

# Assessing the outcomes of native fish stocking in the MDB

David Crook, Damien O'Mahony, Andrew Munro,  
Bronwyn Gillanders, Andrew Sanger





## Introduction

- More than 60 million native fish stocked in the MDB in last 30 years
- The fate of these fish is largely unknown
  - Success of stocking
  - Effects on ecology
- Outcomes of stocking identified as key research need in NFS





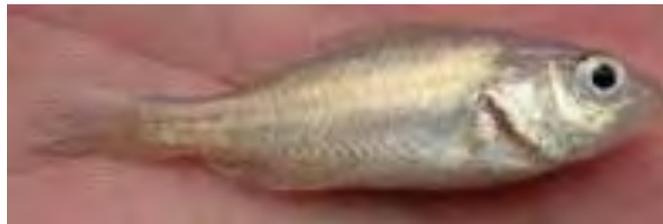
## ▶ **Project MD741 objectives**

- Test and refine protocols for marking fish at hatchery scale
- Gain approvals for use of marking techniques
- Develop non-lethal field detection methods
- Undertake experimental stockings of marked fish to determine the outcomes of native fish stocking in rivers
- Document findings and implications for conservation and rec. fishing

## Methods for discriminating hatchery fish

Golden perch as model species:

1. Broodstock injection
2. Natural otolith chemical signatures
3. Isotope immersion
4. Osmotic induction marking



Golden perch fingerling (40 mm)

## Brood stock injection and natural otolith signatures

- Brood stock injection
  - Female brood fish injected with stable isotope of barium
  - Otoliths (earstones) of progeny chemically analysed to detect mark
- Natural otolith chemical signatures
  - Analyse microchemistry in the core of otoliths to discriminate between hatchery and wild signatures



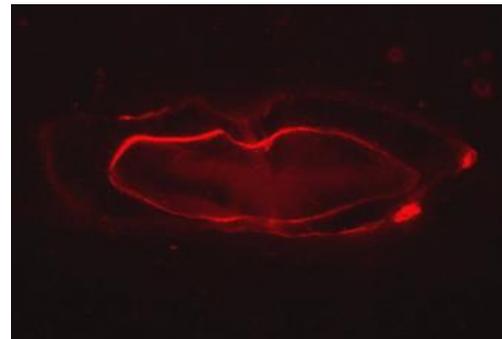
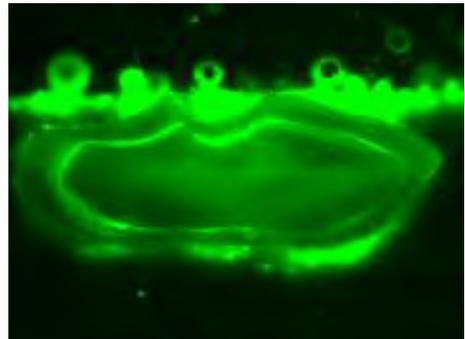
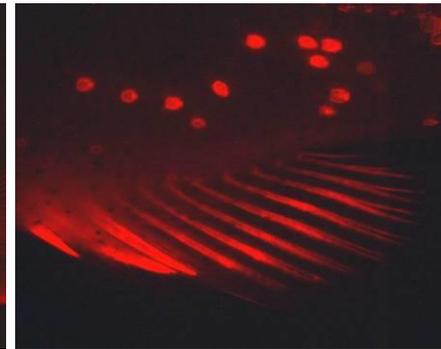
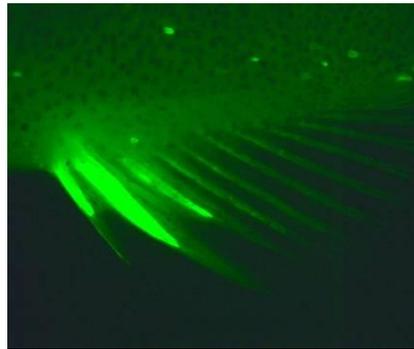
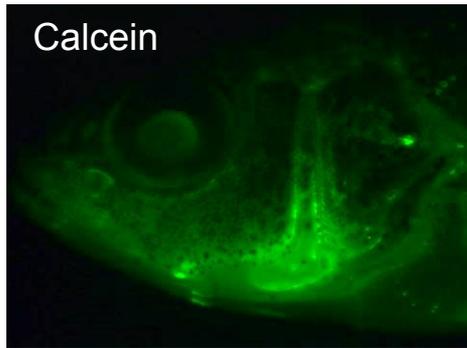
## Isotope immersion

