
# Detecting and tracing farmed salmon with otolith tags: developing and validating mark delivery techniques


## University of Melbourne, Australia

Fletcher Warren-Myers

Associate Prof. Steve Swearer

Dr Tim Dempster

## Institute of Marine Research, Norway

Dr Tom Hansen

Dr Per Gunnar Fjelldal

# Aims

- To evaluate alternate techniques for mass marking farmed Atlantic salmon with alkaline earth elements.
- 1) Marking via injection – Norway vaccinates all Atlantic salmon
- 2) Marking via maternal transfer – 5000 eggs with one injection
- 3) Marking via egg immersion – Immerse 2000 eggs in 1 litre

Main questions for each technique

- Optimization - *marker concentration?*
- Welfare assessment - *side effects?*
- Commercial viability - *applicability, cost?*
- Confirmation – *Guaranteeing 100% differentiation between farm and wild*

# Background
# ratios of alkaline earth elements

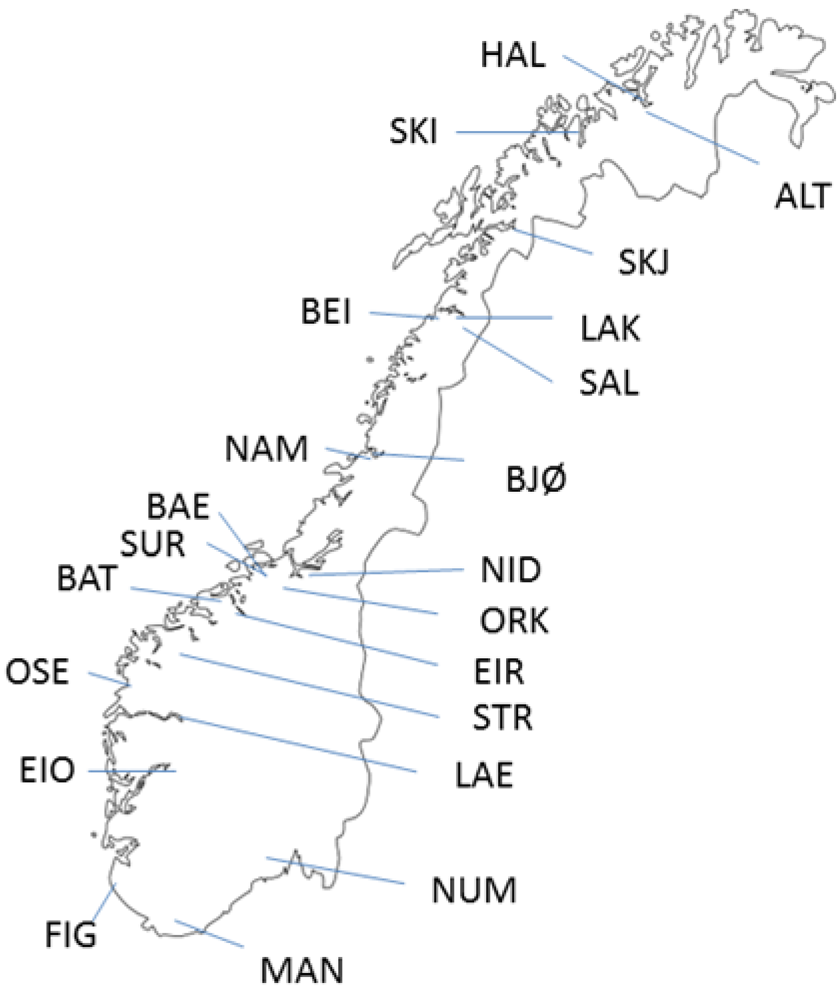
Natural levels of different forms of Ba, Sr & Mg throughout Norwegian wild salmon populations.

**Spatially:** Samples from 22 rivers from north to south

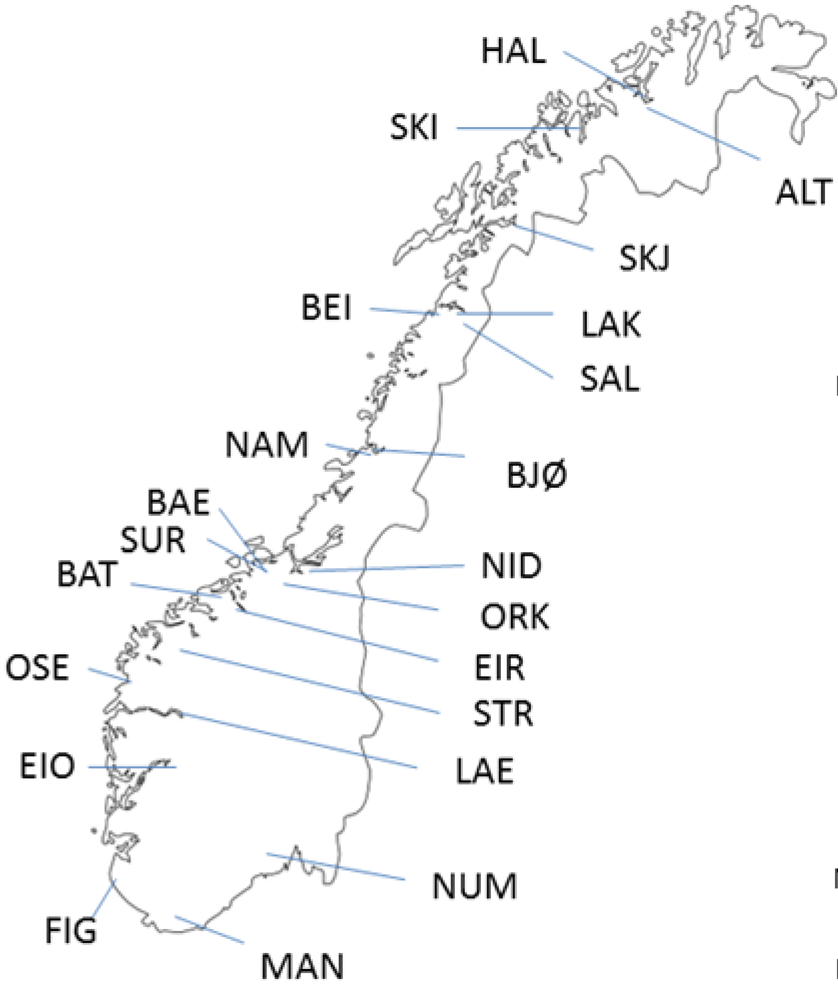
**Temporally**: Samples from 2 rivers spanning from 1990 to 2010

(Otoliths sourced from NINA archive samples, located in Trondheim, Norway)

