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

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OTOLITHS AS DATA LOGGERS

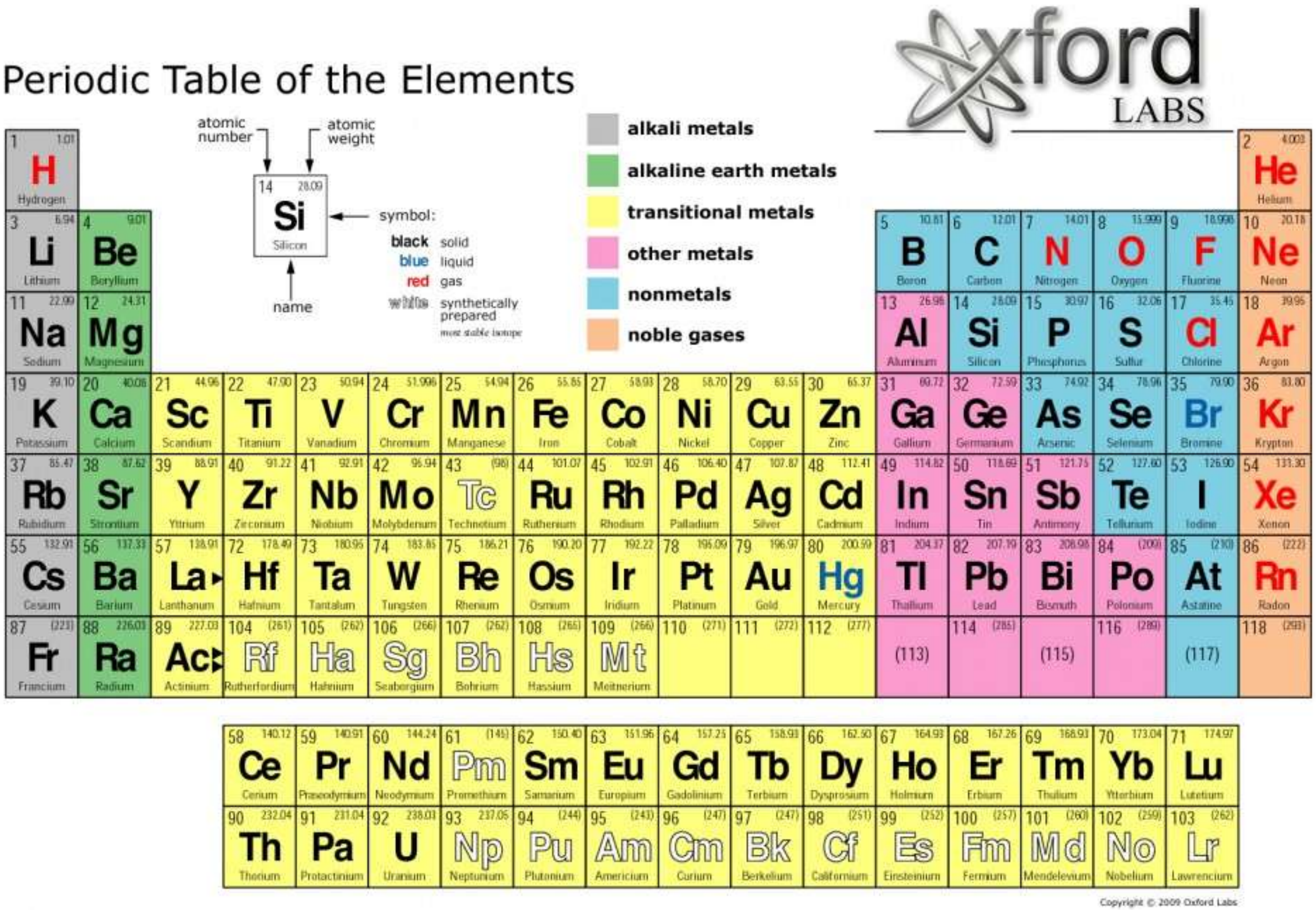
Formed during embryogenesis

