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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.
The effects of habitat on phenotype, growth and fitness in New Zealand triplefin fishes
(Family: Tripterygiidae)

Paul Edward Caiger

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Marine Science

University of Auckland, 2017
Study species: *Notoclinops segmentatus, Forsterygion lapillum, Forsterygion varium,* and *Ruanoho whero*
Abstract

New Zealand triplefins have previously been shown to have diverged in habitat use. This thesis aimed to determine whether they differed in habitat performance, with a view to determine whether processes that are hypothesized to have taken place over evolutionary timescales (i.e. ecological speciation), are evident over ecological timescales. Of particular interest is how variation in the environment can lead to fitness differences that have evolutionary consequences. This was achieved by testing several proximate indicators of fitness in different habitats, and included: (i) describing the patterns and variation in settlement and abundance of the four most common species of triplefins (*Forsterygion lapillum*, *F. varium*, *Ruanoho whero*, and *Notoclinops segmentatus*) among habitats, (ii) testing the effect of habitat on the relationship between male size and clutch size, (iii) utilising the age and growth rate information of two species of triplefin, *F. lapillum* and *F. varium*, as a proxy for post-settlement performance, (iv) testing the relationship between habitat and morphology in *F. lapillum* populations, and (v) testing the hypothesis that the mechanism for the environmentally-induced morphological variation seen in the wild populations (chapter 4) is phenotypic plasticity.

Intraspecific variation was found in all of the ecological traits examined, with phenotype-environment correlations observed in density and reproductive output, growth rates and longevity, and fin and body morphology. The main factors associated with driving selection pressures were wave exposure and microhabitat type, and the former was consistently evident across all traits measured. Additionally, plasticity was shown to be the mechanism responsible for the observed morphological variation in *F. lapillum*, and theoretically has the potential to generate disruptive selection by creating the raw material for natural selection to act upon. By identifying characters under selection, these results suggest a basis for divergent selection in habitat use. This informs our understanding of niche diversification in triplefins and supports the hypothesis that differentiation in habitat use is an important mechanism in the ecological speciation seen in the New Zealand triplefin fish assemblage. As few field studies have examined this process in marine fishes, empirical studies of this nature on marine fishes are important in working towards an understanding of the drivers that promote and maintain diversity in these systems.
Acknowledgements

First I’d like to thank my primary supervisor, Kendall Clements, who has provided me with the opportunity to work on triplefins. Thank you for sharing your sharp scientific expertise and endless knowledge and on all things fish related, but also for your patience and keeping me pointed in the right direction. Also, thanks for involving me in many field trips outside of my research, both temperate and tropical. After all, being in the field and experiencing nature in all its splendour is why we do this. Thank you also to my co-supervisor Richard Taylor for your generous support and good advice towards the thesis. I also immensely enjoyed all the dive and photo missions – I look forward to reading the coastal ID book. Thanks Cécile Croq for the use of some your fish data which was used in chapter four.

This research would also not have been possible were it not for the generous help of many people along the way. Firstly, I would like to thank all of the people who helped with the field work including Anna B, Evan B, Peter B, Kendall C, Brady D, Jan H, Richie H, Christine K, Jared K, Tamsen P, James R, Lauren S, Arie S, Jenni S, Lucy van O, and Valerio V. To anyone I’ve forgotten, I apologise. Also thanks to Brady and Peter for skippering the Hawere on many excellent trips. A huge appreciation to Lauren Shea for the six weeks spent volunteering on the project, even though the lab work often outweighed the diving. I am also extremely grateful to all the staff and students at the Leigh Marine Laboratory for the help along the way, but also making my time in Leigh so enjoyable.

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# Table of Contents

Abstract .......................................................................................................................................... iii  
Acknowledgements ......................................................................................................................... iv  
Table of Contents ............................................................................................................................ v  
List of Tables ................................................................................................................................... viii  
List of Figures .................................................................................................................................. ix  

**Chapter 1 General Introduction** ............................................................................................... 1  
1.1 Ecological speciation: a theoretical background ................................................................. 1  
1.2 Empirical evidence of ecological speciation and adaptive radiations ......................... 3  
1.3 Triplefin fishes ...................................................................................................................... 5  
1.4 Study environment ............................................................................................................... 6  
1.5 Aims of the research .......................................................................................................... 7  

**Chapter 2 Relationships between habitat, abundance and fitness in New Zealand triplefin fishes**................................................................................................................................. 10  
2.1 Introduction ......................................................................................................................... 10  
2.2 Methods .............................................................................................................................. 13  
2.2.1 Sampling locations ....................................................................................................... 13  
2.2.2 Wave exposure analysis ............................................................................................. 13  
2.2.3 Underwater visual censuses ....................................................................................... 15  
2.2.4 Nest photographs ....................................................................................................... 17  
2.3 Results .............................................................................................................................. 20  
2.3.1 Spatial variation in triplefin density ........................................................................... 20  
2.3.2 Spatial densities of triplefin by age class .................................................................. 25  
2.3.3 Spatio-temporal patterns in triplefin density ............................................................... 28  
2.3.4 Spatial variation in nesting for *F. lapillum* and *F. varium* .................................. 35  
2.4 Discussion ......................................................................................................................... 38  
2.4.1 Spatial differences in triplefin abundance ................................................................. 38  
2.4.2 Temporal differences in triplefin abundance ............................................................... 42  
2.4.3 Fecundity .................................................................................................................... 43  
2.4.4 Conclusions ............................................................................................................... 44  

**Chapter 3 Effects of wave exposure on growth in two triplefin species, Forsterygion lapillum and F. varium** ................................................................................................................................. 46  
3.1 Introduction ........................................................................................................................ 46  
3.2 Methods ................................................................................................................................ 49  
3.2.1 Sampling .................................................................................................................... 49
### Table of Contents

3.2.2 Wave exposure analysis ........................................................................................................ 49
3.2.3 Otolith preparation and analyses .......................................................................................... 52
3.2.4 Validation of daily increments using tetracycline.................................................................. 55
3.2.5 Length-based growth estimates ............................................................................................ 56
3.2.6 Data analysis ....................................................................................................................... 57

3.3 Results ........................................................................................................................................ 57
3.3.1 Length-based growth analyses ................................................................................................. 57
3.3.2 Otolith analyses ...................................................................................................................... 62
  3.3.2.1 Fish size ......................................................................................................................... 63
  3.3.2.2 Relationships between otolith growth and somatic growth ................................................. 63
  3.3.2.3 Annual otolith counts ....................................................................................................... 64

3.4.1 Growth rate and size ................................................................................................................ 66
3.4.2 Longevity ................................................................................................................................... 68
3.4.3 Implications of faster growth and increased longevity ............................................................ 69
3.4.4 Conclusions and future directions .......................................................................................... 69

### Chapter 4 Phenotypic variation correlates with wave exposure in the temperate reef fish, *Forsterygion lapillum* ........................................................................................................ 71

4.1 Introduction .................................................................................................................................. 71
4.2 Methods ...................................................................................................................................... 73
  4.2.1 Sampling ................................................................................................................................. 73
  4.2.2 Wave exposure analysis ......................................................................................................... 74
  4.2.3 Body shape morphometrics ................................................................................................... 76
    4.2.3.1 Linear head and body measurements ................................................................................. 76
    4.2.3.2 Geometric morphometrics ............................................................................................ 77
  4.2.4 Pectoral fin morphology ....................................................................................................... 79

4.3 Results ........................................................................................................................................ 80
  4.3.1 Head and body morphology .................................................................................................... 80
  4.3.2 Pectoral fin morphology ........................................................................................................ 83
  4.3.3 Geometric morphometric analyses ......................................................................................... 84

4.4 Discussion .................................................................................................................................. 87
  4.4.1 Functional implications in changes in body morphology ......................................................... 88
  4.4.2 Functional implications to changes in pectoral fin morphology ............................................... 89
  4.4.3 Eye region .............................................................................................................................. 91
  4.4.4 Sexual dimorphism ................................................................................................................. 91
  4.4.5 Conclusions ........................................................................................................................... 92
<table>
<thead>
<tr>
<th>Chapter 5</th>
<th>Phenotypic plasticity evident across a small spatial scale in the temperate marine reef fish <em>Forsterygion lapillum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Introduction</td>
<td>.................................................................................................................................................. 93</td>
</tr>
<tr>
<td>5.2 Methods</td>
<td>.................................................................................................................................................. 95</td>
</tr>
<tr>
<td>5.2.1 Fish collection</td>
<td>.................................................................................................................................................. 95</td>
</tr>
<tr>
<td>5.2.2 Common garden experiment</td>
<td>.................................................................................................................................................. 96</td>
</tr>
<tr>
<td>5.2.3 Water movement experiment</td>
<td>.................................................................................................................................................. 96</td>
</tr>
<tr>
<td>5.2.4 Wave exposure analysis</td>
<td>.................................................................................................................................................. 98</td>
</tr>
<tr>
<td>5.2.5 Linear body measurements</td>
<td>.................................................................................................................................................. 98</td>
</tr>
<tr>
<td>5.2.6 Pectoral fin morphology</td>
<td>.................................................................................................................................................. 99</td>
</tr>
<tr>
<td>5.2.7 Geometric morphometrics</td>
<td>.................................................................................................................................................. 99</td>
</tr>
<tr>
<td>5.3 Results</td>
<td>.................................................................................................................................................. 101</td>
</tr>
<tr>
<td>5.3.1 Common garden experiment</td>
<td>.................................................................................................................................................. 101</td>
</tr>
<tr>
<td>5.3.2 Water movement experiment</td>
<td>.................................................................................................................................................. 103</td>
</tr>
<tr>
<td>5.3.3 Geometric morphometrics</td>
<td>.................................................................................................................................................. 105</td>
</tr>
<tr>
<td>5.4 Discussion</td>
<td>.................................................................................................................................................. 108</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 6</th>
<th>General Discussion</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1 Ecological speciation on contemporary timescales</td>
<td>.................................................................................................................................................. 111</td>
</tr>
<tr>
<td>6.2 Future directions</td>
<td>.................................................................................................................................................. 113</td>
</tr>
<tr>
<td>6.3 Conclusions</td>
<td>.................................................................................................................................................. 115</td>
</tr>
</tbody>
</table>

| List of References | .................................................................................................................................................. 116 |
| Appendix | .................................................................................................................................................. 130 |
List of Tables

Table 2.1. Fetch calculations and coordinates for each sample site......................................................... 15
Table 2.2. Summary statistics ($F$ statistics and $P$-values) of general linear models comparing
density among several habitat variables and among years, and the interactions among
those variables for each species. For more detailed statistics, see tables 2.3-2.6 below...........20
Table 2.3. Comparison of *Forsterygion lapillum* density among several habitat variables and
among years from the general linear model. Significant interactions are in bold......................... 22
Table 2.4. Comparison of *Forsterygion varium* density among several habitat variables and
among years from the general linear model. Significant interactions are in bold......................... 23
Table 2.5. Comparison of *Ruanoho whero* density among several habitat variables and
among years from the general linear model. Significant interactions are in bold......................... 23
Table 2.6. Comparison of *Notoclinops segmentatus* density among several habitat variables
and among years from the general linear model. Significant interactions are in bold.............. 24
Table 2.7. Proportional abundances by habitat and age class over the 3 year sampling period
(e.g. *F. lapillum* 0+ fish at sheltered locations had the highest densities at 17 out of 18
bi-monthly surveys = 0.94). Darker shading indicates higher proportions. .............................. 35
Table 3.1. Fetch and wave exposure calculations and coordinates for each sampling
location........................................................................................................................................... 52
Table 3.2. Growth parameters for *F. lapillum* and *F. varium* estimated from length-based
analyses (Munro’s method).............................................................................................................. 62
Table 4.1. Fetch and wave exposure calculations and coordinates for each sampling site............. 76
Table 4.2. MANOVA results for canonical variate analysis comparing males vs females and
exposure location. ......................................................................................................................... 87
Table 5.1. Results of MANOVA on fish body shape for the common garden experiment
(sheltered vs exposed) and the water movement experiment (low energy vs high
energy)........................................................................................................................................... 106
Appendix table 1. Bonferroni’s pairwise comparisons for wave exposure, microhabitat and
year for *Forsterygion lapillum* density from the general linear model. Significant
interactions are highlighted........................................................................................................... 132
Appendix table 2. Bonferroni’s pairwise comparisons for wave exposure, microhabitat and
year for *Forsterygion varium* density from the general linear model. Significant
interactions are highlighted........................................................................................................... 133
Appendix table 3. Bonferroni’s pairwise comparisons for wave exposure, microhabitat and
year for *Ruanoho whero* density from the general linear model. Significant interactions
are highlighted.............................................................................................................................. 134
Appendix table 4. Bonferroni’s pairwise comparisons for wave exposure, microhabitat and
year for *Notoclinops segmentatus* density from the general linear model. Significant
interactions are highlighted........................................................................................................... 135
List of Figures