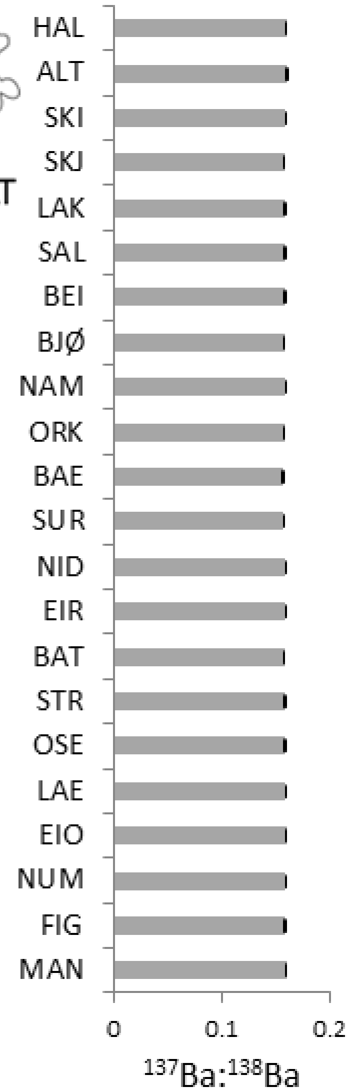
# Background ratios



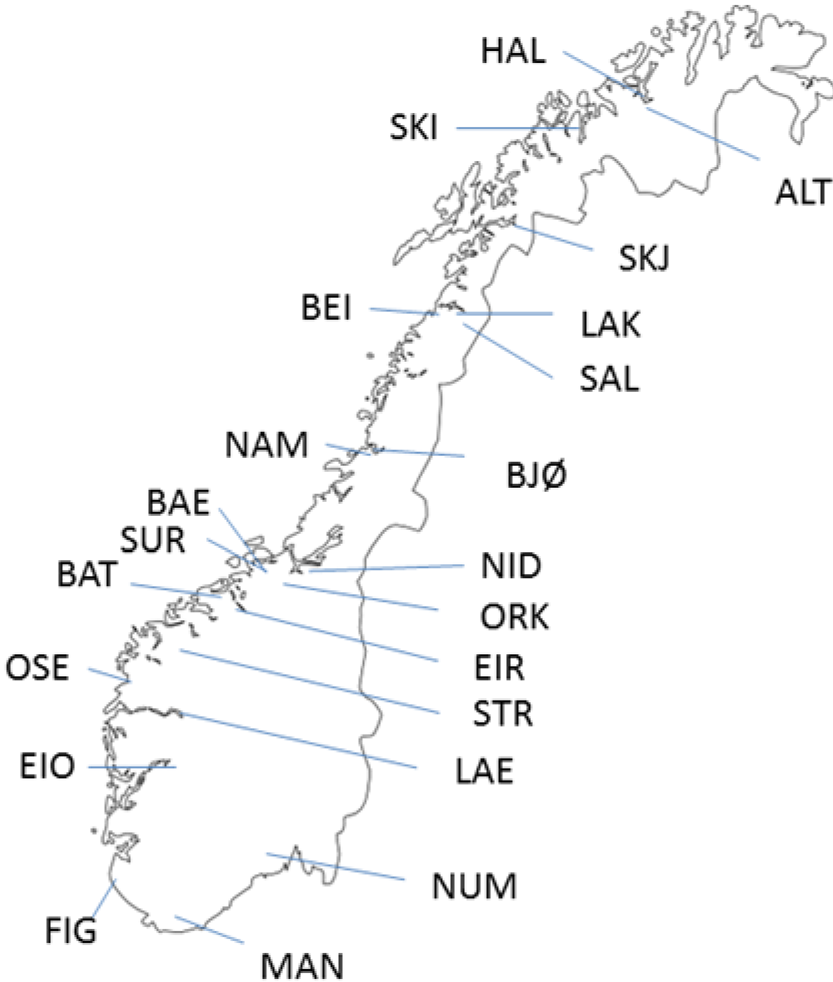
# Background ratios




$^{137}Ba:^{138}Ba$ +/-1%

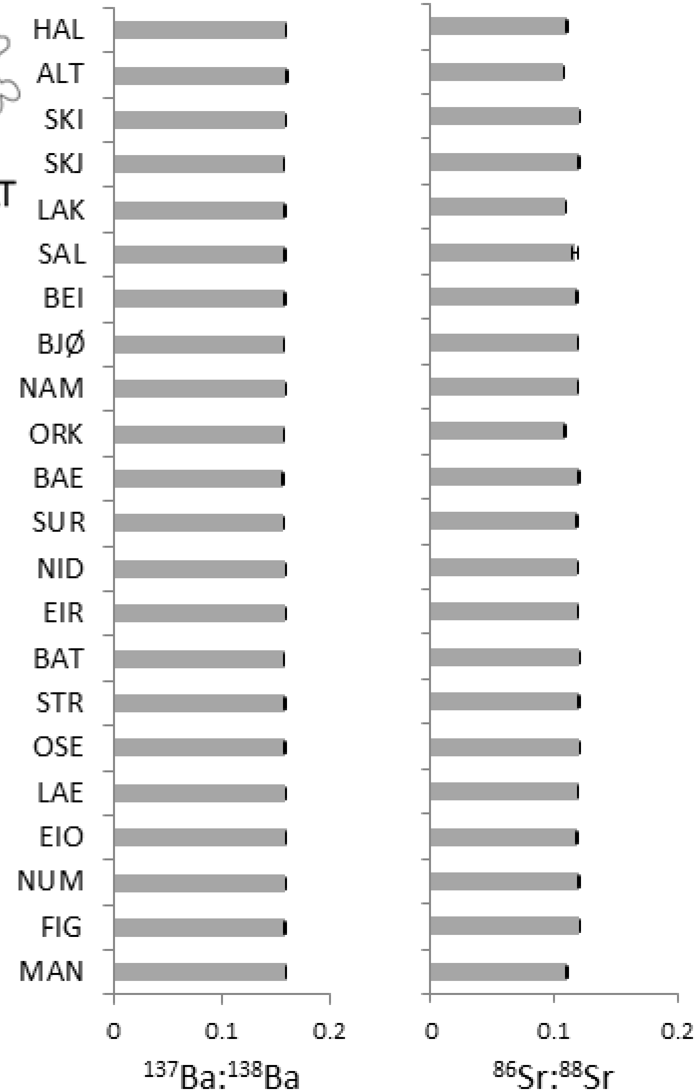


# Background ratios




$^{137}Ba:^{138}Ba$ +/-1%

$^{86}Sr:^{88}Sr$ +/- 5%



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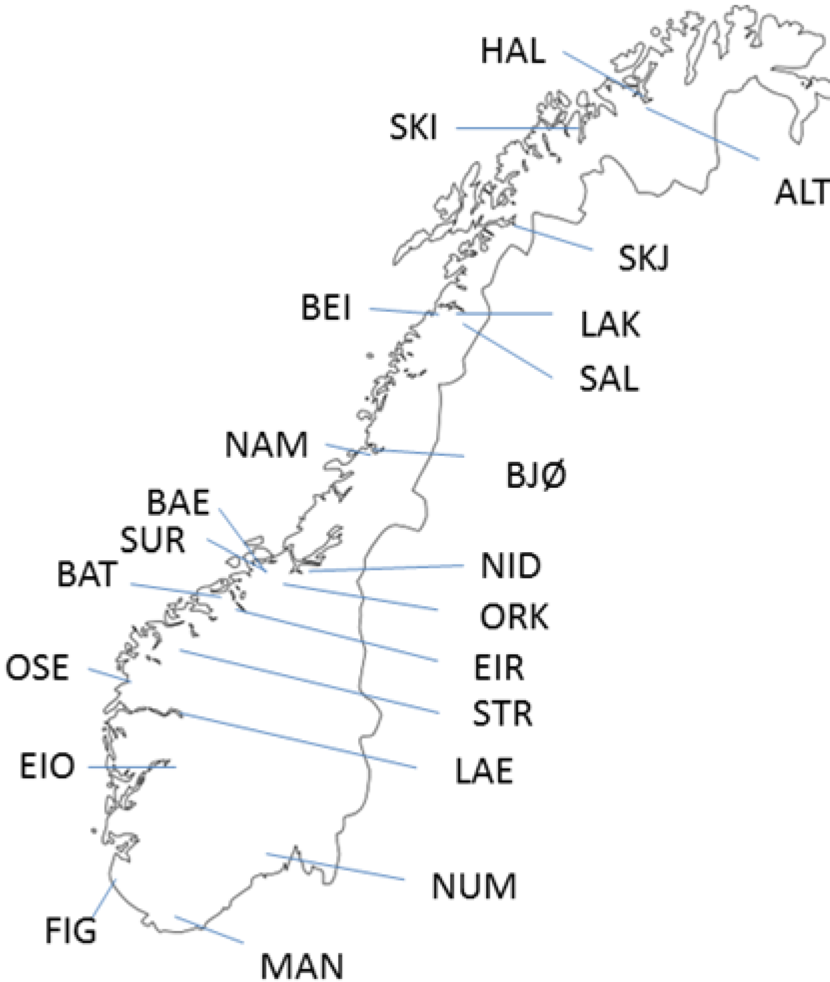
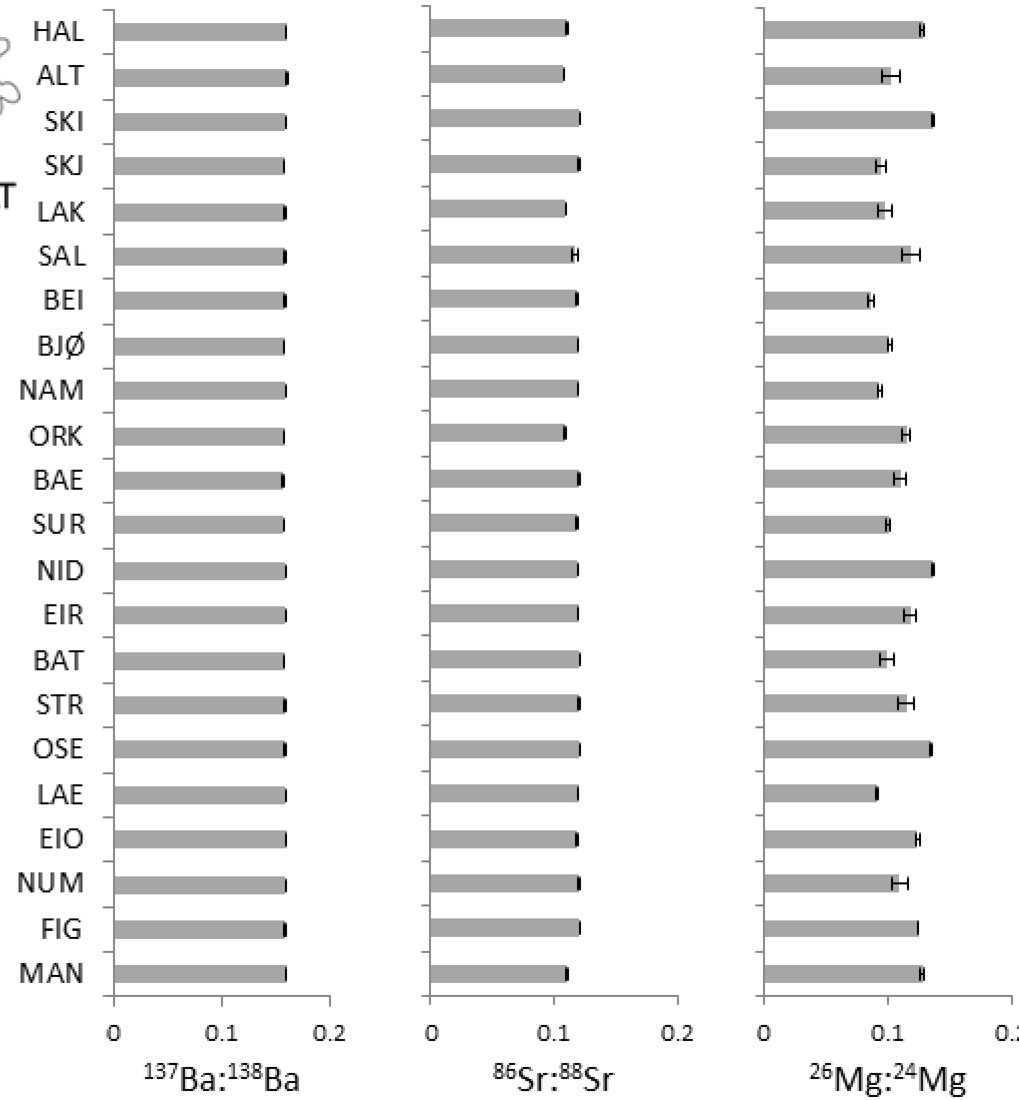


