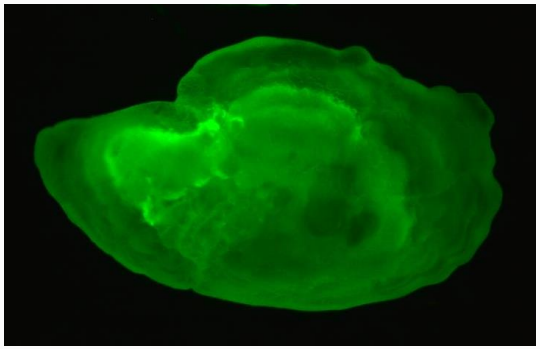
Alternative methods for marking otoliths: enriched stable isotopes and fluorescent dyes

Andrew R. Munro



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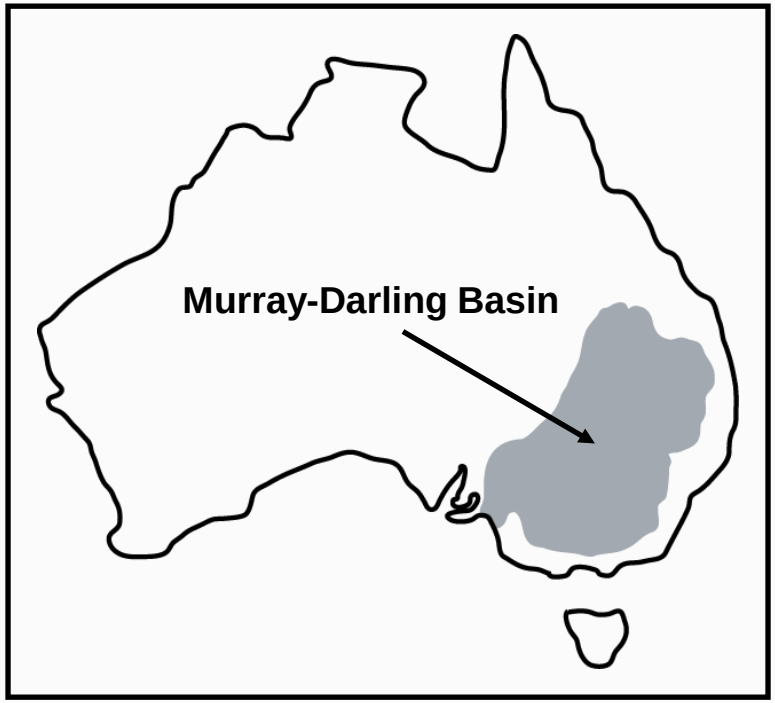
Native fish stocking - Australia

- > 60 million native fish stocked in MDB over past 30 years
- Fate of stocked fish unknown
  - success of stocking
  - effects on ecology
- Lack of suitable methods for marking hatchery fish
  - CWT
  - Alizarin complexone



Developing methods for marking hatchery fish

- **Enriched stable isotopes**
  - Otolith marking experiments
    - Fingerling immersion
    - Larval immersion
    - Broodstock injection (transgenerational marking)

- **Osmotic induction** of fish with fluorescent compounds

Stable isotopes

Barium

natural relative abundances

$^{137}Ba = 11.30\%$ $^{138}Ba = 71.70\%$

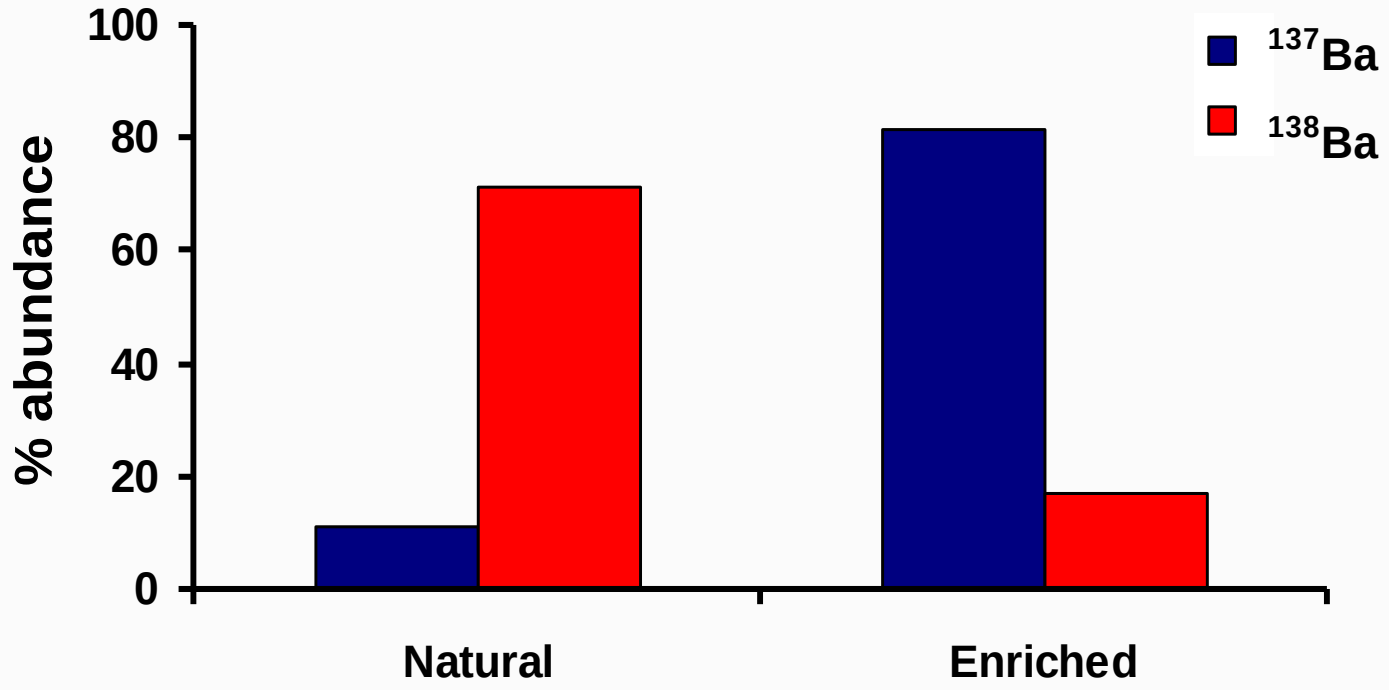
$$\frac{^{138}Ba}{^{137}Ba} = \frac{71.70}{11.30} = 6.38$$