Figure 2.1. Map of sample sites in the outer Hauraki Gulf. Inset 1 shows sites on the coastal mainland: sheltered (Ti, Jo), semi-exposed (No, Ma), exposed (WR, Ta). Mo and GB are offshore islands (very exposed). Inset 2 shows Hauraki Gulf location in the North Island of New Zealand. ................................................................. 14

Figure 2.2. Examples of triplefin nests showing method of photographic analysis of male size and nest area for A) *F. varium*, and B) *F. lapillum*. Note: numbers on figure indicate the tracing tool used during clutch size analysis. ........................................................................................................... 19

Figure 2.3. Mean densities (± S.E.M.) of each species of triplefin (all age classes) averaged over three years across a wave exposure gradient in the Hauraki Gulf. N = number of transects.................................................................................................................. 21

Figure 2.4. Mean densities (± S.E.M.) of each species of triplefin (all age classes) in different microhabitats, averaged over three years across all sites. N = number of transects.................................................................................................................. 22

Figure 2.5. Mean density of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* in different microhabitats and wave exposures. Data averaged across time for all size classes. EFD = *Ecklonia* forest deep; EFS = *Ecklonia* forest shallow; MA = mixed algae; STA = shallow turfing algae; COB = cobbles. Note difference in y-axis scales. .......................................................................................................................... 25

Figure 2.6. Mean densities (± S.E.M.) of 0+ and 1++ age classes across a wave exposure gradient in the Hauraki Gulf for A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus*. Time is averaged over three years. Note difference in y-axis scales. .................................................................................................................. 26

Figure 2.7. Mean densities (± S.E.M.) of 0+ and 1++ age classes between habitats in the Hauraki Gulf for A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus*. Time is averaged over three years. Note difference in y-axis scales. .................................................................................................................. 27

Figure 2.8. Relationship between seasonal recruitment and mortality, as seen by seasonal mean size (TL) ±SEM, and density (fish per m²) of a) *F. lapillum*, b) *F. varium*, c) *R. whero*, and d) *N. segmentatus* across all sites and years combined. Note differences in y-axis scales. .................................................................................................................. 28

Figure 2.9. Seasonal density for three successive years of 0+ cohorts of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* across a wave exposure gradient in the Hauraki Gulf. Shaded bars indicate peak spawning periods. Note differences in y-axis scales. .................................................................................................................. 30

Figure 2.10. Seasonal density for three successive years for adult individuals (1++ cohorts) of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* across a wave exposure gradient in the Hauraki Gulf. Shaded bars indicate peak spawning periods. Note differences in y-axis scales. .................................................................................................................. 31

Figure 2.11. Seasonal density for three successive years of 0+ cohorts of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* in different habitats. EFD = *Ecklonia* forest deep; EFS = *Ecklonia* forest shallow; MA = mixed algae; STA = shallow turfing algae; COB = cobbles. Shaded bars indicate peak spawning periods. Note difference in y-axis scales. .................................................................................................................. 33

Figure 2.12. Seasonal density for three successive years of adult individuals (1++ cohorts) of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* in different habitats. EFD = *Ecklonia* forest deep; EFS = *Ecklonia* forest shallow; MA = mixed algae; STA = shallow turfing algae; COB = cobbles. Shaded bars indicate peak spawning periods. Note differences in y-axis scales. .................................................................................................................. 34
Figure 2.13. Total lengths of nest-guarding males for A) *F. lapillum*, and B) *F. varium* at different wave exposure locations. Dashed lines represent the sample means. 

Figure 2.14. A) 0.5 and B) 0.9 quantile regression estimates for *F. lapillum*, and C) 0.5 and D) 0.9 quantile regression estimates *F. varium* across a wave exposure gradient in the Hauraki Gulf. 

Figure 2.15. A) 0.5 and B) 0.9 quantile regression estimates for *F. varium* across a wave exposure gradient in the Hauraki Gulf. 

Figure 3.1. Map of sample sites in the outer Hauraki Gulf. Inset 1 shows sites on the coastal mainland: sheltered (Ti, Jo), semi-exposed (No, Ma), exposed (WR, Ta). Mo and GB are offshore islands (very exposed). Inset 2 shows Hauraki Gulf location in the North Island of New Zealand. 

Figure 3.2. Example of transverse sagittal otolith section for a *F. lapillum* (27.2 mm TL) under transmitted light at A) 100x, and B) 250x. 

Figure 3.3. Examples of transverse sagittal otolith sections under reflected light for A) *F. lapillum* (59.6 mm TL) and B) *F. varium* (104.1 mm TL). 

Figure 3.4. Example of transverse *F. varium* sagittal otolith section at 400x following tetracycline injection procedure under A) transmitted light, B) ultraviolet light, and C) transmitted and ultraviolet light. 

Figure 3.5. Modal progression of *F. lapillum* populations at A) sheltered, B) semi-exposed, and C) exposed locations from October 2012 through August 2015. 

Figure 3.6. von Bertalanffy growth functions for *F. lapillum* based on growth parameters estimated from the length-based growth (Munro’s method) at sheltered, semi-exposed and exposed locations. 

Figure 3.7. Modal progression of *F. varium* populations at A) sheltered, B) semi-exposed, and C) exposed locations from October 2012 through August 2015. 

Figure 3.8. von Bertalanffy growth functions for *F. varium* based on growth parameters estimated from the length-based growth (Munro’s method) at sheltered, semi-exposed and exposed locations. 

Figure 3.9. Boxplots of fish length for A) *F. lapillum*, and B) *F. varium* for samples collected at different exposure locations in the Hauraki Gulf. Dashed lines represent sample means. 

Figure 3.10. Relationship between otolith length and standard length for A) *F. lapillum*, and B) *F. varium*. Otolith lengths were measured along the rostral-postrostral axis. Relationship between otolith weight and total length for C) *F. lapillum*, and D) *F. varium*. 

Figure 3.11. Otolith annual increment counts for A) *F. lapillum*, and B) *F. varium* across a wave exposure gradient in the outer Hauraki Gulf. von Bertalanffy growth functions estimated from length-based growth (figs. 3.6 & 3.8) are overlaid for comparison (i.e. growth curves here are not created from the annual otolith counts). No offshore growth curves were estimated due to insufficient bi-monthly sampling in the length-based growth. 

Figure 4.1. Map of sample sites in the Hauraki Gulf. Inset 1 shows sites on the coastal mainland: sheltered (Tw, Jo), semi-exposed (No, Ma, Wa), exposed (Ta). Mo and GB are offshore islands (very exposed). Inset 2 shows Hauraki Gulf location in the North Island of New Zealand.
Figure 4.2. A) Linear body measurements, and B) landmark configuration used to analyse variability in body shape of *F. lapillum*..............................................................79

Figure 4.3. Boxplot of total length of adult *F. lapillum* from sites differing in wave exposure. .................................................................................................................................81

Figure 4.4. Mean standardised morphological measurements of the A) eye region, B) mouth region, C) head region, and D) tail region of *F. lapillum* by exposure. Bars represent standard errors of the mean. Abbreviations: total length (TL), eye diameter (ED), interorbital width (IOW), gape width (GW), gape length (GL), maximum body depth (BD), head length (HL), pre-pectoral length (PPL), pre-dorsal length (PDL), post-anal length (PAL), caudal peduncle depth (CPD), and caudal fin length (CFL). ...........................82

Figure 4.5. Mean measurements of pectoral fin morphology for *F. lapillum* across an exposure gradient. (A) Fin size, and (B) aspect ratio. Bars represent standard errors of the mean. ........................................................................................................83

Figure 4.6. A) Canonical variate analysis (CVA) of *F. lapillum* body shape landmarks from sites differing in wave exposure: sheltered (triangles), semi-exposed (crosses), and offshore (stars) locations. Deformation grids with relative displacement vectors for each landmark, visualise the areas of greatest body shape change captured by B) CVA axis 1, and C) axis 2. ...............................................................................................................85

Figure 4.7. Thin-plate spline visualisation of mean shape differences between *F. lapillum* from A) offshore and B) sheltered sites. All differences are linearly extrapolated by a factor of 3. .........................................................................................................................86

Figure 5.1. Map of the Hauraki Gulf, New Zealand, with the sample sites inset: Jones Bay (sheltered) and Takatu Point (exposed). ..................................................................................97

Figure 5.2. A) Linear body measurements, and B) landmark configuration used to analyse variability in body shape of *F. lapillum*..............................................................101

Figure 5.3. Boxplot showing the total lengths of *F. lapillum* at the beginning and conclusion of the common garden experiment, depicting recruits collected from sheltered and exposed sites. Dashed lines represent the sample mean. ......................................................102

Figure 5.4. Mean measurements of A) fin size and B) aspect ratio for pectoral fin morphology of *F. lapillum* at the conclusion of the common garden experiment for sheltered and exposed treatments. Bars represent standard errors of the mean. ........................102

Figure 5.5. Mean morphological measurements of the eye, mouth, head and tail regions of *F. lapillum* from the common garden experiment. Sheltered (black); exposed (grey). Bars represent the standard errors of the mean. Abbreviations: total length (TL), eye diameter (ED), inter-orbital width (IOW), gape width (GW), gape length (GL), maximum body depth (BD), head length (HD), pre-pectoral length (PPL), pre-dorsal length (PDL), post-anal length (PAL), caudal peduncle depth (CPD), and caudal fin length (CFL). .........................................................................................................................103

Figure 5.6. Boxplot describing the total lengths of *F. lapillum* at the conclusion of the water movement tank experiment. Dashed lines represent the sample mean. .................................104

Figure 5.7. Mean measurements of A) fin size and B) aspect ratio for the pectoral fin morphology of *F. lapillum* from the different treatments of the water movement tank experiment. Bars represent standard errors of the mean. ......................................................104

Figure 5.8. Mean morphological measurements of the eye, mouth, head and tail regions of *F. lapillum* from the different treatments of the water movement experiment. Low flow (black); dump bucket (grey). Bars represent the standard errors of the mean. Abbreviations: total length (TL), eye diameter (ED), inter-orbital width (IOW), gape
width (GW), gape length (GL), maximum body depth (BD), head length (HD), prepectoral length (PPL), pre-dorsal length (PDL), post-anal length (PAL), caudal peduncle depth (CPD), and caudal fin length (CFL).

Figure 5.9. Canonical variate analysis depicting all four treatments from both experiments using Procrustes superimposition on landmarks: sheltered (red), exposed (pink), low energy (blue), high energy (black).

Figure 5.10. Deformation grid with vectors of displacement for fish shape along the first CVA axis for A) the common garden experiment, and B) the water movement experiment.

Figure 5.11. Average landmark configurations of specimens from the two experiments, depicting mean shape for A) common garden experiment treatments: sheltered (asterisks, black outline), exposed (triangles, blue outline), and B) water movement experiment: low energy (asterisks, black outline), high energy (triangles, blue outline).

Appendix figure 1. Sea surface temperature from Leigh marine laboratory monitoring, and seasonal density of four species of triplefins (all age classes).

Appendix figure 2. Fifteen year time series of mean monthly wave power for outer Hauraki Gulf coastal vs offshore sites.

Appendix figure 3. Mean monthly wave power, averaged over fifteen years (1998-2012) for outer Hauraki Gulf coastal vs offshore sites.

Appendix figure 4. Seasonal density of all individuals for three successive years of A) F. lapillum, C) F. varium, C) R. whero, and D) N. segmentatus across a wave exposure gradient in the Hauraki Gulf. Shaded bars indicate peak spawning periods.

Appendix figure 5. Seasonal density (fish per m²) of all individuals for three successive years of A) F. lapillum, B) F. varium, C) R. whero, and D) N. segmentatus in different habitats. EFD = Ecklonia forest deep; EFS = Ecklonia forest shallow; MA = mixed algae; STA = shallow turfing algae; COB = cobbles.

Appendix figure 6. Fifteen year time series of mean monthly wave power for Jones Bay vs Takatu Point.

Appendix figure 7. Mean monthly wave power, averaged over fifteen years (1998-2012) for Jones Bay vs Takatu Point.

Appendix figure 8. Relationship between male length and nest size for A) F. lapillum and B) F. varium at different wave exposure locations.

Appendix figure 9. Relationship between male length and nest size for F. varium in different habitats.
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Chapter 4

<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Collection and processing of fish material, all statistical analyses and figure preparation. Author of chapter.</th>
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<tr>
<td>Cécile Croq</td>
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**Certification by Co-Authors**

The undersigned hereby certify that:

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Chapter 1

General Introduction

1.1 Ecological speciation: a theoretical background

Charles Darwin (1859) was the first to suggest that natural selection played an important role in the evolution of new species by favouring those individuals in a population that best matched the environment. However, this was criticised during the ‘modern’ synthesis, where the focus shifted towards geographic barriers limiting gene flow (Dobzhansky 1937, Mayr 1942), and for many years the theory of speciation via the geographical isolation of populations (allopatric speciation) was widely accepted as the default mode of speciation (Coyne and Orr 2004). Allopatric speciation can be either vicariant (species split into isolated populations) or peripatric (a small peripheral population becomes split) (Schluter 2000). In both situations, given enough time, non-adaptive genetic differences accumulate (neutral genetic drift) (Via 2001). When the vicariant barrier collapses, populations that have been isolated may come into contact again, but are either differentiated enough to co-occur without interbreeding, or reproductive isolation may occur via reinforcement, otherwise the two populations introgress back into one (Kirkpatrick 2000, Schluter 2000). However, with the support of numerous examples, species divergence in the presence of gene flow (i.e. parapatry or sympatry) is now widely accepted (Dieckmann and Doebeli 1999, Bolnick and Fitzpatrick 2007, Schluter 2009, Nosil 2012, Bowen et al. 2013).

