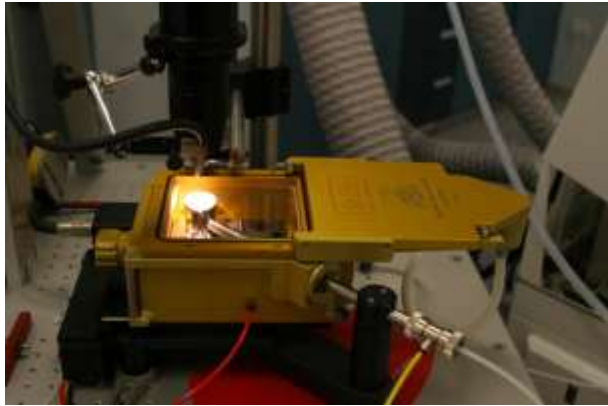
Stable isotopes of Barium

| 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 |

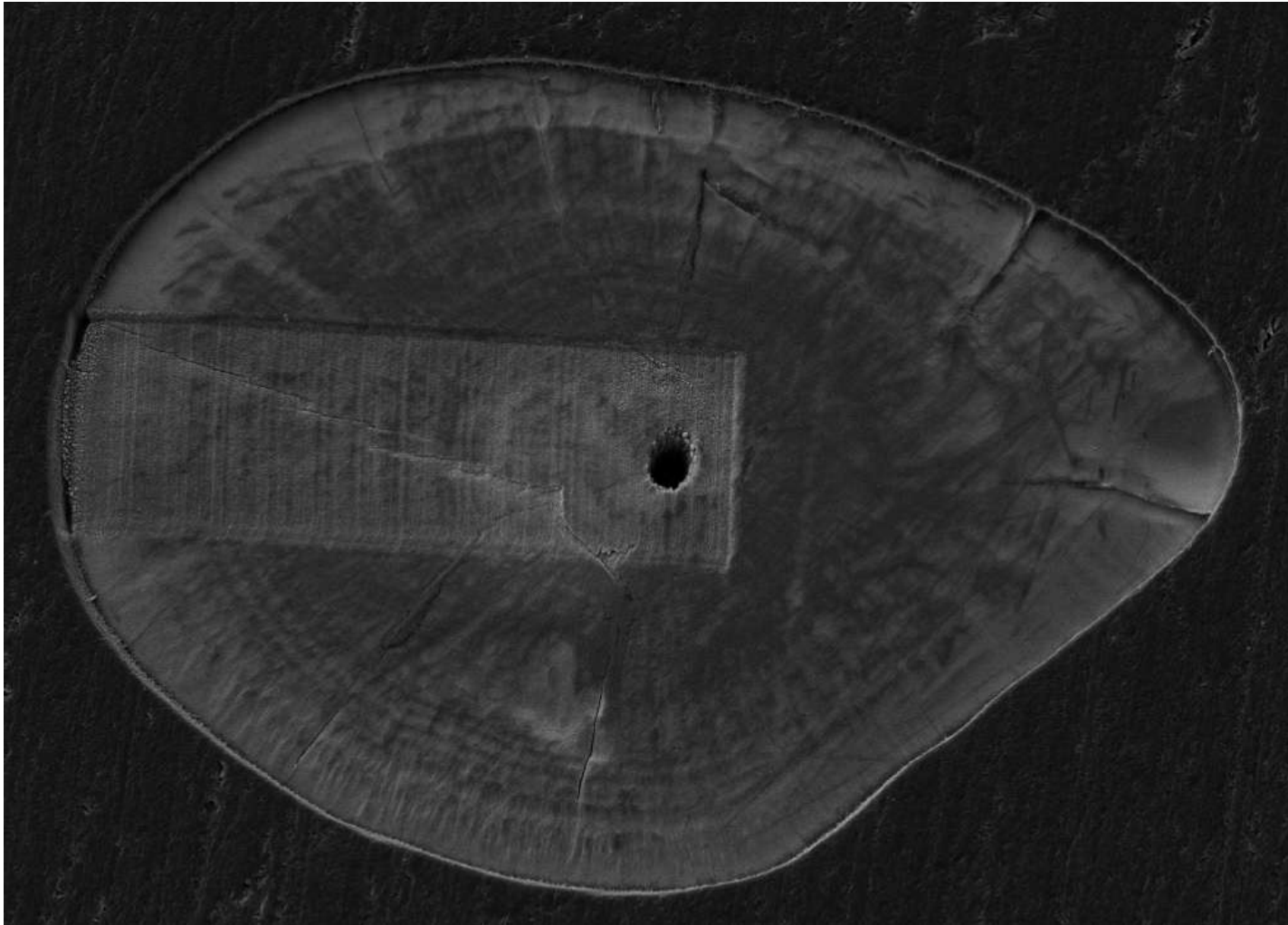