Non radioactive

Enriched stable isotopes

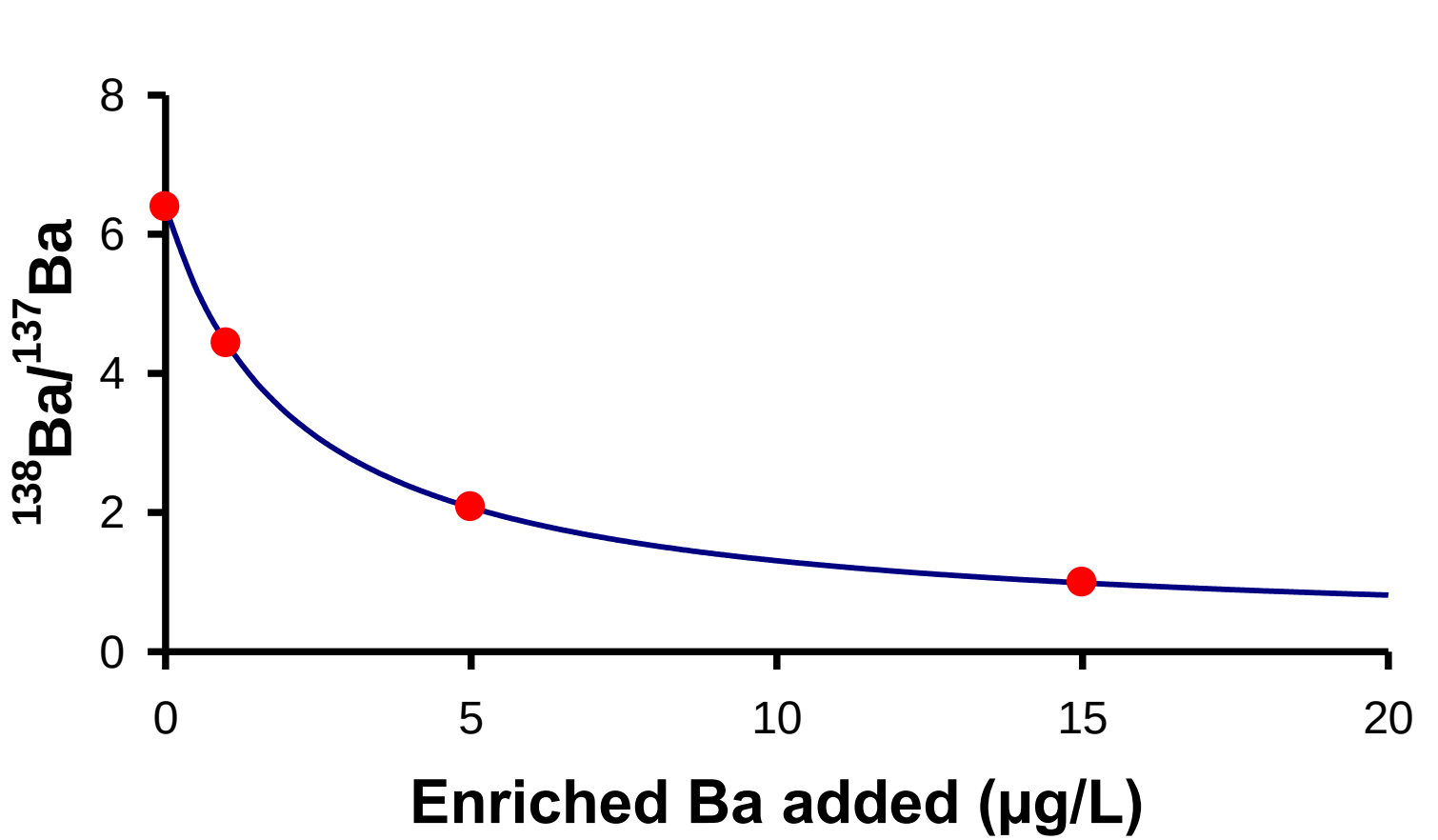
$BaCO_3$ – enriched in ^{137}Ba

11.30% ⇨ **81.90%**



Enriched Isotopes

Hypothesis: can alter otolith isotopic ratios by exposing fish to specific isotopes



Fingerling immersion

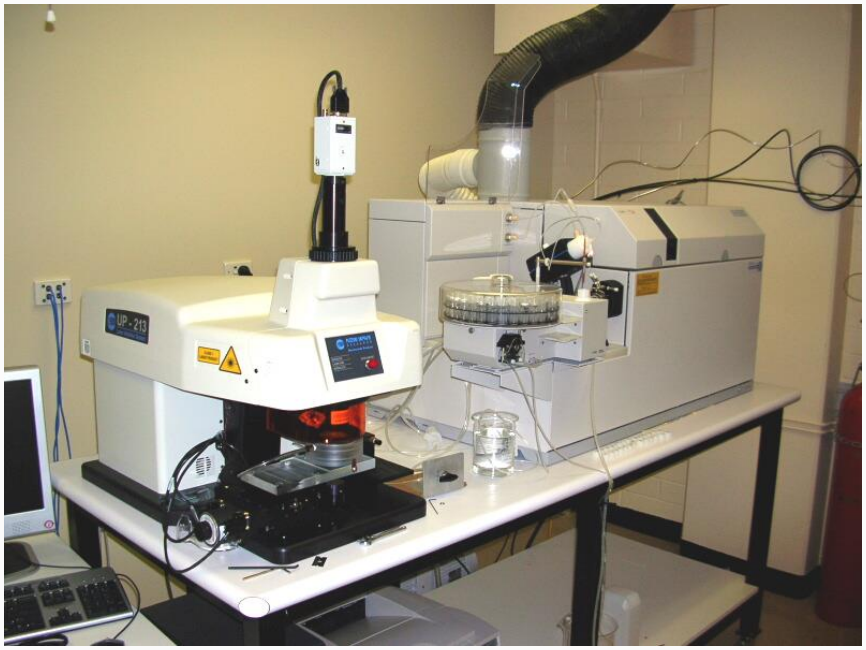
Reared juvenile golden perch in varying levels of enriched Ba for different lengths of time

0 µg/L **1 µg/L** **5 µg/L** **15 µg/L**

1d **4d** **8d** **24d**

Munro et al. (2008) CJFAS

Analysis



Otolith analysis: either whole or sectioned otoliths

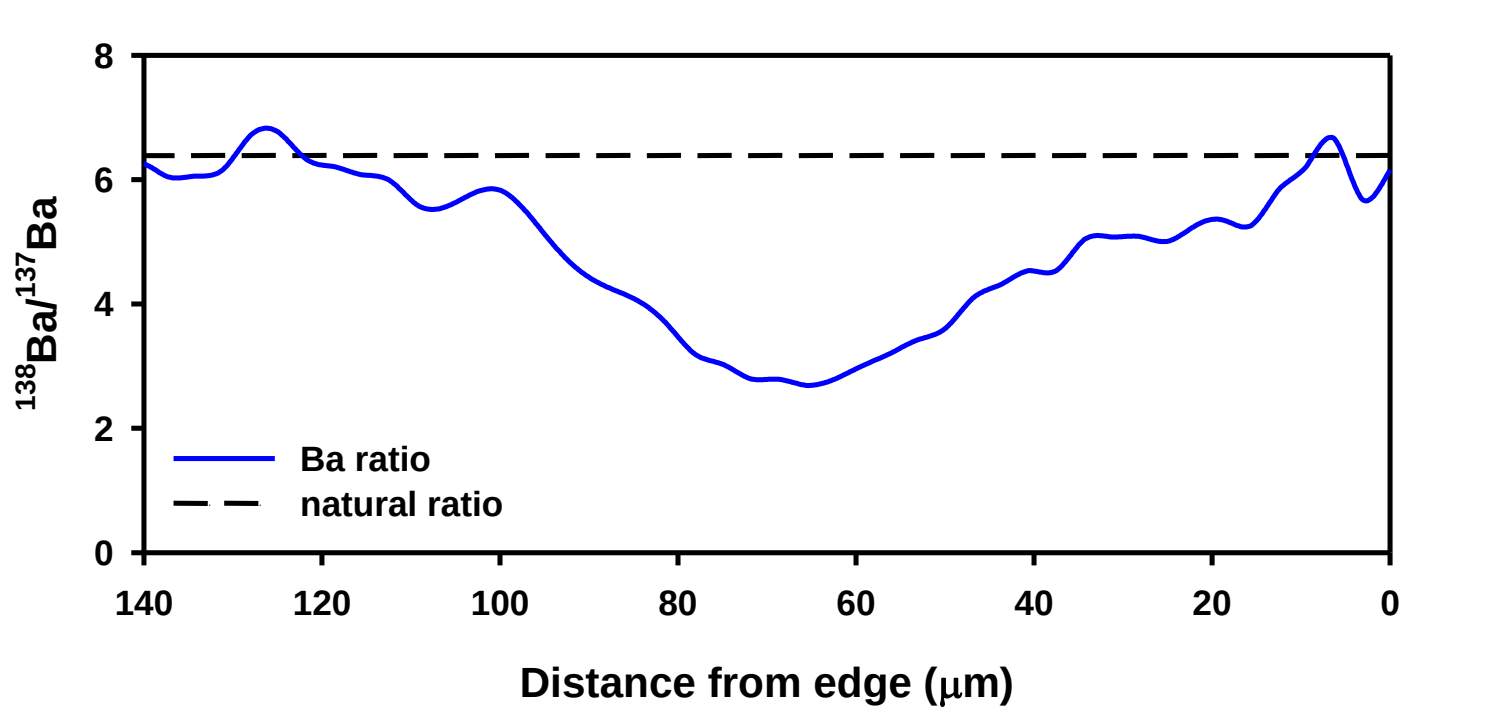
Transects/spot analyses

Measured isotopes of interest (e.g.^{137}Ba & ^{138}Ba)