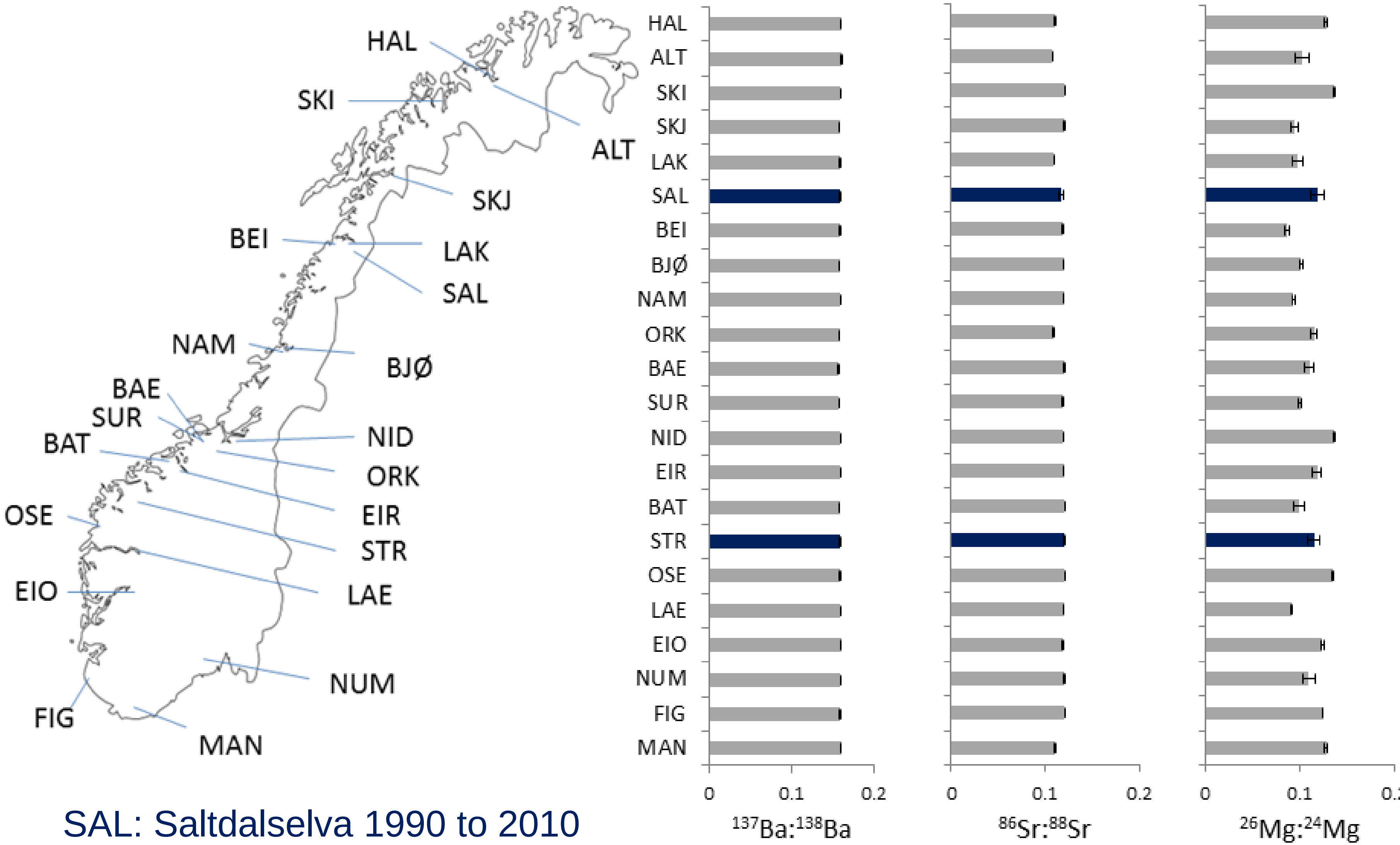




$^{137}Ba:^{138}Ba$ +/-1%

$^{86}Sr:^{88}Sr$ +/- 5%

$^{26}Mg:^{24}Mg$ +/-15%

# Background ratios

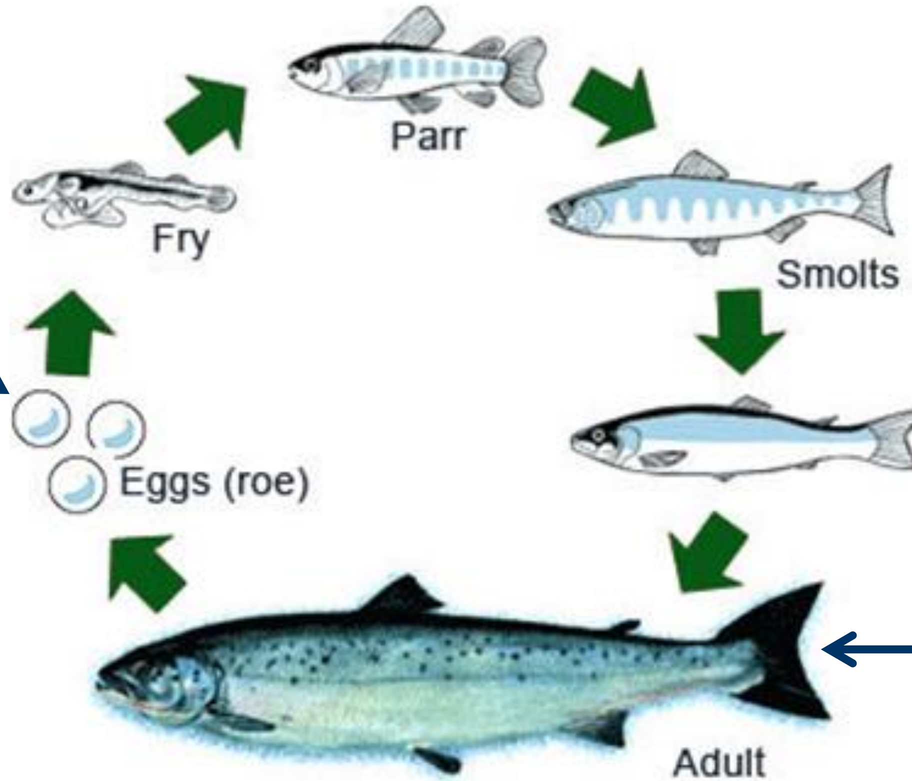



SAL: Saltdalselva 1990 to 2010

STR: Strynselva 1990 to 2009

# Mark application points

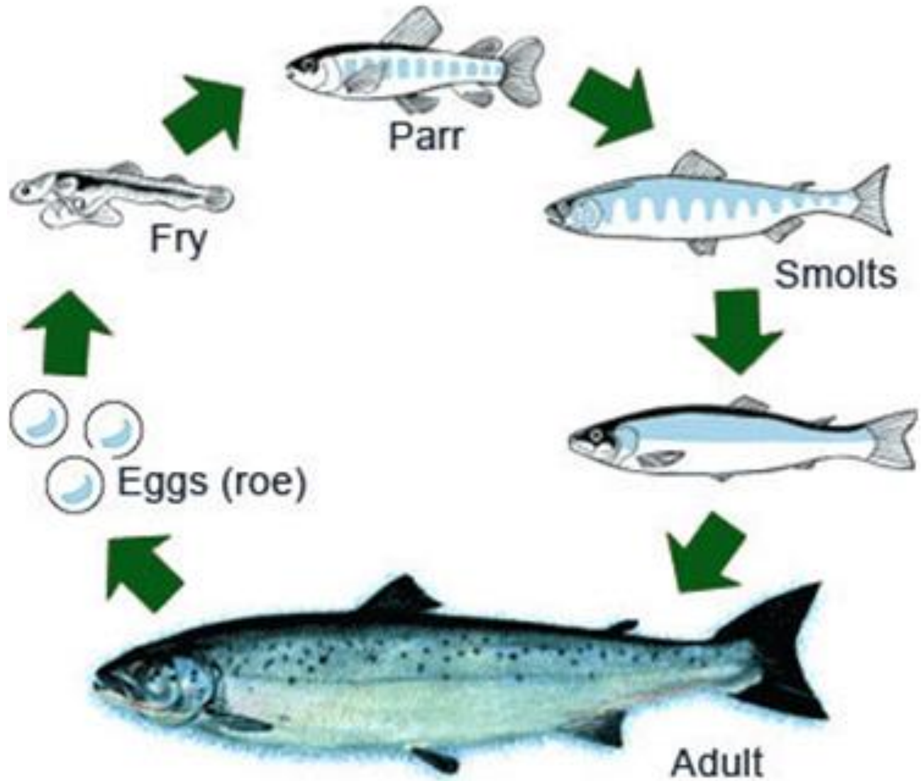
Egg immersion




Vaccination

Maternal Transfer

# Mark application points




Vaccination

# Mass marking via vaccination

# Vaccination 1

**Question**: Is carrier solution or injection site important for marker uptake?

**Method:**

- Fish were pit tagged 2 months prior
- 3 tags used: $^{137}Ba$, $^{86}Sr$, and $^{26}Mg$
- Concentration 2 µg per g fish weight
  (Average weight was 57 grams (SE +/- 0.1 g)
- Otolith samples 2 weeks post injecting

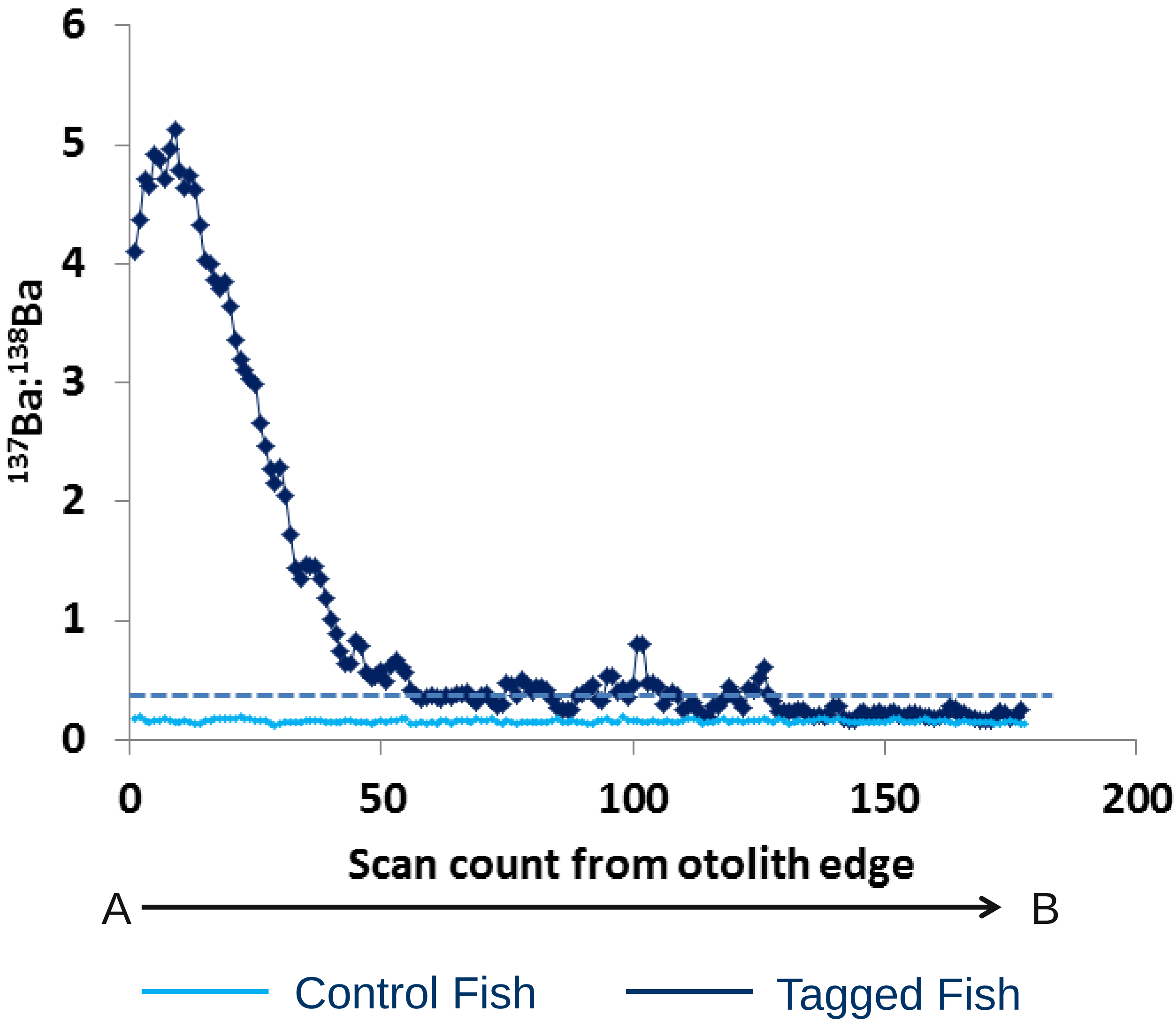
# Experimental design

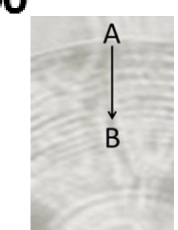
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (2) Injection sites | Intra-peritoneal cavity | | | | | | Muscle | | | | | |
| (3) Solutions | Water | | Vaccine | | Emulsion | | Water | | Vaccine | | Emulsion | |
| (2) Tag/Control | T | C | T | C | T | C | T | C | T | C | T | C |
| Replicate fish | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 |

Total of 144 fish, spread amongst 3 tanks (48 per tank)

