



Stable Isotope Otolith Fingerprint Signatures: A mass marking technique for farmed Atlantic salmon



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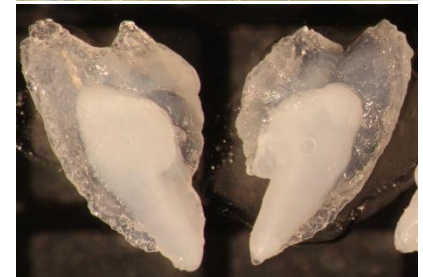
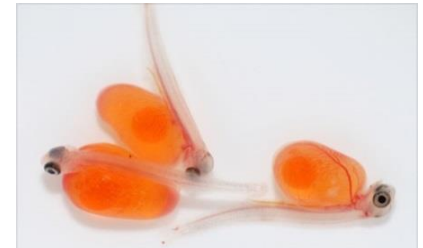
Principal scientist Tom Hansen

Dr Per Gunnar Fjelldal



Identification using stable isotope “otolith fingerprinting”

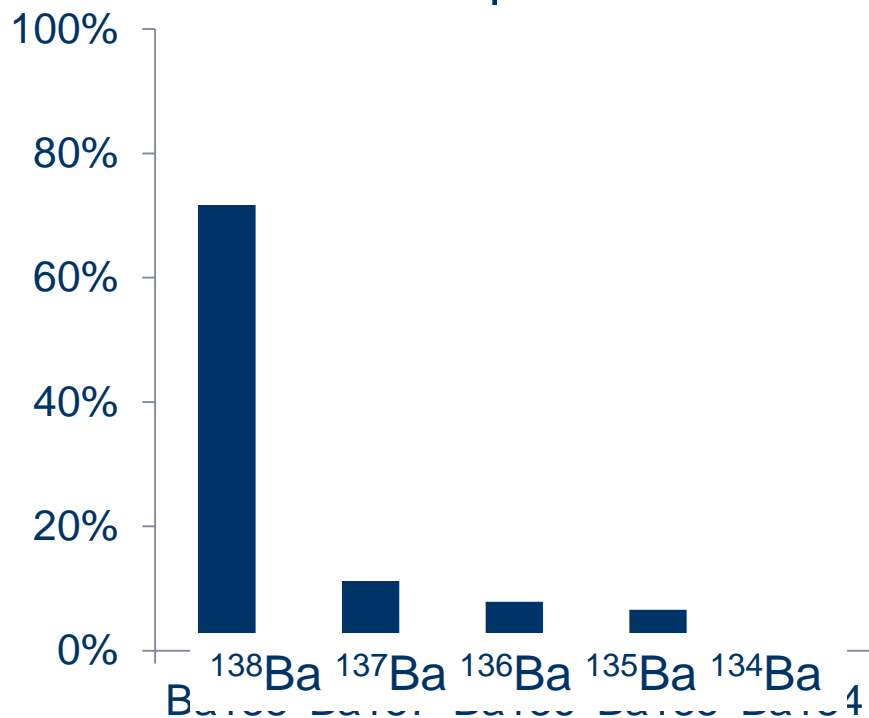
- Otolith formed during embryogenesis
- Grow continuously
- Metabolically inert
- Incorporated impurities into the otolith matrix (e.g. isotopes of Ba, Sr, Mg)
- Used to create permanent, unique isotopic fingerprint signatures



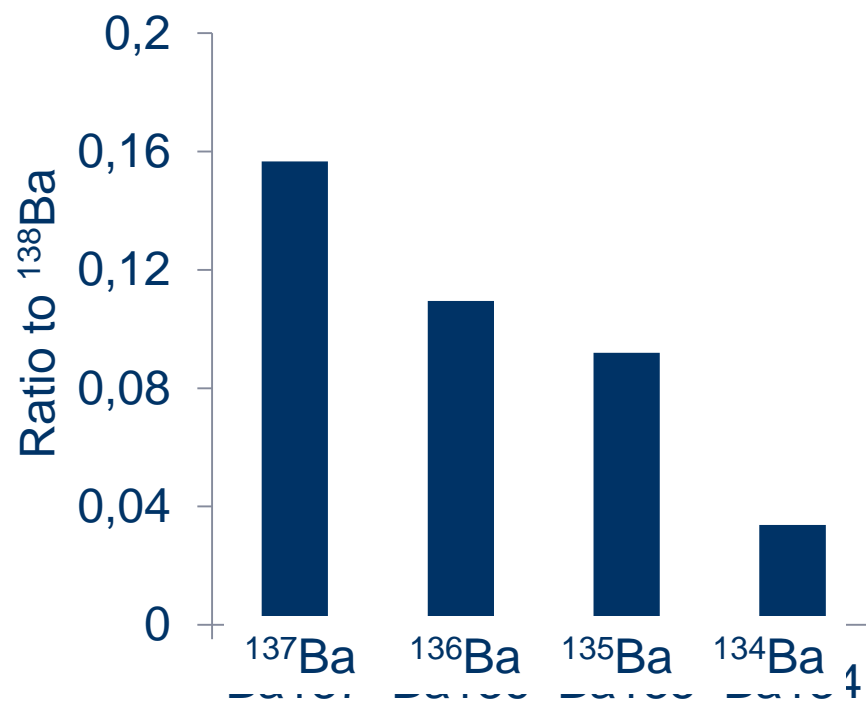


Barium isotopes (Ba)