Sympatric speciation occurs in the absence of any geographical barriers, and involves divergent and/or disruptive natural selection that leads either directly or indirectly to reproductive isolation (Orr and Smith 1998). Host choice in phytophagous insects is an example of a direct mechanism causing divergence in sympatry, where assortative mating evolves as a pleiotropic by-product of adaptation to resources (Berlocher and Feder 2002). Alternatively, reproductive isolation can be favoured indirectly if a tight genetic association between mate choice and preference traits exists, thus leading to the coordinated evolution of mate recognition (Kronforst et al. 2006).

The geographic categories of speciation differ in the extent to which ecological interactions or neutral processes occur. With allopatric speciation, neutral genetic drift is the principal mechanism of divergence, and in sympatric and parapatric populations, disruptive selection caused by ecological interactions or sexual selection are the main mechanisms responsible for diversification (Tregenza and Butlin 1999, Dieckmann et al. 2004). However, there is a
compelling case for the geographic pattern-based framework to be replaced with a process-based pattern. For example, there is now evidence for active selection in allopatric settings being involved in speciation (Gaither et al. 2015).

Two concepts have been developed to explain how species-environment interactions have caused divergence – adaptive speciation and ecological speciation. At first, these two concepts seem very similar, but there are two key differences between them. The first is that sexual selection can be wholly responsible for disruptive selection and thus divergence in adaptive speciation but not in ecological speciation (Nosil 2012). The second is that ecological interactions have to be frequency-dependent for reproductive isolation to occur in adaptive speciation, and thus adaptive speciation cannot take place in allopatry, i.e. adaptive speciation takes place with a level of gene flow, whereas ecological speciation can occur in the presence or absence of gene flow (Dieckmann et al. 2004). Nonetheless, frequency-dependent selection can also be involved at least at some stage in ecological speciation (Nosil 2012). This means that adaptive speciation becomes increasingly unlikely with geographic separation, to the point where adaptive speciation is not possible in the absence of frequency-dependent selection (i.e. allopatry) (Dieckmann et al. 2004). An exception to this is in the very unlikely situation where there is a highly mobile second species interacting with both populations sufficient enough to drive adaptive speciation; though this is only a theoretical possibility and unlikely to be encountered in nature (Dieckmann et al. 2004).

Ecological speciation can involve divergence in two main aspects of the niche: (i) along environmental gradients and between macrohabitats (the β-niche), or (ii) by partitioning resources such as food and microhabitats (the α-niche) (Ackerly et al. 2006). In the ‘habitat-first’ model, the initial divergence during speciation is between β-niches, with any α-niche divergence coming later (Ackerly et al. 2006). One interpretation of this is developed in models of parapatric speciation along environmental gradients, caused by divergent natural selection and assortative mating (Doebeli and Dieckmann 2003). Habitat-first speciation has been inferred from sister-species comparisons in clades such as Lake Victoria cichlids (Seehausen et al. 2008). An alternative to habitat-first speciation is ‘within-habitat’ speciation, which occurs when disruptive selection favours divergence in the α-niche. Theoretical models show that sympatric speciation can be driven by disruptive selection on resource-use traits combined with assortative mating based on ecological traits (Dieckmann and Doebeli 1999). Compelling evidence for ‘within-habitat’ speciation is more scarce, but α-niche divergence can be found in young sympatric sister species (e.g. Barluengsa et al. 2006).

This thesis examines evidence for ecological speciation in New Zealand triplefins. First, empirical examples of ecological speciation and adaptive radiations will be discussed, to add a framework
for this study on ecological speciation in triplefins. This is followed by background information on relevant ecology in New Zealand triplefins, and what is currently known about processes of speciation in this clade. This thesis is based around measuring variables of habitat performance in triplefins. Habitat performance is referred to throughout this thesis in terms of trait utility, i.e. as some measurable phenotypic trait of a fish that is associated with the environment. The penultimate section of this chapter describes the study environment, thus providing context for the habitat variables under test.

1.2 Empirical evidence of ecological speciation and adaptive radiations

Developing theoretical models requires well documented empirical examples. When the evolution of ecological or phenotypic diversity generates a rapidly multiplying lineage, this is broadly termed an adaptive radiation (Schluter 2000). Schluter (2000) lists four criteria that define an adaptive radiation: common ancestry (monophyly), rapid speciation, phenotype-environment correlation, and trait utility. A phenotype-environment correlation is when a significant association exists between environments and the morphological and physiological traits used to exploit those environments. Trait utility refers to the performance or fitness advantages of trait values in their corresponding environments. Adaptive radiations that fit these criteria are evident across many taxa, and include examples in birds (Smith et al. 1997, Palmer and Kronförst 2015), reptiles (Losos and Ricklefs 2009), plants (Robichaux et al. 1990, Savolainen et al. 2006, Givnish et al. 2009) and insects (Filchak et al. 2000, Berlocher and Feder 2002, Gillespie 2004). These species groups are greatly differentiated in aspects of morphology and microhabitat, and in particular, characters such as beak shape, leaf design, and limb size. While terrestrial examples date back to Darwin (Darwin 1859), a growing number of studies have examined ecological speciation and adaptive radiations in fishes.

Fishes are the most speciose group of vertebrates, with over 30,000 extant species (Eschmeyer et al. 2016). The majority of empirical examples of adaptive radiations in fishes involve freshwater lacustrine environments. These include the most well-known of all fish radiations, the cichlids of the east African rift lakes (Kosswig 1947, Fryer and Iles 1972, Genner and Turner 2005, Seehausen 2006), and more recently, the cichlids from South and Central America (Wilson et al. 2000, Schliewen et al. 2001, Barluenga et al. 2006, Dittmann et al. 2012). These cichlids have undergone explosive adaptive radiations, with hundreds of putative species. Each of the Great Lakes in eastern Africa are characterised by these extensive radiations, with endemic species groups sometimes referred to as species flocks or species complexes (Seehausen 2006). The most common feature of these species flocks is the extensive secondary divergence in trophic
morphology, with numerous adaptations for food acquisition (Kocher 2004). Divergence in jaw and mouth morphology has allowed an extensive partitioning of trophic niches and led to herbivorous, omnivorous, detrivorous, planktivorous and carnivorous species (Danley and Kocher 2001, Albertson et al. 2003, Albertson 2008). Trophic resource partitioning is a common trend in many adaptive speciation events in fishes, including other freshwater examples such as Lake Tana barbs (Sibbing et al. 1998, Sibbing and Nagelkerke 2001), lake whitefish (Lu and Bernatchez 1999), Arctic char (Jonsson and Jonsson 2001), electric mormyrids (Feulner et al. 2007), sailfin silversides (Pfaender et al. 2011), pupfishes (technically saline lakes) (Martin and Wainwright 2013), lake trout (Chavarie et al. 2015), and the widely studied stickleback species complex (McKinnon and Rundle 2002). Whilst there are many examples of freshwater adaptive radiations, marine examples are few.

Marine organisms present a strong challenge to models of speciation that require geographic isolation (i.e. allopatry), as many species have large effective population sizes, vast geographic ranges, and long pelagic dispersal phases that have the potential to connect distant habitats (Bowen et al. 2013). Furthermore, there is a paucity of barriers to gene flow in the ocean, and recognised dispersal barriers are often porous and can be sporadically overcome when favourable conditions arise (Briggs and Bowen 2013, Gaither and Rocha 2013). These observations, together with the high biodiversity on coral reefs (Allen 2007), suggest that natural selection must also play an important role in diversification in the oceans.

Empirical evidence of ecological speciation in the sea is increasing, and examples can be seen in invertebrates (Johannesson 2009, Bird et al. 2011, Prada and Hellberg 2013). Of particular interest to this thesis are examples of ecological speciation in marine fishes, and include tropical pufferfish (Alfaro et al. 2007), greenlings (*Hexagramma* spp.) of the north and northeast Pacific (Crow et al. 2010), pipefishes (*Stigmatopora* spp.) in Australia and New Zealand (Dawson 2012), sea horses (*Hippocampus* spp.) (Jones et al. 2003) and coral dwelling gobies (*Gobiodon* spp.) in Australia (Munday et al. 2004), wrasses in the Caribbean (Rocha et al. 2005), and manta rays (*Manta* spp.) in the Indian Ocean (Kashiwagi et al. 2012). However, diverse adaptive radiations (sensu Schluter 2000) in marine fishes are far fewer, and include the Antarctic notothenioids (Eastman and McCune 2000, Rutschmann et al. 2011), the rockfishes (*Sebastes* spp.) of the northwest Pacific (Johns and Avise 1998, Ingram 2011), and triplefins in New Zealand (Wellenreuther et al. 2007, 2008, 2009). In these examples of sympatric or parapatric speciation, many of the speciation events have diverged via selection on a number of traits, and some include sexual selection and/or trophic resource partitioning as well as habitat specialisation. The New Zealand triplefin assemblage has been suggested to be an example of ecological speciation (Wellenreuther et al. 2007), and is the focus of this thesis.
1.3 Triplefin fishes

Triplefins (F. Tripterygiidae) are a family of blennioid fishes with approximately 168 species in 29 genera worldwide (Stewart and Clements 2015). They reach their highest diversity and endemism in New Zealand, with 27 known species in 12 genera (Stewart and Clements 2015). Apart from four species (*Enneapterygius kermadecensis*, *Apopterygion oculus*, *Notoclinus* spp.), triplefins in New Zealand form a monophyletic group, and are almost all sympatrically distributed (Wellenreuther et al. 2007) with no obvious latitudinal trends in abundance (Wellenreuther et al. 2008b). Together with the apparent rapid speciation of this group, as indicated by the relatively short branch lengths between clades, in particular in the genus *Forsterygion* (Hickey and Clements 2005), this suggests an adaptive radiation (sensu Schluter 2000).

Further evidence for an adaptive radiation in New Zealand triplefins is provided by phenotype-environment correlations, resulting from diversification in physiology (Brix et al. 1999, Hickey and Clements 2003, Hilton et al. 2008, 2010), sensory structures (Wellenreuther et al. 2010) and habitat (Syms 1995, Feary and Clements 2006, Wellenreuther et al. 2007, 2009, Wellenreuther and Clements 2008), with exposure and depth being found as the most significant ecological axes of habitat differentiation (Wellenreuther et al. 2007). Conversely, research has shown there to be little specialisation in diet, jaw morphology (Vasques 1999, Feary et al. 2009) and male breeding colouration (Wellenreuther and Clements 2007) in this assemblage, meaning diversification via trophic partitioning or sexual selection on colour is unlikely. Furthermore, gene flow in most species is relatively high (Hickey et al. 2009), and in the Hauraki Gulf *Forsterygion lapillum* has been shown to have almost complete panmixia (Rabone et al. 2015). Together, this suggests that factors other than geographical barriers are responsible for speciation in this clade of fishes, and that diversification driven by selection (i.e. ecological speciation) has been important in the evolution of this group.

After settlement, triplefins establish a small territory (1-4 m²) which they defend year round, and in which all feeding and breeding occurs (Thompson 1986). New Zealand species exhibit high site fidelity, and have been shown to home if displaced from their territory (Thompson 1983, Subedar 2009, Shima et al. 2012). Reproduction takes place during the Austral winter-spring seasons, peaking around August in most species (Wellenreuther and Clements 2007). A polygynandrous mating strategy is adopted, where different clutches are simultaneously present in a nest prepared by the male on hard substrata, and involves intense displaying by the male to lead the female to the nest (Thompson 1986). Females lay eggs in one dense layer, by attaching the eggs to the surface (Anderson 1973, Thompson 1986). Paternal care for the eggs includes defence from inter-
and intraspecific predators, and fanning the eggs to oxygenate them and prevent silt from accumulating (Thompson 1979). During the breeding period, the male remains in close proximity to the nest, with only occasional short-distance feeding forays (Handford 1979). Eggs then hatch and undergo a relatively long pelagic larval duration (PLD) of approximately 2-3 months (Kohn and Clements 2011). The lack of correlation between PLD and gene flow suggests that dispersal is limited by other factors such as larval behaviour and the availability of settlement habitat (Kohn and Clements 2011).