# Results

## $^{137}Ba:^{138}Ba$



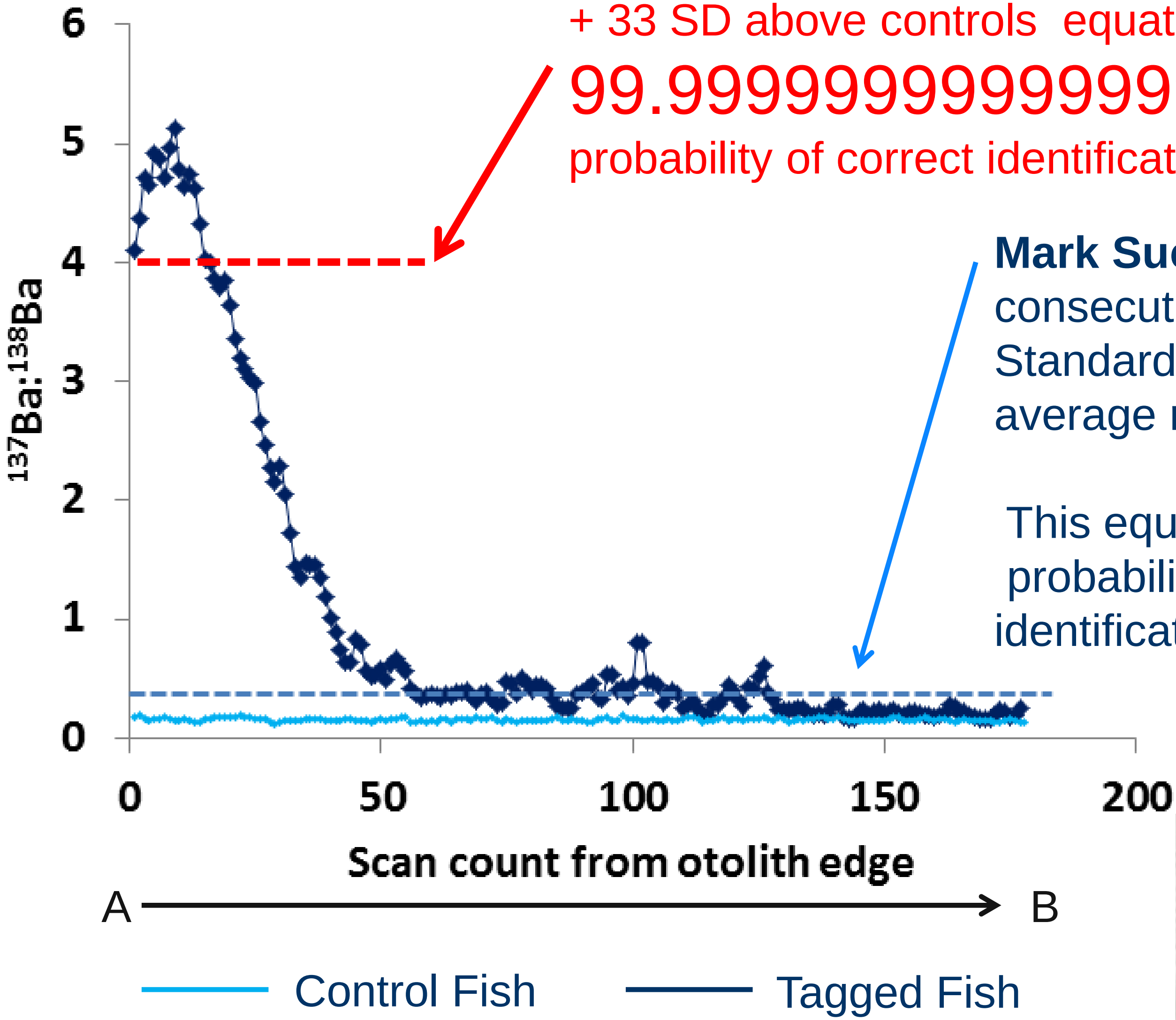



# Results
# $^{137}Ba:^{138}Ba$

+ 33 SD above controls  equates to a

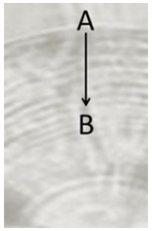
99.9999999999999999%

probability of correct identification



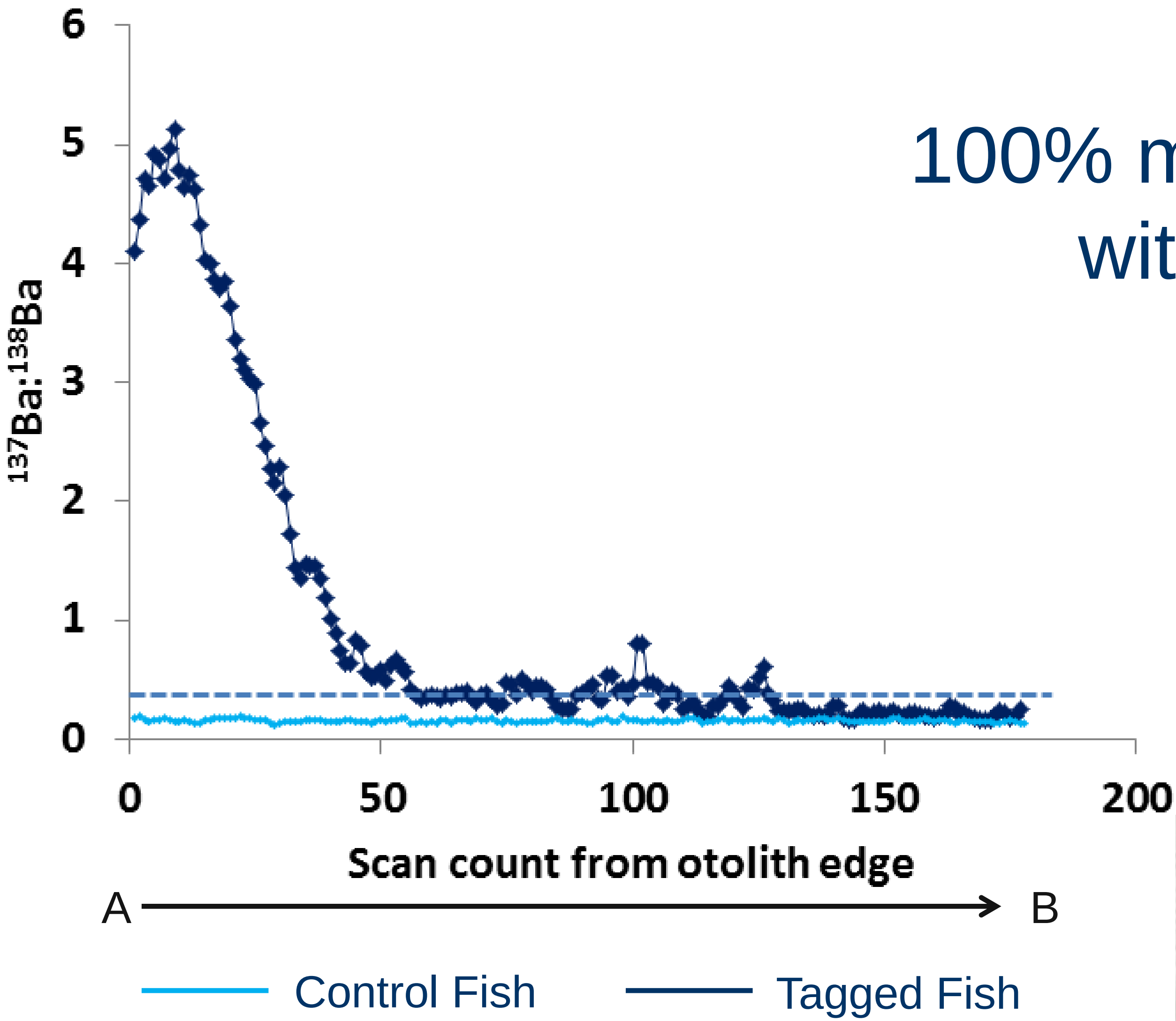

**Mark Success:** defined as 3 consecutive ratios 3.3 Standard Deviations above the average ratio of control fish.

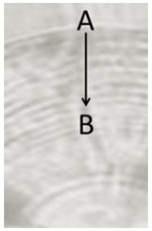
This equates to a 99.94% probability of correct identification of a tagged fish