Grow continuously

Metabolically inert

Incorporate impurities into the matrix

COMMON ENVIRONMENTAL MARKERS



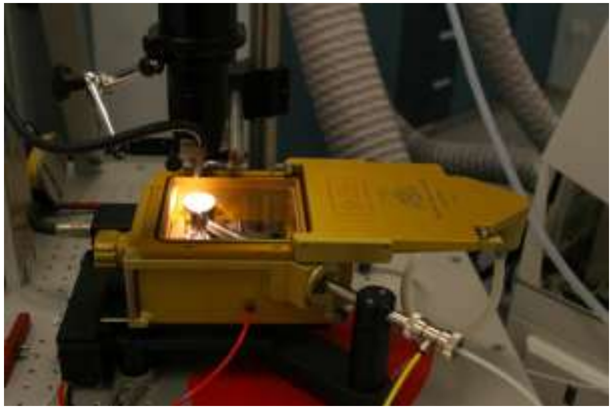
Stable isotopes of Barium

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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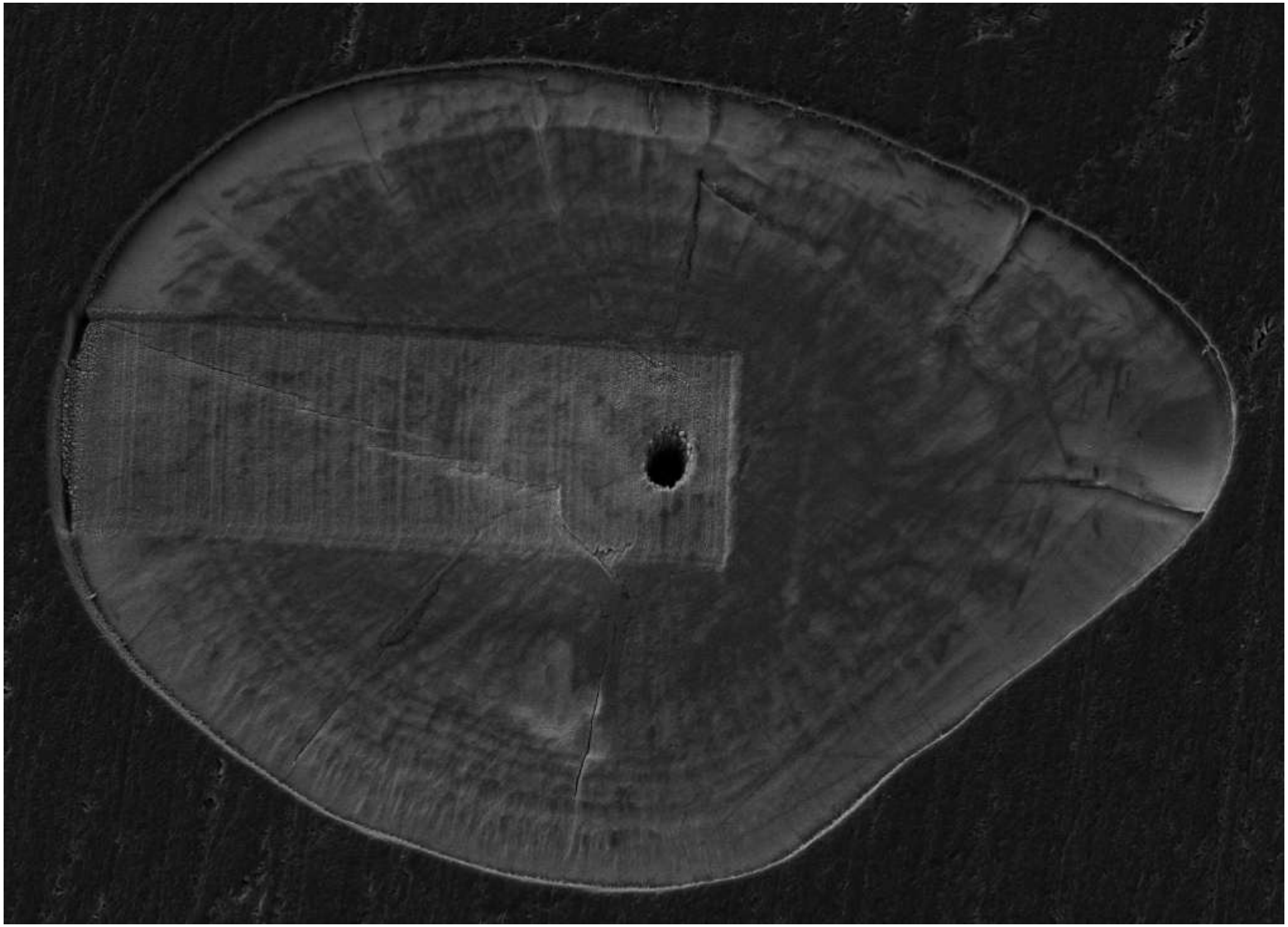
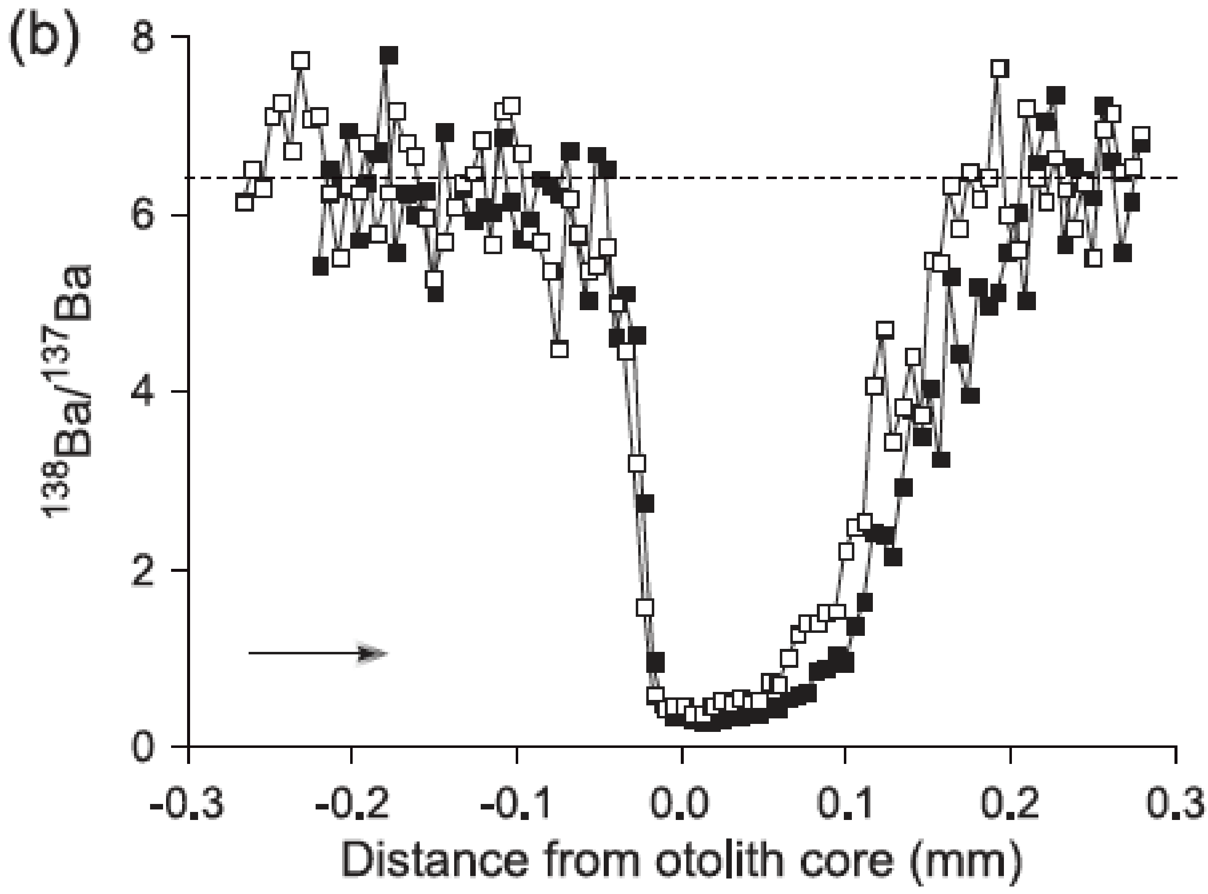