BENEFITS OF ENRICHED STABLE ISOTOPES

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

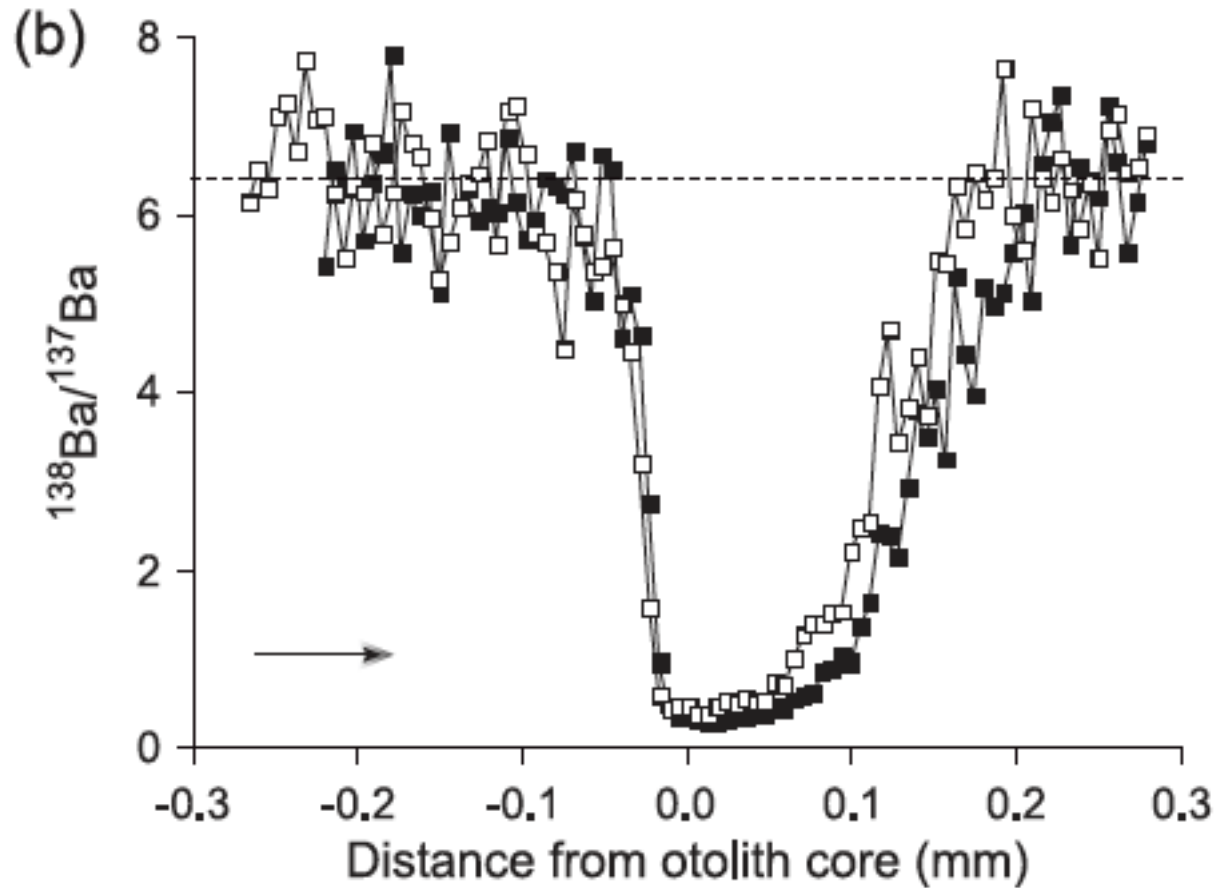
LA-ICPMS ANALYSIS OF FISH OTOLITHS