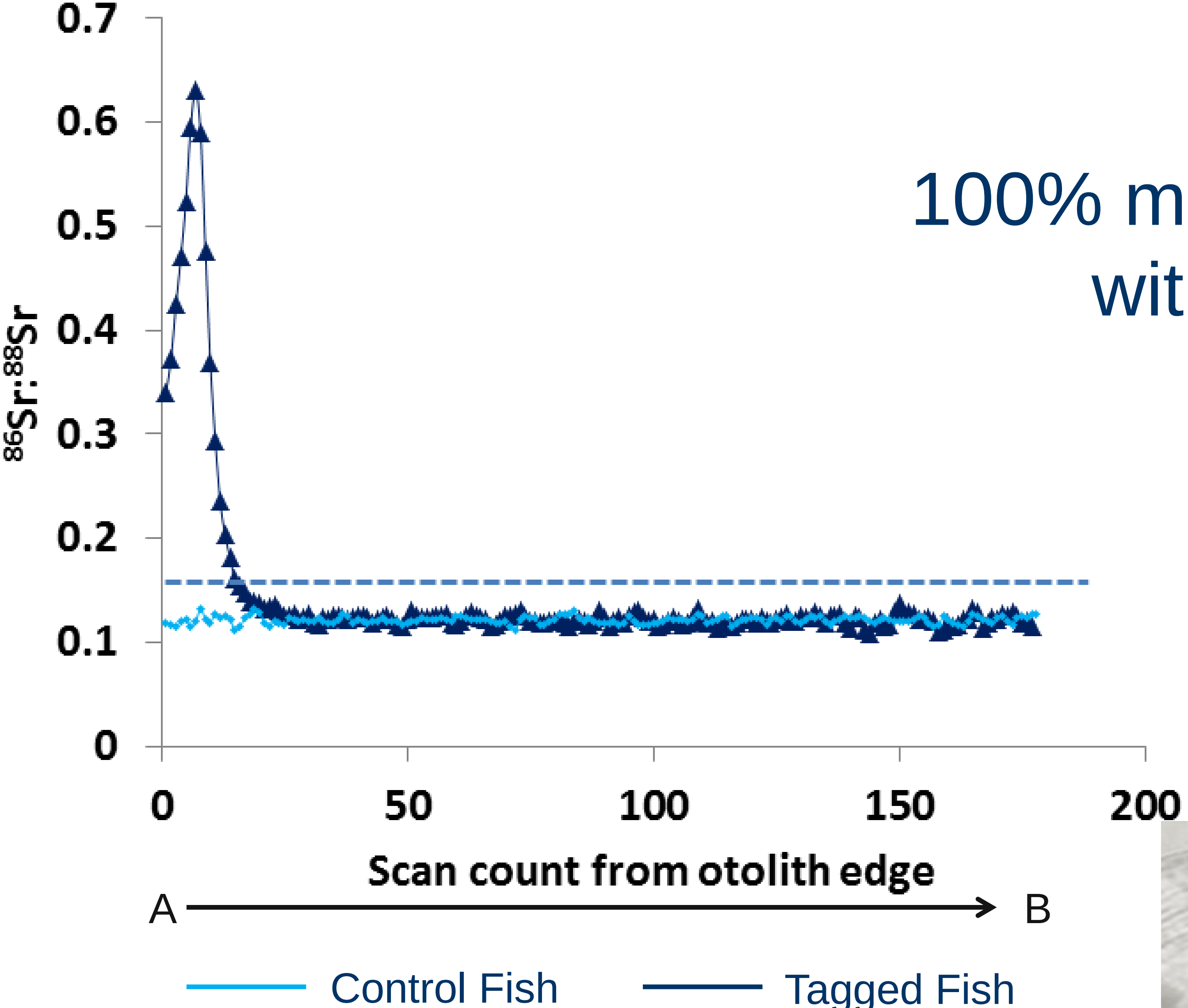
# Mark Success
# $^{137}Ba$:$^{138}Ba$

100% mark uptake with $^{137}Ba$

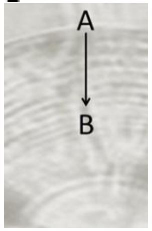


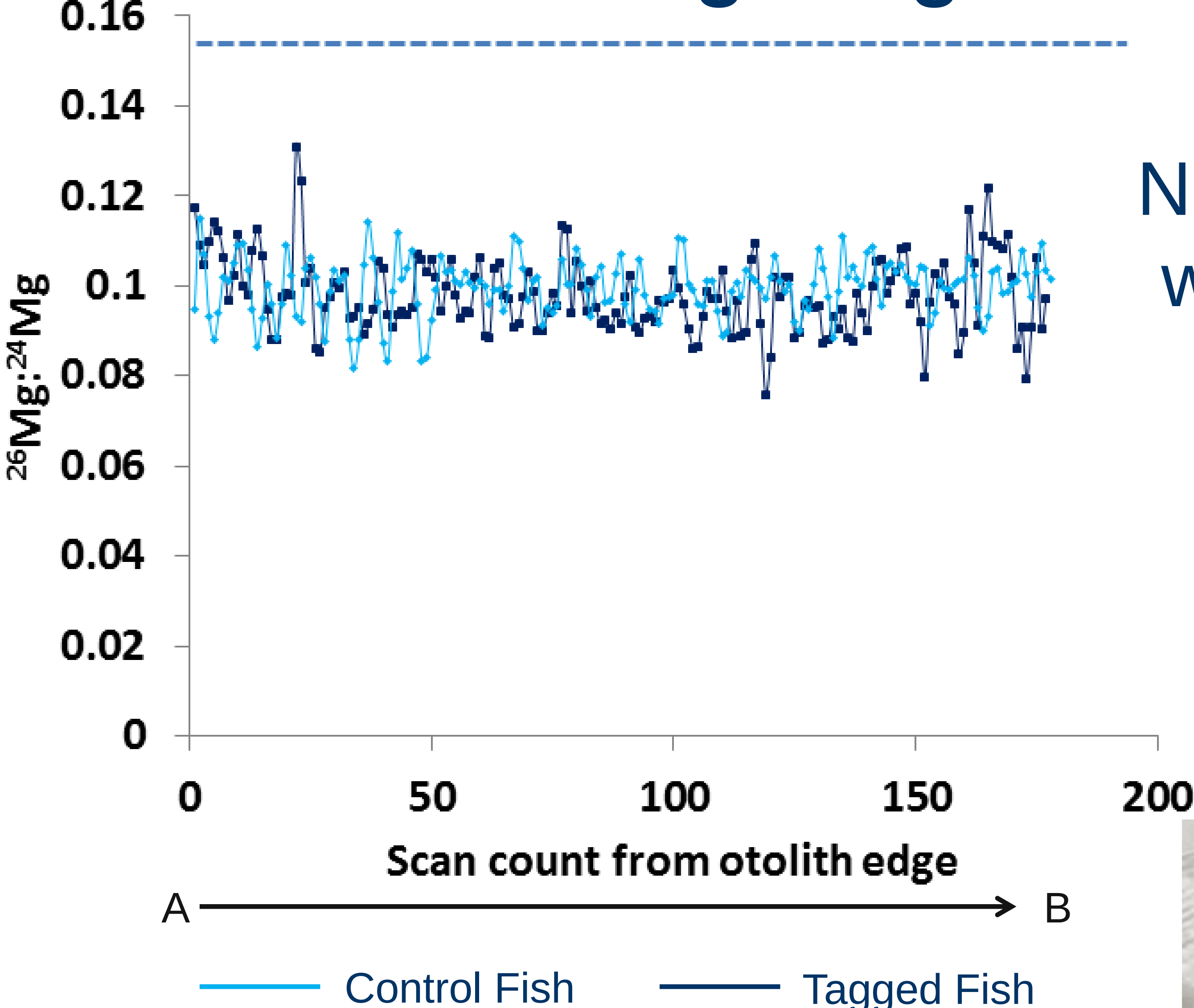
# Mark Success
## $^{86}Sr$:$^{88}Sr$



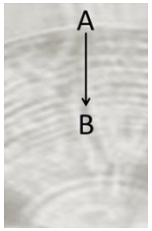

100% mark uptake with $^{86}Sr$



# Mark Success
## $^{26}Mg:^{24}Mg$




No uptake with $^{26}Mg$



# Results

Injection site:

**Intra-peritoneal cavity** overall produced stronger marks compared to muscle injection for **both $^{137}$Ba and $^{86}$Sr**

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**Intra-peritoneal cavity** overall produced stronger marks compared to muscle injection for **both $^{137}$Ba and $^{86}$Sr**

Carrier solution:

**Water and emulsion** solutions produced stronger marks compared to the vaccine solution for **$^{137}$Ba**

# Results

Injection site:

**Intra-peritoneal cavity** overall produced stronger marks compared to muscle injection for **both $^{137}$Ba and $^{86}$Sr**

Carrier solution:

**Water and emulsion** solutions produced stronger marks compared to the vaccine solution for **$^{137}$Ba**

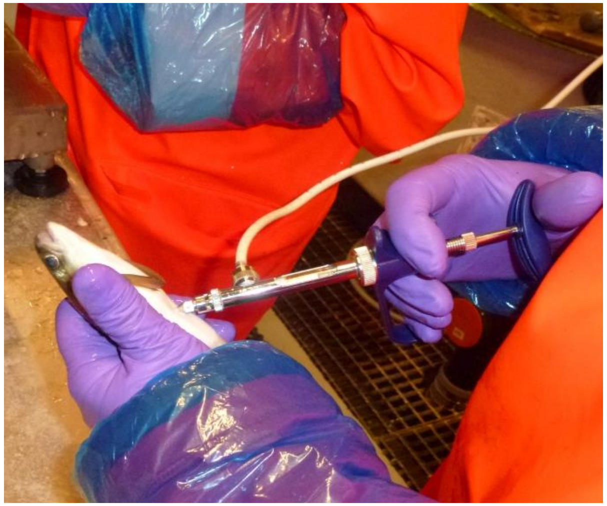
**Vaccine and emulsion** solutions produced stronger marks compared to water for **$^{86}$Sr**

# Conclusions

- Best to inject into the intra-peritoneal cavity
- MINOVA 6 as a carrier is appropriate to use
- $^{137}Ba$ and $^{86}Sr$ markers highly successful

# Vaccination 2

**Method:** Deliver multiple concentrations and combinations of markers via injection



## Combinations:

- **1** : $^{137}$Ba
- **4** : $^{137}$Ba, $^{135}$Ba, $^{136}$Ba, $^{86}$Sr
- **7** : $^{137}$Ba, $^{136}$Ba, $^{135}$Ba, $^{134}$Ba, $^{87}$Sr, $^{86}$Sr &$^{26}$Mg

## Concentrations:

(µg. $g^{-1}$ fish weight)

(Average weight 102 +/- 0.6 g)

1 µg

0.1µg

0.01 µg

0.001 µg

# Experimental design

| | | | |
|---|---|---|---|
| (3) Combinations | Single | Four | Seven |
| (4) Concentrations (µg. g$^{-1}$ fish weight) | 1, 0.1, 0.01, 0.001 | 1, 0.1, 0.01, 0.001 | 1, 0.1, 0.01, 0.001 |
| Replicate fish | 50 50 50 50 | 50 50 50 50 | 50 50 50 50 |

Plus 50 control fish injected with vaccine only

- Fish spread amongst 5 tanks (130 per tank)
- Standard vaccination volume (0.1 ml)
- Otolith samples collected 3 months post vaccination

# Results

| | | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Number of Markers** | **Concentration** (µg. $g^{-1}$ fish) | $^{137}$**Ba** | | | | | | |
| **1** | 1<br>0.1<br>0.01<br>0.001 | | | | | | | |

# Results

| | | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Number of Markers** | **Concentration** (µg. $g^{-1}$ fish) | **$^{137}$Ba** | | | | | | |
| **1** | 1 | **100%** | | | | | | |
| | 0.1 | **100%** | | | | | | |
| | 0.01 | **100%** | | | | | | |
| | 0.001 | **100%** | | | | | | |

# Results

| | | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Number of Markers** | **Concentration** (µg. $g^{-1}$ fish) | $^{137}$**Ba** | $^{136}$**Ba** | $^{135}$**Ba** | $^{86}$**Sr** | | | |
| 1 | 1 | 100% | | | | | | |
| | 0.1 | 100% | | | | | | |
| | 0.01 | 100% | | | | | | |
| | 0.001 | 100% | | | | | | |
| **4** | 1 | | | | | | | |
| | 0.1 | | | | | | | |
| | 0.01 | | | | | | | |
| | 0.001 | | | | | | | |

# Results

| | | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Number of Markers** | **Concentration** ($\mu g. g^{-1}$ fish) | $^{137}Ba$ | $^{136}Ba$ | $^{135}Ba$ | $^{86}Sr$ | | | |
| 1 | 1 | 100% | | | | | | |
| | 0.1 | 100% | | | | | | |
| | 0.01 | 100% | | | | | | |
| | 0.001 | 100% | | | | | | |
| **4** | 1 | **100%** | **100%** | **100%** | **100%** | | | |
| | 0.1 | **100%** | **100%** | **100%** | 30% | | | |
| | 0.01 | **100%** | **100%** | **100%** | 0% | | | |
| | 0.001 | 80% | 20% | 80% | 0% | | | |

# Results

| | | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Number of Markers | Concentration ($\mu g. g^{-1}$ fish) | $^{137}Ba$ | $^{136}Ba$ | $^{135}Ba$ | $^{86}Sr$ | $^{134}Ba$ | $^{87}Sr$ | $^{26}Mg$ |
| 1 | 1 | 100% | | | | | | |
| | 0.1 | 100% | | | | | | |
| | 0.01 | 100% | | | | | | |
| | 0.001 | 100% | | | | | | |
| 4 | 1 | 100% | 100% | 100% | 100% | | | |
| | 0.1 | 100% | 100% | 100% | 30% | | | |
| | 0.01 | 100% | 100% | 100% | 0% | | | |
| | 0.001 | 80% | 20% | 80% | 0% | | | |
| **7** | 1 | | | | | | | |
| | 0.1 | | | | | | | |
| | 0.01 | | | | | | | |
| | 0.001 | | | | | | | |