Natural abundance of Ba isotopes



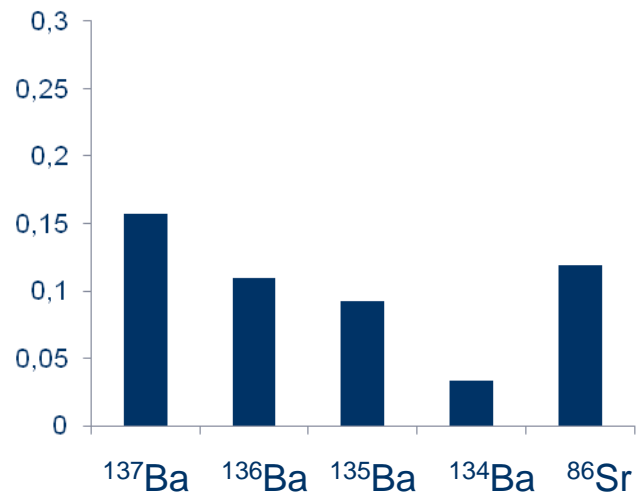
Natural ratios relative to ^{138}Ba





Creating a fingerprint

Natural ratios of Ba and Sr



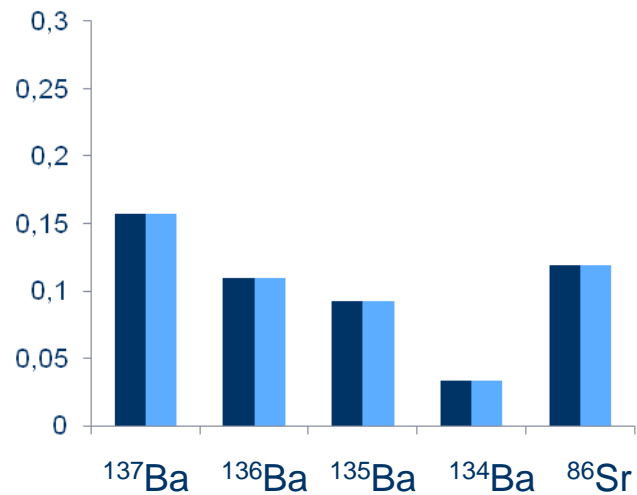
Ba compared to ^{138}Ba
Sr compared to ^{88}Sr

 Wild Salmon



Creating a fingerprint

Fingerprint mark (#marks = 0)



Ba compared to ^{138}Ba
Sr compared to ^{88}Sr

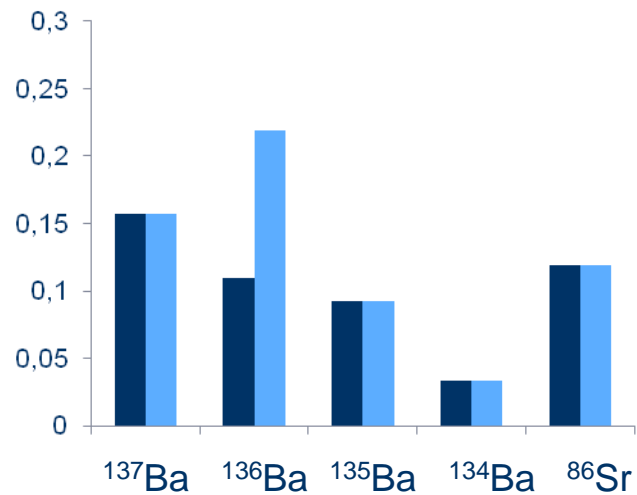
 Wild Salmon

 Farmed Salmon



Creating a fingerprint

Fingerprint mark (#marks = 1)



Ba compared to ^{138}Ba
Sr compared to ^{88}Sr

Adding ^{136}Ba

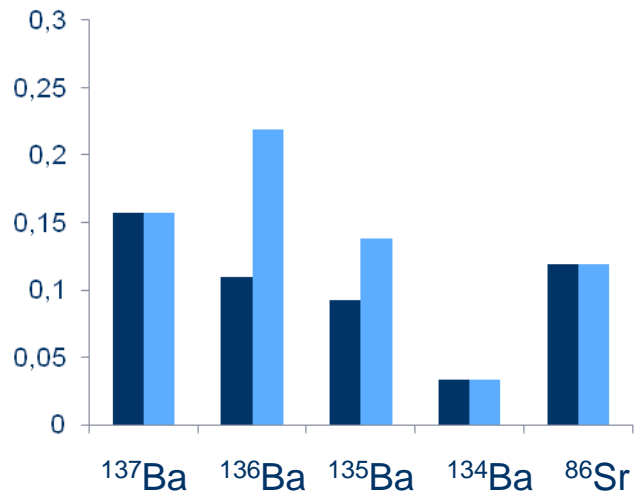
 Wild Salmon

 Farmed Salmon



Creating a fingerprint

Fingerprint mark (#marks = 2)

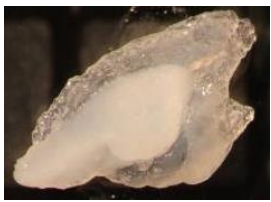


Ba compared to ^{138}Ba
Sr compared to ^{88}Sr

Adding ^{135}Ba

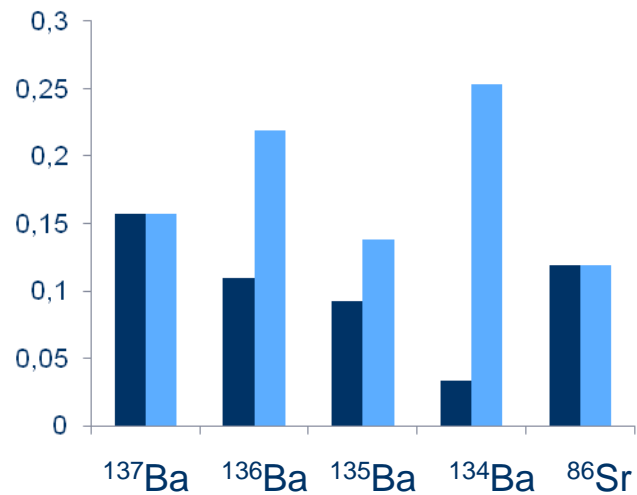
 Wild Salmon

 Farmed Salmon



Creating a fingerprint

Fingerprint mark (#marks = 3)