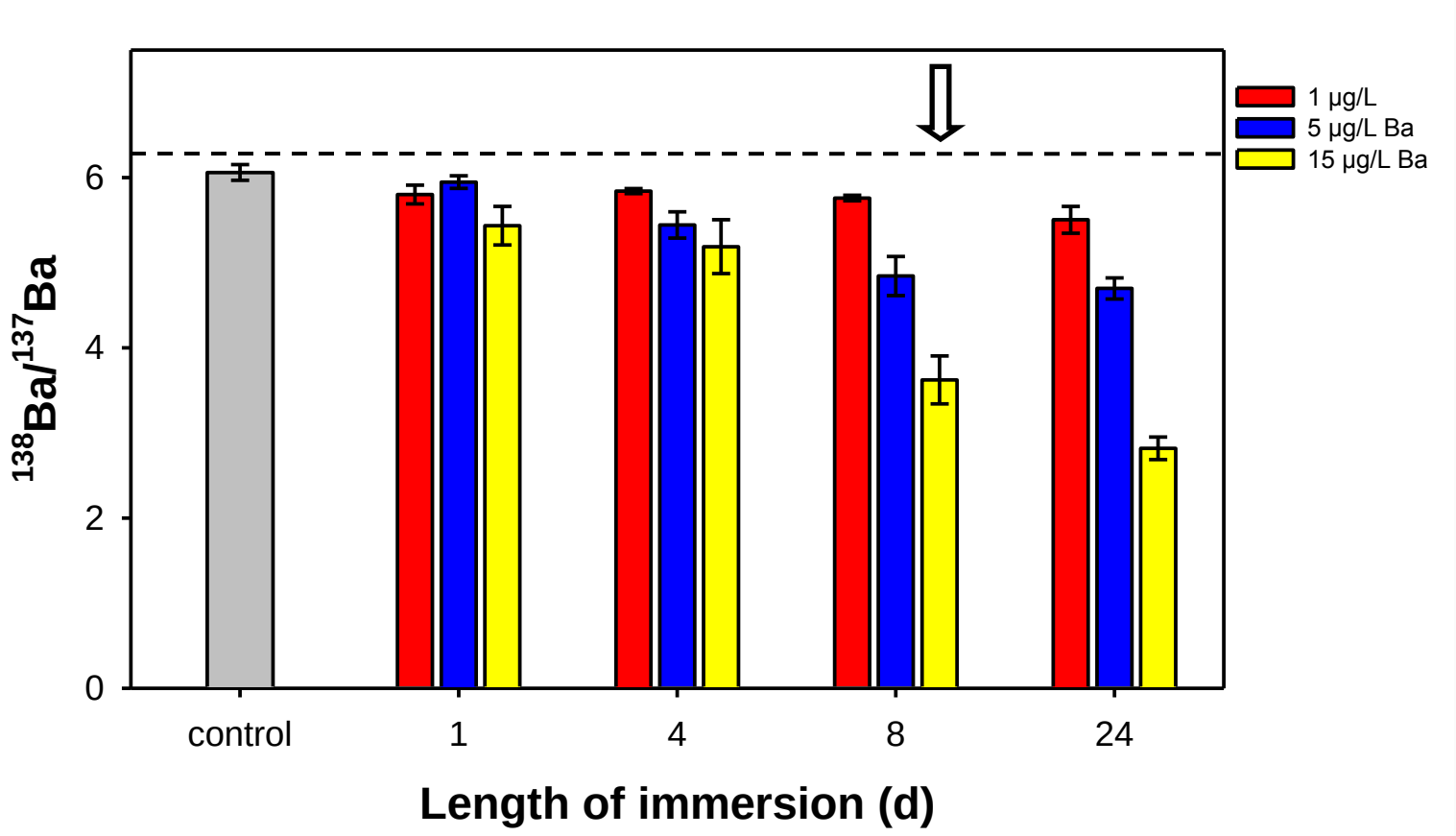
LA-ICPMS (single collector)

Fingerling immersion results

8 d immersion – 15 µg ^{137}Ba/L



Fingerling immersion results



Significant mark 1 d @ 15 µg/L

100% marked 8 d @ 15 µg/L

Base water concentration ~ 15 µg/L

Munro et al. (2008) CJFAS

Fingerling immersion summary

- **Altered otolith Ba isotope ratio**
- **100% mark success – 8 d @ 15µg/L**
- **Unambiguous mark – not natural**
- **Stress free**
- **Requires extended holding time**

Brood stock injection

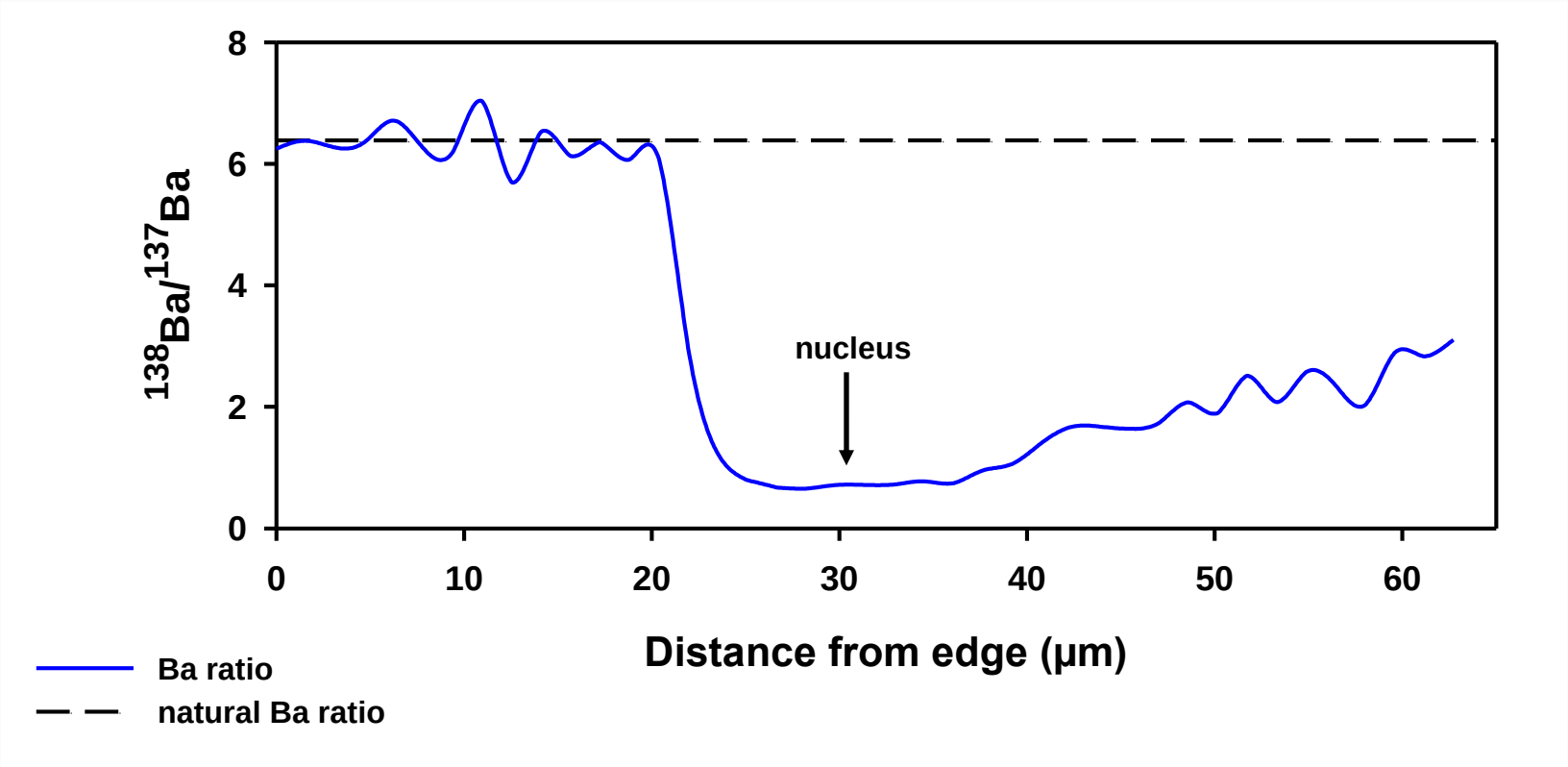
Length of time prior to hormone injection