- Larvae immersed in solutions of stable Ba and Sr isotopes
- Otoliths of recaptured fish chemically analysed to detect mark
- Cheap and easy to administer, permanent hatchery-specific mark



## Osmotic induction marking

- Fingerlings immersed in solutions of fluorescent chemicals
- Long lasting external (non-lethal) mark, plus permanent otolith mark
- Cost effective and practical in hatchery



Photos taken using  
fluorescence dissecting  
microscope

## Osmotic induction marking - non-lethal field detection



- Trial use of GFP fluorometer to detect calcein marks
- Practical and objective way of identifying marks on live fish
- Effective for >2 years in lab, 4 months in field (so far)

## Large scale trials at Narrandera hatchery

- 2007/08: 6,000 golden perch larvae marked by isotope immersion
- 2008: 20,000 golden perch fingerlings marked with calcein and released into Billabong Creek
  - 9 recaptures, 100% detection using fluorometer 4 months post-release





## Proposed hatchery protocol for golden perch

Induce spawning & raise larvae in hatchery for ~5 days

Isotope marking of larval otoliths with unique hatchery signature



Grow out larvae in ponds until 30-40 mm fingerlings



Osmotic induction marking with calcein



Stock fingerlings

A photograph of a wooden bridge with metal railings spanning a river. The bridge is supported by several wooden posts. The river reflects the surrounding trees and the sky. The scene is set in a wooded area with many trees visible in the background.

## Other species

- Murray cod
  - 3,700 successfully marked with calcein in 2008
    - external marks not as good as golden perch
  - isotope immersion trials to begin in 2008/09 (Skye Woodcock)
- Australian bass
  - Trials underway for marking hatchery fish with calcein (Leo Cameron and Lee Baumgartner, NSW DPI)
- Carp gudgeon and pygmy perch
  - Calcein marking evaluated for use in ecological studies (Zoe Squires and Nick Bond, Monash University)
- Atlantic salmon trial of fluorometer (Jerré Mohler, US Fish and Wildlife Service, Pennsylvania, USA)

A photograph of a wooden bridge with metal railings crossing a river. The bridge is supported by several wooden posts. The surrounding area is a dense forest with trees and sunlight filtering through the leaves, creating a dappled light effect on the water and the bridge.

## Registration of chemicals

- Only relevant for osmotic induction marking
- Australian Pesticides and Veterinary Medicines Authority (APVMA)
  - Advice: use of chemicals for fish marking not required to be registered as veterinary chemical on Agvet code
- Food Standards Australia New Zealand (FSANZ)
  - Listing on Australia New Zealand Food Standards Code only required if quantifiable residues present
  - Residue test for calcein and alizarin red S developed by consulting laboratory
  - All tests below limits of detection ( $0.02 \text{ mg kg}^{-1}$ )
  - Advice: no amendment to code required