Ba compared to ^{138}Ba
Sr compared to ^{88}Sr

Adding ^{134}Ba

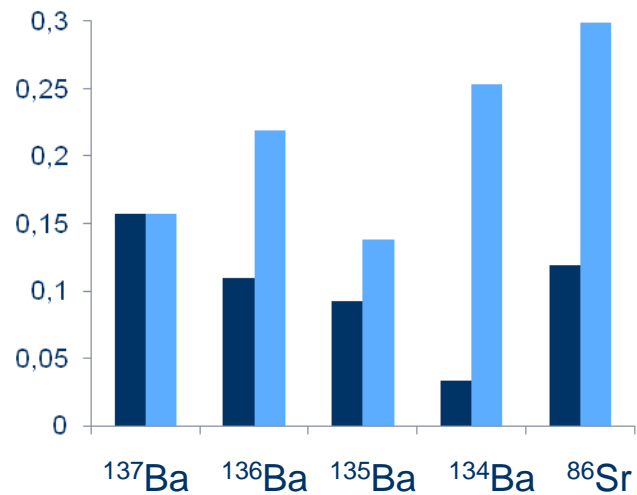
 Wild Salmon

 Farmed Salmon



Creating a fingerprint

Fingerprint mark (#marks = 4)

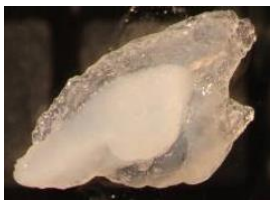


Ba compared to ^{138}Ba
Sr compared to ^{88}Sr

Adding ^{86}Sr

 Wild Salmon

 Farmed Salmon



How many unique fingerprints?

1 isotope ($2^1 - 1$) = 1 mark

2 isotopes ($2^2 - 1$) = 3 markers

3 isotopes ($2^3 - 1$) = 7 markers

7 isotopes ($2^7 - 1$) = 127 markers

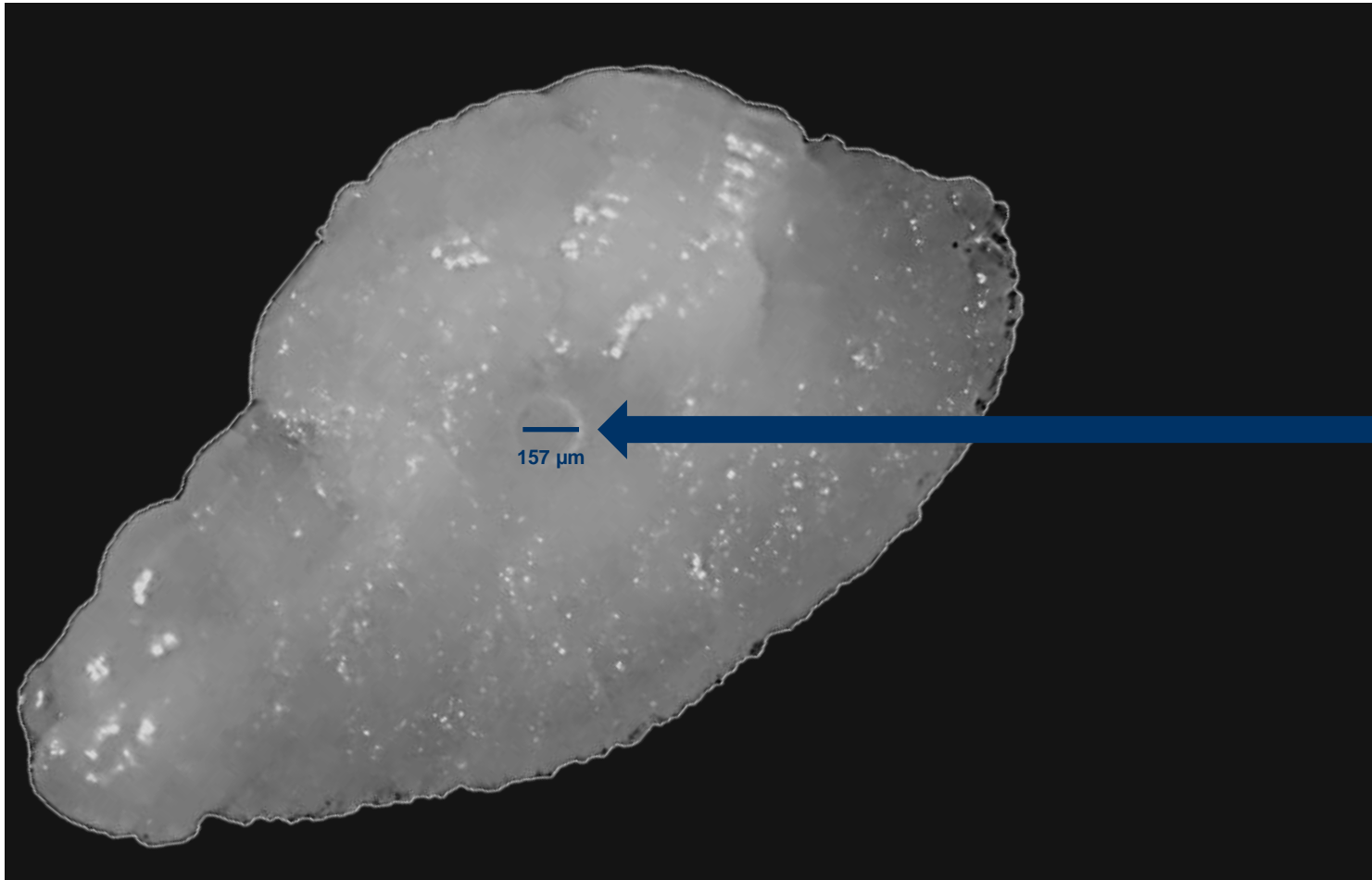
Number of possible markers = ($2^{\text{number of isotopes}} - 1$)