1.4 Study environment

This study was conducted on subtidal reefs in the outer Hauraki Gulf in northeastern New Zealand (fig. 2.1), a region that comprises an area of approximately 4000 km². Reefs provide a structurally complex habitat along the margin of the shore and in isolated patches throughout the gulf (i.e. islands). The geological formation of the reefs vary, and consist of ancient baserocks (greywacke) through large parts of the gulf (e.g. Leigh coast and Tawharanui peninsula), to small pockets of reef caused by volcanic flows (e.g. Ti Point) (Edbrooke 2001). Influences from sedimentation and other terrestrial run off and proximity to rivers and estuaries can cover the baserock with sand and mud (Edbrooke 2001). A wave exposure gradient is formed by the geography of the gulf (i.e. shape of the coast) and the barrier islands in combination with the fetch from the Pacific Ocean. Subsequently, wave energy is a major source of structuring biotic and abiotic variation across the Hauraki Gulf.

Wave exposure has been shown to have a significant effect on both the abiotic and biotic nature of reef systems (Geister 1977, Kilar and McLachlan 1989, Fulton et al. 2013). The mixing of water bodies and consequently the transportation of nutrients is dependent on water movement, leading to variation in the trophic makeup on reefs between areas of differing wave exposure (Madsen et al. 2001). The water motion produced by the wave energy can also prevent the build-up of silt and sediment. Sediment loading has been suggested to influence fish assemblages in the Caribbean (Dennis and Bright 1988) and the composition of fish at sites in the Hauraki Gulf (Kingsford 1989). Furthermore, the softer rock substrata found in more sheltered sites across the Hauraki Gulf provide less topographical complexity than the reefs composed of hard rock (Shears and Babcock 2007). Wave energy-induced gradients are therefore an important determinant of both the community structure of mobile taxa in marine environments (Williams 1982, Denny 1988), and the phenotypic-environment interactions of individual species (e.g. Fulton et al. 2013). As wave exposure has been shown to be a principal component of habitat diversification in the New Zealand triplefin assemblage (Wellenreuther et al. 2007), and changes markedly across the
geographical range of most species, this abiotic factor was the main environmental variable examined in this thesis.

1.5 Aims of the research

It is well established that New Zealand triplefin species have diverged in habitat use, and habitat is a major component in the adaptive radiation in this clade of fishes (Syms 1995, Feary and Clements 2006, Wellenreuther et al. 2007, 2008a). However, the mechanisms that have led to this high diversity remain largely unknown – although diversification in habitat selection (Wellenreuther and Clements 2008) and mate selection (Wellenreuther et al. 2008a) appear to be two of the potential mechanisms responsible. Furthermore, the relationships between habitat variables and fitness in individual species has not been examined previously. This predominantly sympatric group provides an ideal situation to study the mechanisms that drive habitat diversification due to their abundance, short generation times, and the small territory in which breeding and feeding occurs (Wellenreuther and Clements 2007). Morphology, abundance and growth are all important characters in the context of ecological speciation (Hatfield and Schluter 1999, McKinnon and Rundle 2002). Given the small territory and extreme philopatry in this assemblage, the environmental variation that individuals are exposed to should be relatively consistent following settlement, permitting testable measures of habitat performance.

The work proposed here aims to test several proximate indicators of fitness in different locations and habitats, with a view to determine whether processes that are hypothesized to have taken place over evolutionary timescales (e.g. ecological speciation) are evident over ecological timescales. Of particular interest is how variation in the environment can lead to fitness differences that have evolutionary consequences. More specifically, this was approached by examining habitat performance from two different contexts. First, by looking at direct and indirect (i.e. proximate) fitness measures – for example, do some individuals of a species exhibit higher growth or larger nest sizes in some habitats relative to others. And secondly based on an “engineer’s criterion of good design”, e.g. does a certain morphology suit a particular habitat based on what is known from the literature? In doing so, these two different contexts complement each other to give a more complete understanding of the mechanisms of habitat performance. Therefore, this thesis addresses two of the four criteria of ecological speciation (sensu Schluter 2000), namely phenotype-environment correlations and trait utility. Together, this would lead to a better understanding of the mechanisms responsible for habitat diversification in triplefins, and also provide insights into ecological speciation in general, as few field studies have examined this process in marine fishes. This thesis contains four data chapters, achieved by a combination of
field, laboratory and experimental work. We know that New Zealand triplefins have diverged in habitat use, but this thesis tests whether these four triplefin species differ in habitat performance.

The main hypothesis for chapter 2 is that there are intraspecific variations in abundance and reproductive success among habitats, and that this correlates with individual fitness. This chapter describes the patterns and variation in settlement and abundance of four species of triplefins (*Forsterygion lapillum*, *F. varium*, *Ruanoho whero*, and *Notoclinops segmentatus*). This was examined among sites varying in wave exposure, and within sites at the level of microhabitat, and the interaction between the two. These species were chosen as they are the four most common species in the study region, and are found in all habitats and locations (Feary and Clements 2006, Wellenreuther 2007, Wellenreuther and Clements 2007). Furthermore, these species represent a coverage of the phylogenetic diversity of New Zealand triplefins (three different genera) (Hickey and Clements 2005), a congeneric comparison (*F. varium* and *F. lapillum*), and a broad range of sizes, from one of the smallest species (*N. segmentatus*) to one of the largest (*F. varium*).

However, the real interest in these habitat patterns is the intraspecific differences, and simply by recording four different species’ size-class abundance, we increase our information available to answer these hypotheses. In addition, male reproductive fitness was estimated across the same spatial scales in the two *Forsterygion* species, by measuring clutch size, and examined in the context of male size. These two species are the focus for the remainder of the thesis, as they are the species with the most existing previous knowledge, based upon previous literature, in addition to their high abundance in these study locations.

The main hypothesis of chapter 3 is that variation in growth and age will vary with habitat and serve as proxies for post-settlement performance. This chapter describes age and growth of two species of triplefin, *F. lapillum* and *F. varium* across the wave exposure gradient in the Hauraki Gulf.

Chapter 4 tests the relationship between wave exposure and morphology in *F. lapillum* populations throughout the Hauraki Gulf, i.e. over spatial scales that we know lack genetic structure in this species (Rabone et al. 2015). Phenotypic variation in body shape and pectoral fin morphology was examined using both traditional linear morphological measurements and a geometric morphometric approach. The hypothesis is that morphology will be differ in habitats varying in wave exposure to best deal with the oscillatory flows in each location.

Chapter 5 follows on from the previous chapter and uses reciprocal common garden experiments to test phenotype-environment interactions in *F. lapillum*. The main hypothesis under test is that the mechanism for the environmentally-induced morphological variation seen in the wild populations (chapter 4) is phenotypic plasticity. This hypothesis was tested by collecting newly-
settled fish and rearing them in simulated water movement environments in aquaria until adulthood, and then measuring morphology as per the previous chapter.
Chapter 2

Relationships between habitat, abundance and fitness in New Zealand triplefin fishes

2.1 Introduction

Habitat variation is widely viewed as a precursor to biological diversity (Tews et al. 2004). This is likely to be particularly important for reef environments, which are characterized by high spatial heterogeneity in composition and complexity (Williams 1991). Diversification of habitat use has been extensively studied in coral reef fish assemblages, documenting distinct patterns of habitat utilisation in closely related species, such as gobies (Munday et al. 1997, 2004), clownfishes (Litsios et al. 2012), and wrasses (Rocha et al. 2005). However, relative to tropical fishes, habitat specialisation in temperate reef fishes is poorly understood (Rocha and Bowen 2008). Measuring variables of habitat performance, such as abundance and reproductive output, are viable ways to test the proximate mechanisms ultimately responsible for habitat diversification. Are the processes driving inter-specific diversification over evolutionary timescales evident from intra-specific variation in habitat performance over ecological (i.e. contemporary) timescales?

Physical barriers in marine environments are few, promoting extensive larval dispersal. However, there is increasing evidence that individuals can differ in dispersal capacity (Cowen and Sponaugle 2009, Bowen et al. 2013) and habitat preference (Montgomery et al. 2001 and references therein). If the distribution of genotypes is non-random due to these active processes, then fine-scale population differentiation can arise rapidly and be facilitated rather than impeded by gene flow (Bolnick and Otto 2013). Subsequently, if this involves matching phenotypes with habitats conferring higher fitness, microgeographic adaptation can result (Richardson et al. 2014). For this to occur the habitats must be within the individual’s normal dispersal range, as they must sample alternate habitats to choose the one conferring the highest fitness (Richardson et al. 2014). Active habitat selection therefore offers some of the greatest potential for promoting microgeographic adaptation (Richardson et al. 2014).

There is mounting evidence that active habitat selection plays an important role in triplefin distributions in New Zealand. Previous research on Forsterygion varium showed movement by adults between habitats is extremely rare (Connell and Jones 1991). Triplefins also exhibit active pre-settlement processes, as the larvae are known to have strong swimming abilities (Kingsford and Choat 1989), are actively attracted to environmental cues (Tolimieri et al. 2000), and exhibit
species-specific distributions both in relation to distance from shore and depth in the water column (Hickford and Schiel 2003). Furthermore, *F. lapillum* larvae are known to exhibit active habitat selection under both field and experimental conditions (McDermott and Shima 2006). Wellenreuther and Clements (2008) demonstrated significant inter-specific differences in habitat preference by new recruits. Furthermore, by showing triplefin recruits had the same habitat distributions as conspecific adults, and in light of the high philopatry in these species, also suggests habitat preferences at settlement (Syms 1995, Wellenreuther and Clements 2008). Conversely, Connell and Jones (1991) found that newly settled *F. varium* larvae were found in a much wider range of habitats than adults, and concluded that higher post-settlement mortality in some habitat patches led to the narrower habitat distributions in adults. However, strong selection pressure is likely to favour settling into habitats that do not experience heavy mortality rates. Therefore, it can be concluded that the predominant species-specific habitat associations in triplefins are established at the time of settlement.

It is well established that New Zealand triplefin species have diverged in habitat use (Syms 1995, Feary and Clements 2006, Wellenreuther et al. 2007, 2008a). This sympatric group provides an ideal situation to study the mechanisms that drive habitat diversification, as after settlement all species occupy small territories where spawning also occurs (Wellenreuther and Clements 2007). Furthermore, there is little movement of individuals between habitats (Connell and Jones 1991), and extreme philopatry is illustrated by homing capabilities evident over several hundred metres (Thompson 1983, Subedar 2009, Shima et al. 2012). Wave exposure and depth are the two main environmental factors that define habitat use among New Zealand triplefins (Syms 1995, Wellenreuther et al. 2007), with several distinct groups categorized by different depth strata across a wave exposure gradient (Feary and Clements 2006). At finer scales, species-specific distributions are evident based on microhabitats and macroalgal community structure (Syms 1995, Feary and Clements 2006, Wellenreuther et al. 2007). The partitioning of triplefins into different microhabitats, as in other closely related fishes (e.g. Ormond et al. 1996, Munday 2004), provides fitness advantages by providing greater access to resources (Clarke 1992, Edgar and Shaw 1995), mediating the effects of competition and predation through varying degrees of shelter (Behrens 1987, Hixon and Beets 1989, Steele 1999), and/or providing nesting substrata (Koppel 1988).

As spawning success is an inherent indicator of fitness, relationships between male fitness and habitat can provide a direct measure of habitat performance. Furthermore, the linkage between habitat and mate choice has been identified as a powerful mechanism of speciation in parapatry or sympatry, and has been referred to as a ‘magic trait’, one that is subject to disruptive selection and simultaneously controls non-random mating (Gavrilets 2004). Male body size has been shown to positively correlate with spawning success in *F. varium* (Thompson 1986). Selection generally
favours female mate choice of larger males, as large males generally indicate better access to
territory and resources, good health, and higher stores of energy and sperm for spawning (Howard
et al. 1998). This positive correlation is evident in several other small benthic-nesting fishes with
very similar reproductive strategies to triplefins (Cote and Hunte 1989, Hamilton et al. 1997,
Oliveira et al. 2000). Nesting quality (i.e. substrate, size, aspect) is an important factor in female
mate choice in substrate-spawning fishes (Takahashi and Kohda 2002), and can even be
preferential to male size (Ludlow et al. 2001). Furthermore, the females of some species choose to
mate based on the presence of eggs or females already in the nest (Marconato and Bisazza 1986,
Kraak and Videler 1991, Ruchon et al. 1999), including in triplefins (Petersen 1989, Geertjes and
Videler 2002). Variation in male spawning success across spatial scales should thus be a direct
indicator of habitat performance operating over an ecological timescale, and provide further
insights into the mechanisms that drive habitat diversification in the speciose triplefin fish
assemblage in New Zealand.