Maternal dose rate (^{137}Ba)	0 h	1 d	21 d
20 µg/g	2 fish	2 fish	X
40 µg/g	2 fish	2 fish	2 fish

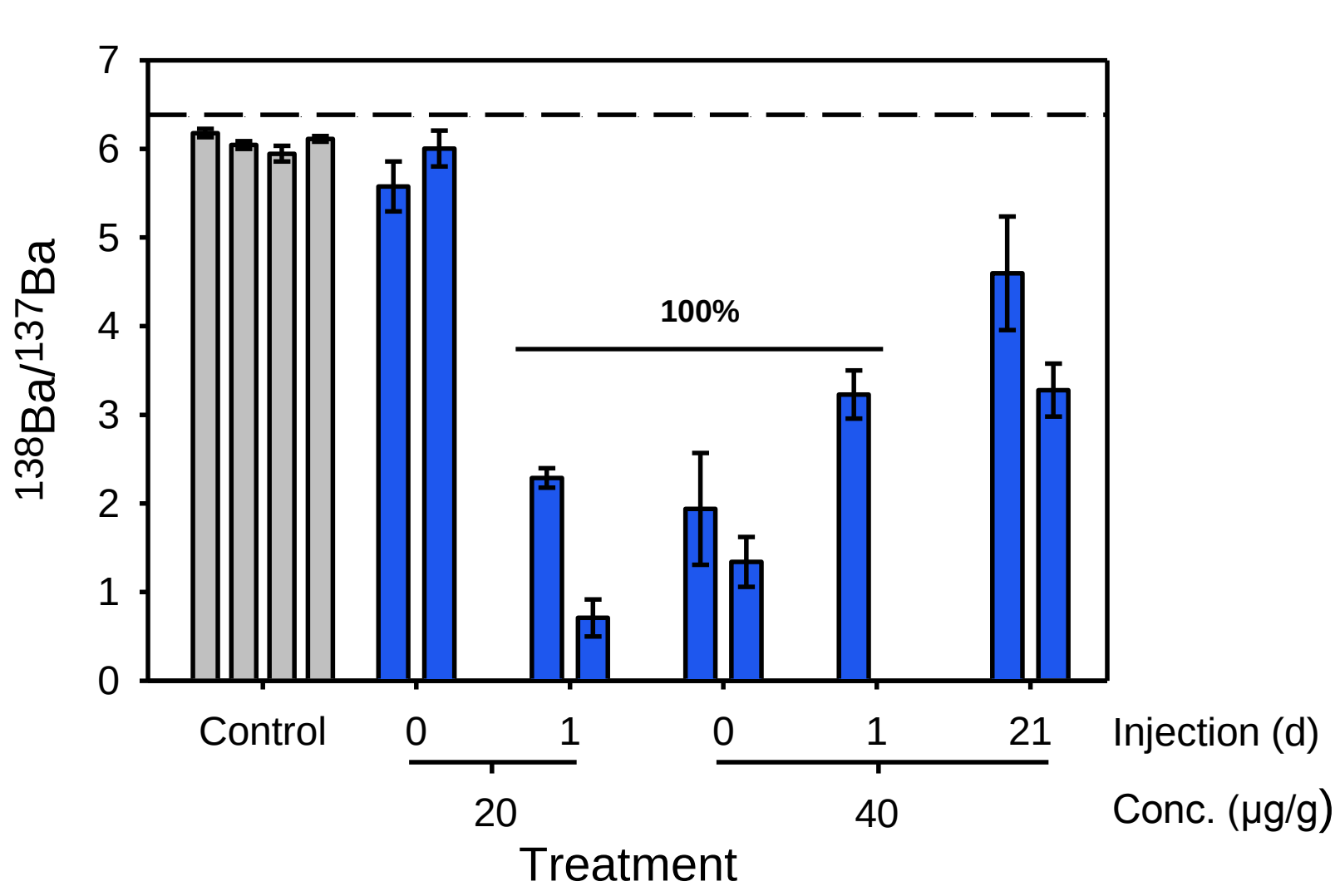
Munro et al. (2009) JFB

Brood stock injection results

Maternal parent injected with 40 µg/g of enriched ^{137}Ba at same time hormone



Brood stock injection results



Munro et al. (2009) JFB

Brood stock injection summary

- Altered otolith Ba isotope ratio
- 40 µg/g at time of hormone 100% mark
- Fits in with standard hatchery practices
- Variable spawning success of injected fish

Larval immersion

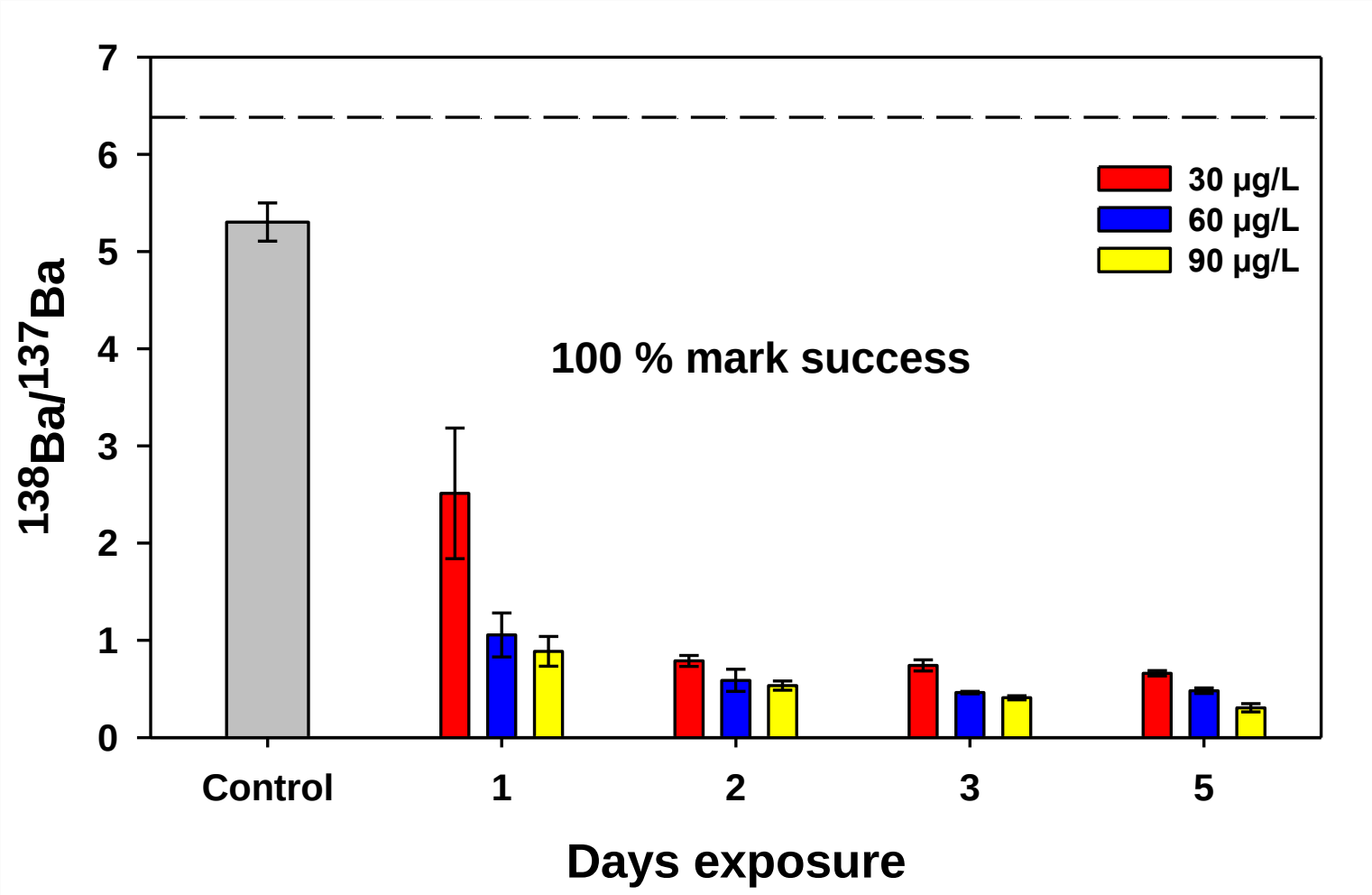
Reared larval golden perch in varying levels of enriched Ba for different lengths of time