Possible to create > 1000 combinations

Enough for each fish farm to have its own individual unique fingerprint marker



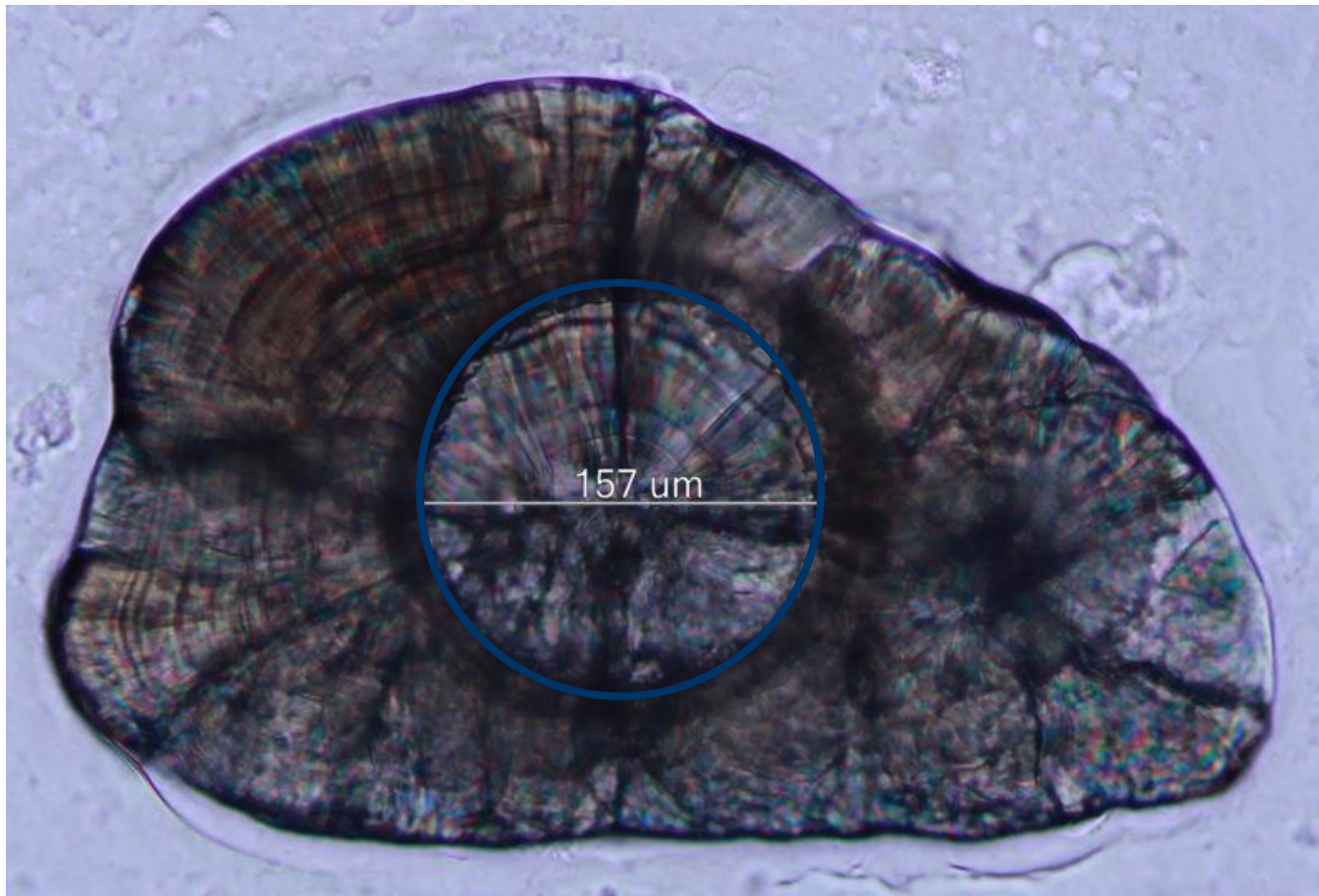
LA-ICP-MS analysis of fish otoliths



Spot
ablation



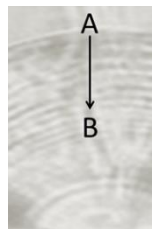
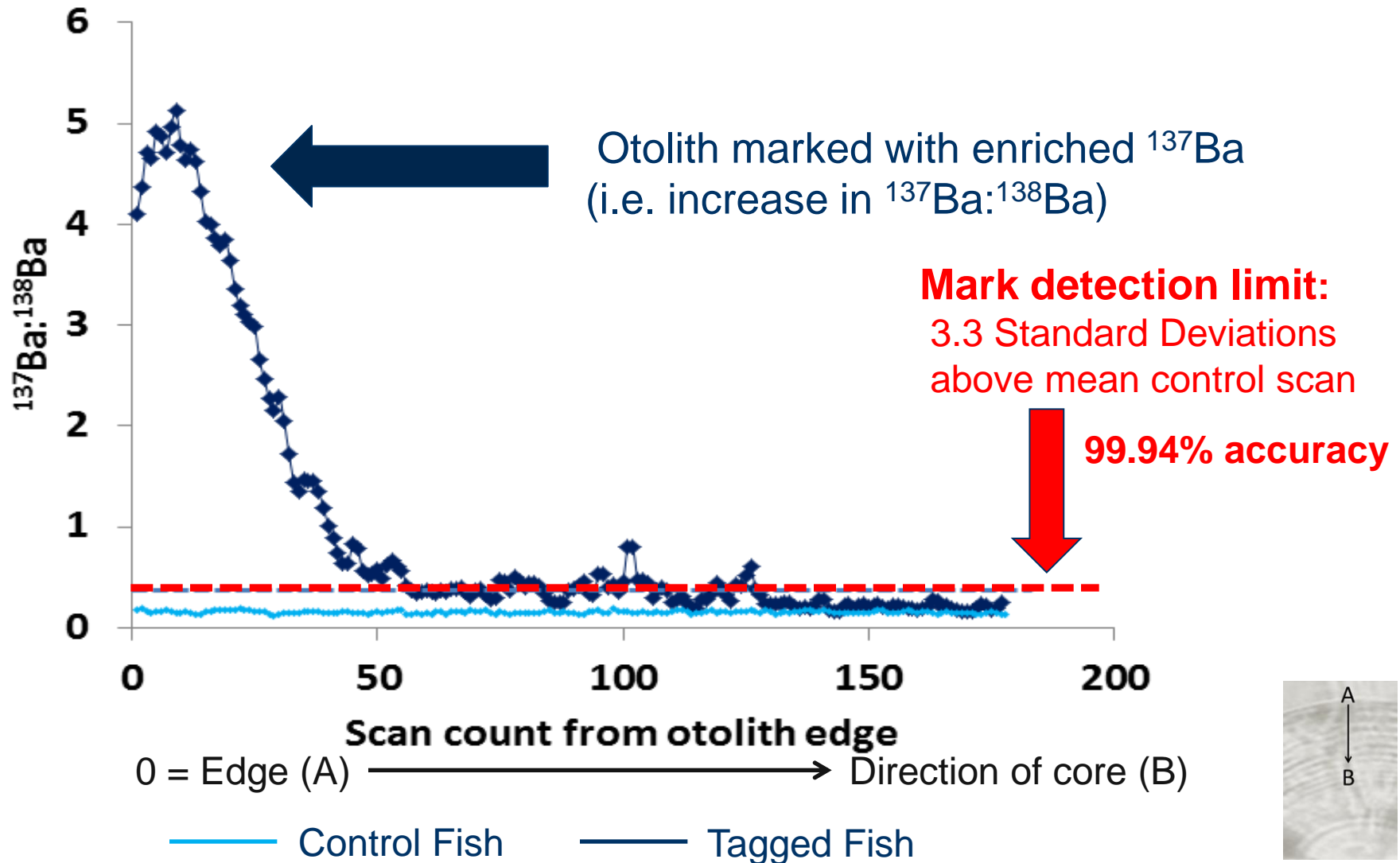
LA-ICP-MS analysis of fish otoliths