Habitat associations are complex to quantify, and mechanistically, processes operate at a range of
scales (Syms 1995). At fine spatial scales, fish species distributions can be strongly associated
2003), algal cover (Carr 1994, Levin and Hay 1996, La Mesa et al. 2006), and slope (Hofrichter
and Patzner 2000, La Mesa and Vacchi 2005). Across broader spatial scales, water temperature
(i.e. latitudinal gradients) (Meekan and Choat 1997, Feary et al. 2014), reef geology (Syms 1995)
and wave exposure (Thorman 1986, Syms 1995, La Mesa et al. 2004) are environmental factors
associated with shaping fish assemblages. Therefore, strength of habitat associations are only
interpretable in the context of the spatial scale at which it is measured. The same is true for the
temporal scale. Temporal and seasonal variation in fish habitat studies have received less attention
than spatial variation, and for fast-growing short-lived fishes, the temporal scale can be vitally
important. In this thesis, the habitat associations of four species of triplefins (\textit{F. lapillum}, \textit{F.
varium}, \textit{Ruanoho whero}, and \textit{Notoclinops segmentatus}) are assessed across two different spatial
scales, over a reasonably high temporal resolution, and repeated for three successive years. These
species were chosen as they are the four most common subtidal triplefin species in northern New
Zealand (Wellenreuther et al. 2007), and are found throughout the majority of available habitats in
the Hauraki Gulf (Feary and Clements 2006). Furthermore, these four species display
considerable overlap in depth distribution (Feary and Clements 2006).

The main hypothesis for this chapter is that there are intraspecific differences in abundance along
an environmental gradient (wave exposure), and also among microhabitats, and that this correlates
with individual fitness. The chapter also aims to document the seasonal fluctuations in abundance
of each species and relate this to temporal patterns of settlement and mortality. In what follows,
reproductive output is used as a direct indicator of fitness, and mate choice and habitat are collectively considered to be as a ‘magic trait’ (Gavrilets 2004). It is hypothesised here that there will be differentiation in the relationship between male size and nest size among habitats and locations.

2.2 Methods

2.2.1 Sampling locations

Sampling was undertaken within the Hauraki Gulf in northeastern New Zealand (fig. 2.1), a region that comprises an area of approximately 4000 km², and covers a wide range of exposures, depths and habitats, including both coastal and offshore locations. Sampling sites spanned a wave exposure gradient, based on fetch and wind/wave energy (see below and table 2.1 for details of wave exposure calculations), with replicate sites for sheltered, semi-exposed, exposed to offshore (i.e. very-exposed) wave exposure groupings. Survey sites included Ti Point (Ti), Jones Bay (Jo), Mathesons Bay (Ma), Nordic (No), Waterfall Reef (WR), Takatu Point (Ta), Mokohinau Islands (Mo), and Great Barrier Island (GB).

2.2.2 Wave exposure analysis

A combination of wave power data, fetch, and wind-derived exposure were used to verify the wave exposure groupings used in the present study. Empirical wave energy data derived from MetOcean Wavewatch (NOAA 2016) was used to calculate wave exposure. Wave power was chosen as the variable of interest, as it incorporates wave height and period. Spatial resolution from the grid cells (5 x 5 km) of these data was not high enough to permit delineation between closely situated sites, such as along the Leigh coast (WR, No, Ma, Ti, Ta, Jo). However, it did permit accurate wave power comparisons between broader locations, such as the Leigh coast and the offshore islands (see appendix for graphic representation).

More precise levels of wave exposure at each site were approximated using an index of potential fetch, which is defined as the area of the sea surface over which waves are generated by wind with a constant direction and speed (Denny 2005). The exposure of each site was calculated as the total sum of the fetch based on defined settings, using the program ‘Fetch Effect Analysis’ (Villouta 2000). For each site location, each 10º on a compass rose a vector was projected a distance of 300 km, or until land is encountered, and all distances summed. The use of fetch as a proxy for exposure does not take into account differences in wave spectra among areas (Denny 1988),
however, it does permit tentative conclusions to be made (Thomas 1986). Furthermore, in north-eastern New Zealand it is suitable, as the longest fetch and largest swells are from the east/northeast (Denny 2005).

To further verify the wave exposure groupings, wind-derived exposure calculations were performed (e.g. Burrows et al. 2008). Historical wind data was attained for a 10 year period (2005-2015) at a three hourly resolution for maximum wind speed and direction (NIWA 2016). These were recorded from wind stations closest to the representative sites (outer gulf coast=Leigh; offshore islands=Mokohinau Islands). Within each data set, maximum speed was averaged for each $10^\circ$ vector, and multiplied by the corresponding vector for fetch calculations for each site. Subsequently, all 36 vector calculations were summed to get a site total (see table 2.1).

Figure 2.1. Map of sample sites in the outer Hauraki Gulf. Inset 1 shows sites on the coastal mainland: sheltered (Ti, Jo), semi-exposed (No, Ma), exposed (WR, Ta). Mo and GB are offshore islands (very exposed). Inset 2 shows Hauraki Gulf location in the North Island of New Zealand.
Table 2.1. Fetch calculations and coordinates for each sample site.

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<th>Location</th>
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<th>Latitude</th>
<th>Longitude</th>
<th>Sum of Fetch (km)</th>
<th>Max Wind*Fetch</th>
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<td>Ti Point wharf (Ti)</td>
<td>Sheltered</td>
<td>36°19’03.80”S</td>
<td>174°47’01.97”E</td>
<td>38</td>
<td>916</td>
<td></td>
</tr>
<tr>
<td>Jones Bay (Jo)</td>
<td>Sheltered</td>
<td>36°22’49.59”S</td>
<td>174°47’01.97”E</td>
<td>415</td>
<td>11545</td>
<td></td>
</tr>
<tr>
<td>Mathesons Bay (Ma)</td>
<td>Semi-exposed</td>
<td>36°18’13.87”S</td>
<td>174°47’01.97”E</td>
<td>1110</td>
<td>32266</td>
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<tr>
<td>Nordic (No)</td>
<td>Semi-exposed</td>
<td>36°17’35.56”S</td>
<td>174°48’38.58”E</td>
<td>925</td>
<td>25890</td>
<td></td>
</tr>
<tr>
<td>Waterfall Reef (WR)</td>
<td>Exposed</td>
<td>36°16’04.86”S</td>
<td>175°48’05.75”E</td>
<td>2282</td>
<td>67163</td>
<td></td>
</tr>
<tr>
<td>Takatu Pt. (Ta)</td>
<td>Exposed</td>
<td>36°22’02.95”S</td>
<td>174°52’07.61”E</td>
<td>2227</td>
<td>62376</td>
<td></td>
</tr>
<tr>
<td>Great Barrier Isld. (GB)</td>
<td>Offshore</td>
<td>36°03’11.89”S</td>
<td>175°24’10.98”E</td>
<td>4965</td>
<td>186925</td>
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</tr>
<tr>
<td>Mokohinau (Mo)</td>
<td>Offshore</td>
<td>35°54’30.48”S</td>
<td>175°06’25.93”E</td>
<td>4674</td>
<td>174821</td>
<td></td>
</tr>
</tbody>
</table>

2.2.3 Underwater visual censuses

Underwater visual censuses (UVCs) were performed on SCUBA bi-monthly for three years, from October 2012 through August 2015. The four most commonly encountered triplefin species were recorded (Forsterygion lapillum, F. varium, Notoclinops segementatus, Ruanoho whero).

Sampling was undertaken during the first week of every other month (only rarely was it moved to the following week due to weather restrictions), to keep spatial and temporal comparisons as consistent as possible. This seasonal work was focused mainly on the coastal exposure gradient (sheltered, semi-exposed and exposed). However, three research trips to offshore sites were undertaken in 2014 to get a representative snapshot of offshore habitat distributions, where both size-class abundance (UVCs) and triplefins nests were censused (see below).

Within each site, up to ten transects were performed per dive (i.e. per site) in representative microhabitats defined a priori. Microhabitats were decided based on previous subtidal ecological studies in the Hauraki Gulf (Ayling 1978, Syms 1995, Shears et al. 2004, Feary and Clements 2006). Microhabitats included Ecklonia forest (EF), mixed algae (MA), shallow turfing algae/rock flats (STA), and cobbles/open rock (COB). These categories encompassed all areas of hard substrata (i.e. available reef habitat) at each site. To eliminate depth as a factor, all transects were performed at a constant depth (between 3-7 m). Additionally, a deeper EF microhabitat (7-12 m) was added for comparisons. The four study species overlap considerably in depth distribution (Feary and Clements 2006), therefore the majority (~80%) of transects were performed in this well documented 3-7m depth zone. By keeping depth constant (except EF deep), this permitted comparisons across microhabitats and the wave exposure gradient. COB microhabitat was rare at sheltered sites. Underwater visual censuses consisted of 5 m line transect surveys, where a weighted line was placed randomly in the pre-assigned microhabitat zones, and the four species of interest were recorded 1 m either side of the line, giving a 10 m² transect. The size of each fish
was assessed visually, by placing a ruler close to the stationary fish, and total length estimated to the nearest 5 mm. As triplefins exhibit high site fidelity (Thompson 1983, Subedar 2009, Shima et al. 2012), they remain in the defined microhabitat zones post-settlement. Furthermore, transects were performed away from boundaries of microhabitat zones so the home range of recorded triplefins was completely encompassed within designated microhabitats. All together 13,401 triplefins were recorded over 863 transects.

Spatial differences in abundance were examined separately for each species using a general linear model, where wave exposure, microhabitat, year, site and transect were all fixed factors. Density (fish per m²) was the dependent factor. Month was not tested in the GLM, as density clearly shifts seasonally, and is examined further with respect to temporal differences. Where significant interactions were found, Bonferroni’s pairwise comparisons were made. To test for any spatial differences in settlement of triplefins, each species was categorised into two main size classes, young (0+ cohort) and older (1++ cohorts), where 0+ cohorts were tracked throughout the first year of life (i.e. 0-1 year), and 1++ all other fish (i.e. older than 1 year, also referred to hereafter as adults). Size ranges for these two categories were determined by length frequency distributions for each species, and with information from annual counts and growth work (from chapter 3). Spatial differences in density were tested using one way ANOVA on ranks, with post-hoc tests performed using Dunn’s method (significance level $\alpha = 0.05$). The data was unsuitable for parametric testing, due to the high zero counts and the very high positive skewness in the data distributions. Several attempts at data transformation, including log $(x+c)$, were unsuccessful in normalising the data, therefore a rank based analysis was deemed the most appropriate. Consequently, non-parametric two-way ANOVA comparing different age classes, time and habitat was not possible. However, the main interest was spatial differences, which were successfully tested.

For examination of the spatio-temporal patterns (seasonal), density was calculated as the sum of triplefins divided by the area across all applicable transects. This was due to replication per sampling period per location or habitat not being high enough to permit rigorous statistical analyses. Spatio-temporal density results are listed in table 2.2, and are presented as proportions of the habitat with the highest density each sampling period out of all sample periods (i.e. $x$ out of 18 samples). Peak spawning periods (shaded bars on figures 2.9-2.12) were based on personal observations during UVCs, and previous research on peak spawning (Wellenreuther and Clements 2007) and PLD (Kohn and Clements 2011) for each species.
2.2.4 Nest photographs

New Zealand triplefins are polygynous benthic nest spawners, where females are enticed into the territory of a male where they lay eggs attached by sticky filaments onto a prepared nest surface (Thompson 1979, Clements 2003). The highly philopatric males remain with and vigorously defend the nests from other fishes and invertebrates. Utilising spawning data to test habitat fitness was performed on only two species, *F. lapillum* and *F. varium*. This is because the nests of these species are often laid on the top and side of rocky substratum, or under smaller rocks (i.e. cobbles) that could be overturned (Wellenreuther and Clements 2007), therefore being more accessible to censusing (see details below). Males in spawning condition adopt a spawning colouration, which in the two species of interest is almost jet black (Wellenreuther and Clements 2007). The black colouration and nest guarding behaviour make it easily discernible which nest belongs to which male. It was thus possible to record both nest size (total nest area) and male size (total length) for each nest found.

Underwater photography was used as a non-invasive means of recording nest size and male size. All photographs were recorded *in situ* on SCUBA at an average of 5 m depth (± 3 m), from the eight different sites situated along the wave exposure gradient (fig. 2.1, table 2.1). Nest photographs were recorded during the spawning periods (June-October) of 2013 and 2014. Each photograph was taken with a scale on a plane parallel to the nest surface (figs. 2.2 A, B). Where egg clutches were laid on multiple surfaces, separate photographs were taken for each different plane. Where nests were not on flat planes, these nests were not photographed or excluded from further analyses. Nest photographs were analysed using the image analysis software program ImageJ (version 1.46r). Random subsamples of nests were measured directly for egg density per unit area and for egg size (diameter), and these were consistent with the data from photographs for all locations. Nest area was thus deemed a useful proxy for reproductive output. Male size was measured from the same photographs (e.g. figs. 2.2 A, B). To test for differences in nest-guarding male size across the exposure gradient, one-way ANOVAs were performed, with post-hoc analyses performed using Tukey’s test where significant interactions were present.