# Results

| | | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Number of Markers** | **Concentration** (µg. $g^{-1}$ fish) | $^{137}$Ba | $^{136}$Ba | $^{135}$Ba | $^{86}$Sr | $^{134}$Ba | $^{87}$Sr | $^{26}$Mg |
| 1 | 1 | 100% | | | | | | |
| | 0.1 | 100% | | | | | | |
| | 0.01 | 100% | | | | | | |
| | 0.001 | 100% | | | | | | |
| 4 | 1 | 100% | 100% | 100% | 100% | | | |
| | 0.1 | 100% | 100% | 100% | 30% | | | |
| | 0.01 | 100% | 100% | 100% | 0% | | | |
| | 0.001 | 80% | 20% | 80% | 0% | | | |
| **7** | 1 | **100%** | **100%** | **100%** | **100%** | **100%** | **100%** | 0% |
| | 0.1 | **100%** | **100%** | **100%** | 20% | **100%** | 60% | 0% |
| | 0.01 | **100%** | **100%** | **100%** | 0% | **100%** | 0% | 0% |
| | 0.001 | 70% | 20% | 70% | 0% | 0% | 0% | 0% |

# Conclusions

- **$^{137}$Ba as a single marker** can be used at concentrations as low as **0.001 µg** per gram of fish

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- **$^{137}$Ba as a single marker** can be used at concentrations as low as **0.001 µg** per gram of fish

- Combinations of **$^{134}$Ba, $^{135}$Ba $^{136}$Ba** and **$^{137}$Ba** can be used at concentrations as low as **0.01 µg** per gram of fish

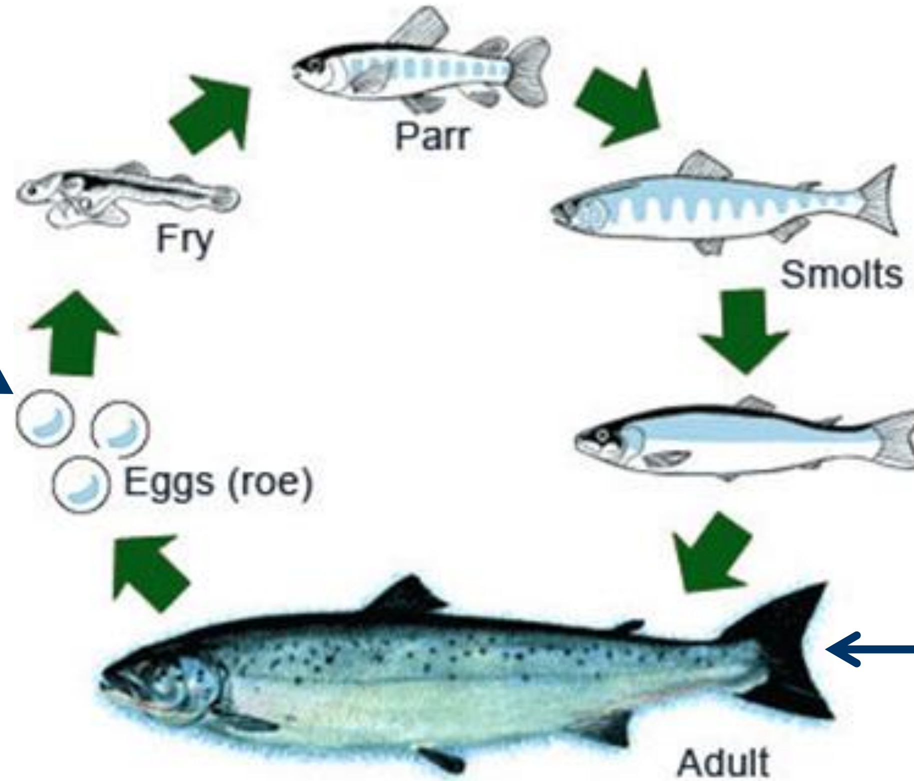
# Conclusions

- **$^{137}$Ba as a single marker** can be used at concentrations as low as **0.001 µg** per gram of fish
- Combinations of **$^{134}$Ba, $^{135}$Ba $^{136}$Ba** and **$^{137}$Ba** can be used at concentrations as low as **0.01 µg** per gram of fish
- Combinations using **$^{86}$Sr and $^{87}$Sr** can be used at concentrations as low as **1 µg** per gram of fish.

# Mass Marking Via Maternal Transfer

# Mark application points

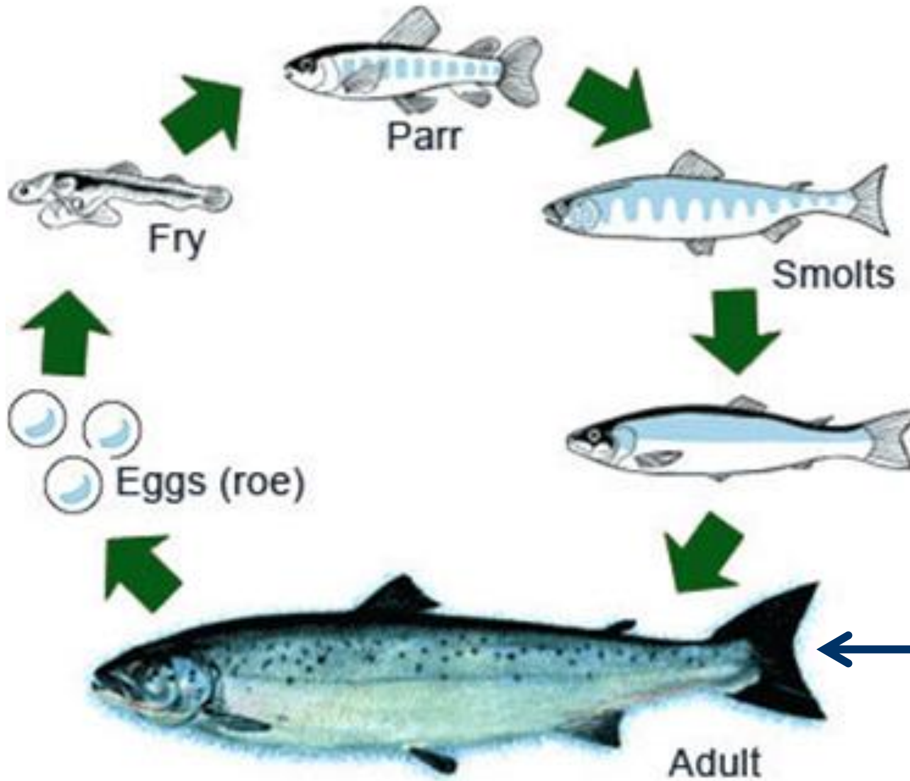
Egg immersion




Vaccination

Maternal
Transfer

# Mark application points




**Maternal Transfer**

# Maternal Transfer

**Method**: Multiple concentrations using a seven marker combination

- Injected 30 female brood stock

- Standard injection volume of 60 ml

- Combination of $^{137}Ba$, $^{136}Ba$, $^{135}Ba$, $^{134}Ba$, $^{87}Sr$, $^{86}Sr$ & $^{26}Mg$

# Experimental design

(1) Combination — Seven markers

(4) Concentrations — 1, 0.1, 0.01, 0.001

(µg isotope per g brood fish weight)

Replicate fish — 6 6 6 6

Plus 6 control fish injected with saline solution .

~1500 eggs per brood fish were stripped and fertilised

First samples were taken before first feeding

# Results

| Spawning Date | # Brood fish Spawned | Concentration µg. g$^{-1}$ brood fish | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $^{137}Ba$ | $^{136}Ba$ | $^{135}Ba$ | $^{134}Ba$ | $^{87}Sr$ | $^{86}Sr$ | $^{26}Mg$ |
| Week 1 | 4 | | | | | | | | |
| Week 2 | 0 | 2 | | | | | | | |
| Week 3 | 2 | | | | | | | | |

# Results

| Spawning Date | # Brood fish Spawned | Concentration µg. $g^{-1}$ brood fish | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $^{137}Ba$ | $^{136}Ba$ | $^{135}Ba$ | $^{134}Ba$ | $^{87}Sr$ | $^{86}Sr$ | $^{26}Mg$ |
| Week 1 | 4 | 2 | 100% | 100% | 100% | 100% | 15% | 3% | 10% |
| Week 2 | 0 | | | | | | | | |
| Week 3 | 2 | | 100% | 100% | 100% | 100% | 100% | 100% | 30% |

# Results

| Spawning Date | # Brood fish Spawned | Concentration µg. $g^{-1}$ brood fish | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $^{137}Ba$ | $^{136}Ba$ | $^{135}Ba$ | $^{134}Ba$ | $^{87}Sr$ | $^{86}Sr$ | $^{26}Mg$ |
| Week 1 | 4 | 2 | 100% | 100% | 100% | 100% | 15% | 3% | 10% |
| Week 2 | 0 | | | | | | | | |
| Week 3 | 2 | | 100% | 100% | 100% | 100% | 100% | 100% | 30% |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Week 1 | 1 | 0.2 | 95% | 10% | 100% | 5% | 0% | 0% | 0% |
| Week 2 | 4 | | 100% | 98% | 100% | 90% | 5% | 5% | 8% |
| Week 3 | 1 | | 100% | 100% | 100% | 100% | 10% | 0% | 0% |

# Results

| Spawning Date | # Brood fish Spawned | Concentration $\mu g. g^{-1}$ brood fish | Mark uptake $^{137}Ba$ | $^{136}Ba$ | $^{135}Ba$ | $^{134}Ba$ | $^{87}Sr$ | $^{86}Sr$ | $^{26}Mg$ |
|---|---|---|---|---|---|---|---|---|---|
| Week 1 | 4 | | 100% | 100% | 100% | 100% | 15% | 3% | 10% |
| Week 2 | 0 | 2 | | | | | | | |
| Week 3 | 2 | | 100% | 100% | 100% | 100% | 100% | 100% | 30% |
| Week 1 | 1 | | 95% | 10% | 100% | 5% | 0% | 0% | 0% |
| Week 2 | 4 | 0.2 | 100% | 98% | 100% | 90% | 5% | 5% | 8% |
| Week 3 | 1 | | 100% | 100% | 100% | 100% | 10% | 0% | 0% |
| Week 1 | 2 | | 95% | 0% | 100% | 0% | 0% | 0% | 0% |
| Week 2 | 1 | 0.02 | 100% | 10% | 100% | 10% | 0% | 0% | 10% |
| Week 3 | 0 | | | | | | | | |

# Results

| Spawning Date | # Brood fish Spawned | Concentration μg. g$^{-1}$ brood fish | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $^{137}$Ba | $^{136}$Ba | $^{135}$Ba | $^{134}$Ba | $^{87}$Sr | $^{86}$Sr | $^{26}$Mg |
| Week 1 | 4 | | 100% | 100% | 100% | 100% | 15% | 3% | 10% |
| Week 2 | 0 | 2 | | | | | | | |
| Week 3 | 2 | | 100% | 100% | 100% | 100% | 100% | 100% | 30% |
| Week 1 | 1 | | 95% | 10% | 100% | 5% | 0% | 0% | 0% |
| Week 2 | 4 | 0.2 | 100% | 98% | 100% | 90% | 5% | 5% | 8% |
| Week 3 | 1 | | 100% | 100% | 100% | 100% | 10% | 0% | 0% |
| Week 1 | 2 | | 95% | 0% | 100% | 0% | 0% | 0% | 0% |
| Week 2 | 1 | 0.02 | 100% | 10% | 100% | 10% | 0% | 0% | 10% |
| Week 3 | 0 | | | | | | | | |
| Week 1 | 0 | | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| Week 2 | 4 | 0.002 | 30% | 0% | 65% | 0% | 0% | 0% | 8% |
| Week 3 | 2 | | 75% | 0% | 80% | 0% | 0% | 0% | 0% |