Spot
ablation



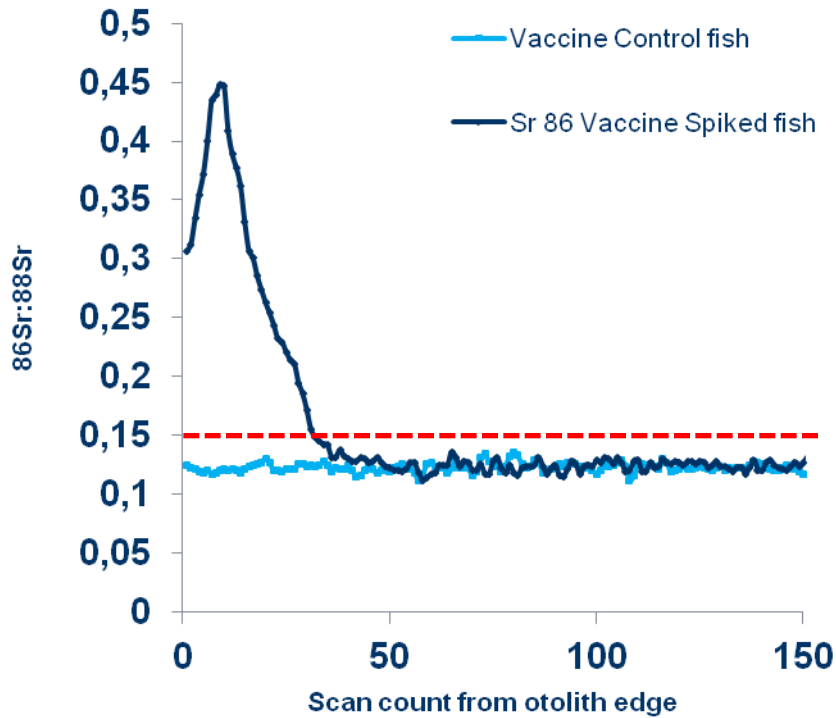
Detecting a successful mark



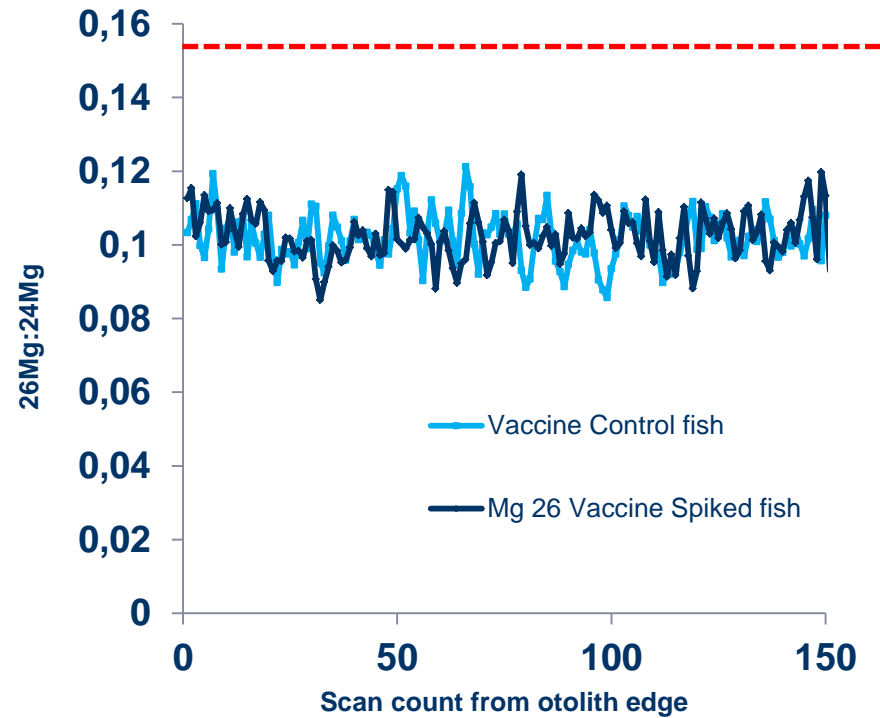


Sr and Mg example

$^{86}\text{Sr}:^{88}\text{Sr}$



$^{26}\text{Mg}:^{24}\text{Mg}$



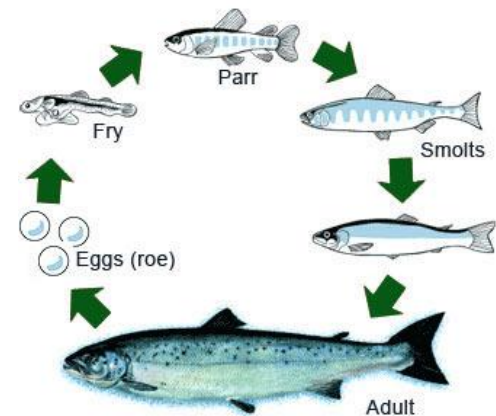
— Control Fish — Tagged Fish - - - - - Detection limit



Aims

To develop 3 different stable isotope mark delivery techniques for Atlantic salmon

- 1) Vaccination (parr stage)
- 2) Transgenerational (brood stock)
- 3) Egg immersion (fertilised eggs)



- Confirmation – *Can we create unique marks?*
- Optimization – *how well does each technique work?*
- Welfare - *side effects?*
- Commercial viability - *applicability, cost?*

Application points



Vaccination

Isotopes

^{137}Ba ,

Application points



Vaccination

Isotopes

^{137}Ba , ^{136}Ba ,
 ^{135}Ba ,
 ^{86}Sr ,

Application points

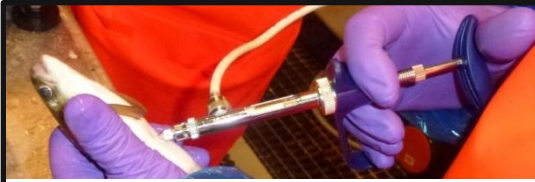


Vaccination

Isotopes

^{137}Ba , ^{136}Ba ,
 ^{135}Ba , ^{134}Ba , ^{87}Sr ,
 ^{86}Sr , ^{26}Mg

Application points



Vaccination

Isotopes

^{137}Ba , ^{136}Ba ,
 ^{135}Ba , ^{134}Ba , ^{87}Sr ,
 ^{86}Sr , ^{26}Mg

Concentrations

1

0.1

0.01

0.001