0 µg/L **30 µg/L** **60 µg/L** **90 µg/L**

1d **2d** **3d** **5d**

Woodcock et al. (2011) EFF

Larval immersion results

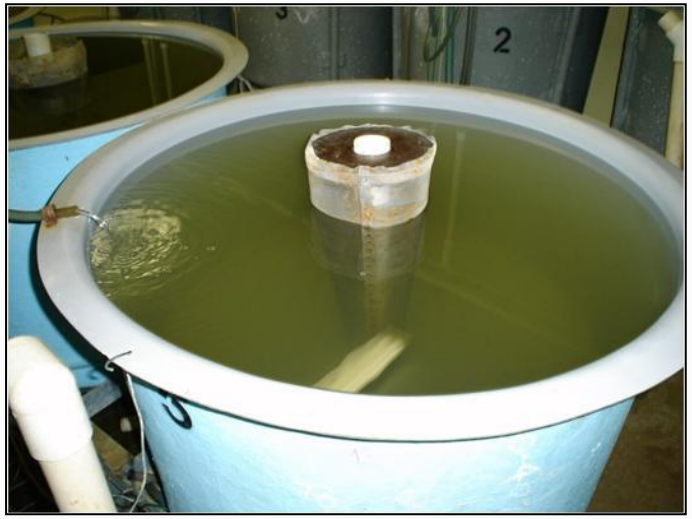


Base water concentration ~ 10 µg/L

Woodcock et al. (2011) EFF

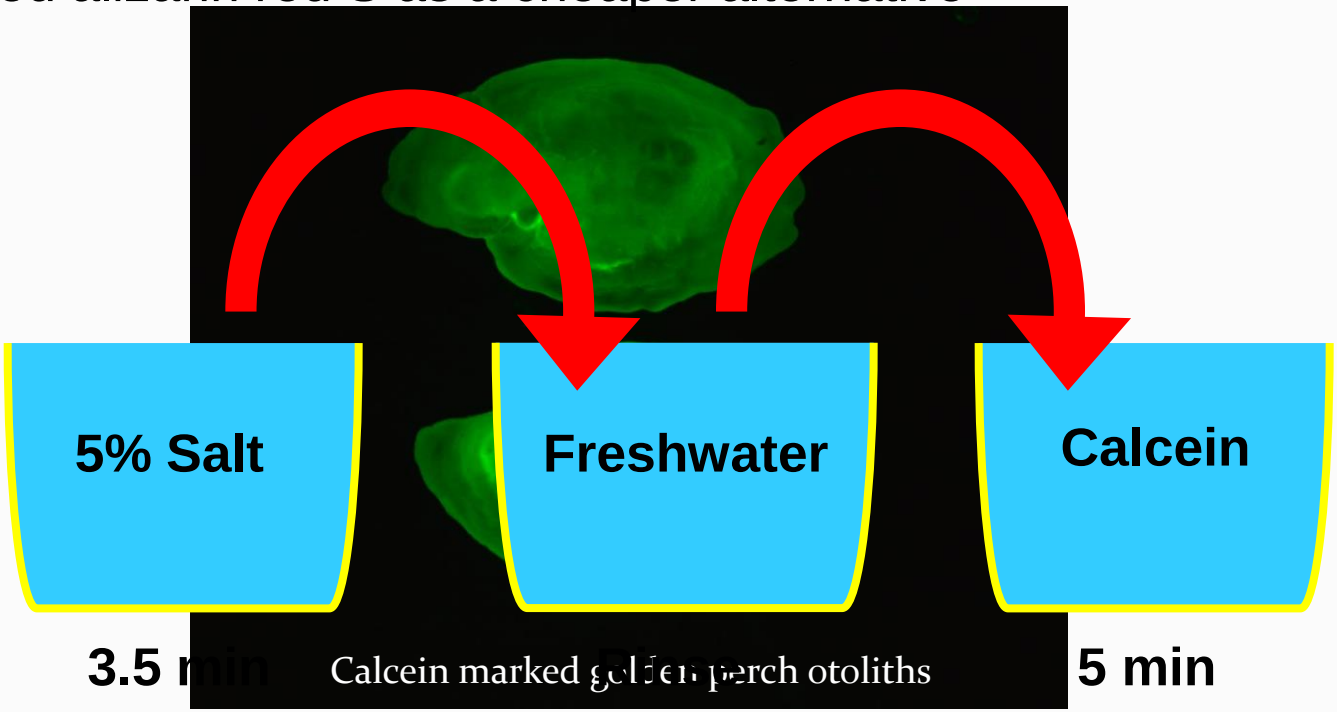
Larval immersion summary

- compatible with hatchery procedures
- 100% mark success
- high density = less isotope
- mark location known
- most cost effective
- variable survival to stocking
  - 30–50%



Osmotic induction marking

- Method developed for marking Atlantic salmon with calcein (Mohler 2003)
- Also trialed alizarin red S as a cheaper alternative