Quantile regression estimates were used to test the relationships between fish length, nest area and environmental variables (wave exposure and microhabitat), as it was hypothesised that a limiting effect existed, based on patterns in the data and the discrete manner of sampling triplefin spawning (i.e. our measurements are a snapshot in time, not the complete reproductive output of an individual). Quantile regression (Koenker and Bassett 1978) is a method for estimating functional relationships between variables for all portions of a probability distribution (Cade and Noon 2003), not just the centre or mean, as in more common linear regression models (i.e. least
squares regression). This can provide a more complete view of possible causal relationships between variables in ecological processes, as there may be a weak or no predictive relationship between the mean of the response variable distribution ($Y$) and the measured predictive factor ($X$), but perhaps stronger relationships in other parts of the distribution (Cade and Noon 2003). Furthermore, this method is useful in modelling the edge of data distributions, and is particularly applicable in ecology at the upper boundaries of the data (Cade et al. 1999).

In the present study, we modelled the approximately maximum (e.g. upper quantiles) nest sizes across body sizes. This takes into account the fact that nests may range in size quite widely below the maximum sizes, because the fish had not finished laying when they were censused, or they had finished laying but predators ate some of the eggs before they were photographed (i.e. both limiting and/or unknown factors). Furthermore, Thompson (1986) suggested there may be a threshold size for female mate choice in *F. varium*, and that males below about 80 mm obtained very few spawnings. Quantile regression estimates were tested using the statistical software SAS. Note: for *F. lapillum* we did not analyse the fish length/nest area relationship for microhabitat as >90% of the nests were found in COB microhabitats.
Figure 2.2. Examples of triplefin nests showing method of photographic analysis of male size and nest area for A) *F. varium*, and B) *F. lapillum*. Note: numbers on figure indicate the tracing tool used during clutch size analysis.
2.3 Results

2.3.1 Spatial variation in triplefin density

*Forsterygion lapillum* was the most abundant of the four species over the entire sampling period, with 5585 individuals recorded, compared with *F. varium* (3536), *R. whero* (1943) and *N. segmentatus* (2337). Both the major habitat variables examined – wave exposure and microhabitat – had a significant effect on mean density for each species overall (figs. 2.3, 2.4 table 2.2).

Table 2.2. Summary statistics ($F$ statistics and $P$-values) of general linear models comparing density among several habitat variables and among years, and the interactions among those variables for each species. For more detailed statistics, see tables 2.3-2.6 below.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th><em>F. lapillum</em></th>
<th></th>
<th></th>
<th><em>F. varium</em></th>
<th></th>
<th></th>
<th><em>R. whero</em></th>
<th></th>
<th></th>
<th><em>N. segmentatus</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F$</td>
<td>$P$</td>
<td></td>
<td>$F$</td>
<td>$P$</td>
<td></td>
<td>$F$</td>
<td>$P$</td>
<td></td>
<td>$F$</td>
<td>$P$</td>
</tr>
<tr>
<td>Exposure</td>
<td>20.367</td>
<td><strong>0.000</strong></td>
<td>4.652</td>
<td><strong>0.003</strong></td>
<td>9.098</td>
<td><strong>0.000</strong></td>
<td>23.053</td>
<td><strong>0.000</strong></td>
<td>23.053</td>
<td><strong>0.000</strong></td>
<td></td>
</tr>
<tr>
<td>Microhabitat</td>
<td>20.487</td>
<td><strong>0.000</strong></td>
<td>3.482</td>
<td><strong>0.008</strong></td>
<td>9.587</td>
<td><strong>0.000</strong></td>
<td>18.574</td>
<td><strong>0.000</strong></td>
<td>18.574</td>
<td><strong>0.000</strong></td>
<td></td>
</tr>
<tr>
<td>Year</td>
<td>0.036</td>
<td>0.964</td>
<td>27.475</td>
<td><strong>0.000</strong></td>
<td>15.710</td>
<td><strong>0.000</strong></td>
<td>1.801</td>
<td>0.166</td>
<td>1.801</td>
<td>0.166</td>
<td></td>
</tr>
<tr>
<td>Exposure * Microhabitat</td>
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<td><strong>0.000</strong></td>
<td>2.927</td>
<td><strong>0.001</strong></td>
<td>2.973</td>
<td><strong>0.001</strong></td>
<td>3.331</td>
<td><strong>0.000</strong></td>
<td>3.331</td>
<td><strong>0.000</strong></td>
<td></td>
</tr>
<tr>
<td>Site * Exposure</td>
<td>5.595</td>
<td><strong>0.001</strong></td>
<td>3.930</td>
<td><strong>0.009</strong></td>
<td>32.292</td>
<td><strong>0.000</strong></td>
<td>5.552</td>
<td><strong>0.001</strong></td>
<td>5.552</td>
<td><strong>0.001</strong></td>
<td></td>
</tr>
<tr>
<td>Site * Microhabitat</td>
<td>0.614</td>
<td>0.817</td>
<td>0.665</td>
<td>0.772</td>
<td>1.463</td>
<td>0.141</td>
<td>1.467</td>
<td>0.139</td>
<td>1.467</td>
<td>0.139</td>
<td></td>
</tr>
<tr>
<td>Transect * Exposure</td>
<td>0.164</td>
<td>0.976</td>
<td>1.491</td>
<td>0.191</td>
<td>1.373</td>
<td>0.223</td>
<td>1.092</td>
<td>0.363</td>
<td>1.092</td>
<td>0.363</td>
<td></td>
</tr>
<tr>
<td>Transect * Microhabitat</td>
<td>0.440</td>
<td>0.852</td>
<td>0.421</td>
<td>0.834</td>
<td>1.239</td>
<td>0.284</td>
<td>0.630</td>
<td>0.707</td>
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<tr>
<td>Year * Exposure</td>
<td>3.321</td>
<td><strong>0.006</strong></td>
<td>3.185</td>
<td><strong>0.008</strong></td>
<td>1.754</td>
<td>0.120</td>
<td>9.362</td>
<td><strong>0.000</strong></td>
<td>9.362</td>
<td><strong>0.000</strong></td>
<td></td>
</tr>
<tr>
<td>Year * Microhabitat</td>
<td>0.977</td>
<td>0.453</td>
<td>1.463</td>
<td>0.167</td>
<td>0.778</td>
<td>0.622</td>
<td>4.372</td>
<td><strong>0.000</strong></td>
<td>4.372</td>
<td><strong>0.000</strong></td>
<td></td>
</tr>
</tbody>
</table>

Mean densities of *F. lapillum* ($F_{3, 858} = 20.367, P < 0.001$) and *R. whero* ($F_{3, 856} = 9.10, P < 0.001$) differed among wave exposures, and both species had significantly higher densities at sheltered sites than all other wave exposure sites (see appendix tables 1-4 for details of pairwise comparisons). Mean densities of *F. varium* differed among wave exposure sites ($F_{3, 843} = 4.65, P < 0.01$), and had significantly lower densities at offshore sites. Mean density of *Notoclinops segmentatus* correlated positively with wave exposure ($F_{3, 855} = 25.05, P < 0.001$), with significantly higher densities at offshore sites relative to elsewhere.
The highest density per microhabitat of any species was *F. lapillum* in COB. The other three species all had highest densities in *Ecklonia* forest microhabitats. Mean densities of *F. lapillum* ($F_{4, 858} = 20.487, P < 0.001$) differed among microhabitat, with all microhabitats significantly different from each other in mean density, except EFD and EFS, and MA and STA (fig. 2.3, table 2.3). Mean densities of *F. varium* differed among microhabitats ($F_{4, 843} = 3.482, P < 0.01$), with descending order of mean density from EFD through EFS, MA, STA and COB (fig. 2.4, table 2.3). *Ruanoho whero* showed similar patterns of microhabitat density to *F. varium*, with mean density being different among microhabitats ($F_{4, 856} = 9.587, P < 0.001$), and densities in EFD and EFS significantly higher than MA, STA and COB (fig. 2.4, table 2.5). Mean density of *N. segmentatus* differed among microhabitats ($F_{4, 855} = 18.574, P < 0.001$), with all microhabitats significantly different from each other in mean density, except COB and STA (fig. 2.4, table 2.6).
Figure 2.4. Mean densities (± S.E.M.) of each species of triplefin (all age classes) in different microhabitats, averaged over three years across all sites. N = number of transects.

Table 2.3. Comparison of *Forsterygion lapillum* density among several habitat variables and among years from the general linear model. Significant interactions are in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>229.66</td>
<td>223</td>
<td>1.030</td>
<td>2.802</td>
<td><strong>0.000</strong></td>
</tr>
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<td>Intercept</td>
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<td>34.145</td>
<td>92.893</td>
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<tr>
<td>Exposure</td>
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<td>7.487</td>
<td>20.367</td>
<td><strong>0.000</strong></td>
</tr>
<tr>
<td>Microhabitat</td>
<td>30.122</td>
<td>4</td>
<td>7.530</td>
<td>20.487</td>
<td><strong>0.000</strong></td>
</tr>
<tr>
<td>Year</td>
<td>0.027</td>
<td>2</td>
<td>0.013</td>
<td>0.036</td>
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<tr>
<td>Exposure * Microhabitat</td>
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<tr>
<td>Site * Exposure</td>
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<td>5.595</td>
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<td>Site * Microhabitat</td>
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<tr>
<td>Transect * Microhabitat</td>
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<tr>
<td>Year * Exposure</td>
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<td>Year * Microhabitat</td>
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<td>8</td>
<td>0.359</td>
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<tr>
<td>Error</td>
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<td>Total</td>
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<td>858</td>
<td>0.368</td>
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<tr>
<td>Corrected Total</td>
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R Squared = 0.496
### Table 2.4. Comparison of *Forsterygion varium* density among several habitat variables and among years from the general linear model. Significant interactions are in bold.

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<th>Mean Square</th>
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<td>Corrected Model</td>
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<td>Year</td>
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<tr>
<td>Exposure * Microhabitat</td>
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<td>0.372</td>
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</tr>
<tr>
<td>Site * Exposure</td>
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<tr>
<td>Site * Microhabitat</td>
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<td>11</td>
<td>0.085</td>
<td>0.665</td>
<td>0.772</td>
</tr>
<tr>
<td>Transect * Exposure</td>
<td>0.948</td>
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</tr>
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<td>Transect * Microhabitat</td>
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<td>0.054</td>
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</tr>
<tr>
<td>Year * Exposure</td>
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<td>0.405</td>
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</tr>
<tr>
<td>Year * Microhabitat</td>
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<td>8</td>
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<td>0.167</td>
</tr>
<tr>
<td>Error</td>
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<tr>
<td>Total</td>
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<tr>
<td>Corrected Total</td>
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R Squared = 0.399

### Table 2.5. Comparison of *Ruanoho whero* density among several habitat variables and among years from the general linear model. Significant interactions are in bold.

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<th>Mean Square</th>
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</tr>
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<td>7.001</td>
<td>143.177</td>
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</tr>
<tr>
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</tr>
<tr>
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<tr>
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<td>1.536</td>
<td>2</td>
<td>0.768</td>
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</tr>
<tr>
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<td>11</td>
<td>0.145</td>
<td>2.973</td>
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</tr>
<tr>
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<td>1.579</td>
<td>32.292</td>
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</tr>
<tr>
<td>Site * Microhabitat</td>
<td>0.787</td>
<td>11</td>
<td>0.072</td>
<td>1.463</td>
<td>0.141</td>
</tr>
<tr>
<td>Transect * Exposure</td>
<td>0.403</td>
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<td>0.067</td>
<td>1.373</td>
<td>0.223</td>
</tr>
<tr>
<td>Transect * Microhabitat</td>
<td>0.364</td>
<td>6</td>
<td>0.061</td>
<td>1.239</td>
<td>0.284</td>
</tr>
<tr>
<td>Year * Exposure</td>
<td>0.429</td>
<td>5</td>
<td>0.086</td>
<td>1.754</td>
<td>0.120</td>
</tr>
<tr>
<td>Year * Microhabitat</td>
<td>0.304</td>
<td>8</td>
<td>0.038</td>
<td>0.778</td>
<td>0.622</td>
</tr>
<tr>
<td>Error</td>
<td>31.001</td>
<td>634</td>
<td>0.049</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>110.92</td>
<td>856</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>67.585</td>
<td>855</td>
<td></td>
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</tr>
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</table>

R Squared = 0.541
Table 2.6. Comparison of *Notoclinops segmentatus* density among several habitat variables and among years from the general linear model. Significant interactions are in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P-value</th>
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</thead>
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<td>220</td>
<td>0.251</td>
<td>4.536</td>
<td>0.000</td>
</tr>
<tr>
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<td>11.869</td>
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<td>Exposure</td>
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<td>3</td>
<td>1.275</td>
<td>23.053</td>
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</tr>
<tr>
<td>Microhabitat</td>
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<td>1.027</td>
<td>18.574</td>
<td>0.000</td>
</tr>
<tr>
<td>Year</td>
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<td>2</td>
<td>0.100</td>
<td>1.801</td>
<td>0.166</td>
</tr>
<tr>
<td>Exposure * Microhabitat</td>
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<td>11</td>
<td>0.184</td>
<td>3.331</td>
<td>0.000</td>
</tr>
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<td>Site * Exposure</td>
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<td>3</td>
<td>0.307</td>
<td>5.552</td>
<td>0.001</td>
</tr>
<tr>
<td>Site * Microhabitat</td>
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<td>11</td>
<td>0.081</td>
<td>1.467</td>
<td>0.139</td>
</tr>
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<td>0.060</td>
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</tr>
<tr>
<td>Transect * Microhabitat</td>
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<td>6</td>
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<td>0.630</td>
<td>0.707</td>
</tr>
<tr>
<td>Year * Exposure</td>
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<td>5</td>
<td>0.518</td>
<td>9.362</td>
<td>0.000</td>
</tr>
<tr>
<td>Year * Microhabitat</td>
<td>1.935</td>
<td>8</td>
<td>0.242</td>
<td>4.372</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>35.073</td>
<td>634</td>
<td>0.055</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td>855</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>90.272</td>
<td>854</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R squared = 0.611