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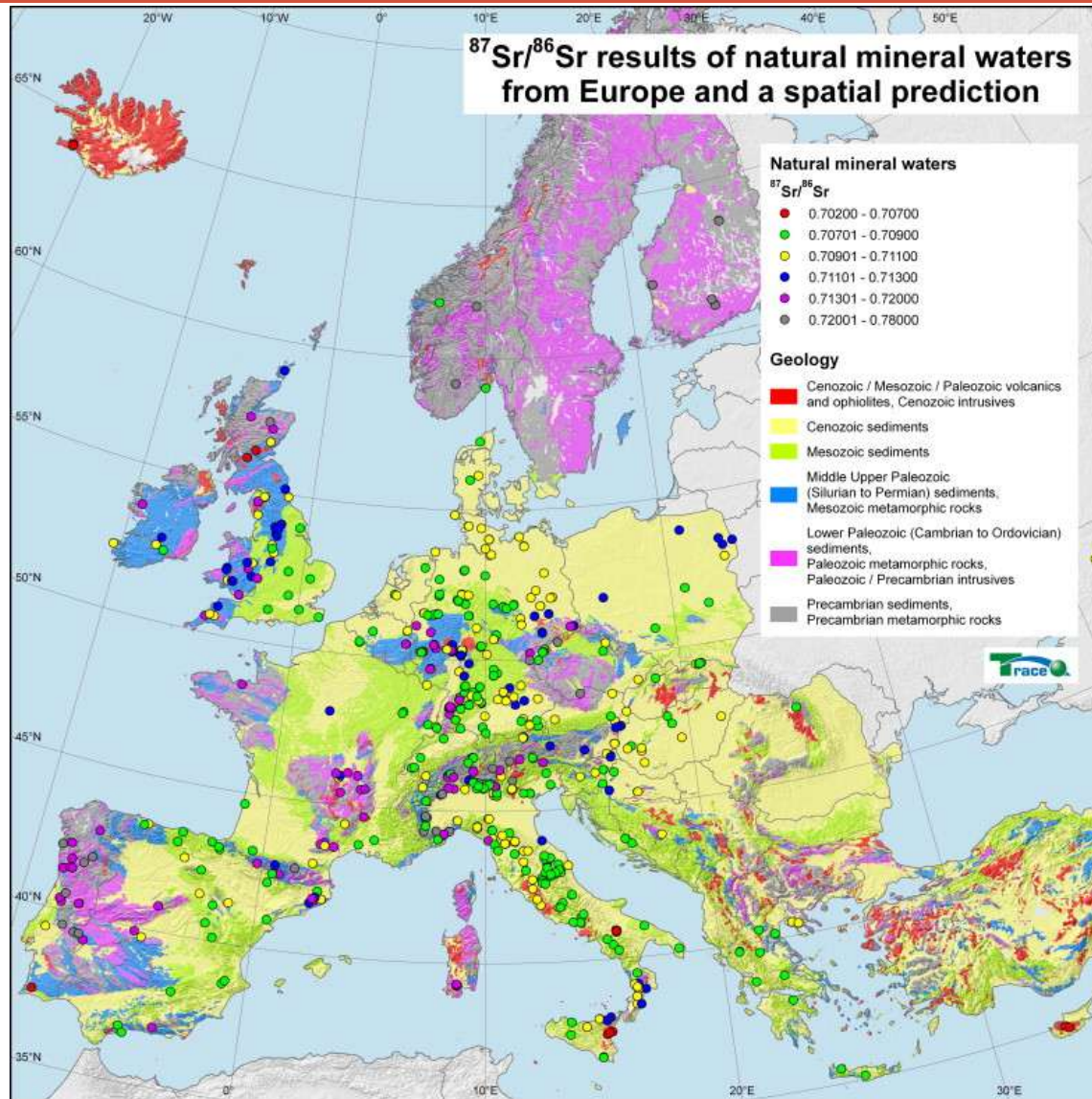
DETECTING ISOTOPIC TAGS



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.

