



# Otolith Fingerprint Signatures: A mass marking technique for marking farmed Atlantic Salmon *Salmo salar*



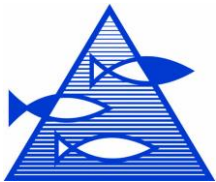
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# Farmed fish escapee

*“Escapees can have **detrimental genetic and ecological effects** on populations of wild conspecifics, and the present level of escapees is regarded as a **problem for the future sustainability of sea-cage aquaculture**” (Naylor et al., 2005).*

To understanding the impacts

**Identify** and **trace**



# Identification of escapees

**Genetic markers** - ID 60-90%, Traceability 60-90%

(continuous data library required)

**Adipose fin clipping** – ID 100%, Traceability not possible

(welfare issues)

**Scale readings** – ID 90%, Traceability not possible

(data library required)

**Physical tags** – coded wire; t-bar tags ID 95%, Traceability possible

(welfare issues, and costly)

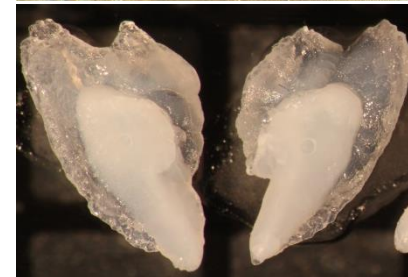
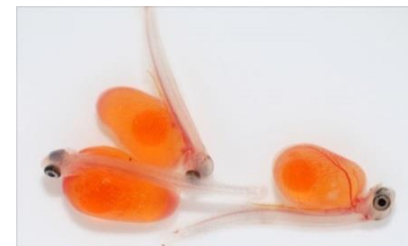
**Fluorescence markers** – ID 95%, Traceability not possible

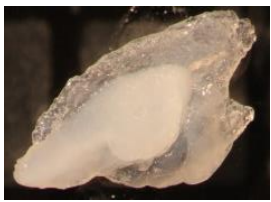
*Current methods fail either in 100% mark detectability, traceability, have welfare issues, or are costly*



# Identification using stable isotope “otolith fingerprinting”

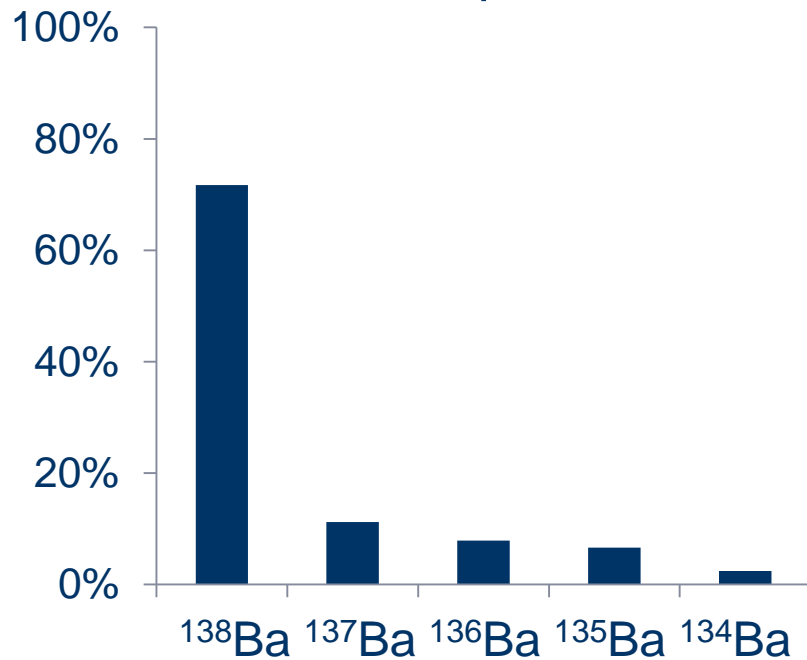
- 100% marking and traceability of escapees
- Otolith formed during embryogenesis
- Grow continuously
- Metabolically inert
- Incorporated impurities into the otolith matrix (e.g. isotopes of Ba, Sr, Mg)
- Used to create permanent, unique isotopic fingerprint signatures