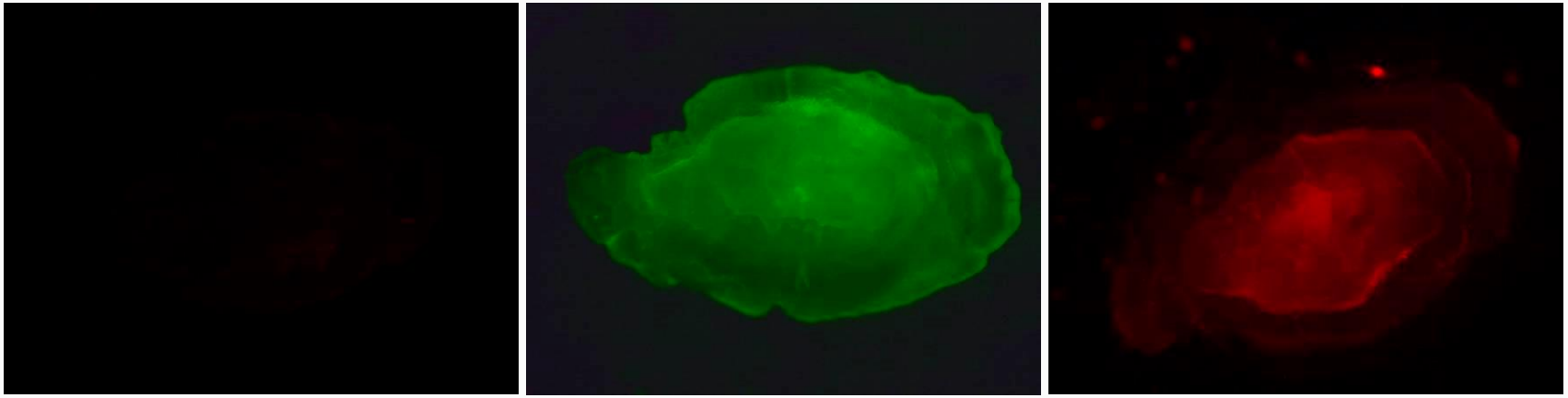


Crook et al. (2007, 2009) NAJFM

9 months post-marking

Dissecting microscope - white light

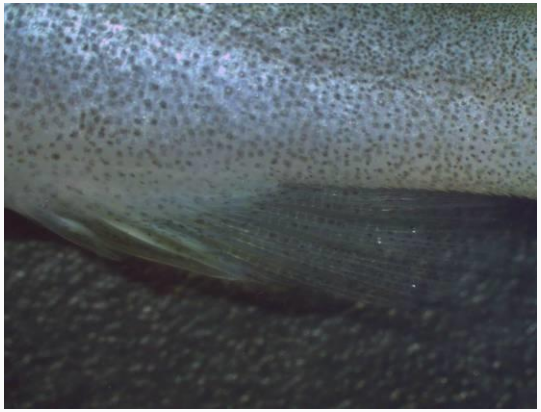
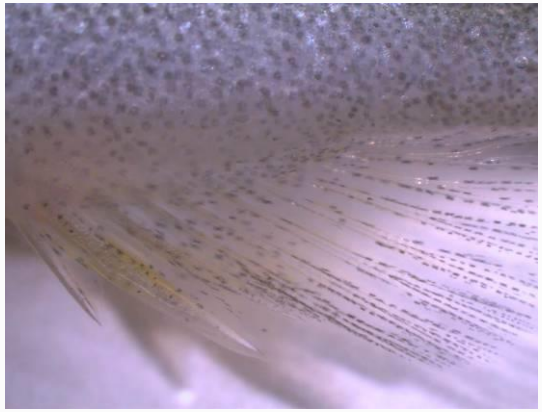
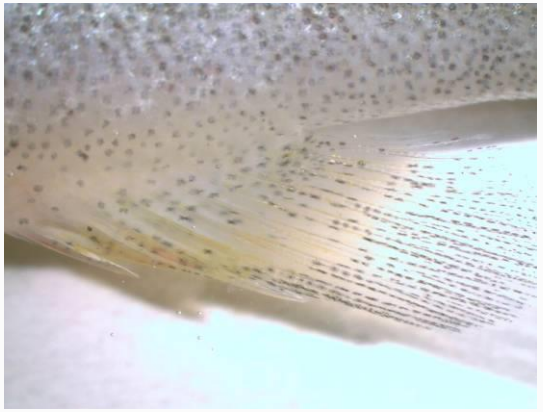
Dissecting microscope - fluorescence filters



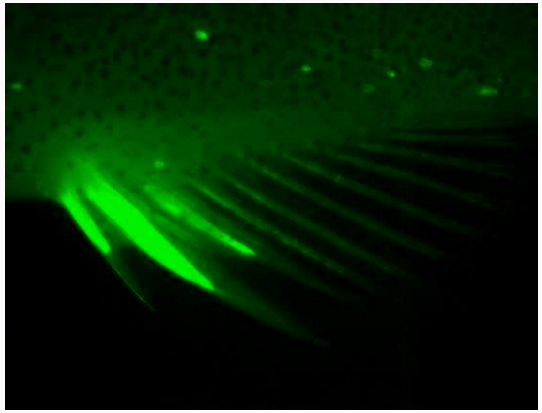
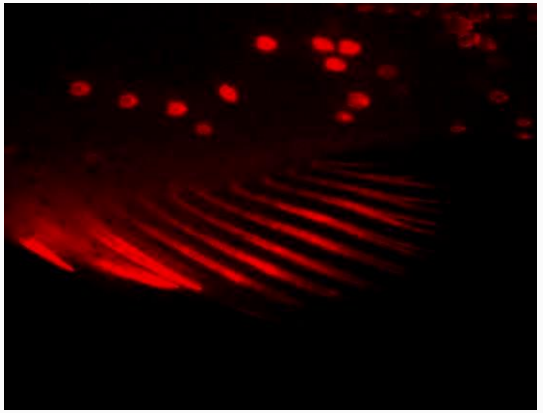
Control | Calcein treatment | ARS treatment

9 months post-marking

Dissecting microscope - white light

Dissecting microscope - fluorescence filters

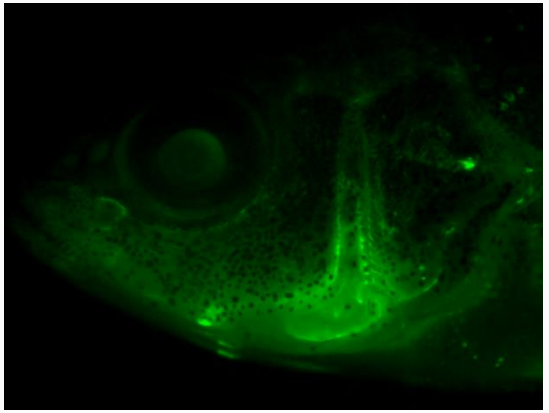
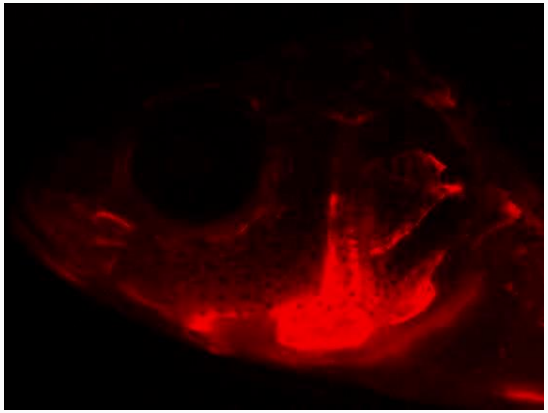
Control | Calcein treatment | ARS treatment

9 months post-marking

Dissecting microscope - white light



Dissecting microscope - fluorescence filters

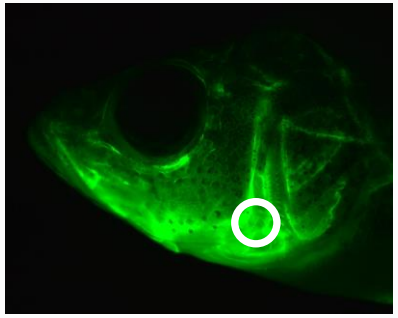
Control | Calcein treatment | ARS treatment