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

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 |

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

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

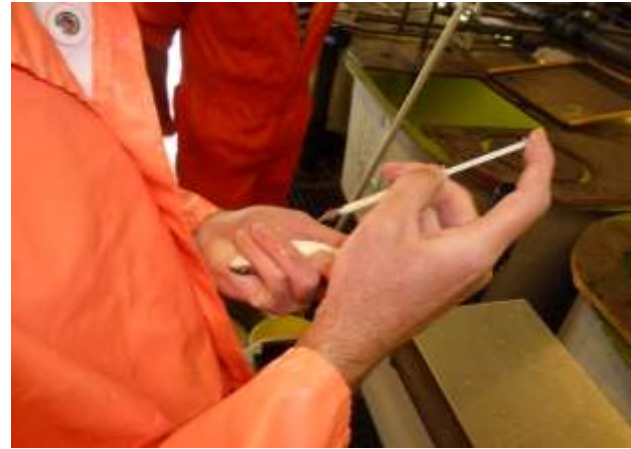
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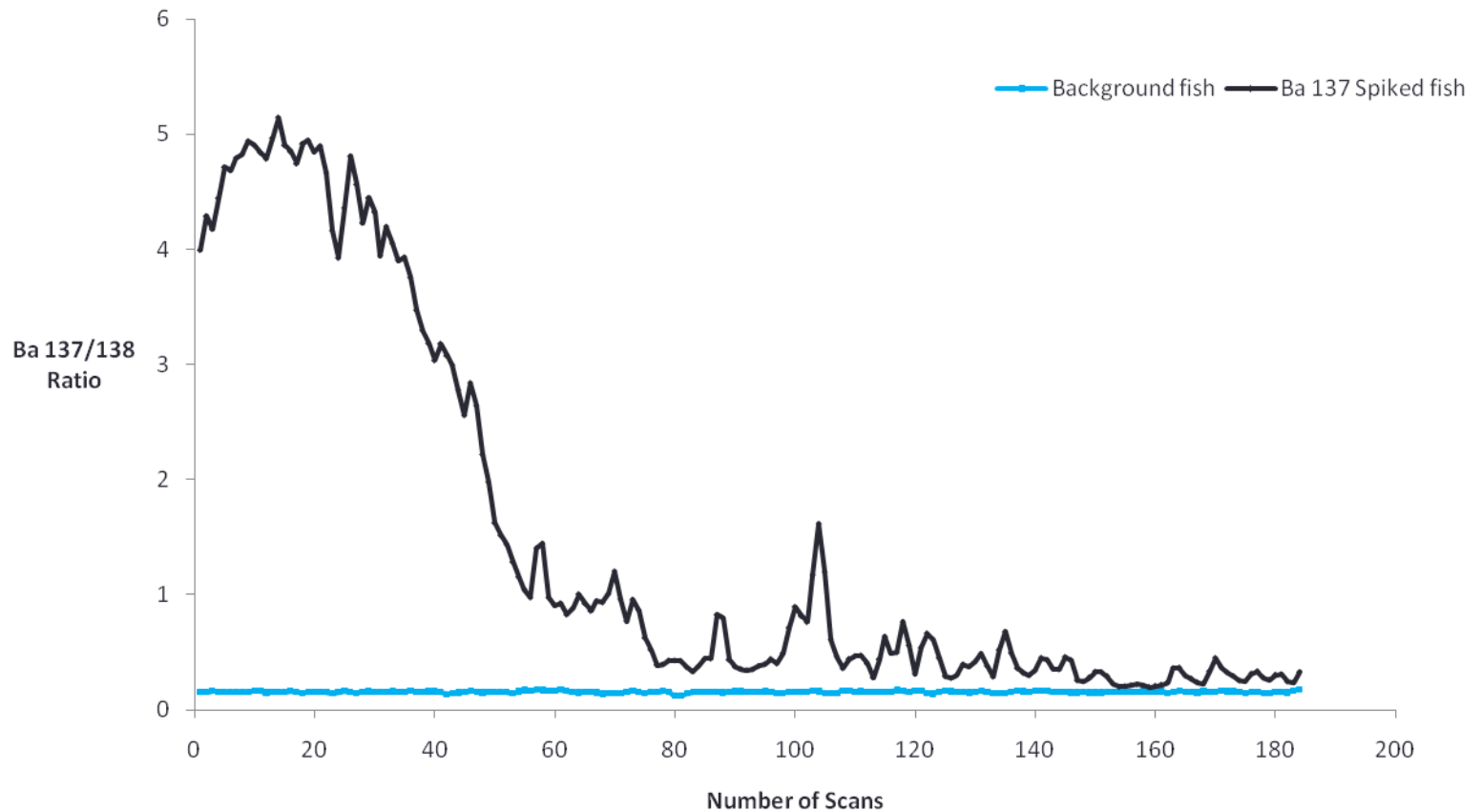
Vaccination Pilot Results