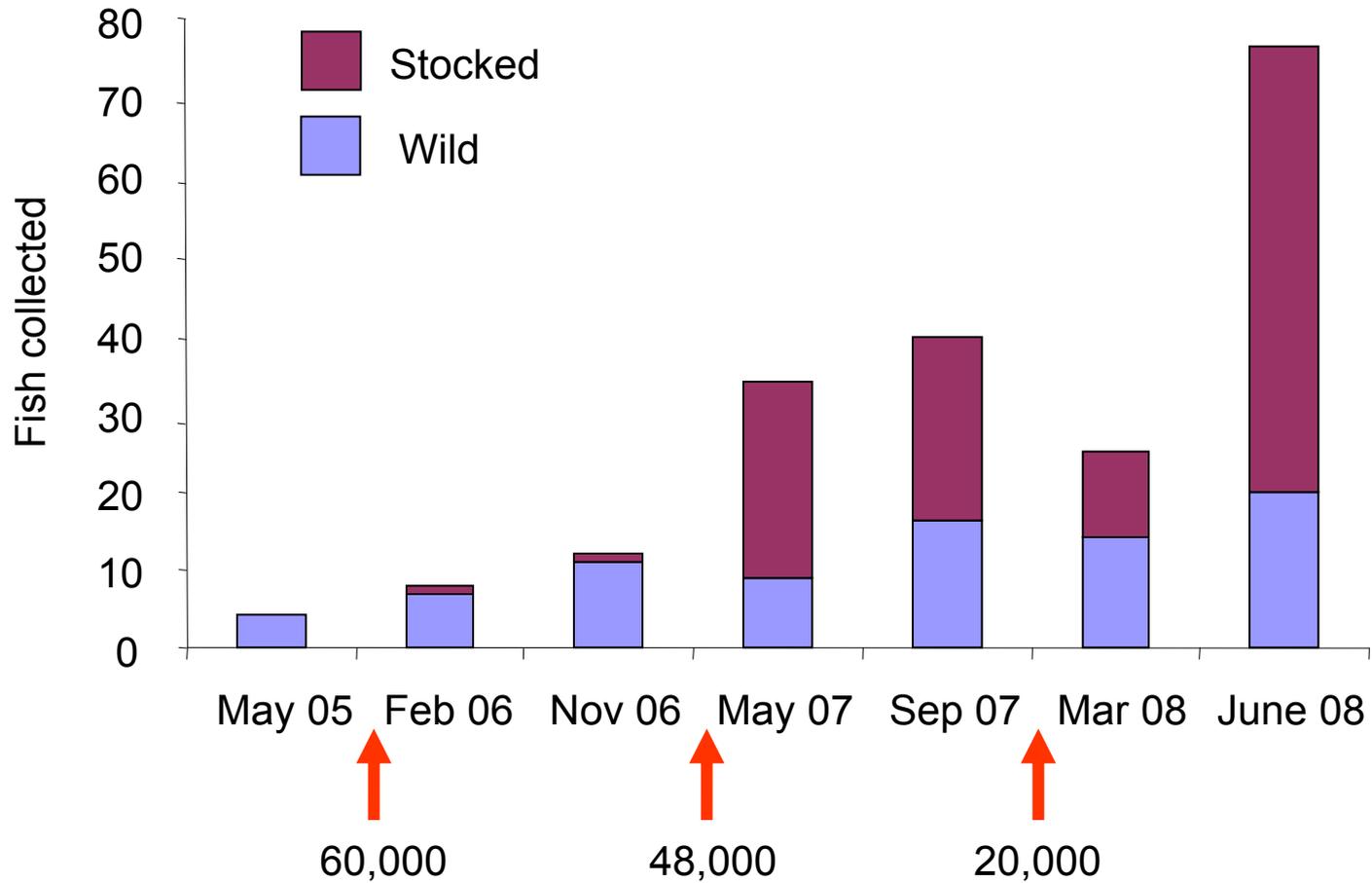


## Experimental stockings – survey results

<b>River</b>	<b>Year class</b>	<b># stocked</b>	<b>% stocked</b>
Murrumbidgee R	2002	50,000	37% (n=30)
	2004	50,000	32% (n=38)
Edward R	2004	50,000	20% (n=74)
	2005	50,000	66% (n=3)
Billabong Ck	2006	60,000	100% (n=54)
	2007	48,000	100% (n=7#)
	2008	20,000	100% (n=9#)

# Experimental stockings – Billabong Creek

## Golden perch



A photograph of a wooden bridge with metal railings crossing a river. The bridge is supported by several wooden posts. The river is calm, reflecting the surrounding trees and the bridge. The background is a dense forest of trees with green and yellow foliage, suggesting an autumn setting. The text "Activities for the next year..." is overlaid in white on the left side of the image.