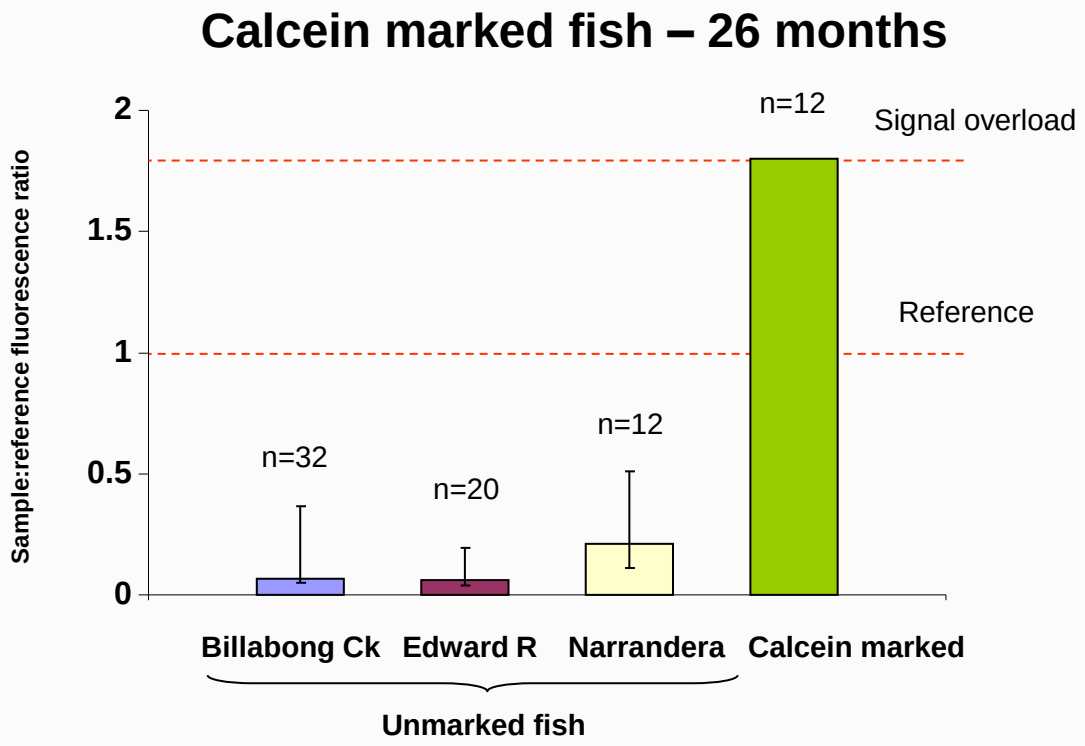
Non-lethal field detection

- Practical and objective way of identifying marks on live fish in the field

Non-lethal field detection

GFP portable fluorometer





Osmotic induction marking summary

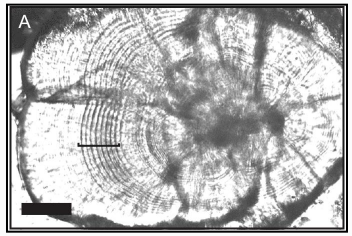
- Advantages:
  - Marking procedure is easy and quick (15 min)
  - Detectable on live fish in the field
  - Excellent accuracy (100% after 18 months in field, 26 months in lab)
- Disadvantages:
  - Adjustments to hatchery protocols required
  - Chemicals must be disposed of appropriately
  - Unknown longevity of external marks

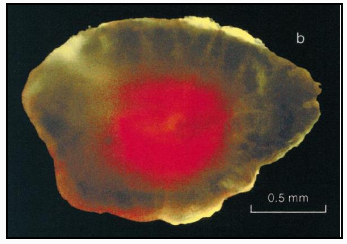
Marking Costs

Method	$ per 1,000 fingerling	Notes
Isotope immersion (^{137}Ba)		
fingerlings	9.80	15 µg/L @ 10 fish/L
larvae	1.60	30 µg/L @ 250 fish/L
Isotope injection (^{137}Ba)	0.66 - 19.14	20 µg/g
Osmotic induction		
Calcein	37.00	0.5% @ 800 fish/L
ARS	0.50	0.05% @ 800 fish/L

Cost comparison for marking

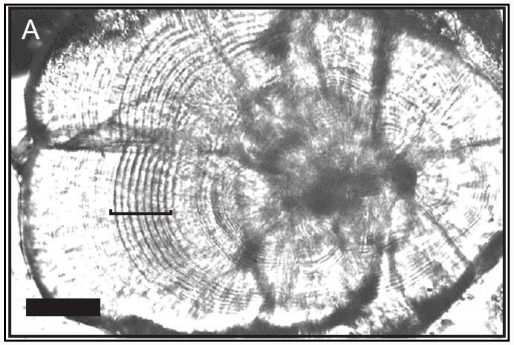
Method	$ per 1,000
Immersion	
fingerling	9.80
larvae	1.60
Injection	0.66 - 19.14
Calcein (OI)	37.00
ARS (OI)	0.50
Thermal	6.25
CWT	83
ALC (10-400 mg/L)	11.48 - 459

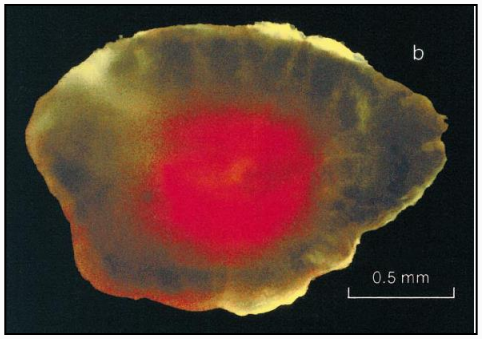




Cost comparison for reading marks

	reading cost/fish	marking/1,000
Enriched isotopes	$14.50 - $45.00	$1.60
Calcein & field detector	$0.00	$37.00
Thermal marking	$5.00 - $14.00	$6.25
Coded wire tags	$2.14	$83.00
Alizarin complexone	$3.75	$11.48





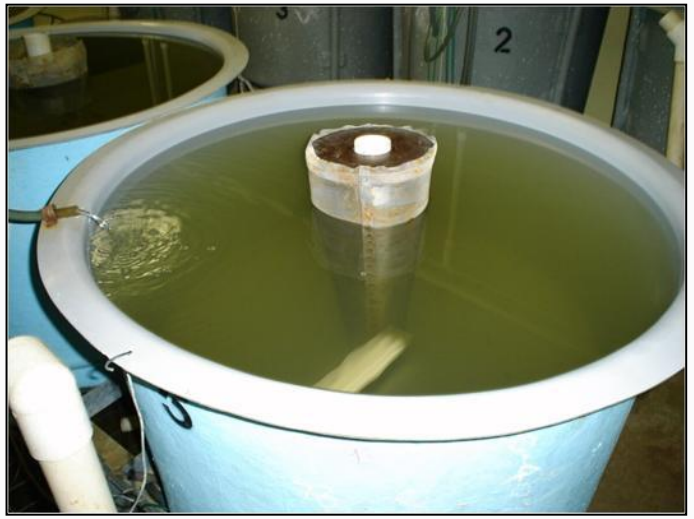
Scaling up

Osmotic induction Calcein marking

- 60,000 fish
- batches ~4,000 – 5,000 fish

Larval marking w/isotopes

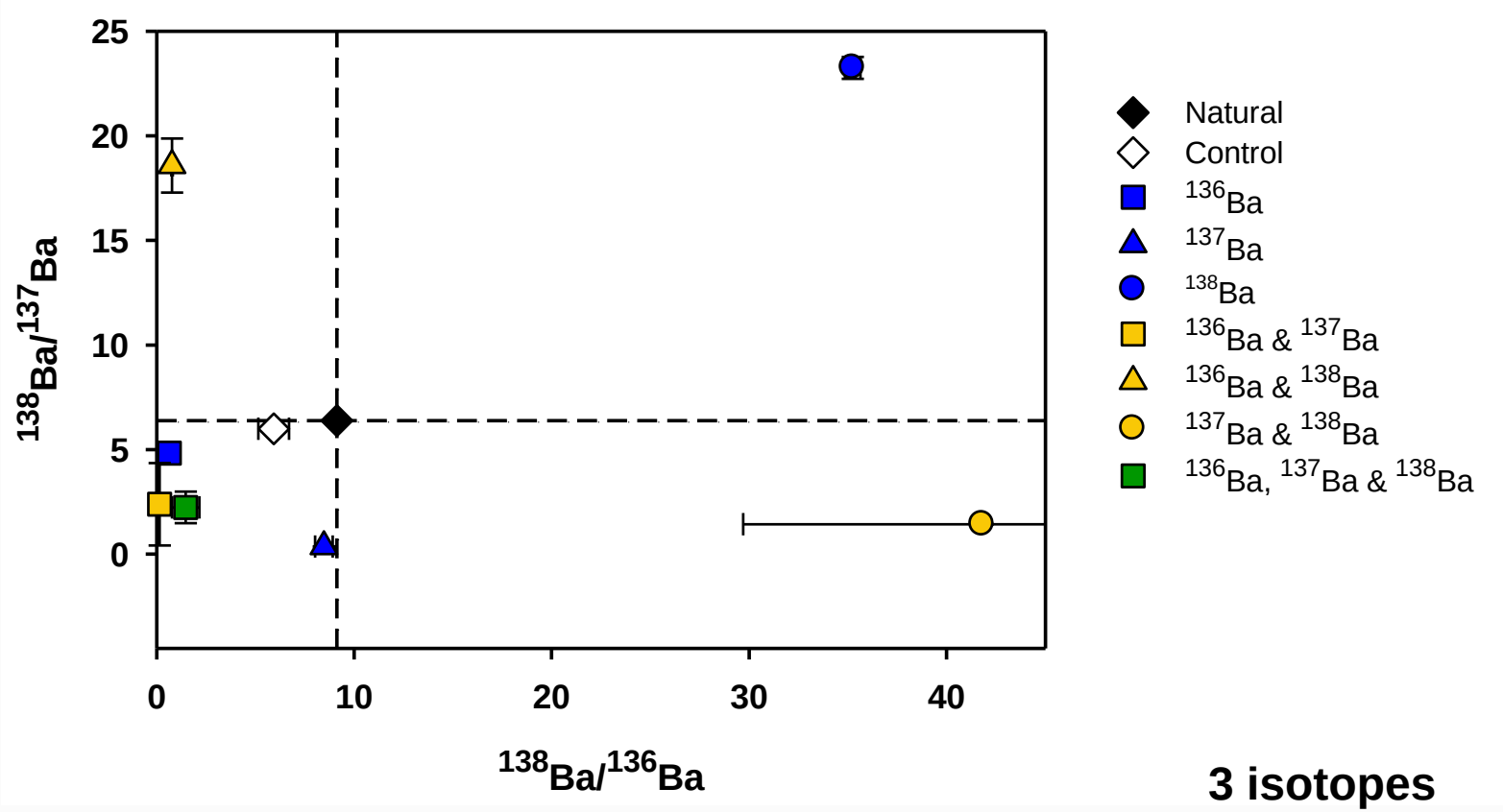
- ~100,000 fish
- few ml isotope solution