- 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)



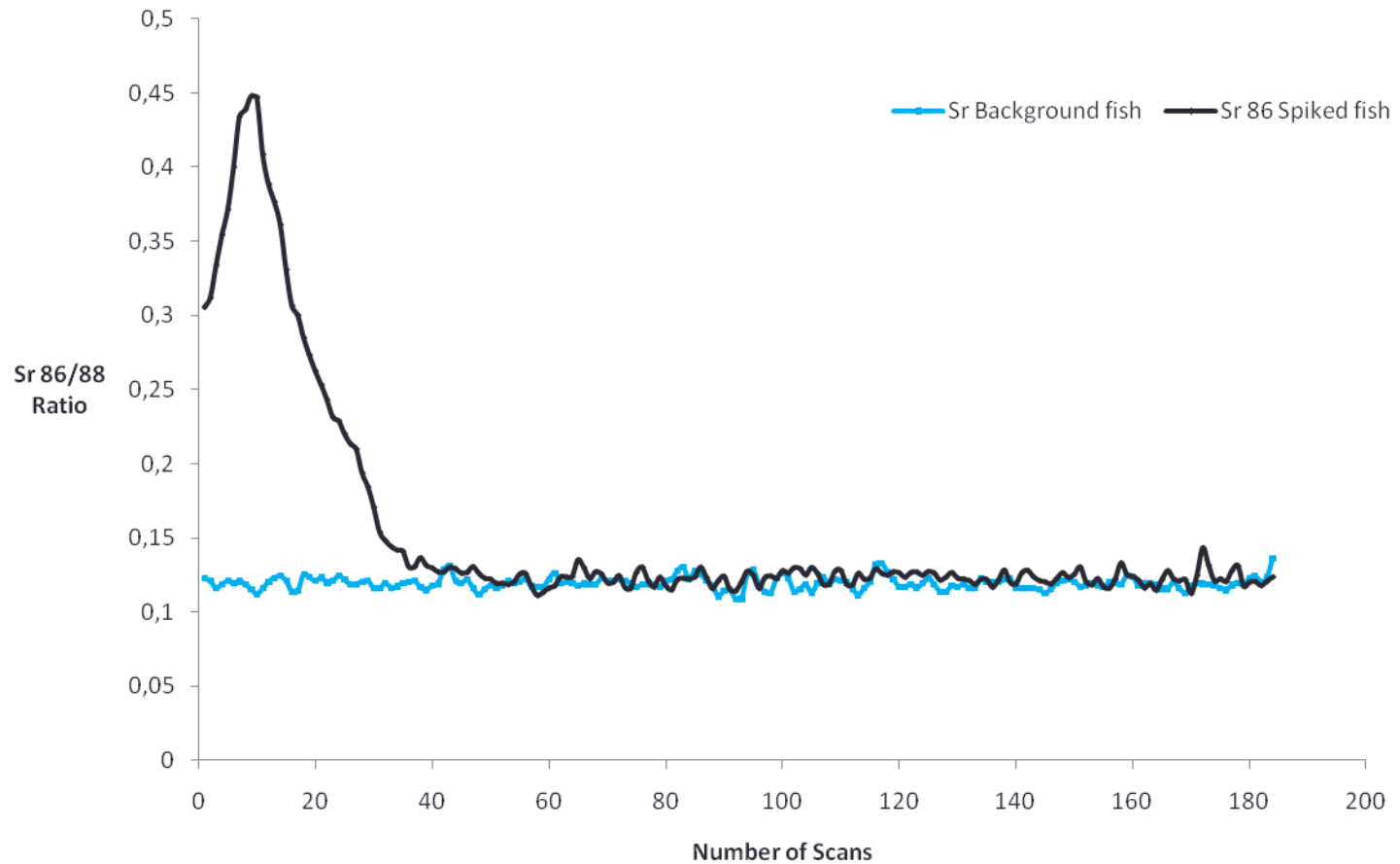
Marking successful with Barium 137