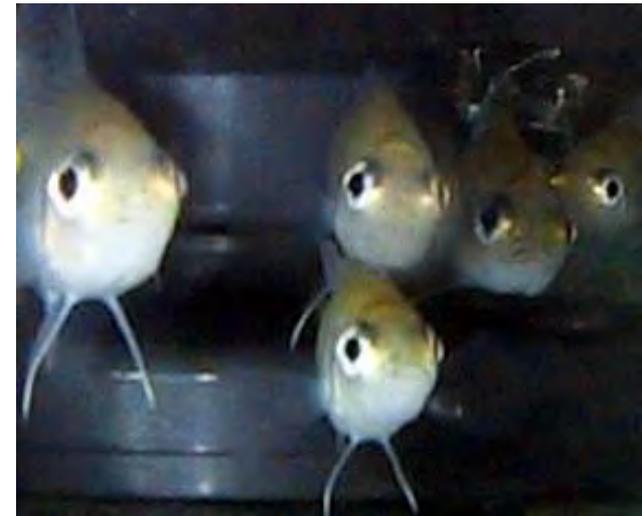
## Activities for the next year...

- Implement full marking protocol at commercial scale at Narrandera
  - 50,000 golden perch to be stocked into Murrumbidgee (08/09)
- Further develop and implement communication strategy
  - liaison with agencies and hatchery managers
- Begin process of rolling-out marking protocol in private hatcheries
- Continue sampling of stocked rivers
  - assess longevity of calcein marks under field conditions
  - begin analysis of stocked rivers data

A photograph of a wooden bridge with metal railings spanning a river. The bridge is supported by several wooden posts. The surrounding area is a dense forest with trees and fallen branches. The water in the river is calm, reflecting the light from the trees and the sky.

## Acknowledgments

- Matt Barwick, Jim Barrett, Dean Ansell, Mark Lintermans (MDBC)
- Lee Baumgartner, Mark Stimson, Stephen Thurstan, Cameron Westaway, Alistair McBurnie (NSW DPI)
- Skye Woodcock, Angus Netting, Justin Rowntree, Travis Elsdon (Adelaide Uni)
- Justin O'Mahony, Jed Macdonald, Fern Hames (ARI)
- Larry Taitelbaum (Opti-Sciences, USA)
- Brett Ingram (Vic DPI)
- Bruce Malcolm (Uarah Fisheries)
- Noel Penfold (Murray Darling Fisheries)
- Greg Semple (Murray Cod Hatcheries)





## References

- Crook *et al.* (2007). Production of external fluorescent marks on golden perch fingerlings through osmotic induction marking with alizarin red S. *North American Journal of Fisheries Management* 27, 670-675.
- Munro *et al.* (2008). Enriched stable isotope marking of juvenile golden perch *Macquaria ambigua* otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* 65, 276-285.
- Crook *et al.* (*in press*). Development and evaluation of methods for osmotic induction marking of golden perch (*Macquaria ambigua*) with calcein and alizarin red S. *North American Journal of Fisheries Management*.
- Crook *et al.* (*in review*). Quantitative measurement of calcein fluorescence for non-lethal, field based discrimination of hatchery and wild fish. *Advances in Fish Tagging and Marking Technology*, American Fisheries Society, Bethesda, Maryland, USA.
- Munro *et al.* (*in prep.*). Development of the transgenerational marking method in freshwater fish: implications for assessing connectivity and stock enhancement programs.
- Munro *et al.* (*in prep.*). Discriminating among sources of golden perch in the Murray-Darling Basin, Australia using natural otolith chemical signatures.
- Gillanders *et al.* (*in prep.*). Distinguishing hatchery from wild produced fish based on elemental chemistry of otoliths: a test using known source fish followed by unknown source fish.
- Woodcock *et al.* (*in prep.*). Using stable isotopes to produce multiple marks on the otolith of larval golden perch *Macquaria ambigua* stocked into the Murray Darling Basin, Australia.