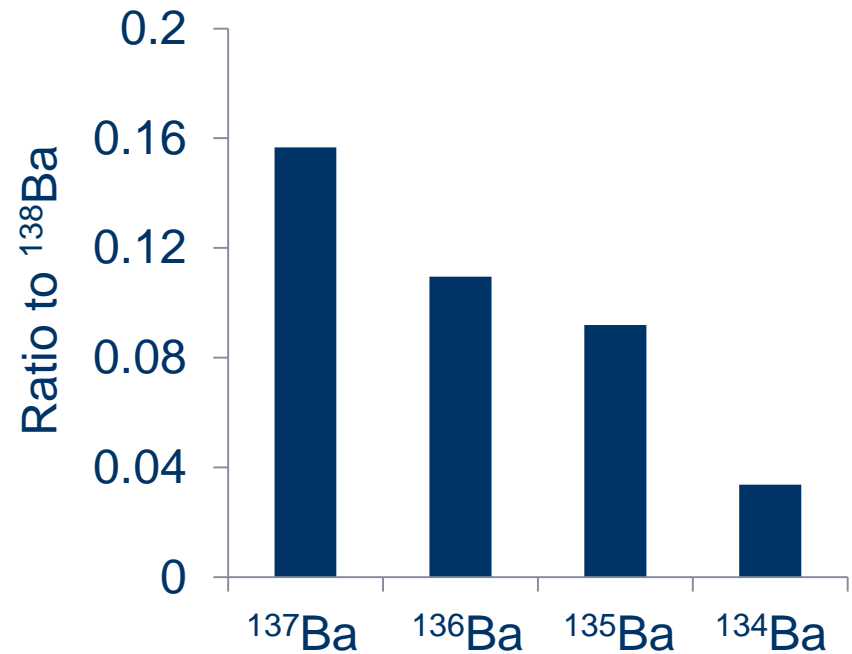


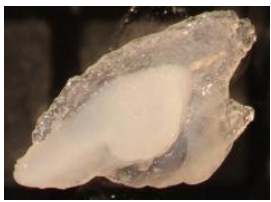
# Barium isotopes (Ba)

Natural abundance of Ba isotopes



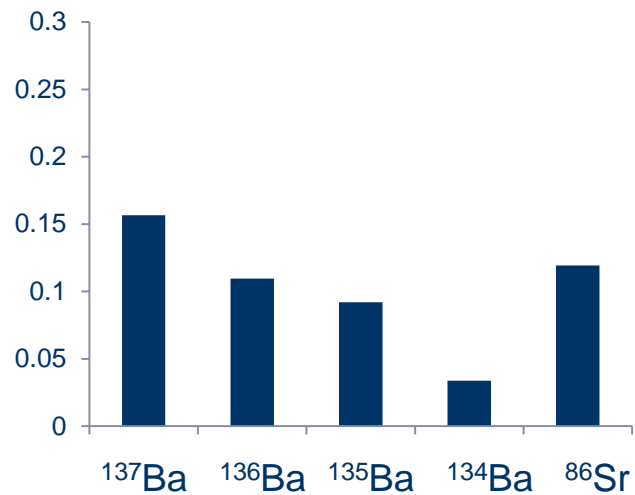
Natural ratios relative to  $^{138}\text{Ba}$





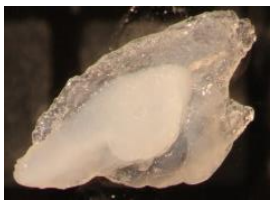
# Creating a fingerprint

## Natural ratios of Ba and Sr



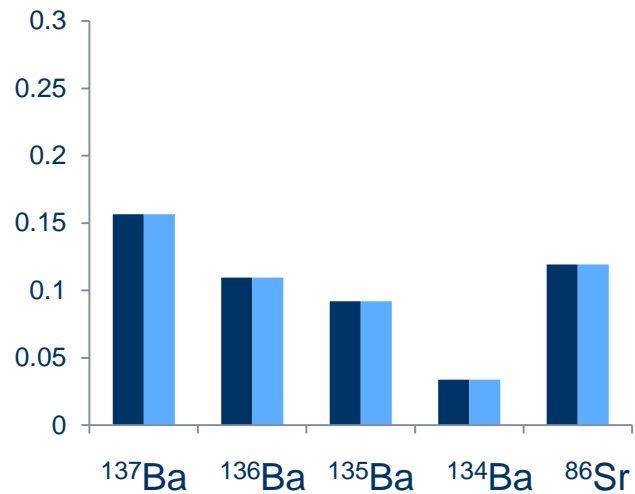
Ba compared to  $^{138}\text{Ba}$   
Sr compared to  $^{88}\text{Sr}$

 Wild Salmon



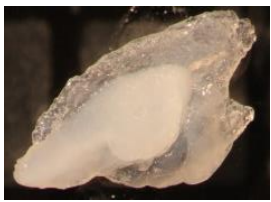
# Creating a fingerprint

Fingerprint mark (#marks = 0)