($\mu\text{g. g}^{-1}$ parr weight)

Mark success



Vaccination

Isotopes

^{137}Ba , ^{136}Ba ,
 ^{135}Ba , ^{134}Ba , ^{87}Sr ,
 ^{86}Sr , ^{26}Mg

Concentrations

1

0.1

0.01

0.001

($\mu\text{g. g}^{-1}$ parr weight)

^{137}Ba 100% at 0.001 $\mu\text{g. g}^{-1}$

Mark success



Vaccination

Isotopes

^{137}Ba , ^{136}Ba ,
 ^{135}Ba , ^{134}Ba , ^{87}Sr ,
 ^{86}Sr , ^{26}Mg

Concentrations

1

0.1

0.01

0.001

($\mu\text{g. g}^{-1}$ parr weight)

^{137}Ba 100% at $0.001 \mu\text{g. g}^{-1}$

^{137}Ba

^{136}Ba 100% at $0.01 \mu\text{g. g}^{-1}$

^{135}Ba

^{134}Ba

Mark success



Vaccination

Isotopes

^{137}Ba , ^{136}Ba ,
 ^{135}Ba , ^{134}Ba , ^{87}Sr ,
 ^{86}Sr , ^{26}Mg

Concentrations

1

0.1

0.01

0.001

($\mu\text{g. g}^{-1}$ parr weight)

^{137}Ba 100% at 0.001 $\mu\text{g. g}^{-1}$

^{137}Ba

^{136}Ba 100% at 0.01 $\mu\text{g. g}^{-1}$

^{135}Ba

^{134}Ba

^{86}Sr 100% at 1 $\mu\text{g. g}^{-1}$

^{87}Sr

UNIQUE CODES



Vaccination: 63 unique fingerprints
Cost **\$0.0002** to **\$0.13** per fish (\$US)

Transgenerational marking

Mature fish sorted and transferred to freshwater tanks several months prior to spawning



