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

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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 ($O_{2\text{ crit}}$) in 12 species of triplefin examined. $O_{2\text{ crit}}$ 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 led to divergence in $O_{2\text{ crit}}$ 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.

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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 <i>c</i> oxidase
CCO _c	Cytochrome <i>c</i> oxidase in the presence of exogenous cytochrome <i>c</i>
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
V _f	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