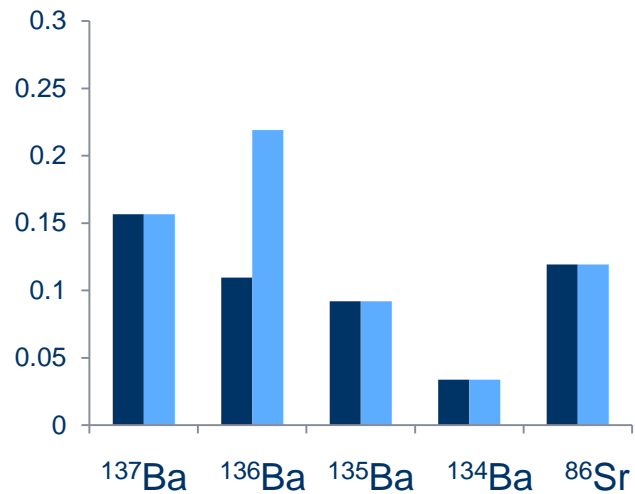
Ba compared to  $^{138}\text{Ba}$   
Sr compared to  $^{88}\text{Sr}$

-  Wild Salmon
-  Farmed Salmon



# Creating a fingerprint

Fingerprint mark (#marks = 1)



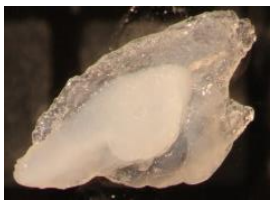
Ba compared to  $^{138}\text{Ba}$   
Sr compared to  $^{88}\text{Sr}$

Adding  $^{136}\text{Ba}$

 Wild Salmon

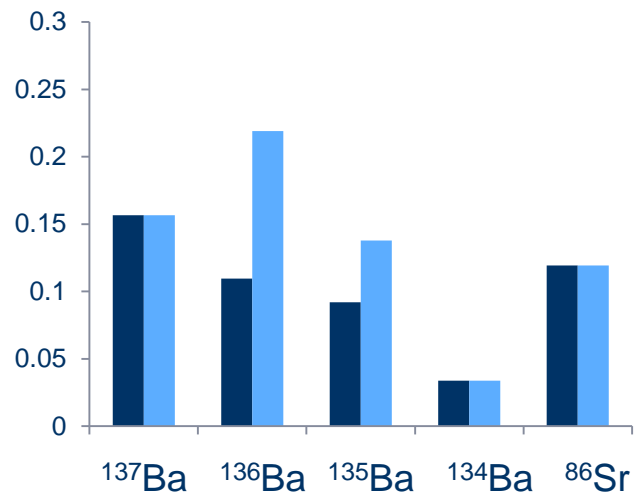
 Farmed Salmon





# Creating a fingerprint

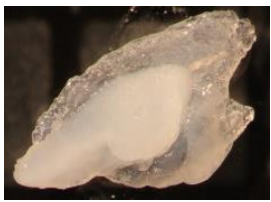
Fingerprint mark (#marks = 2)



Ba compared to  $^{138}\text{Ba}$   
Sr compared to  $^{88}\text{Sr}$

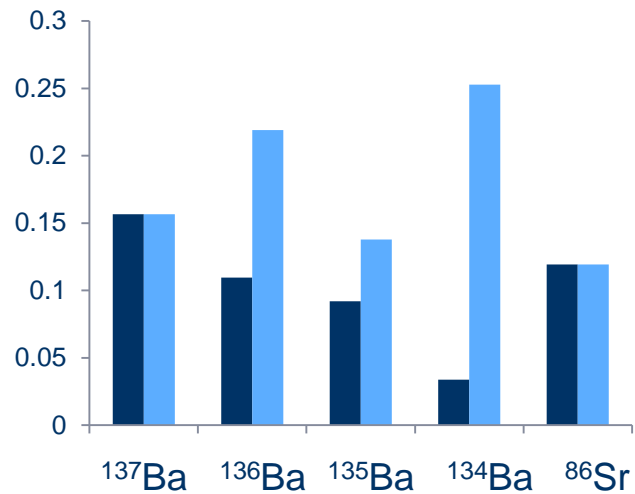
Adding  $^{135}\text{Ba}$

-  Wild Salmon
-  Farmed Salmon



# Creating a fingerprint

Fingerprint mark (#marks = 3)