There was a significant interaction between wave exposure and microhabitat for triplefin density (fig. 2.5, tables 2.2-2.6). *Ecklonia* forest microhabitat negatively correlated with wave exposure for *R. whero*, and positively correlated with wave exposure for *N. segmentatus*. *Forsterygion lapillum* had low densities in both *Ecklonia* forest microhabitats in all but sheltered locations, where densities were as high as all other microhabitats. Overall, COB microhabitat was the environmental variable with the most variation associated with it, with notably high densities of *F. varium* and *R. whero* at sheltered COB microhabitats, and offshore COB microhabitats for *R. whero*. Elsewhere, some of the wave exposure patterns held true across all microhabitats, such as *F. lapillum* with higher densities at sheltered sites, *F. varium* lowest densities at offshore sites, and *N. segmentatus* highest densities at offshore sites. There were no transect or site effects on mean densities in microhabitats (table 2.2). Likewise, there was no effect of different transects on mean densities by wave exposure. However, site did have a significant on wave exposure for all species (table 2.2).
2.3.2 Spatial densities of triplefin by age class

When separated by 0+ and 1++ cohorts, there were also intraspecific differences in mean densities across the wave exposure gradient (fig. 2.6). Young cohorts (0+) of *F. lapillum* differed in mean density across sites varying in wave exposure ($H_3 = 114.94, P < 0.001$), with sheltered locations having significantly higher densities than all other wave exposures (fig. 2.6 A). Older cohorts (1++) of *F. lapillum* did not differ in density across the wave exposure gradient ($H_3 = 7.61, P = 0.06$). *Forsterygion varium* 0+ cohorts differed in mean density across sites varying in wave exposure ($H_3 = 42.31, P < 0.001$), with offshore densities significantly lower than all other wave exposures. Older cohorts (1++) also differed in mean density differed in mean density across sites...
varying in wave exposure ($H_3 = 15.10$, $P = 0.002$), with densities at sheltered and exposed, and exposed and offshore sites being significantly different (fig. 2.6 B). *Notoclinops segmentatus* 0+ cohorts had significantly higher mean densities offshore ($H_3 = 42.32$, $P < 0.001$) than in all other wave exposures (fig. 2.6 D).

![Figure 2.6](image)

**Figure 2.6.** Mean densities (± S.E.M.) of 0+ and 1++ age classes across a wave exposure gradient in the Hauraki Gulf for A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus*. Time is averaged over three years. Note difference in y-axis scales.

When separated by 0+ and 1++ cohorts, there were also intraspecific differences in mean densities between different microhabitats for all species (fig. 2.7). Mean density of *F. lapillum* 0+ cohorts differed between habitats ($H_4 = 51.76$, $P < 0.001$), with both *Ecklonia* habitats having significantly lower densities than all other microhabitats (fig. 2.7 A). Older *F. lapillum* cohorts (1++) followed the same patterns as the 0+ cohorts, differing in density between microhabitats ($H_4 = 218.75$, $P < 0.001$), with all microhabitats significantly different from each other, except EFD and EFS. Interestingly, adult individuals (1++) of *F. lapillum* had higher densities than 0+ in COB habitats (fig. 2.7 A). *Forsterygion varium* showed the biggest decline in density between 0+ and 1++ cohorts of the four species (fig. 2.7 B). There were differences between microhabitats for 0+
cohorts of *F. varium* (*H*₄ =57.86, *P* < 0.001), with EFD and COB, MA and STA, and EFS versus COB and MA all significantly differing in mean density. Older *F. varium* cohorts (1++) also differed in mean density (*H*₄ =102.67, *P* < 0.001), with densities in all microhabitats significantly different from each other, except EFS and EFD, and STA and COB. *Ruanoho whero* 0+ cohort mean density differed among habitats (*H*₄ = 126.84, *P* < 0.001), with mean densities different between all habitats, except EFD and EFS, and COB versus MA and STA. Mean density of older cohorts (1++) of *R. whero* differed between microhabitat (*H*₄ = 104.73, *P* < 0.001), with EFS versus EFD and MA, and COB versus STA and MA significantly differing in mean density. Lastly, mean density of 0+ *N. segmentatus* cohorts differed in all microhabitats (*H*₄ = 163.58, *P* < 0.001), except EFS and EFD, and STA versus COB (fig. 2.6 D). Older *N. segmentatus* cohorts (1++) also differed in mean density between habitat (*H*₄ =112.90, *P* < 0.001), with mean densities significantly different from each other between all microhabitats, except EFS and EFD, and STA versus MA and COB.

![Figure 2.7. Mean densities (± S.E.M.) of 0+ and 1++ age classes between habitats in the Hauraki Gulf for A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus*. Time is averaged over three years. Note difference in y-axis scales.](image-url)
2.3.3 Spatio-temporal patterns in triplefin density

All four species displayed similar seasonal patterns of settlement (fig. 2.8), due to winter/early spring spawning peaks in all species. Settlement peaked in all species during summer (December to February), as seen by an abrupt increase in density, coinciding with a decrease in mean size. Settlement was a more defined event in *F. varium* and *N. segmentatus*, with main settlement peaks in December and February, respectively. *Forsterygion lapillum* and *R. whero* had wider settlement windows, with peaks in density extending from December through February.

![Figure 2.8](image)

Figure 2.8. Relationship between seasonal recruitment and mortality, as seen by seasonal mean size (TL) ±SEM, and density (fish per m²) of a) *F. lapillum*, b) *F. varium*, c) *R. whero*, and d) *N. segmentatus* across all sites and years combined. Note differences in y-axis scales.

There were very strong seasonal fluctuations in density for each species across the wave exposure gradient, for both younger (fig. 2.9) and older (fig. 2.10) cohorts. *Forsterygion lapillum* and *R. whero* showed the strongest effects of wave exposure on density of 0+ cohorts, with more fish settling into sheltered habitats (i.e. density following peak spawning) (fig. 2.9 A & C). *Forsterygion lapillum* and *R. whero* 0+ fish at sheltered habitats had the highest densities in 17 out of 18, and 15 out of 18 bi-monthly samples, respectively (table 2.7). Densities of 0+ *N. segmentatus* were relatively equal among wave exposures coastally (table 2.7), however, by far
the highest 0+ densities were found at offshore sites during April and December 2014, around double that of mainland locations (fig. 2.9 D). There were no obvious patterns in 0+ abundance of *F. varium* across the wave exposure gradient, with density highest at semi-exposed habitats during 2012 recruitment, and exposed habitats during the 2014 recruitment event (fig. 2.9 B).

The adult densities (fig. 2.10) show the relative effects of post-settlement processes across the wave exposure gradient on species distributions, when compared with the 0+ densities (fig. 2.10). The strongest associations with wave exposure and adult density were *R. whero* and *N. segmentatus*. *Ruanoho whero* maintained highest adult densities in sheltered habitats (15/18 sampling periods), and *N. segmentatus* the lowest adult densities in sheltered habitats (2/18 sampling periods), and highest densities most often at exposed habitats (fig. 2.10 C & D; table 2.7). To a lesser extent, density of adult *F. varium* showed a positive correlation with wave exposure, with densities lowest in sheltered habitats and highest density in exposed habitats (fig. 2.10 B; table 2.7). No consistent pattern was seen for adult *F. lapillum* density and wave exposure (fig. 2.10 A), and if anything, had slightly higher densities at more exposed locations (table 2.7).

General temporal patterns between wave exposure and density were consistent year to year, however, there was significant variation in overall numbers of fishes between years (tables 2.2-2.6). The second year (2013/2014) of sampling exhibited the lowest densities of triplefins overall. This coincides with summer maximum sea surface temperatures, which that year were 1-1.5°C lower than during sampling years one (2012/2013) and three (2014/2015) (see appendix for SST).
Figure 2.9. Seasonal density for three successive years of 0+ cohorts of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* across a wave exposure gradient in the Hauraki Gulf. Shaded bars indicate peak spawning periods. Note differences in y-axis scales.
Figure 2.10. Seasonal density for three successive years for adult individuals (1++ cohorts) of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* across a wave exposure gradient in the Hauraki Gulf. Shaded bars indicate peak spawning periods. Note differences in y-axis scales.
There were also very strong seasonal fluctuations in density for each species among microhabitats, for both 0+ fish (fig. 2.11) and adults (fig. 2.12). *Forsterygion lapillum, R. whero* and *N. segmentatus* all showed higher densities in particular microhabitats immediately following spawning (fig. 2.11). *Forsterygion lapillum* new settlers were more abundant in COB, STA and MA microhabitats (fig. 2.11 A; table 2.2), whereas *R. whero* and *N. segmentatus* settlers had higher densities in *Ecklonia* forest microhabitats, and lowest densities in the two open microhabitats (i.e. no kelp) COB and STA (fig. 2.11 C, D; table 2.2). *Forsterygion varium* exhibited no strong patterns in density of new settlers among microhabitats (fig. 2.11 B; table 2.2).

Adult density of *F. lapillum* was consistently highest in COB microhabitat, with densities in this microhabitat the highest 13 out of 18 months sampled (table 2.2). Adult densities of *F. varium* showed much clearer habitat patterns than the 0+ cohorts, with highest densities in the *Ecklonia* forest microhabitats, followed by the other kelp microhabitat MA. Lowest densities for *F. varium* were in open microhabitats (COB, STA) (fig. 2.12; table 2.2). The microhabitat-density patterns of adult *R. whero* and *N. segmentatus* were generally similar to those of new settlers (fig. 2.12 C, D).
Figure 2.11. Seasonal density for three successive years of 0+ cohorts of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* in different habitats. EFD = *Ecklonia* forest deep; EFS = *Ecklonia* forest shallow; MA = mixed algae; STA = shallow turfing algae; COB = cobbles. Shaded bars indicate peak spawning periods. Note difference in y-axis scales.
Figure 2.12. Seasonal density for three successive years of adult individuals (1++ cohorts) of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* in different habitats. EFD = *Ecklonia* forest deep; EFS = *Ecklonia* forest shallow; MA = mixed algae; STA = shallow turfiging algae; COB = cobbles. Shaded bars indicate peak spawning periods. Note differences in y-axis scales.
Table 2.7. Proportional abundances by habitat and age class over the 3 year sampling period (e.g. *F. lapillum* 0+ fish at sheltered locations had the highest densities at 17 out of 18 bi-monthly surveys = 0.94). Darker shading indicates higher proportions.

<table>
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<th>Age Class</th>
<th>Species</th>
<th>Wave Exposure</th>
<th>Microhabitat</th>
</tr>
</thead>
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<td></td>
<td></td>
<td>Sheltered</td>
<td>Semi-exp.</td>
</tr>
<tr>
<td>0+</td>
<td><em>F. lapillum</em></td>
<td>0.94</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td><em>F. varium</em></td>
<td>0.17</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td><em>R. whero</em></td>
<td>0.83</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td><em>N. segmentatus</em></td>
<td>0.28</td>
<td>0.39</td>
</tr>
<tr>
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<td><em>F. lapillum</em></td>
<td>0.22</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>F. varium</em></td>
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<td>0.22</td>
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<tr>
<td></td>
<td><em>R. whero</em></td>
<td>0.83</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td><em>N. segmentatus</em></td>
<td>0.11</td>
<td>0.28</td>
</tr>
</tbody>
</table>

2.3.4 Spatial variation in nesting for *F. lapillum* and *F. varium*

The length of *F. lapillum* males guarding nests did not differ between habitats varying in wave exposure ($F_3 = 0.385, P = 0.764$), with average lengths of 53.1, 54.7, 53.7, and 54.3 mm TL for sheltered, semi-exposed, exposed and offshore habitats, respectively (fig. 2.13 A). Both the largest nest size and the largest guarding male of *F. lapillum* were both found at exposed locations (fig. appendix fig. 8 A). The length of nest-guarding males for *F. varium* negatively correlated with wave exposure ($F_3 = 3.973, P = 0.01$), with average lengths of 103.1, 94.8, 97.8, and 93.2 mm TL for sheltered, semi-exposed, exposed and offshore habitats, respectively (fig. 2.13 B). Pairwise comparisons showed male length differed significantly between sheltered habitats and both semi-exposed ($P = 0.01$) and offshore habitats ($P = 0.014$). Consistently the smallest *F. varium* nests were seen at sheltered (appendix fig. 8 B).
Figure 2.13. Total lengths of nest-guarding males for A) *F. lapillum*, and B) *F. varium* at different wave exposure locations. Dashed lines represent the sample means.
Quantile regression estimates were used to analyse the nest data. In addition to the ecological reasons for selecting the use of quantile regressions over standard linear regressions (see methods), the wedge-shaped distributions (for both wave exposure and microhabitat) and unequal variation implies that there is more than a single slope (rate of change) describing the relationships between fish size and nest area for both wave exposure and microhabitat. This is indicative of a data set with limiting factors (sensu Cade and Noon 2003). Therefore analyses focused on rates of change in quantiles near the maximum response (0.9 quantiles), and this upper distribution (0.9 quantiles) is compared with the middle of the distribution (median/0.5 quantiles) for *F. lapillum* and *F. varium* at different wave exposure locations (fig. 2.14).

