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

Thesis submitted by
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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 Frish 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 *Nationoal Sttement 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)

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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}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}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}Ba (0.5, 1.0 and 2.5 µg ^{137}Ba/g fish weight) and the effectiveness of the mark was quantified by measuring the ^{138}Ba/^{137}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 µg ^{137}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 µg ^{137}Ba/g fish weight, the ^{138}Ba/^{137}Ba ratio returned to the natural barium ratio of 6.385 after 2 clutches (≈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}Ba dose, 2.5 µg ^{137}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}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 µg and 4 µg ^{137}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}Ba/^{137}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

List of tables

List of figures