DETECTING ISOTOPIC TAGS

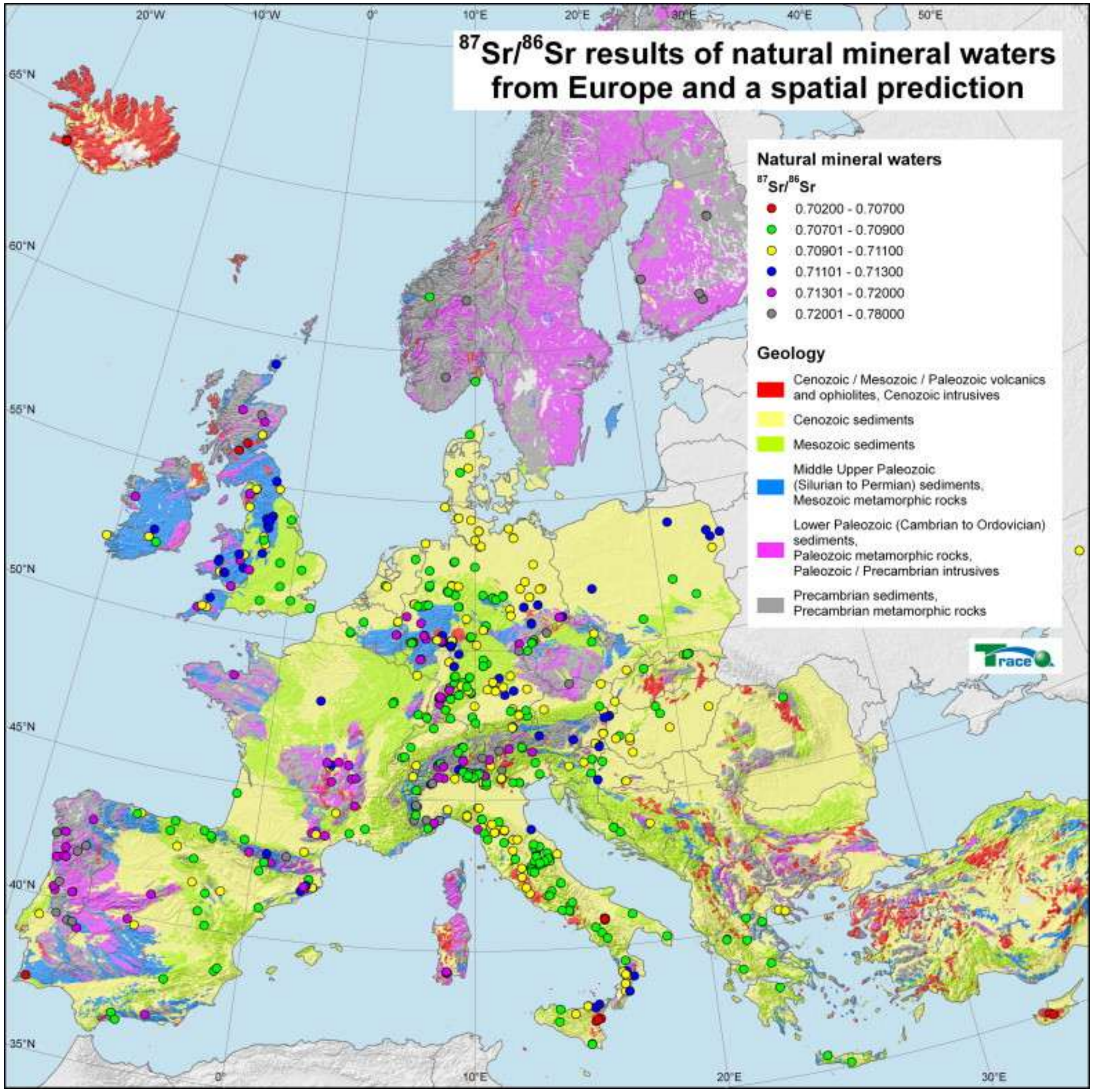


Thorrold et al.*CJFAS* (2006)