Stripping → fertilisation and placed in hatchery



Females injected with isotope



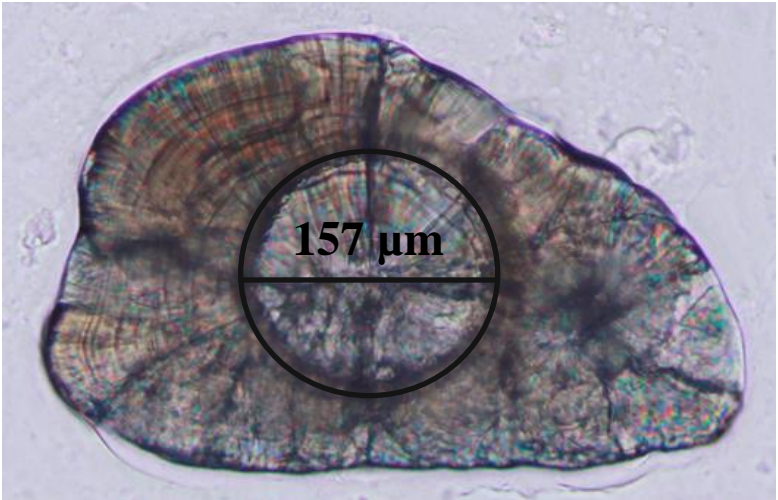
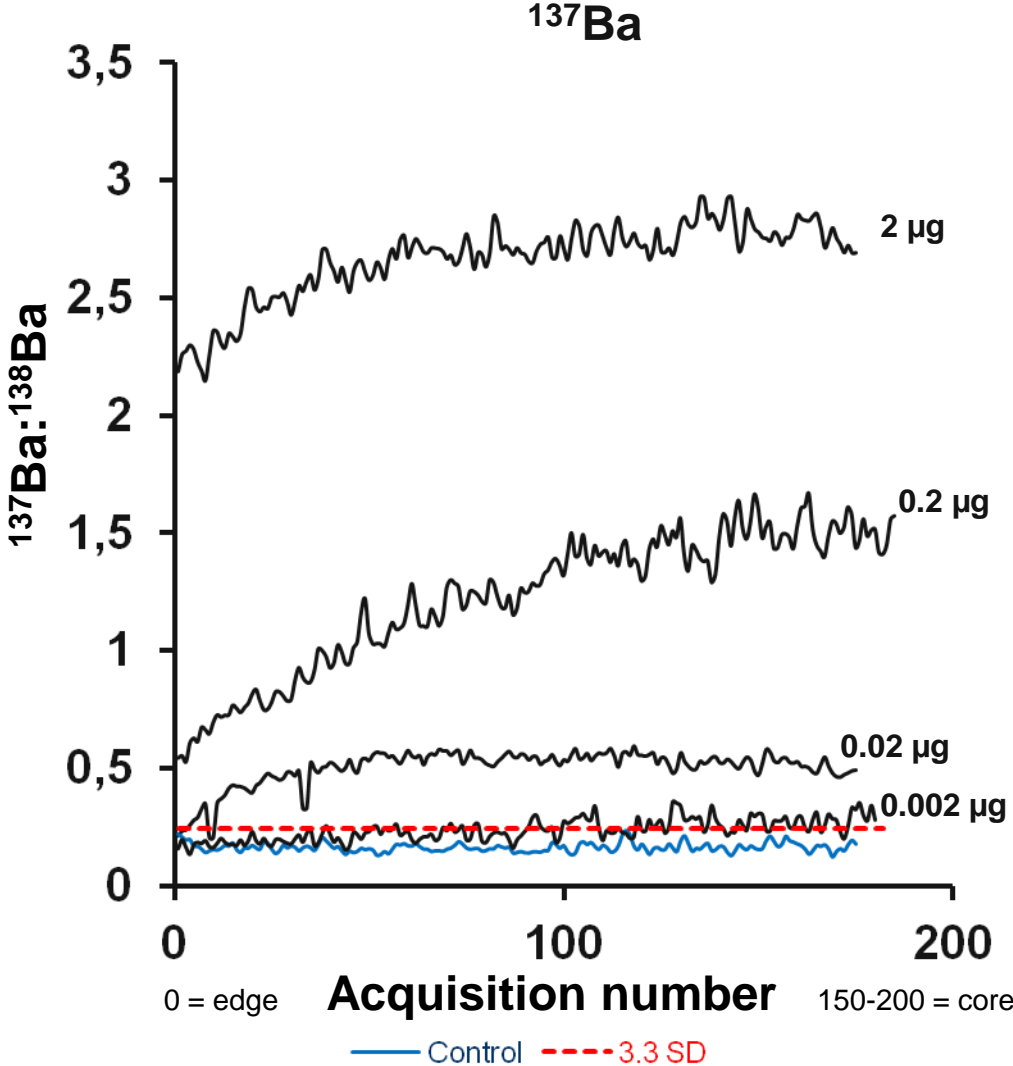
Marks passed from mother to egg *in situ*



Mark Success



Transgenerational



UNIQUE CODES



Vaccination: 63 unique fingerprints

Cost \$0.0002 to \$1.03 per fish (\$US)

Transgenerational: 63 unique fingerprints

Cost **\$0.0002** to **\$0.13** per fish (\$US)