# Conclusions

- Mark uptake depends on:

  A) Concentration of marker

  B) Time between injection and spawning

# Conclusions

- Mark uptake depends on:

  A) Concentration of marker

  B) Time between injection and spawning

- Combinations using **$^{137}Ba$** and **$^{135}Ba$** can be created at concentrations as low as **0.02 µg**. $g^{-1}$ brood stock

# Conclusions

- Mark uptake depends on:

  A) Concentration of marker

  B) Time between injection and spawning

- Combinations using $^{137}$**Ba** and $^{135}$**Ba** can be created at concentrations as low as **0.02 µg**. $g^{-1}$ brood stock

- Combinations using $^{136}$**Ba** and $^{134}$**Ba** can be created at a concentrations as low as of **0.2 µg**. $g^{-1}$ brood stock

# Conclusions

- Mark uptake depends on:

  A) Concentration of marker

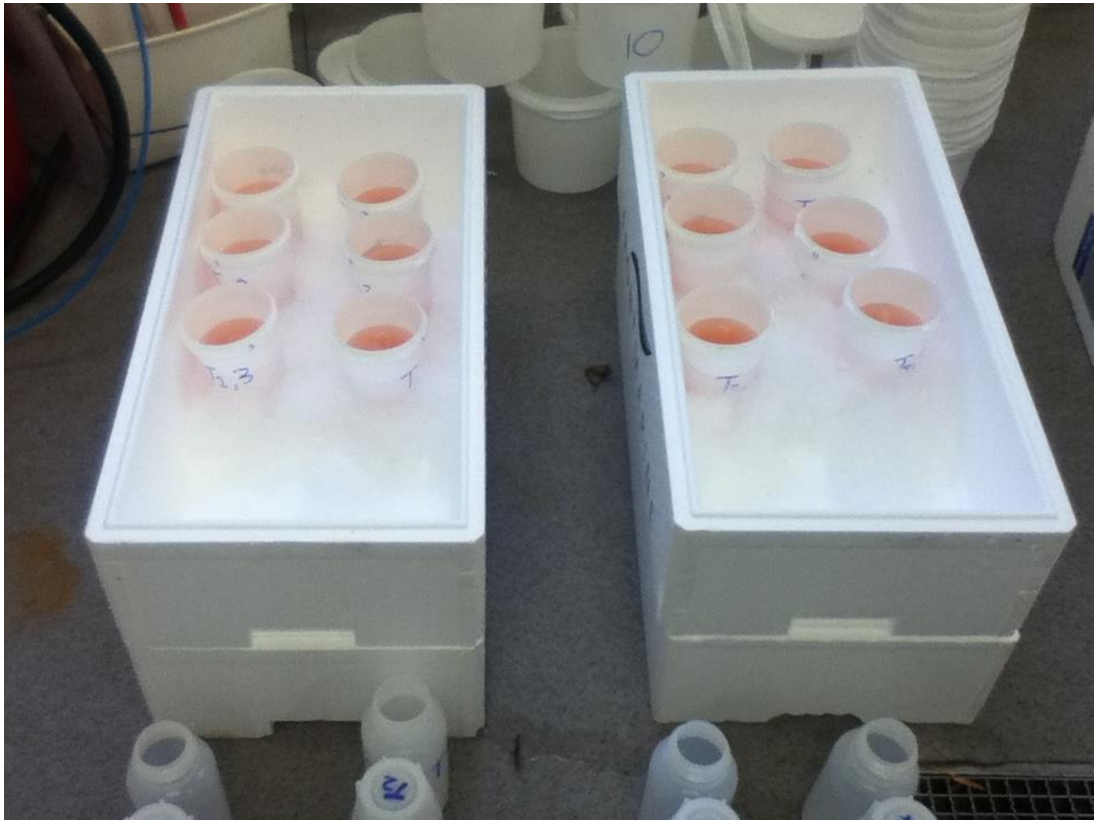
  B) Time between injection and spawning

- Combinations using $^{137}$**Ba** and $^{135}$**Ba** can be created at concentrations as low as **0.02 µg**. g$^{-1}$ brood stock

- Combinations using $^{136}$**Ba** and $^{134}$**Ba** can be created at a concentrations as low as of **0.2 µg**. g$^{-1}$ brood stock

- Combinations using $^{87}$**Sr** and $^{86}$**Sr** can be created at a concentration as low as **2 µg**. g$^{-1}$ brood stock

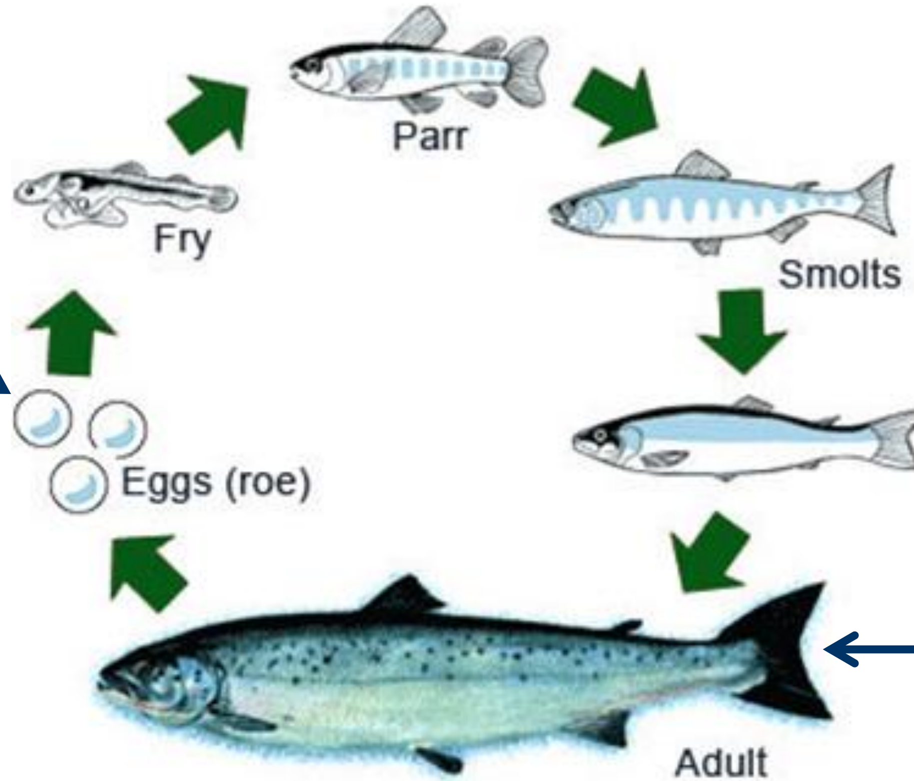
# Mass Marking Via Egg Immersion



# Mark application points

Vaccination

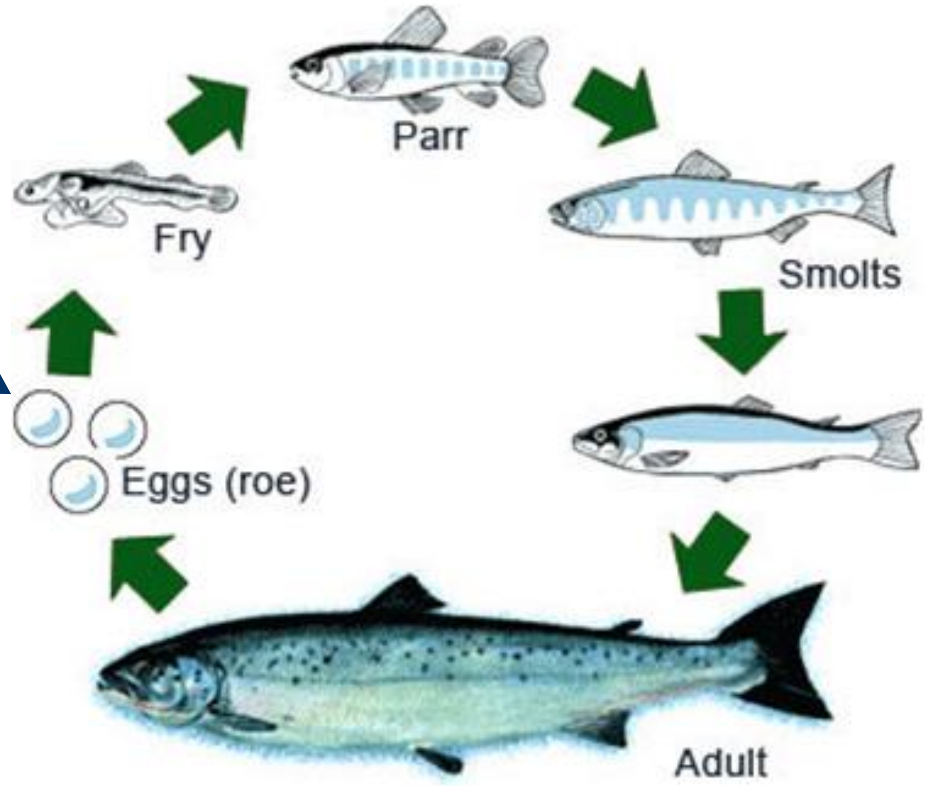
Egg immersion




Maternal Transfer

# Mark application points

**Egg immersion**



# Egg Immersion

**Method**: Multiple concentrations using a seven marker combination.

- Standard immersion volume (300 ml)
- Standardised egg volume (175 ml)
- Combination of $^{137}Ba$, $^{136}Ba$, $^{135}Ba$, $^{134}Ba$, $^{87}Sr$, $^{86}Sr$ & $^{26}Mg$
- 2 hour immersion time

# Experimental design

| | | | | | |
|---|---|---|---|---|---|
| (1) Combination | | Seven markers | | | |
| (4) Concentrations | Ba | 1000 | 100 | 10 | 1 |
| (µg per litre water) | Mg & Sr | 2500 | 250 | 25 | 2.5 |
| Replicate batches | | 3 | 3 | 3 | 3 |

Plus 3 control batches immersed in pure water

Each batch contained ~1000 fertilised eggs

First otolith samples taken before first feeding

# Results

| Marker Concentrations (µg. L$^{-1}$) | | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $^{137}$Ba, $^{136}$Ba, $^{135}$Ba, $^{134}$Ba | $^{87}$Sr, $^{86}$Sr, $^{26}$Mg | $^{137}$Ba | $^{136}$Ba | $^{135}$Ba | $^{134}$Ba | $^{87}$Sr | $^{86}$Sr | $^{26}$Mg |
| 1000 | 2500 | | | | | | | |
| 100 | 250 | | | | | | | |
| 10 | 25 | | | | | | | |
| 1 | 2.5 | | | | | | | |