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: 2µg per g female weight (n=6)
- T2: 0.2µg per g female weight (n=6)
- T3: 0.02µg per g female weight (n=6)
- T4: 0.002µg 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 1000µg 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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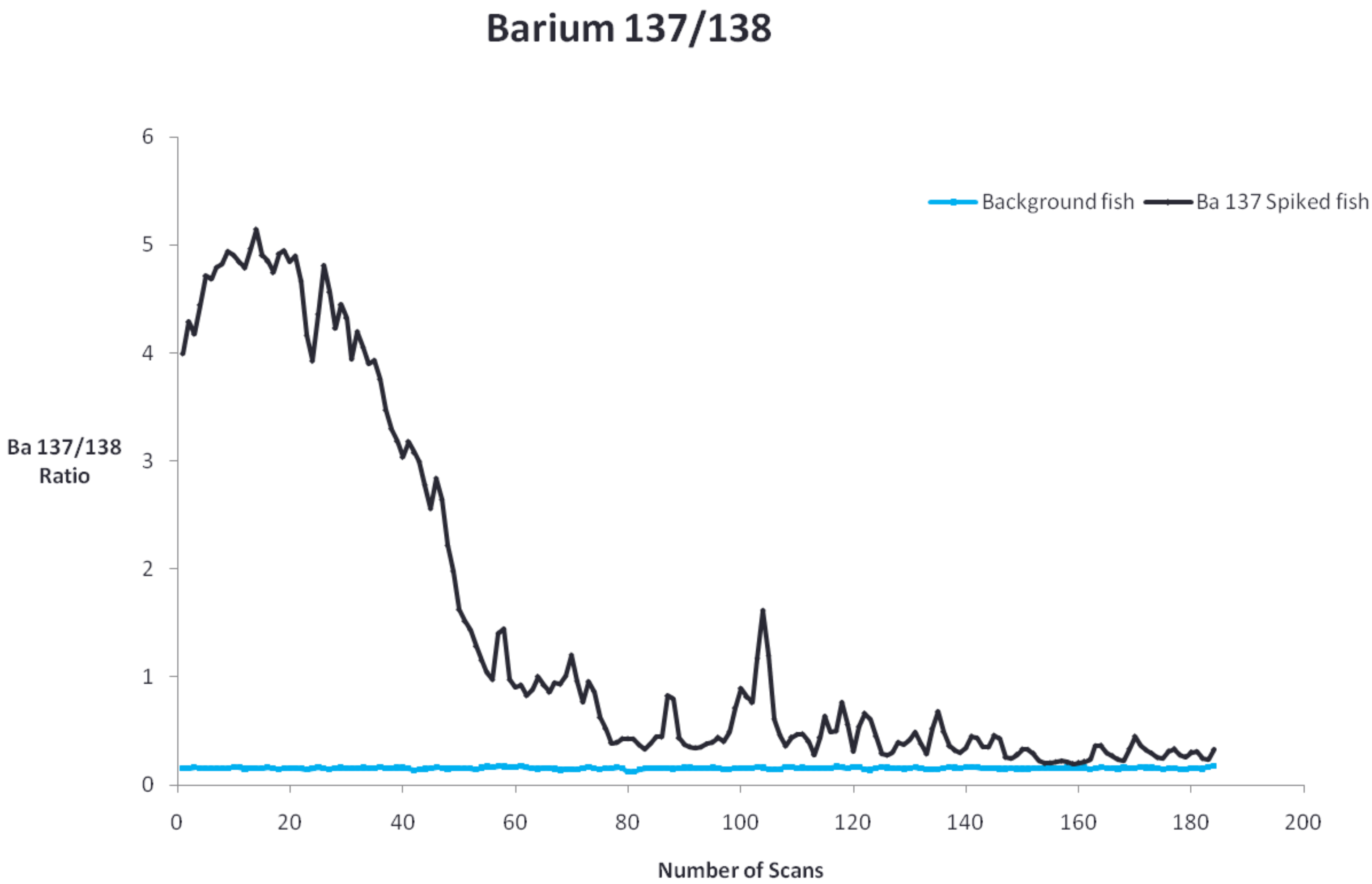
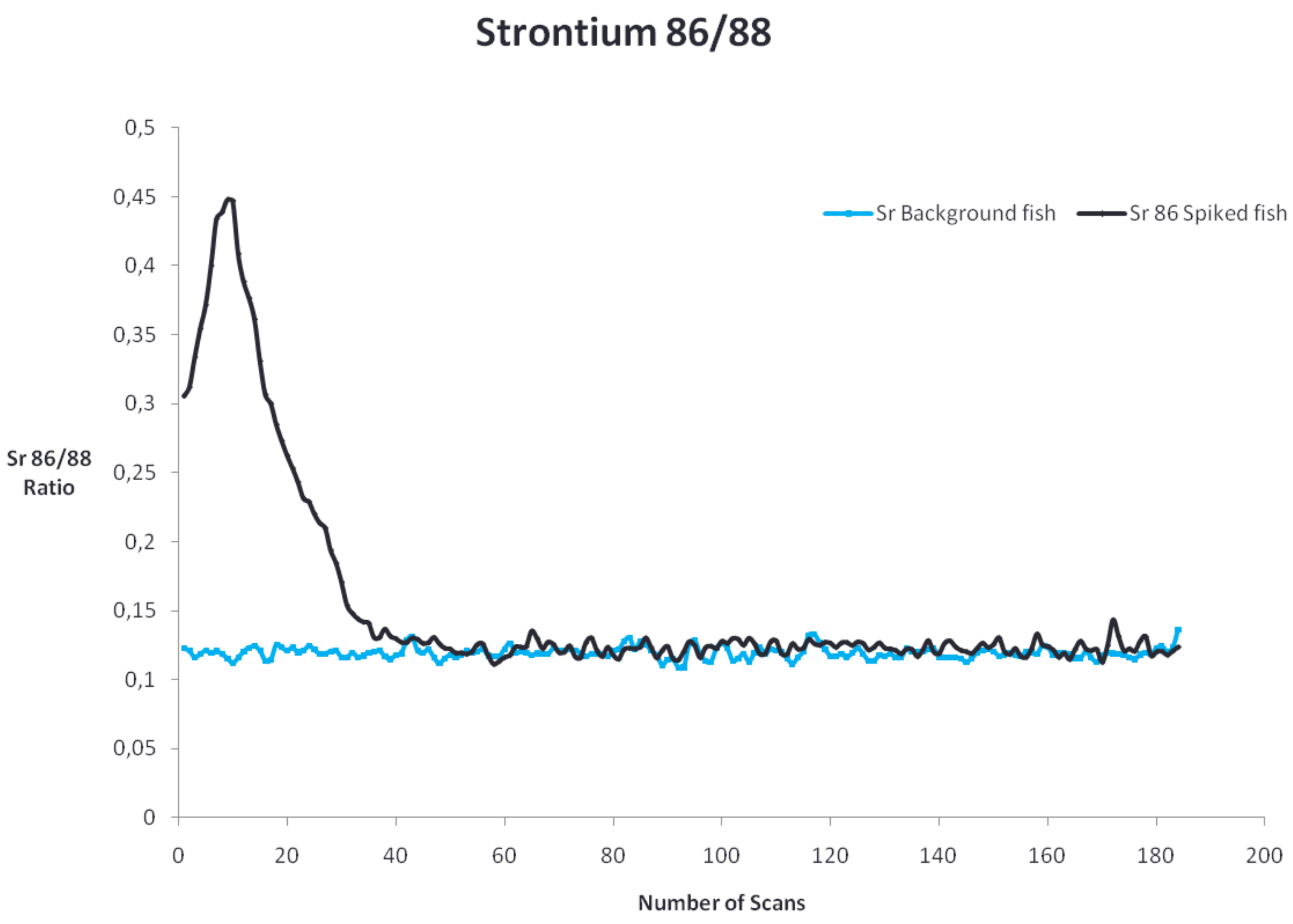
Vaccination Pilot Results