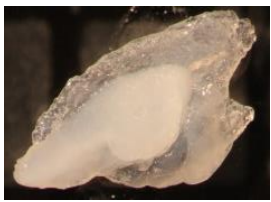


Ba compared to  $^{138}\text{Ba}$   
Sr compared to  $^{88}\text{Sr}$

Adding  $^{134}\text{Ba}$

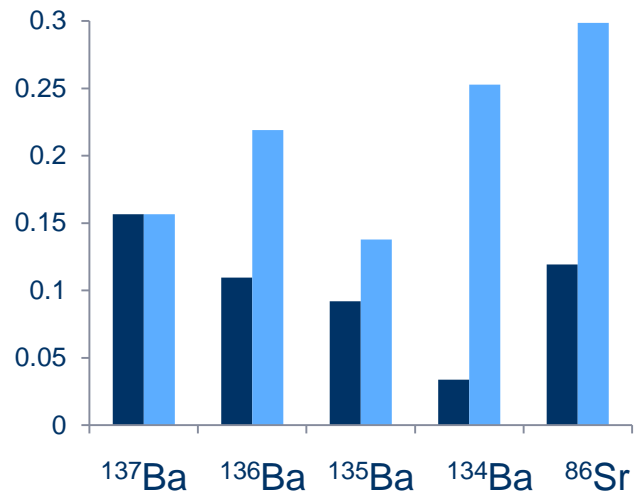
 Wild Salmon

 Farmed Salmon



# Creating a fingerprint

Fingerprint mark (#marks = 4)

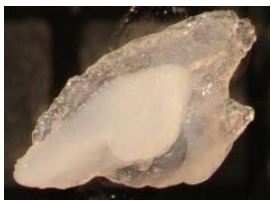


Ba compared to  $^{138}\text{Ba}$   
Sr compared to  $^{88}\text{Sr}$

Adding  $^{86}\text{Sr}$

 Wild Salmon

 Farmed Salmon



# How many unique fingerprints?

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

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

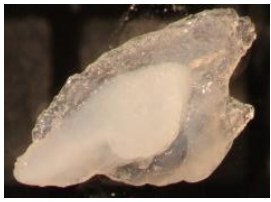
3 isotopes ( $2^3 - 1$ ) = 7 markers

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

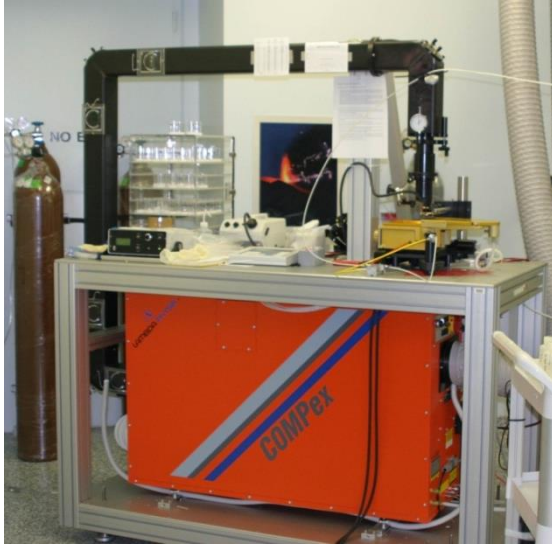
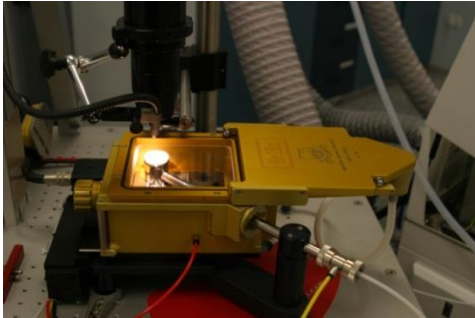
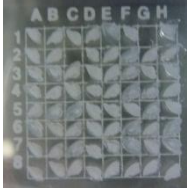
Number of possible markers =  $(2^{\text{number of isotopes}} - 1)$

