

# JCU ePrints

This file is part of the following reference:

**Roy, Alexandra-Sophie (2008) *Trans-generational marking of clownfish larvae via maternal transmission of stable isotopes*. Masters (Research) thesis, James Cook University.**

Access to this file is available from:

<http://eprints.jcu.edu.au/3257>



**Trans-generational marking of clownfish larvae  
via maternal transmission of stable isotopes**

Thesis submitted by  
Alexandra-Sophie ROY, BSc (QC, CAN)  
In february 2008

Supervisor  
Professor Geoffrey P. Jones

For the degree of Master of Science  
School of Marine and Tropical Biology  
James Cook University

## STATEMENT OF ACCESS

I, the undersigned, the author of this work, understand that James Cook University will make this thesis available for the use within the University Library and, via the Australian Digital Theses network, for the use elsewhere.

I understand that, as an unpublished work, a thesis has significant protection under the Copyright Act and;

I do not wish to place any further restriction on access to this work.

-----  
Signature

18.02.08  
-----  
Date

STATEMENT OF SOURCES  
DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution or tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given. Also, all research procedures reported in the thesis received the approval of the relevant Ethics Committees.

-----  
Signature

18.02.08  
-----  
Date

## STATEMENT OF CONTRIBUTION OF OTHERS

This thesis included some collaborative work with my supervisor Professor Geoffrey P. Jones, Dr. Simon R. Thorrold from the Woods Hole Oceanographic Institute, and Ashley Frisch from James Cook University. Overall, I was primarily responsible for the project concept and design, carrying out the experiments, their analysis and interpretation. Professor Jones assisted in the revision of chapters into a format suitable for publication. Dr. Thorrold carried out the ICP-MS analyses for Chapter 2 at Woods Hole. Ashley Frisch assisted with the hormone analyses in Chapter 3.

STATEMENT ON ETHICS  
DECLARATION

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research involving Human* (1999), the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A1134)

-----  
Signature

18.02.08  
Date

ELECTRONIC COPY

I, the undersigned, the author of this thesis, declare that the electronic copy of this thesis provided to the James Cook University Library is an accurate copy of the print thesis submitted, within the limits of the technology available.

-----  
Signature

18.02.08  
-----  
Date

## Acknowledgements

Many people were involved in the progress and the completion of this thesis. In particular, I would like to thank:

- Professor Geoffrey P. Jones for his supervision, financial support, comments on the manuscript and encouragement
- Dr. Craig Syms for his help with statistical analyses and comments on the manuscript
- Simon, John and Greg from MARFU for all their help, good advice and their moral support
- Simon Thorrold for his advice and otolith analysis
- Ashley Frisch for his involvement in the hormone analysis and all his hard work.
- Kathryn Markey for her help with larval rearing
- Heidi Luter and Laura Castell for their great comments during the review
- My friends for their moral support
- Special thanks to my parents, Gilles and France, my brother, Jean-Mathieu and my partner, Dirk for their constant and incredible support, their strength and their belief in me.

This research was supported by a grant to Professor G. P. Jones from the Australian Research Council Center of Excellence Coral Reef Studies. Additional financial support was provided by Dr. Simon Thorrold for otolith ICP-MS analyses.

Thank you



## Abstract

Recent studies on coral reef fishes have successfully employed chemical tagging techniques to quantify local patterns of larval retention and dispersal. Experiments in which larvae were marked via tetracycline immersion of embryos have shown larval dispersal to be more limited than previously thought. However, this technique is limited to fishes that lay eggs on artificial substrata. More recently, a new chemical marking technique has been developed which can be applied to all reef fishes. Females are injected with enriched stable isotopes, such as  $^{137}\text{Ba}$ , and the chemical signature is maternally transmitted to embryos and is deposited at the core of the otoliths of larvae. While this technique has been validated for a few species and applied in the field to estimate local dispersal patterns, further laboratory experiments are necessary to determine appropriate injection concentrations and assess any negative effects on larval and adult condition.