Barium 137/138



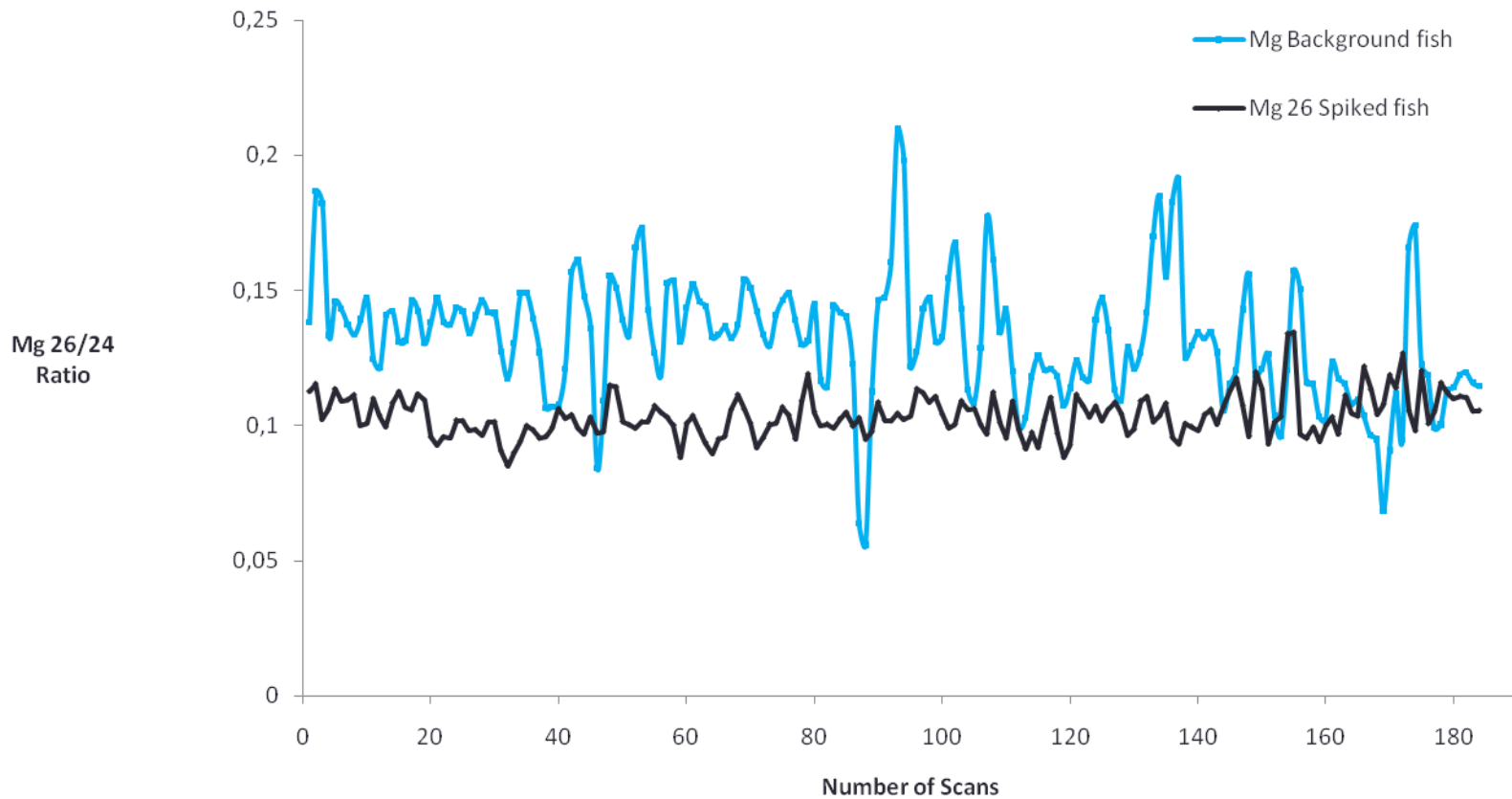
Mark successful with Strontium 86

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