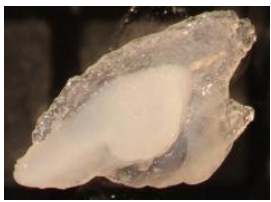
Possible to create > 1000 combinations

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

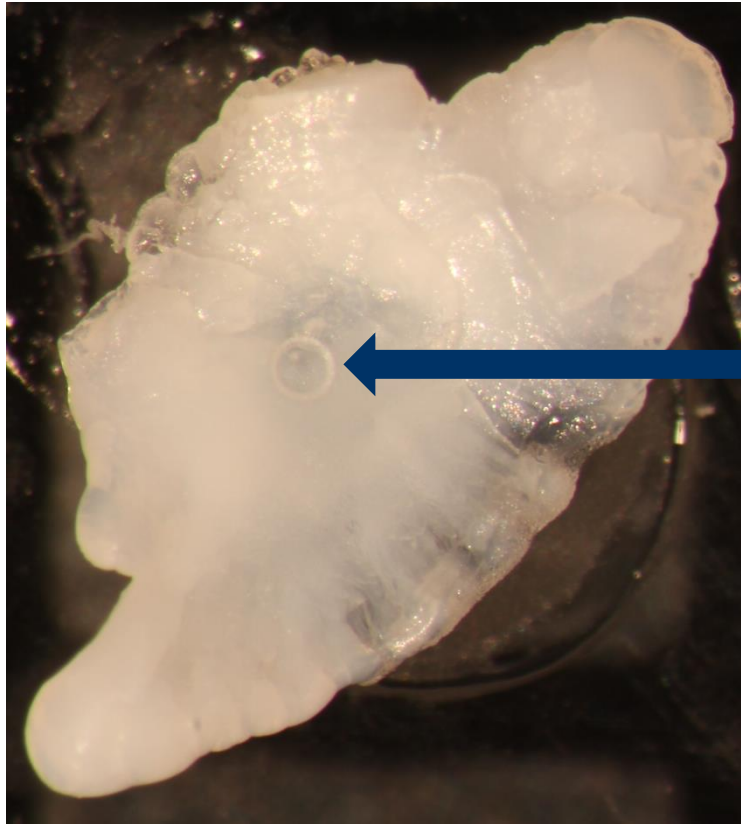


# LA-ICP-MS analysis of fish otoliths

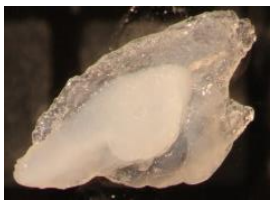




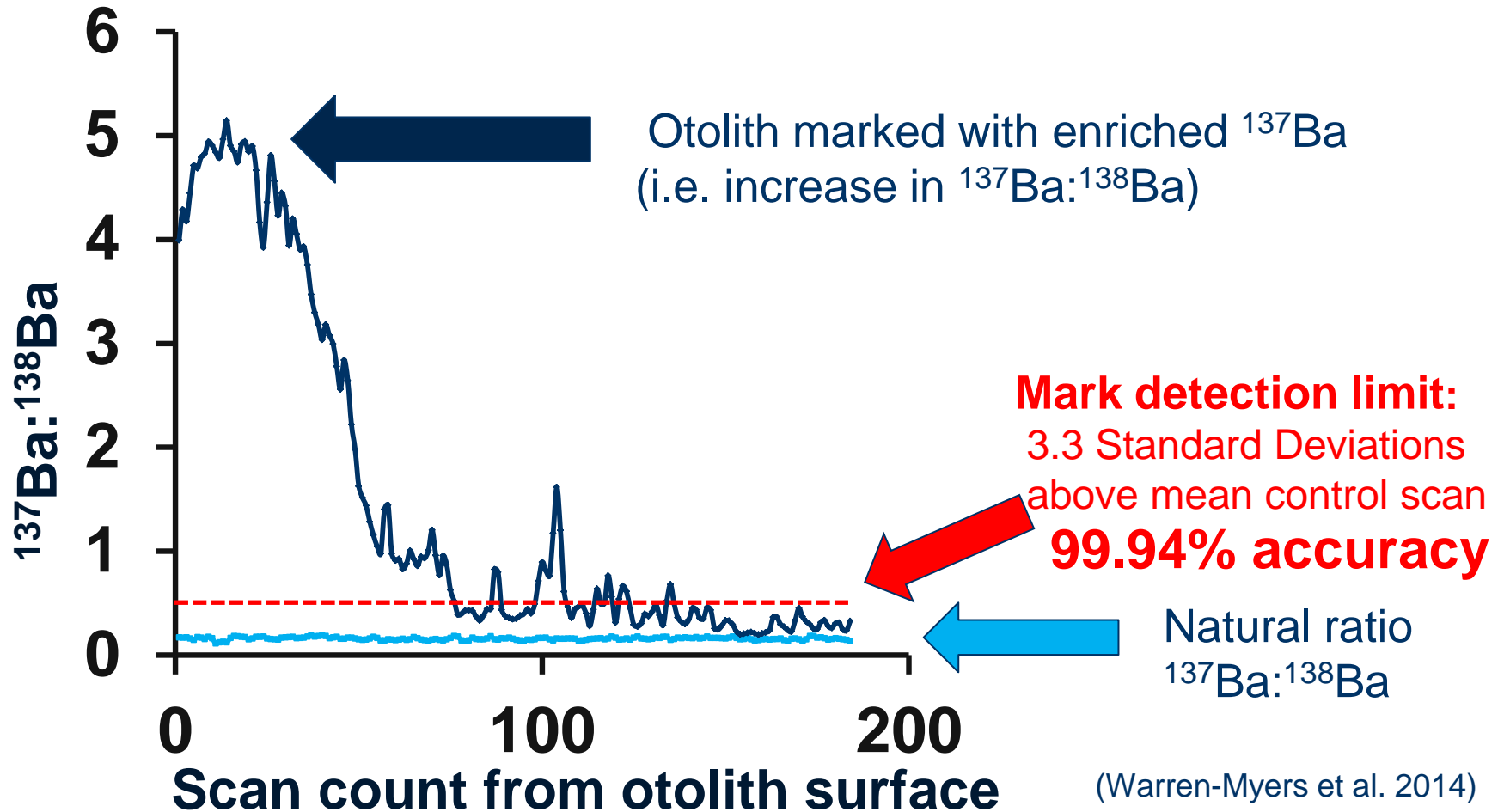
# LA-ICP-MS analysis of fish otoliths



Spot ablation



# Detecting a successful mark ( $^{137}\text{Ba}$ )

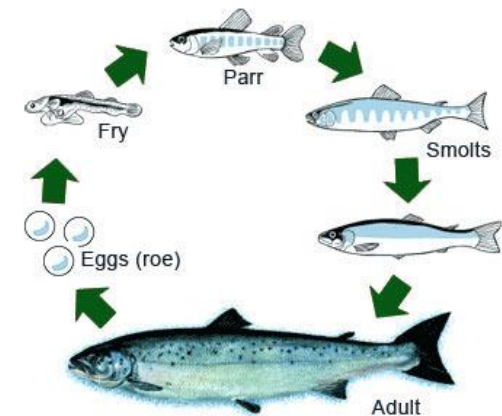




# Aims

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

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

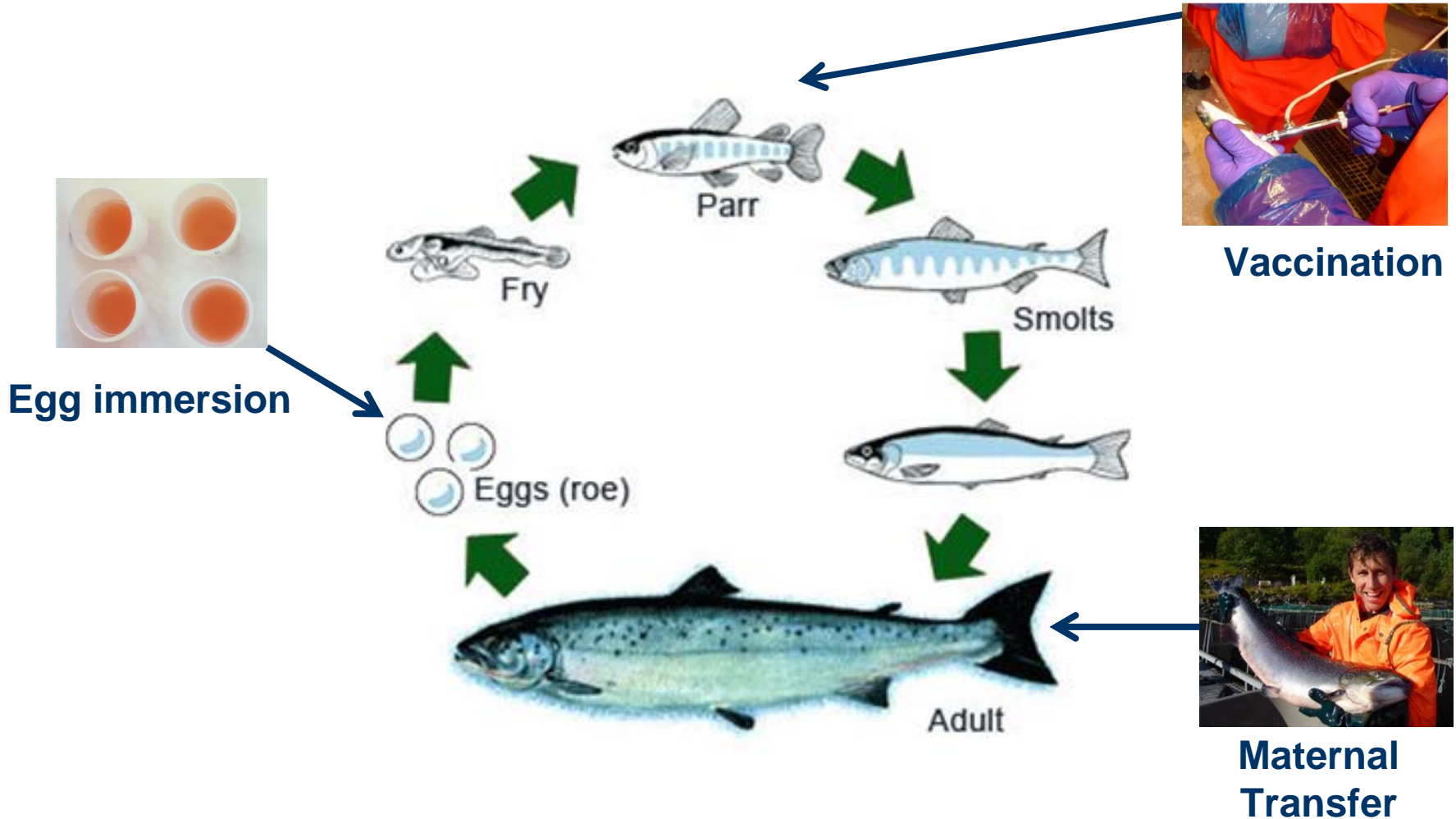


- Confirmation – *Can we create unique marks?*
- Optimization – *how well does each technique work?*
- Welfare - *side effects?*
- Commercial viability - *applicability, cost?*





# Mark application points



# Application points



## Vaccination

### Isotopes

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

### Concentrations

1

0.1

0.01

0.001

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



## Maternal transfer

### Isotopes

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

### Concentrations

2

0.2

0.02

0.002

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



## Egg immersion

### Isotopes

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