The goal of this study was to conduct a series of laboratory experiments to validate the use of trans-generational marking in clownfishes (genus *Amphiprion*). In the first experiment, the minimum dose of  $^{137}\text{BaCl}$  for successful marking of *Amphiprion percula* larvae and the period over which females continue to produce marked larvae were evaluated. The effects of barium injections on clutch size, clutch area, size at hatching and subsequent larval growth were also assessed. The fish were subject to three dose levels of  $^{137}\text{Ba}$  (0.5, 1.0 and 2.5  $\mu\text{g }^{137}\text{Ba/g}$  fish weight) and the effectiveness of the mark was quantified by measuring the  $^{138}\text{Ba}/^{137}\text{Ba}$  ratio at the core of the otoliths of recently metamorphosed larvae. All dose levels were 100% successful in providing unequivocal chemical signatures on offspring otoliths. The two highest dose levels, 1.0 and 2.5  $\mu\text{g }^{137}\text{Ba/g}$  fish weight, continued to mark larvae over 6 consecutive clutches, extending over a period of 80 days after a single injection. In females injected with the lowest concentration, 0.5  $\mu\text{g }^{137}\text{Ba/g}$  fish weight, the  $^{138}\text{Ba}/^{137}\text{Ba}$  ratio returned to the natural barium ratio of 6.385 after 2 clutches ( $\approx 40$  days). Therefore, while all dose levels could be used to mark larvae, the low dose may require females to be re-injected if longer-term

marking is required. Barium injections had no consistent effects on the clutch size (number of eggs) or the clutch area. A significant interaction between treatment and time was detected for both the length and weight of larvae. The females with the highest  $^{137}\text{Ba}$  dose,  $2.5 \mu\text{g } ^{137}\text{Ba/g}$  fish weight, produced smaller larvae, but the effect disappeared after the fourth generation. As larval size may be a critical parameter affecting survival, this dosage is not recommended for field studies.

In the second experiment, the effects of  $^{137}\text{Ba}$  injections on levels of barium in the tissues of adult females and the period over which barium levels remained elevated were assessed for *Amphiprion melanopus*. In addition, potential effects on adult condition and stress were also evaluated using plasma cortisol analysis. The barium ratios in four tissues (gonads, liver, muscles and bones) were analysed to determine the retention period of barium following two injection doses ( $2 \mu\text{g}$  and  $4 \mu\text{g } ^{137}\text{Ba/g}$  fish weight). The retained barium level was higher in the bones than in the soft tissues (gonads, liver and muscles) for most sampling times (2, 21 and 56 days). Following the initial elevated barium levels, the ratio of  $^{138}\text{Ba}/^{137}\text{Ba}$  gradually approached the natural ratio of 6.835, although there was some retention even at 56 days post-injection. The plasma cortisol analysis showed that neither the injection nor the chemical induced any stress to females.

In conclusion, these results suggest barium marking will be 100% effective for marking clownfishes larvae, and provided dose levels are kept to a minimum, there will be no adverse effects on adult females or their offspring.

## Table of contents

STATEMENT OF ACCESS.....	II
STATEMENT OF SOURCES.....	III
STATEMENT OF CONTRIBUTION OF OTHERS.....	IV
STATEMENT ON ETHICS.....	V
ELECTRONIC COPY.....	VI

<b>Acknowledgements.....</b>	<b>VII</b>
------------------------------	------------

<b>Abstract.....</b>	<b>VIII</b>
----------------------	-------------

<b>Table of contents.....</b>	<b>X</b>
-------------------------------	----------

<b>List of tables.....</b>	<b>XI</b>
----------------------------	-----------

<b>List of figures.....</b>	<b>XII</b>
-----------------------------	------------

<b>1. General introduction.....</b>	<b>1</b>
-------------------------------------	----------

<b>2. Evaluation of the effects of different injected doses of stable barium isotopes on larvae of the clownfish <i>Amphiprion percula</i>.....</b>	<b>5</b>
---	----------

2.1 Synopsis.....	5
-------------------	---

2.2 Introduction.....	6
-----------------------	---

2.3 Methods.....	8
------------------	---

2.4 Results.....	11
------------------	----

2.5 Discussion.....	13
---------------------	----

2.6 Figures and tables.....	16
-----------------------------	----

<b>3. Incorporation of enriched barium isotopes in tissues of female <i>Amphiprion melanopus</i> and assessment of physiological stress.....</b>	<b>25</b>
--	-----------