Egg Immersion



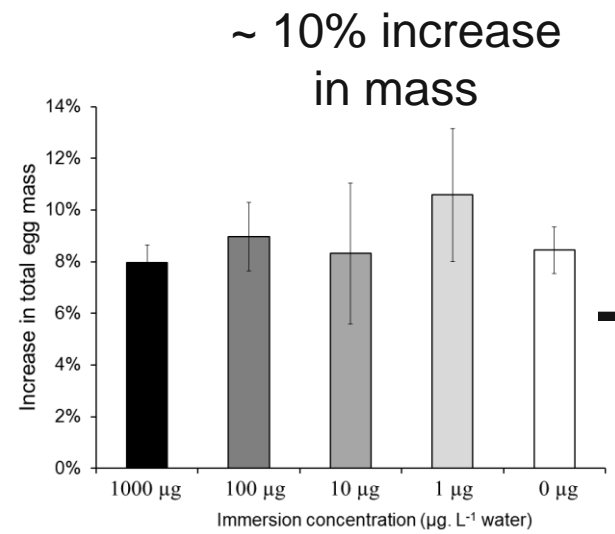
Stripping



Fertilised
add isotope
solution



2 hr

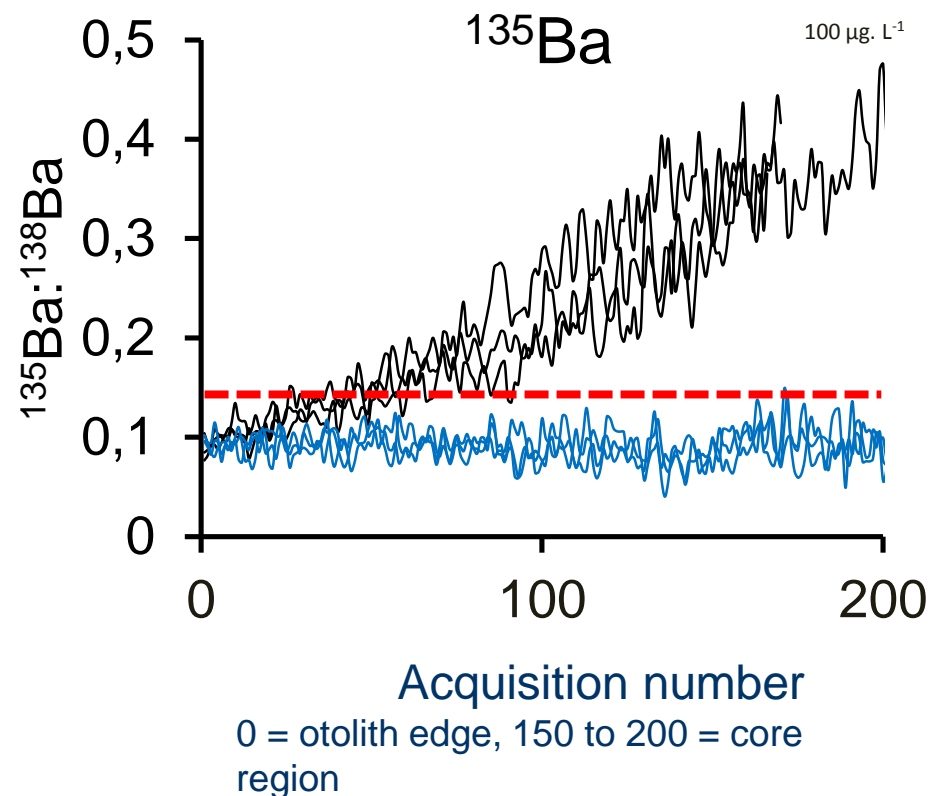
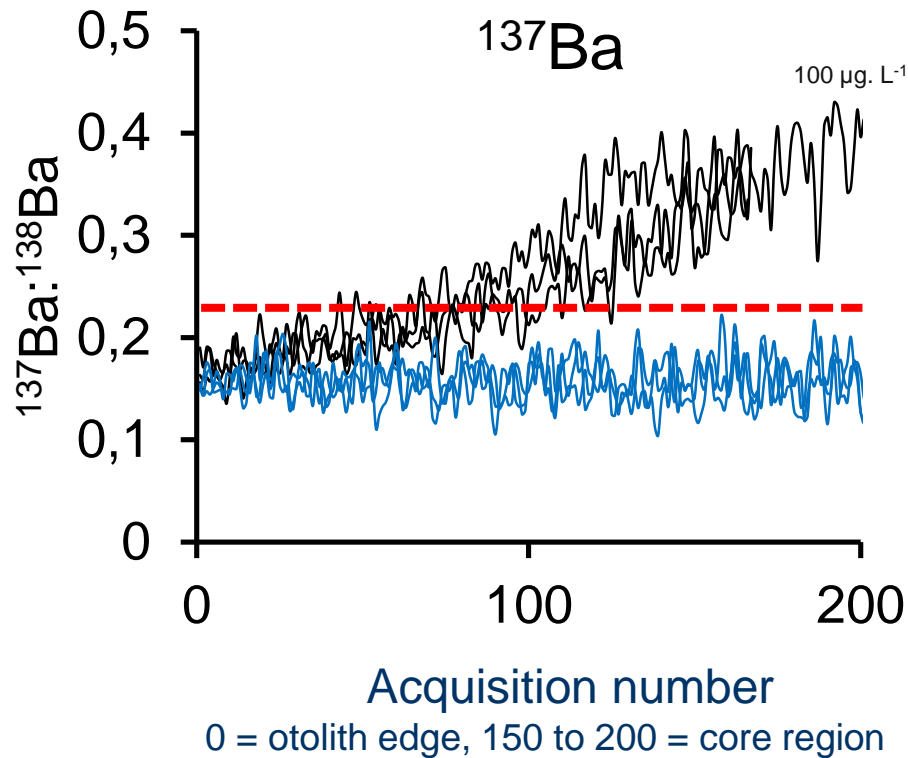
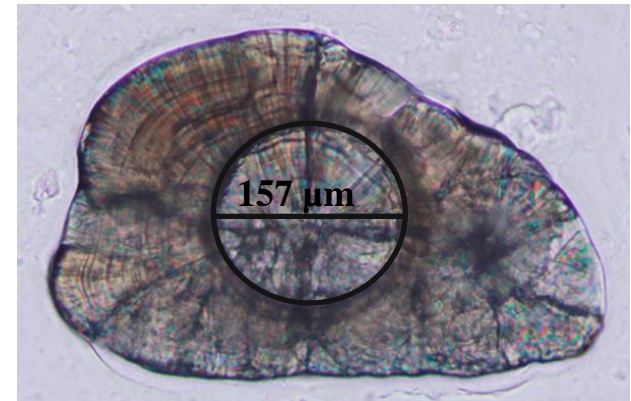


Warren-Myers et al. 2015b

Transfer to hatchery



Mark success



UNIQUE CODES



Vaccination: 63 unique fingerprints

Cost **\$0.0002** to **\$1.03** per fish (\$US)

Transgenerational: 63 unique fingerprints

Cost **\$0.0002** to **\$0.13** per fish (\$US)

Egg immersion: 7 unique fingerprints

Cost **\$0.0001** to **\$0.0017** per fish (\$US)



Fish Health

Monitoring of fish health parameters
(from mark date to harvest size)

No effect on Growth

No effect on Condition

No effect on Mortality

Summary

All three techniques could be used for mass marking Atlantic salmon with 100% mark success

Vaccination: 63 unique fingerprints

Transgenerational: 63 unique fingerprints

Egg immersion: 7 unique fingerprints

Ba markers are cheaper and require less isotope than Sr markers to achieve 100% mark success



Acknowledgements

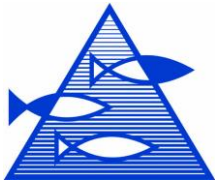
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