# Results

| Marker Concentrations (μg. $L^{-1}$) | | Mark uptake | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $^{137}Ba$, $^{136}Ba$, $^{135}Ba$, $^{134}Ba$ | $^{87}Sr$, $^{86}Sr$, $^{26}Mg$ | $^{137}Ba$ | $^{136}Ba$ | $^{135}Ba$ | $^{134}Ba$ | $^{87}Sr$ | $^{86}Sr$ | $^{26}Mg$ |
| 1000 | 2500 | 100% | 100% | 100% | 93% | 7% | 0% | 4% |
| 100 | 250 | 100% | 3% | 100% | 0% | 0% | 0% | 0% |
| 10 | 25 | 3% | 0% | 21% | 0% | 0% | 0% | 3% |
| 1 | 2.5 | 0% | 0% | 0% | 3% | 0% | 0% | 0% |

# Conclusions

- Concentration of marker important

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- $^{137}$**Ba** and $^{135}$**Ba** 100% mark uptake at a concentration of **100 µg. L$^{-1}$**

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- $^{137}$**Ba** and $^{135}$**Ba** 100% mark uptake at a concentration of **100 µg. L$^{-1}$**
- $^{136}$**Ba** 100% mark uptake at a concentration of **1000 µg. L$^{-1}$**

# Conclusions

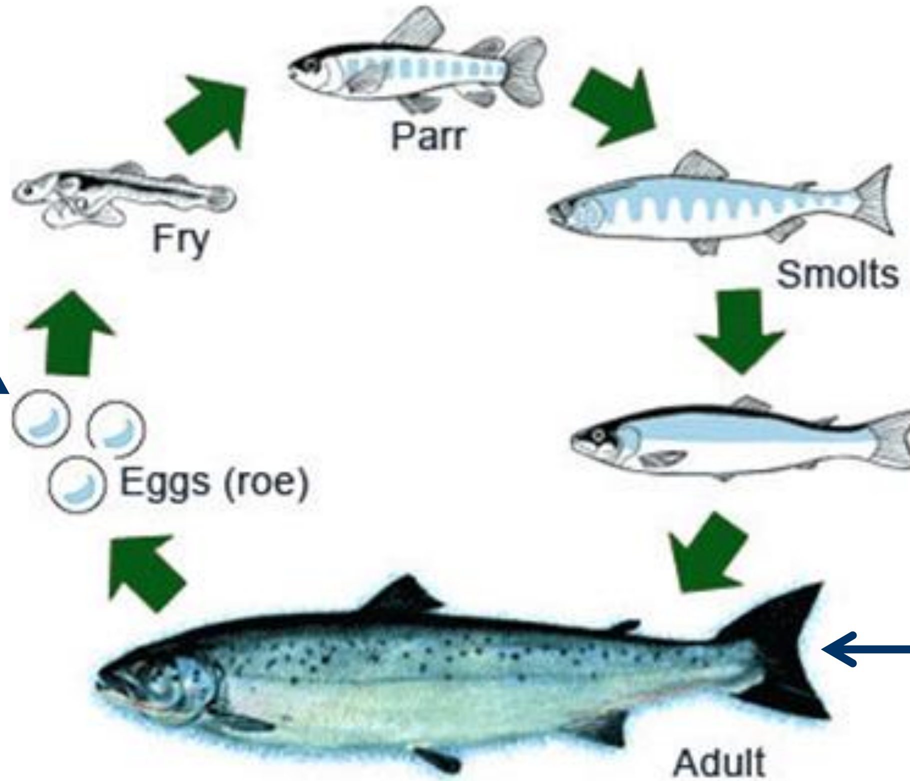
- Concentration of marker important
- **$^{137}$Ba** and **$^{135}$Ba** 100% mark uptake at a concentration of **100 μg. L$^{-1}$**
- **$^{136}$Ba** 100% mark uptake at a concentration of **1000 μg. L$^{-1}$**
- Length of immersion time requires further investigation

# Summary

- All three techniques could be used for mass marking Atlantic salmon with 100% mark success
- Vaccination: 63 codes, Maternal Transfer: 63 codes, Egg immersion: 7 codes
- 100% mark uptake is easiest to achieve using Ba markers
- 100% mark uptake with Sr markers is possible at higher concentrations compared to Ba markers

# Mark application points

Egg immersion




Vaccination

Maternal Transfer

# Fish Health

Monitoring of mortality and growth between tagged and control fish is being undertaken for all three marking techniques

**Monitoring of production parameters**

| | Spawning | Fertilsation | Hatch success | First feeding | Vaccination | Smoltifaction | Sea Transfer | Production Size |
|---|---|---|---|---|---|---|---|---|
| **Vaccination**<br>Mortality<br>Growth | | | | | No difference | No difference | No difference | |
| **Egg immersion**<br>Mortality<br>Growth | | No difference | No difference | No difference | No difference | | | |
| **Maternal Transfer**<br>Mortality<br>Growth | No difference | No difference | No difference | No difference | No difference | | | |

# Cost projections

**Scenario 1**: Marking 100% of production with 1 marker

*(achievable)*

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*(achievable)*

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*(achievable)*

**Scenario 3**: Marking 100% of production (54 company's)
*(achievable)*

# Cost projections

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

**Scenario 2**: Marking 80% of production (24 company's)
*(achievable)*

**Scenario 3**: Marking 100% of production (54 company's)
*(achievable)*

**Scenario 4**: Marking all farm locations (500-1000 sites)
*(Individual codes possible, but currently restrained by cost, and would require further optimisation of techniques)*

# Cost projections
# Scenario 1

## Marking 300 million farmed Atlantic salmon with 1 Ba code

| Vaccination<br>(50 g fish) | Material Cost<br>($US) | Total |
|---|---|---|
| $^{137}$Ba @ 0.001 µg. g$^{-1}$ fish weight<br>(15 g for 300 million parr) | $4.36 per mg<br>(~ $0.0006 per parr) | **$65400** |

# Cost projections
# Scenario 1

## Marking 300 million farmed Atlantic salmon with 1 Ba code

| Vaccination (50 g fish) | Material Cost ($US) | Total |
|---|---|---|
| $^{137}$Ba @ 0.001 µg. g$^{-1}$ fish weight<br>(15 g for 300 million parr) | $4.36 per mg<br>(~ $0.0006 per parr) | **$65400** |

| Egg immersion (2000 eggs L$^{-1}$) | Material Cost ($US) | Total |
|---|---|---|
| $^{137}$Ba @ 100 µg. L$^{-1}$<br>(150,000 L for 300 million eggs) | $4.36 per mg<br>(~ $0.44 per litre) | **$65400** |

# Cost projections
# Scenario 1

## Marking 300 million farmed Atlantic salmon with 1 Ba code

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

| Egg immersion (2000 eggs $L^{-1}$) | Material Cost ($US) | Total |
|---|---|---|
| $^{137}Ba$ @ 100 µg. $L^{-1}$<br>(150,000 L for 300 million eggs) | $4.36 per mg<br>(~ $0.44 per litre) | $65400 |

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

# Cost projections Scenario 2

## Marking 80% of production (24 largest companies, 24 codes)

**Method:** Marking fish once via vaccination or once via maternal transfer with Ba codes

**Vaccination:** Marks the region of the otolith developing at the parr/pre-smolt stage

**Maternal Transfer:** Marks the core of the otolith developing at the eyed egg stage

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.

Data is sourced from:
http://marineharvest.com/PageFiles/1296/2013%20Salmon%20Handbook%2027-04-13.pdf

# Cost projections Scenario 2

## Marking 80% of production (24 biggest companies, 24 codes)

| Company | Production (%) | Production (n fish) | Code number | Marker cost per fish | Cost per company |
|---|---|---|---|---|---|
| Marine Harvest | 22% | 66000000 | 2MT | 0.0002 | 11510 |
| Lerøy Seafoods | 13% | 39000000 | 1V | 0.0002 | 8502 |
| Salmar | 9% | 27000000 | 3V | 0.0003 | 8910 |
| Cermaq | 5% | 15000000 | 4MT | 0.0005 | 7746 |
| Grieg Seafoods | 4% | 12000000 | 5V | 0.0006 | 7746 |
| 6 | 1.42% | 4263158 | 16MT | 0.0007 | 2945 |
| 7 | 1.42% | 4263158 | 7V | 0.0010 | 4272 |
| 8 | 1.42% | 4263158 | 6MT | 0.0026 | 11255 |
| 9 | 1.42% | 4263158 | 15MT | 0.0028 | 11998 |
| 10 | 1.42% | 4263158 | 18MT | 0.0032 | 13456 |
| 11 | 1.42% | 4263158 | 26MT | 0.0033 | 14200 |
| 12 | 1.42% | 4263158 | 9V | 0.0055 | 23362 |
| 13 | 1.42% | 4263158 | 8MT | 0.0080 | 34173 |
| 14 | 1.42% | 4263158 | 17MT | 0.0082 | 34917 |
| 15 | 1.42% | 4263158 | 20MT | 0.0085 | 36375 |
| 16 | 1.42% | 4263158 | 10V | 0.0086 | 36812 |
| 17 | 1.42% | 4263158 | 28MT | 0.0087 | 37118 |
| 18 | 1.42% | 4263158 | 12V | 0.0098 | 41587 |
| 19 | 1.42% | 4263158 | 19MT | 0.0107 | 45428 |
| 20 | 1.42% | 4263158 | 29MT | 0.0108 | 46172 |
| 21 | 1.42% | 4263158 | 27MT | 0.0112 | 47630 |
| 22 | 1.42% | 4263158 | 30MT | 0.0113 | 48373 |
| 23 | 1.42% | 4263158 | 21V | 0.0119 | 50881 |
| 24 | 1.42% | 4263158 | 11V | 0.0122 | 52011 |
| | | | Average cost | **$0.0059** | $26557 |
| | | | Total cost for marking 80% of production | | **$663937** |

# Cost projections Scenario 3

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 3

## 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 |
| **Grieg** | 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 tag

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

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

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

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

# Analysis costs Scenario 1

## Monitoring program sampling 10000 fish per year

| Analysis of 10000 samples per year | Days required | Equipment cost per Day | Labour cost | Totals |
|---|---|---|---|---|
| Sample preparation (50 per day) | 200 | | 200 Days | ? |
| Laser ablation (50 per day) | 200 | $2400 | 200 Days | $480000 + L |
| Data analysis (50 per day) | 200 | | 200 Days | ? |
| | | | Total Estimated Cost | ? |

Sample analysis costs based on standard processing costs

# Analysis costs
# Scenario 2

## Rapid response to an escape event

| Analysis of 50 samples | Days required | Equipment cost | Labour cost | Totals |
|---|---|---|---|---|
| Sample preparation | 2 | | 2 Days | ? |
| Laser ablation | 1 | $2400 | 1 Day | $2400 + L |
| Data analysis and report | 2 | | 2 Days | ? |
| | | | Total Estimated Cost | ? |

Sample analysis and report can be completed in 1 week from the day otoliths are delivered to the laboratory

# Conclusion

Sample analysis and report can be completed in 1 week from the day otoliths are delivered to the laboratory