3.1 Synopsis.....	25
-------------------	----

3.2 Introduction.....	26
-----------------------	----

3.3 Methods.....	28
------------------	----

3.4 Results.....	31
------------------	----

3.5 Discussion.....	33
---------------------	----

3.6 Figures and tables.....	38
-----------------------------	----

<b>4. General conclusions.....</b>	<b>43</b>
------------------------------------	-----------

<b>5. References.....</b>	<b>45</b>
---------------------------	-----------

## List of tables

Table 2.1	Mixed model, hierarchical analysis of variance for the effect of barium dosage and reproductive pair on the clutch size (number of eggs) of <i>Amphiprion percula</i> , for successive clutches at time zero (prior to injection) and for 6 successive clutches post-injection.....	20
Table 2.2	Mixed model, hierarchical analysis of variance for the effect of barium dosage and reproductive pair on the clutch area of <i>Amphiprion percula</i> , for successive clutches at time zero (prior to injection) and for 6 successive clutches post-injection.....	20
Table 2.3	Summary of ANOVA testing the effect of treatment, pair and clutch on the mean length of <i>Amphiprion percula</i> larvae at 3 successive post hatching time a) day 1, b) day 14 and c) day 21. ....	22
Table 2.4	Summary of ANOVA testing the effect of treatment, pair and the clutch on the mean weight of <i>Amphiprion percula</i> larvae at 21 days post-hatching.....	24
Table 3.1	Summary of ANOVA presenting the test of the effect of treatment (2 µg <sup>137</sup> Ba/g fish weight and 4 µg <sup>137</sup> Ba/g fish weight) and sampling time (2, 7 21 and 56 days after injection) on the level of plasma cortisol in <i>Amphiprion melanopus</i> .....	40
Table 3.2	Summary of ANOVA presenting the test of the effect of treatment (2 µg <sup>137</sup> Ba/g fish weight and 4 µg <sup>137</sup> Ba/g fish weight) and time (2, 7, 21, 56 days post injection) on the Ba ratio in a) gonads b) muscles c) liver d) bones of <i>Amphiprion melanopus</i> females.....	41

## List of figures

Figure 2.1	Injection technique; picture taken of <i>Amphiprion percula</i> .....	16
Figure 2.2	Mean $^{138}\text{Ba}/^{137}\text{Ba}$ ratios +/- SE in the core of the otoliths of juvenile <i>Amphiprion percula</i> raised from different clutches laid by females subject to three different doses of $^{137}\text{BaCl}_2$ . The mean ratio for all control fish was 6.385.....	17
Figure 2.3	Clutch size (number of eggs) +/- SE produced by <i>Amphiprion percula</i> pairs, treated with different concentration of $^{137}\text{Ba}$ in function of the number of clutch post injection. ....	18
Figure 2.4	Clutch area ( $\text{mm}^2$ ) +/- SE produced by <i>Amphiprion percula</i> pairs, treated with different concentration of $^{137}\text{Ba}$ in function of the number of clutch post injection.....	19
Figure 2.5	Mean larvae length (mm) $\pm$ SE of <i>Amphiprion percula</i> pairs, treated with different concentration of $^{137}\text{Ba}$ , sampled at 3 successive post hatching intervals. a) day 1 post hatching b) day 14 post hatching c) day 21 post hatching. ....	21
Figure 2.6	Mean weight (mg) $\pm$ SE of <i>Amphiprion percula</i> larvae at 21 days post-hatching pairs, treated with different concentration of $^{137}\text{Ba}$ . ....	23
Figure 3.1	Sex determination of <i>Amphiprion melanopus</i> adult's.....	38
Figure 3.2	Cortisol concentration (ng/ml) $\pm$ SE in function of time and $^{137}\text{Ba}$ concentration ( $\mu\text{g } ^{137}\text{Ba/g}$ fish weight) injected in female <i>Amphiprion melanopus</i> . ....	39
Figure 3.3	Mean $^{138}\text{Ba}/^{137}\text{Ba}$ ratio $\pm$ SE in a) gonads b) muscles c) liver d) bones of <i>Amphiprion melanopus</i> control females or injected females with $2 \mu\text{g } ^{137}\text{Ba/g}$ fish weight or $4 \mu\text{g } ^{137}\text{Ba/g}$ fish weight sampled at four different times post injection. ....	42