LIBRARY
Te Tumu Herenga
THE UNIVERSITY OF AUCKLAND

RESEARCHSPACE@AUCKLAND

http://researchspace.auckland.ac.nz

*ResearchSpace@Auckland*

**Copyright Statement**

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of this thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from their thesis.

To request permissions please use the Feedback form on our webpage. http://researchspace.auckland.ac.nz/feedback

**General copyright and disclaimer**

In addition to the above conditions, authors give their consent for the digital copy of their work to be used subject to the conditions specified on the Library Thesis Consent Form and Deposit Licence.

**Note : Masters Theses**

The digital copy of a masters thesis is as submitted for examination and contains no corrections. The print copy, usually available in the University Library, may contain corrections made by hand, which have been requested by the supervisor.

# Physiological adaptation in the radiation of New Zealand triplefin fishes (Family Tripterygiidae)

**Zoë Hilton**

A thesis submitted in partial fulfilment of the requirements

for the degree of Doctor of Philosophy

in

Biological Sciences

The University of Auckland

2010

# Abstract

Physiological adaptation to divergent environments is a poorly understood factor in adaptive radiation. New Zealand (NZ) triplefin fishes (Tripterygiidae) have undergone a radiation associated with habitat diversification within NZ's coastal waters, where 26 closely-related endemic species occur in overlapping but divergent habitats, partitioned by depth and exposure. By investigating the relationship between respiratory physiological traits and habitat in these fishes, this thesis examines whether there is evidence in this group to support two proposed criteria for adaptive radiation; phenotype-environment correlation and trait utility.

Significant interspecific differences were observed in rates of oxygen consumption ($VO_2$) and critical oxygen concentration ($O_{2\ crit}$) in 12 species of triplefin examined. $O_{2\ crit}$ correlated with species' habitat depth, with intertidal species displaying greater hypoxia tolerance than subtidal species, thus demonstrating phenotype-environment correlation and trait utility in relation to hypoxia exposure. Interspecific differences in $VO_2$ were significantly influenced by phylogeny, indicating a lack of strong environmental selection on $VO_2$. However, there was some indication of lower $VO_2$ in species occupying more exposed habitats. Mitochondrial respiration was also examined in three species; the intertidal species displayed higher cytochrome *c* oxidase activity and was able to maintain efficient oxidative phosphorylation at higher temperatures than the two subtidal species, further indicating phenotype-environment correlation and trait utility.

Haemoglobin (Hb) isoform expression was examined in 23 species. Isoform multiplicity declined with habitat depth, supporting the hypothesis that higher multiplicity may be associated with greater environmental variability. A lack of phylogenetic signal in Hb expression, and latitudinal variation in the relative isoform abundance in some species, indicated potential selection on this trait. However, there was no pattern in expression of cathodal Hbs, and the trait utility of this multiplicity is unknown.

Overall, there is strong evidence that differences between intertidal and subtidal environments in exposure to high temperatures and hypoxia may have lead to divergence in $O_{2\ crit}$ and mitochondrial function between intertidal and subtidal species. Therefore physiological adaptation may have enabled the expansion of species into the more demanding habitats such as the intertidal zone. Hb isoform multiplicity and $VO_2$ were correlated with habitat in both intertidal and subtidal species, however the trait utility associated with these correlations is unknown and thus there remains a lack of evidence to support a direct role of physiological adaptation in habitat divergence of subtidal species − and therefore for adaptive radiation of the group as a whole.

# Acknowledgements

First and foremost I would like to thank my supervisors. I am eternally grateful to my primary supervisor, Assistant Professor Kendall Clements, for the opportunity to be involved in this project. The collecting trips to Fiordland and NZ's offshore islands were the highlight of my PhD and through these I discovered a whole new world, and gained a new appreciation of the beauty and diversity of marine life. I feel incredibly privileged to have had that opportunity. Thank you for sharing your incredible enthusiasm for triplefins, and fishes in general, but most of all thank you for your enduring patience and support when things got in the way of my PhD - for not giving up on me and not letting me give up! I also owe a debt of gratitude to my co-supervisor Dr Tony Hickey for his input into the initial ideas for this project, help with the mitochondrial work, discussions on triplefin physiology and phylogeography, and above all else for his good humour and boundless enthusiasm. I would also like to thank Professor Rufus Wells for his supervision of the early stages of this work, and most of all for being such a great lecturer and inspiring me to study ecophysiology.

I am extremely grateful for the financial support received from the Marsden Fund of the Royal Society of NZ, and the University of Auckland, which enabled me to carry out this research.

This research would also not have been possible were it not for the generous help of many other people along the way. Firstly, I would like to thank all of the people who helped with the field work including Will Allardice, Bill Bell, Kendall Clements, Lisa Clements, Jim Hilton, Megan Marcotte, Damian Moran, Dean Ngorotata, David Raubenheimer, Andrew Stewart, and Victoria Vincent. In particular I would like to thank Maren Wellenreuther who worked on triplefin ecology as a fellow PhD student. She took me under her wing as a relatively novice diver and was my dive buddy in conditions from the most cold bleak muddy estuaries to the most beautiful offshore islands, and everything in between during many collecting trips over several years. She helped me check in to several backpackers hostels around the country despite my perfectly legitimate but extremely suspicious looking luggage of liquid nitrogen dewar, centrifuge, and boxes of hypodermic needles! I would also like to thank Professor Bill Davison of the University of Canterbury, and staff of the Edward Percival Field Station, Kaikoura, for the kind use of their facilities to collect blood samples and enable me to bring live specimens from Banks

Peninsula to Auckland. Thanks also to the crew of the Breaksea Girl who hosted us in Fiordland and Stewart Island. Finally a very special thank you to Brady Doak and Murray Birch, skippers of the RV Haware, for their incredible skills, knowledge and good natures on all of our collecting trips in the Hauraki Gulf and to the Three Kings Islands and Fiordland.