### Concentrations

1000 - 2500

100 - 250

10 - 25

1 - 25

$\mu\text{g. L}^{-1}$  water



# Vaccination

## 100% mark success

**$^{137}\text{Ba}$**  Require **0.001  $\mu\text{g}$**  per g of parr

**$^{134}\text{Ba}$**

**$^{135}\text{Ba}$**  Require **0.01  $\mu\text{g}$**  per g of parr

**$^{136}\text{Ba}$**

**$^{137}\text{Ba}$**

**$^{86}\text{Sr}$**

Require **1  $\mu\text{g}$**  per g of parr

**$^{87}\text{Sr}$**



# Maternal transfer 100% mark success

**$^{137}\text{Ba}$**

Require  **$0.02 \mu\text{g}$**  per g of brood fish

**$^{135}\text{Ba}$**

**$^{134}\text{Ba}$**

Require  **$0.2 \mu\text{g}$**  per g of brood fish

**$^{136}\text{Ba}$**

**$^{86}\text{Sr}$**

Require  **$2 \mu\text{g}$**  per g brood fish

**$^{87}\text{Sr}$**



# Egg immersion 100% mark success

**$^{137}\text{Ba}$**

Require **100  $\mu\text{g}$**  per litre

**$^{135}\text{Ba}$**

**$^{136}\text{Ba}$**

Require **1000  $\mu\text{g}$**  per litre



# Fish Health

Monitoring of fish health parameters found:

**No effect on Growth**

**No effect on Condition**

**No effect on Mortality**



# Cost projections

**Scenario 1:** Marking 100% of production with 1 marker  
*(achievable)*



# Cost projections

## Scenario 1

Marking 300 million farmed Atlantic salmon with 1 Ba code