Multiple unique batch marks

Multiple enriched stable isotopes

4 Ba isotopes → 15 unique combinations



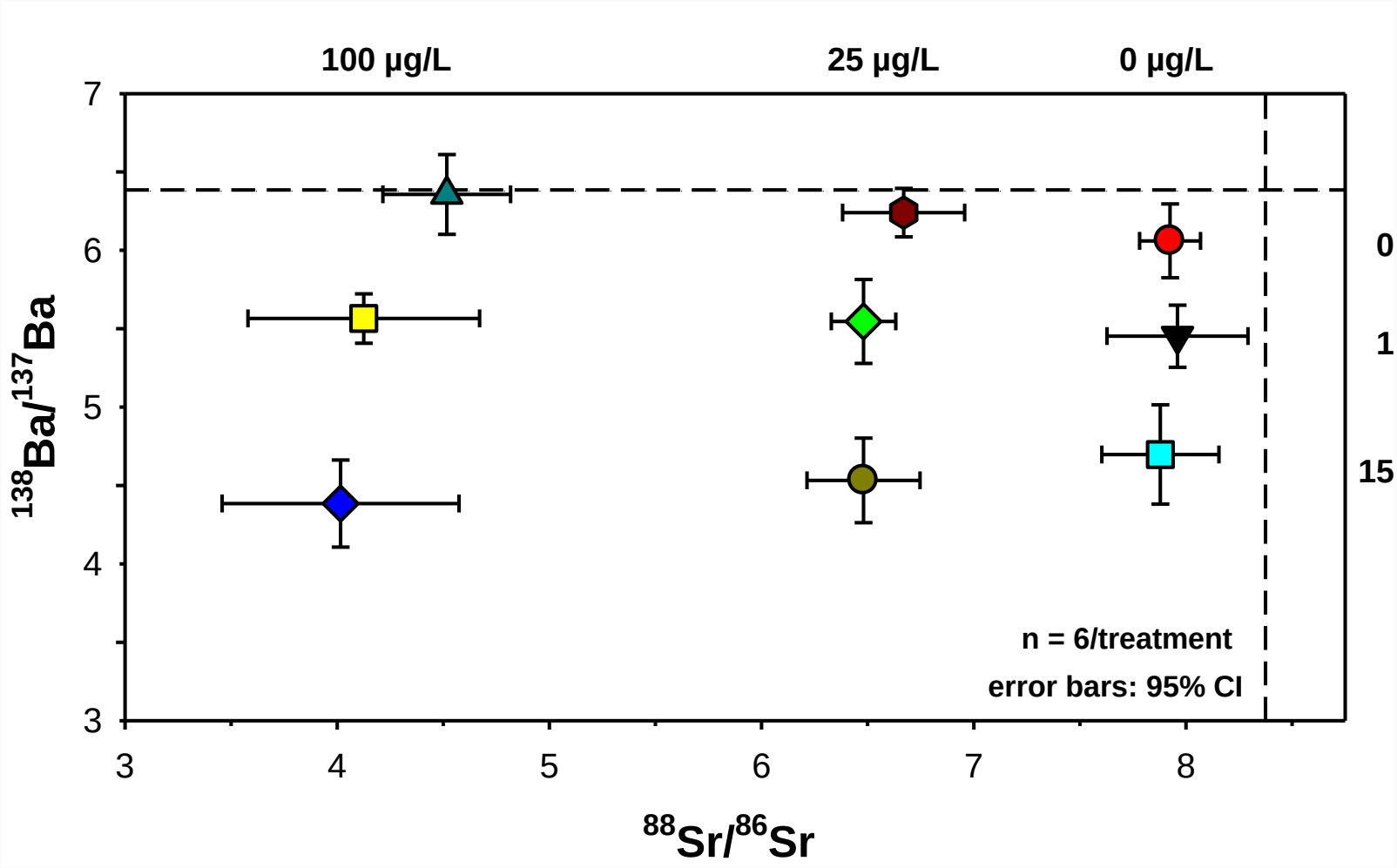
3 isotopes

7 unique marks

Woodcock et al. (2011) EFF

Multiple unique batch marks

Many more if include isotopes of other elements (e.g. Sr, Mg)



8 unique marks; 96% mark success

Munro et al. (2008) CJFAS

Summary

- All methods able to produce distinctive mark in fish otoliths
- Most cost effective method larval immersion in enriched isotopes
- Combine with osmotic induction at fingerling stage

  → external & internal mark
- Investigate effects on growth & survival
- Investigate survival & dispersal of stocked fish, & impacts of stocking
- Methods have potential for use in other areas (e.g. larval dispersal)

Acknowledgements

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And many others who have helped.

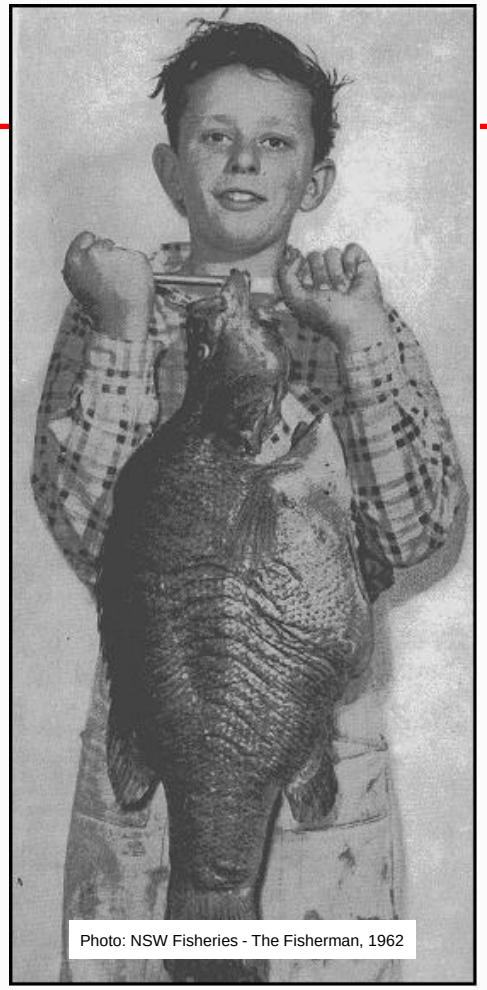

Photo: NSW Fisheries - The Fisherman, 1962

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