For help with the fish maintenance, lab work, and data analysis, many thanks go to: Simrana Clayton, Alfred Degenkolbe and Schannel Van Dijken for assistance with the U of A seawater facilities; Ryan Chai for help in the mitochondria lab; Dr Simon Greenhill for help with the phylogenetic analyses; and Dr Damien deVienne for help with the MAST calculations. I wish to thank Professor Tom Brittain, Ass. Professor Angela Fago, and Kristen Henty for their discussions on haemoglobin analyses, Dr Mary Sewell for generously giving her advice and equipment for the whole animal respirometry work, Iain MacDonald for photographic assistance and Vivian Ward for preparing the beautiful illustrations of the triplefin species. I am very grateful to Kathy Ruggiero, Craig Millar, Emma Marks, Jethro Johnson, Liz Trip, Megan Marcotte and Maren Wellenreuther for statistical and software advice. In the preparation of the thesis I would especially like to thank Jennifer Murray and Prof. Howard Choat for their constructive comments on the writing.

I would like to thank my colleagues at SBS Silver Bishop, Brendan Dunphy, Berit Finkennest, Ramón Gallego, Joshua Guilbert, Jethro Johnson, Elizabeth Laman Trip, Megan Marcotte, Damian Moran, Matt Rayner, David Seldon, Tim Sippell, Gretchen Skea, Maren Wellenreuther, Shane Windsor, and Erica Zarate, for your friendship, advice, support and inspiration.

Last but not least, this thesis would never have been submitted were it not for the moral and practical support of my family, and Jim, Tony and Val, Sasha, Damien, and Gretchen. Words can never express my gratitude to you for your love, support and encouragement, most of all during the times when I felt like I couldn't even see the tunnel let alone the light at the end of it! Thank you…

# Table of Contents

# List of Tables

# List of Figures

# Abbreviations*

| | |
|---|---|
| ACD | Above chart datum |
| ADP | Adenosine-5'-diphosphate |
| ANCOVA | Analysis of covariance |
| ANOSIM | Analysis of similarity |
| ANOVA | Analysis of variance |
| ATP | Adenosine-5'-triphosphate |
| BSA | Bovine serum albumin |
| BPM | Beats per minute |
| CCO | Cytochrome *c* oxidase |
| CCO*c* | Cytochrome *c* oxidase in the presence of exogenous cytochrome *c* |
| DO | Dissolved oxygen concentration |
| EGTA | Ethylene glycol tetraacetic acid |
| ESI-MS | Electrospray ionisation mass spectrometry |
| ETS | Electron transport system |
| $FADH_2$ | Flavin adenine dinucleotide (reduced) |
| FCCP | Carbonyl cyanide p-(trifluoro-methoxy) phenyl-hydrazone |
| FPLC | Fast protein liquid chromatography |
| GLS | Generalised least squares |
| GTR | Generalised time reversible |
| Hb | Haemoglobin |
| HEPES | Na N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid |
| HPLC | High performance liquid chromatography |
| IEF | Isoelectric focusing |
| $JO_2$ | Rate of mitochondrial oxygen consumption |
| LSLR | Least squares linear regression |
| MCCT | Maximum clade credibility tree |
| MES | 2-(N-morpholino) ethanesulfonic acid |
| NADH | Nicotinamide adenine dinucleotide (reduced) |
| $O_2$ | Oxygen |
| $O_{2\ crit}$ | Critical oxygen concentration |
| OXPHOS | Oxidative phosphorylation |

| | |
|---|---|
| PCA | Principal component analysis |
| PC1 | Principal component 1 |
| PC2 | Principal component 2 |
| pI | Isoelectric point |
| $Q_{10}$ | Temperature quotient |
| RBC | Red blood cell |
| RCR | Respiratory control ratio |
| ROS | Reactive oxygen species |
| SD | Standard deviation |
| SE | Standard error |
| SIMPER | Similarity of percentages test |
| SST | Sea surface temperature |
| TCA | Trichloroacetic acid |
| TMPD | N, N, N', N'-tetramethyl-p-phenyldiamine |
| UPGAMA | Un-weighted pair-group with arithmetic means clustering algorithm |
| $Vf$ | Ventilation frequency |
| $VO_2$ | Whole-animal rate of oxygen consumption |

* For species name abbreviations see Table 1.1

**Collection location abbreviations**

| | |
|---|---|
| 3K | Three Kings Islands |
| BL | Bluff |
| BP | Banks Peninsula |
| FL | Fiordland |
| HG | Hauraki Gulf |
| KK | Kaikoura |
| NL | Nelson |
| NP | Napier |
| SI | Stewart Island |
| WT | Wellington |