Vaccination (50 g fish)	Material Cost (\$US)	Total
$^{137}\text{Ba}$ @ 0.001 $\mu\text{g. g}^{-1}$ fish weight (15 g for 300 million parr)	\$4.36 per mg (~ \$0.0006 per parr)	<b>\$65400</b>

Egg immersion (2000 eggs L <sup>-1</sup> )	Material Cost (\$US)	Total
$^{137}\text{Ba}$ @ 100 $\mu\text{g. L}^{-1}$ (150,000 L for 300 million eggs)	\$4.36 per mg (~ \$0.44 per litre)	<b>\$65400</b>

Maternal Transfer (5000 eggs per 10 kg brood fish)	Material Cost (\$US)	Total
$^{137}\text{Ba}$ @ 0.02 $\mu\text{g. g}^{-1}$ brood fish weight (60000 brood fish for 300 million eggs)	\$4.36 per mg (~ \$0.872 per brood fish)	<b>\$52320</b>





# Cost projections

**Scenario 2: Marking 100% of production (54 Companies)**  
*(achievable)*



# Cost projections

## Scenario 2

54 companies, 300 million salmon, 2 delivery methods, 54 codes

**Method:** Marking fish with Ba codes either via vaccination or maternal transfer or marking with a combination of maternal transfer and vaccination.

5 largest companies make up 53% of production: Marine Harvest 22%, Lerøy Seafoods 13%, Salmar 9%, Cermaq 5% and Grieg Seafoods 4%.

19 medium companies make up a further 27% of production:  
Average size 1.43% each.

30 small companies make up the final 20% of production:  
Average size 0.67% each.