- 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)

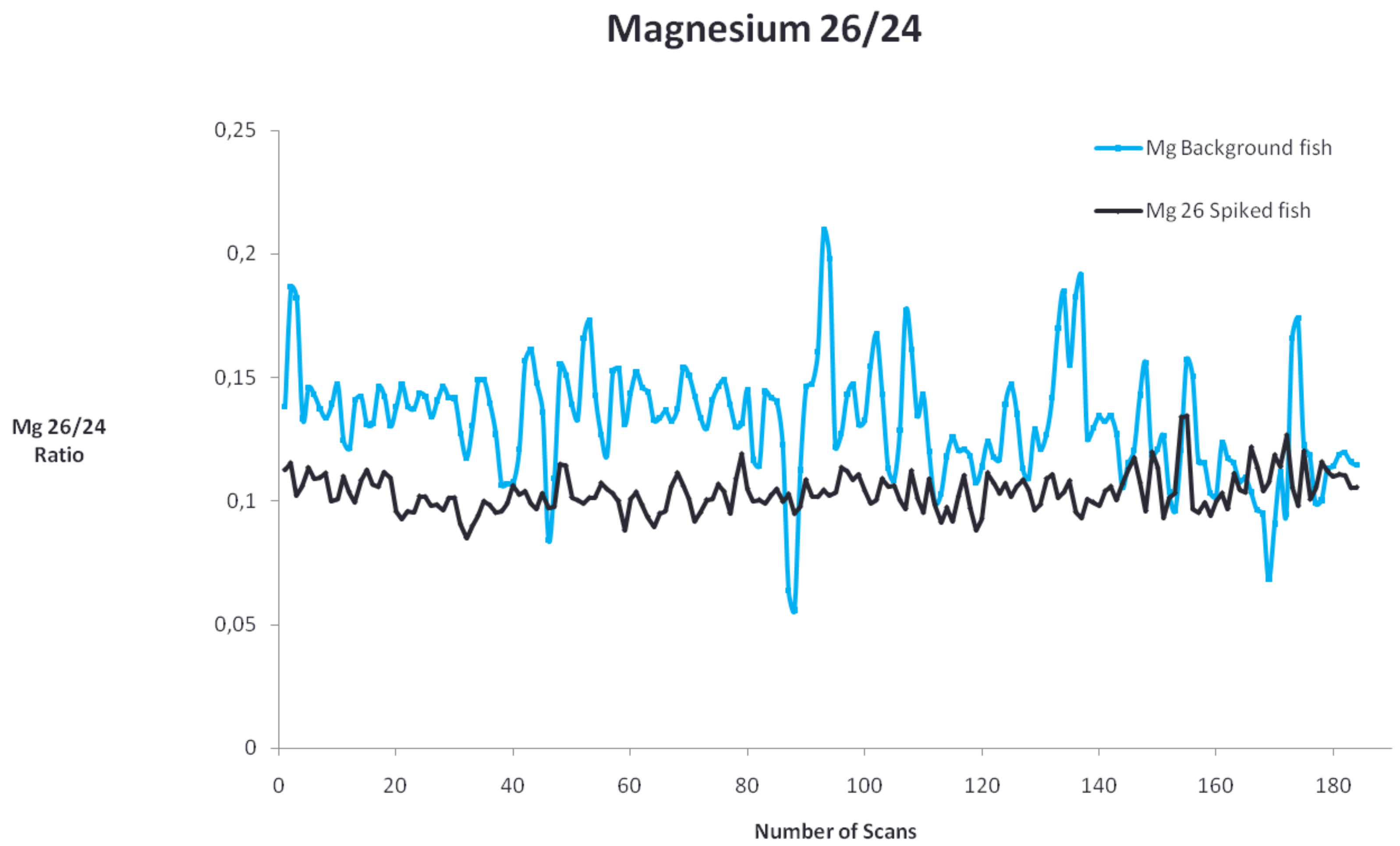
Marking successful with Barium 137



Mark successful with Strontium 86

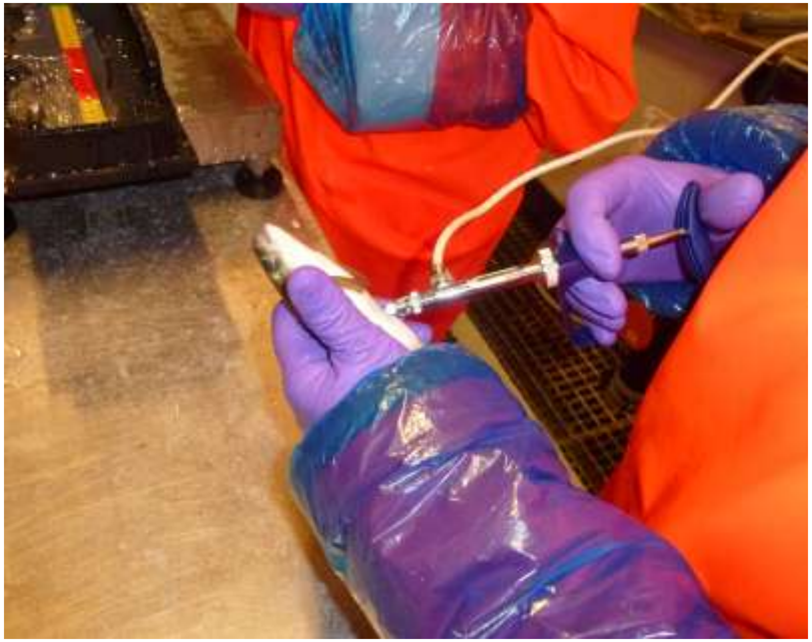


Mark not successful with Magnesium 26



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