# Cost projections Scenario 2

54 companies, 300 million salmon, 2 delivery methods, 54 codes

Company Number	Company (% size)	Production (n fish)	Code number	Cost per fish	Cost per company
Marine Harvest	22%	66000000	2MT	\$0.0002	\$11,510
Lerøy	13%	39000000	1V	\$0.0002	\$8,502
Salmar	9%	27000000	3V	\$0.0003	\$8,910
Cermaq	5%	15000000	1V2MT	\$0.0004	\$5,886
Grier	4%	12000000	3V2MT	\$0.0005	\$6,053
6	1.42%	4263158	4MT	\$0.0005	\$2,201
7	1.42%	4263158	5V	\$0.0006	\$2,752
8	1.42%	4263158	16MT	\$0.0007	\$2,945
9	1.42%	4263158	1V4MT	\$0.0007	\$3,131
10	1.42%	4263158	5V2MT	\$0.0008	\$3,495
11	1.42%	4263158	3V4MT	\$0.0008	\$3,608
12	1.42%	4263158	1V16MT	\$0.0009	\$3,874
13	1.42%	4263158	7V	\$0.0010	\$4,272
14	1.42%	4263158	5V4MT	\$0.0010	\$4,352
15	1.42%	4263158	5V4MT	\$0.0012	\$4,953
16	1.42%	4263158	7V2MT	\$0.0012	\$5,015
17	1.42%	4263158	5V16MT	\$0.0013	\$5,697
18	1.42%	4263158	7V4MT	\$0.0015	\$6,473
19	1.42%	4263158	7V16MT	\$0.0017	\$7,217
20	1.42%	4263158	6MT	\$0.0026	\$11,255
21	1.42%	4263158	15MT	\$0.0028	\$11,998
22	1.42%	4263158	1V6MT	\$0.0029	\$12,184
23	1.42%	4263158	3V6MT	\$0.0030	\$12,662
24	1.42%	4263158	1V15MT	\$0.0030	\$12,928
25	0.67%	2000000	3V15MT	\$0.0031	\$6,289
26	0.67%	2000000	18MT	\$0.0032	\$6,313
27	0.67%	2000000	5V6MT	\$0.0033	\$6,571
28	0.67%	2000000	26MT	\$0.0033	\$6,662
29	0.67%	2000000	1V18MT	\$0.0034	\$6,749
30	0.67%	2000000	5V15MT	\$0.0035	\$6,920
31	0.67%	2000000	3V18MT	\$0.0035	\$6,973
32	0.67%	2000000	1V26MT	\$0.0035	\$7,098
33	0.67%	2000000	7V6MT	\$0.0036	\$7,284
34	0.67%	2000000	3V26MT	\$0.0037	\$7,322
35	0.67%	2000000	5V18MT	\$0.0038	\$7,604
36	0.67%	2000000	7V15MT	\$0.0038	\$7,633
37	0.67%	2000000	5V26MT	\$0.0040	\$7,953
38	0.67%	2000000	7V18MT	\$0.0042	\$8,317
39	0.67%	2000000	7V26MT	\$0.0043	\$8,666
40	0.67%	2000000	9V	\$0.0055	\$10,960
41	0.67%	2000000	9V2MT	\$0.0057	\$11,309
42	0.67%	2000000	9V4MT	\$0.0060	\$11,993
43	0.67%	2000000	9V16MT	\$0.0062	\$12,342
44	0.67%	2000000	8MT	\$0.0080	\$16,032
45	0.67%	2000000	9V6MT	\$0.0081	\$16,240
46	0.67%	2000000	17MT	\$0.0082	\$16,381
47	0.67%	2000000	1V8MT	\$0.0082	\$16,468
48	0.67%	2000000	9V15MT	\$0.0083	\$16,589
49	0.67%	2000000	3V8MT	\$0.0083	\$16,692
50	0.67%	2000000	20MT	\$0.0085	\$17,065
51	0.67%	2000000	10V	\$0.0086	\$17,270
52	0.67%	2000000	9V18MT	\$0.0086	\$17,273
53	0.67%	2000000	5V8MT	\$0.0087	\$17,323
54	0.67%	2000000	28MT	\$0.0087	\$17,414

5 largest companies (53% production)  
\$0.0003 per fish to mark

19 medium companies (27% production)  
\$0.0015 per fish to mark

30 small companies (20% of production)  
\$0.0057 per fish to mark

In total 54 companies (100% of production)  
Average cost of \$0.0017 per fish to mark.

**Total material cost: \$500,000**



## Summary

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

**Vaccination:** 63 unique fingerprints

**Maternal Transfer:** 63 unique fingerprints

**Egg immersion:** 7 unique fingerprints

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



**Thank you**