Quantile regression estimates show wave exposure to have an effect on the relationship between male size and nest size (fig. 2.14). Estimates show heterogeneous distributions in the nest data (i.e. slope estimates differ between wave exposures). Rates of change in the relationship between fish length and nest size positively correlated with coastal wave exposure at the upper end of the distribution, with slope estimates increasing from sheltered to exposed habitats (figs. 2.14 B, D). However, offshore data had lower slope coefficients for both species, especially *F. lapillum*.

**Figure 2.14.** A) 0.5 and B) 0.9 quantile regression estimates for *F. lapillum*, and C) 0.5 and D) 0.9 quantile regression estimates *F. varium* across a wave exposure gradient in the Hauraki Gulf.
More than 90% of nests censused for *F. lapillum* were found in COB microhabitats, therefore no analyses was done on microhabitats for this species. Forty seven % of nests censused for *F. varium* were also found in COB microhabitats (fig. appendix 9). Quantile regression estimates at the upper edge of the distribution (0.9 quantiles) are compared with the middle of the distribution (median/0.5 quantiles) for *F. varium* in different microhabitats (figure 2.15). Estimates show heterogeneous distributions in the nest data (i.e. slope estimates differ between habitats). Rates of change in the relationship between fish length and nest size are approximately consistent for EFS, COB and STA for the middle (0.5) compared to the upper edge (0.9) of the data. However, MA habitats showed a negative relationship between male size and nest size at the higher edge of the distribution. Considerably the strongest relationship between fish size and nest size was in EFD habitats, where the largest nests were also seen.

![Figure 2.15. A) 0.5 and B) 0.9 quantile regression estimates for *F. varium* in different habitats.](image)

### 2.4 Discussion

#### 2.4.1 Spatial differences in triplefin abundance

*Variation by habitat*

The main focus of this chapter was to examine factors generating variation in habitat performance in triplefins, with relative abundance being a key measure of fitness performance. Intraspecific differences in the habitat density distributions were evident, and indicate that certain habitats were more suitable than others. Increased abundance in a given habitat is generally considered to be
beneficial to overall fitness, due mostly to the numbers of individuals contributing the next
generation (although this depends on the carrying capacity of the environment - Bradshaw and
McMahon 2008). Furthermore, species-specific and consistent habitat use across a gradient would
be expected where fitness trade-offs in alternative habitats are high (Rosenfeld and Boss 2001).
Therefore, habitats that consistently have the highest densities of a particular species are likely a
result of phenotypes best suited to that habitat, providing a fitness advantage.

Intraspecific differences in density were observed in all species. In general, the largest differences
in density for each species were seen at the two ends of the wave exposure gradient. *Forsterygion
lapillum* and *R. whero* were both most abundant at sheltered sites, i.e. the sites in this study
receiving the least physical disturbance from the water movement associated with wind and swell
driven fetch. Sheltered habitats differ from exposed sites in more than just wave energy. Firstly,
sheltered habitats are composed of softer sandstone geological formations, such as is the case for
Jones Bay (Edbrooke 2001). Ti Point is also unusual in that its volcanic origins have created a
subtidal landscape of large subtidal boulders. Secondly, sheltered habitat zones are more
compressed due to the shallower depths at these sites, providing less available space. And lastly,
the proximity to estuaries of the sheltered sites in the current study increases sediment loading,
and sediment levels are unlikely to decrease linearly with distance from the estuary, especially for
complex coastlines (Hume et al. 2007). All of these factors could conceivably influence species
fitness in sheltered habitats.

We know there is considerable gene flow between the coast and offshore islands in the Hauraki
Gulf in *F. lapillum*, *F. varium* and *R. whero* (Hickey et al. 2009, Rabone et al. 2015). However,
there were large differences in abundance between these habitats, such as the lowest densities of
*F. varium* and the highest densities of *N. segmentatus* cohorts found offshore. Whilst we can see
there were density differences, the mechanisms behind this are less clear. There is enough larval
mixing to keep high levels of gene flow between the mainland coast and offshore islands;
however, it is likely that recruitment will be less consistent offshore. The larvae of some triplefin
species are distributed further offshore than many other reef fish larvae (Hickford and Schiel
2003). The larvae from the coastal mainland will therefore be constrained by land and will either
drive up on the coast or have a chance of hitting the islands, whereas much of the offshore larvae
can potentially be lost to the open ocean during the early offshore larval periods. Consequently,
there is likely to be more exchange of larvae from the coast to offshore islands than the reverse.
This would result in a larger proportion of maladaptive individuals at offshore sites relative to
coastal sites, thus providing greater disruptive selection offshore, and explaining some of the large
variation in intraspecific densities on offshore reefs in this study.
There were also differences in abundance at the level of microhabitat. This shows evidence of habitat-specific performance (i.e. abundance) across varying spatial scales. Consistent patterns included no significant differences in density between COB and STA, or between EFD and EFS for any species. This gives some idea of the important habitat characteristics favoured by certain triplefin species. The similarity between COB and STA is likely in the small – albeit different – refuges, such as under cobbles and small boulders (COB) compared to cracks and fissures in the bedrock (STA). This is supported by the distribution of certain species (e.g. *F. lapillum* and *R. whero*) (Syms 1995), and by the presence of nests in these microhabitats (Feary and Clements 2006, Wellenreuther and Clements 2007). Additionally, depth has been shown to be a strong axis of species divergence in triplefins (Wellenreuther et al. 2007). However, in the current study the three dimensional structure of the kelp forest largely outweighed depth, based on the similarity in densities between EFS and EFD.

Densities of *R. whero* are likely biased by sampling design, and thus are possibly a little misleading. Previous research has shown *R. whero* to have higher densities in deeper reef habitats at more exposed sites (Wellenreuther et al. 2007), due in part to being replaced by the sister species *R. decemdigitatus* in shallower sheltered habitats (Wellenreuther et al. 2008a). This suggests that we would have seen higher densities at more exposed locations if we had sampled deeper habitats, as is suggested by the slightly higher densities in EFD relative to EFS microhabitats. However, as previously stated, the aim was to sample the area of highest species overlap (which was deemed 3-7m), which still permitted testing intraspecific differences in abundance between habitats.

There was a significant interaction effect between wave exposure and microhabitat in terms of triplefin density. Several habitat-abundance interactions between these differing spatial scales were seen, such as *F. lapillum* exhibiting consistently low densities in *Ecklonia* forests yet high in *Ecklonia* forests at sheltered sites, and *R. whero* densities in *Ecklonia* forests decreased with increasing wave exposure. Overall, COB microhabitat was the environmental variable associated with the most variation. This is the only habitat that is not a hard, fixed substratum, and is therefore subject to the most disruption by wave energy. Furthermore, the underside of cobble surfaces provide available habitat for refuge and nesting. Conversely, in some cases the habitat-density patterns are strongest for one of the habitat variables over the other. For example, *F. varium* is consistently lowest offshore no matter the microhabitat. Ultimately, both of these habitat variables (wave exposure and microhabitat) are major factors driving differences in abundance of these species, but the interactions are complex.
Cohort data and habitat selection

Whether habitat selection and abundance are determined actively (i.e. larval behaviour) or passively (i.e. post-settlement mortality) is an important question, as the latter would imply that habitat divergence is the product of ecological interactions within generations rather than natural selection. Answering this question requires methods that discriminate between active and passive mechanisms of settlement. In the present study, overall densities were partitioned into different cohorts (0+ and 1++) in an attempt to discriminate between these mechanisms, where densities of adult triplefins show the relative effects of post-settlement processes among habitats when compared with 0+ densities. If the habitat distributions of adult triplefins were completely different to those of the 0+ cohorts, then this would indicate that the patterns of density at settlement seen in 0+ cohorts were largely as a result of post-settlement processes. This would be a maladaptive strategy, as there would be great selective pressure to avoid settling where predation is highest (Doyle 1975, Whitman 1980, Levin et al. 1997). However, the adult density patterns generally mirrored those of 0+ cohorts at both the level of wave exposure and microhabitat, thus suggesting some level of habitat selection during settlement. Furthermore, given that some of the sample sites varying in wave exposure are very close in proximity (~3-4 km apart), and triplefins exhibit around a 2-3 month pelagic larval duration (i.e. have the potential to disperse much further than these distances), then the density distributions of 0+ fish are unlikely to be random with respect to habitat. The corroboration of these two aspects suggests that active processes of habitat preference at settlement are primarily responsible for the overall density of triplefin distributions.

Accomplishing active habitat settlement necessitates identifying chemical, visual, and/or acoustic cues from islands, reef patches, microhabitats, conspecifics, or predators (Arvedlund and Kavanagh 2009), and subsequently being capable of physically following these cues. There is a growing body of evidence that marine larvae do have the sensory and locomotive capabilities to accomplish successful active recruitment, and at a range of spatial scales, from broad range at the level of an island or continental shelf amongst the open ocean (Gerlach et al. 2007, Dixson et al. 2008, Stanley et al. 2012), a suitable reef patch within an island or piece of coast (Montgomery et al. 2006, Huijbers et al. 2008, Lecchini et al. 2013), and even appropriate microhabitat (Munday et al. 2009, Igulu et al. 2011, Devine et al. 2012), conspecifics (Lecchini et al. 2010, Lecchini 2011) and predators (Dixson et al. 2012) within these small patches. Settlement stage triplefin larvae have also been shown to possess active orientation to habitat cues (Tolimieri et al. 2000). Therefore, there is ample evidence that marine fish larvae and indeed triplefins have the capabilities to actively select habitats at settlement, and the data in this study support that.
The differences in abundance of 0+ relative to 1++ cohorts does not provide unequivocal evidence of active habitat choice at settlement, as high mortality in fishes immediately following settlement can confound subsequent patterns (Almany and Webster 2006). In order to completely understand the extent of active vs passive settlement, one would have to greatly increase the intensity of sampling, such as surveying daily during the settling period. This is well beyond both the means and original scope of this project. However, the patterns of active habitat selection are still clearly evident when we look at 0+ densities following peak spawning times, where there are intraspecific differences in density between habitats. Importantly, these data provide support rather than disagree with previous research that proposes habitat selection as a mechanism of habitat diversification (e.g. Syms 1995; McDermott and Shima 2006; Wellenreuther and Clements 2008) incrementally adding weight to the argument that active habitat selection is the primary cause of the density distributions seen in adult triplefins. There were however some changes in the density patterns of adults relative to 0+ cohorts. This is not wholly unexpected, as even if active selection is primarily responsible, there are no doubt still post-settlement pressures regulating density on top of active selection, and competition and predation might be more prevalent in certain habitats. However, the majority of 0+ cohorts mirrored those of adults, indicating that genotypes of these species can differ in their dispersal ability (e.g. via larval behaviour).

### 2.4.2 Temporal differences in triplefin abundance

In order to make spatial comparisons in habitat use, one must also consider any temporal variation. With many short lived species the size distribution and abundance will change dramatically throughout the year. Consequently, many studies may falsely conclude that a certain population or species is most abundant at a given habitat, yet have only sampled at a certain time of the year, and this may not be representative. Hence there is a need for understanding this variation before conclusions can be made, and the repetitive sampling in this study was an attempt to address this as best possible. Furthermore, this is the first such study to document triplefin size and abundance on such a high resolution, and provides good documentation of the seasonal patterns of settlement and mortality in each of these four species.

The patterns of settlement mortality follow closely a winter/early-spring spawning season in triplefins, with the peak of abundance during December and February surveys. Additionally, the sharp declines in mean size during these months is a combination of the influx of recruits, but also the senescence of very large proportions of the adult populations post-spawning (e.g. *F. lapillum* usually only live for two winter spawning periods – see ageing data chapter 3).
This study was also the first to assess triplefin abundance at this resolution across multiple years. The results demonstrated that the patterns of density by habitat were consistent across the three year study. However, there were significant differences in absolute abundance among years, with year 2 having generally lower overall numbers of triplefins relative to years 1 and 3. This coincided with a cooler year (see appendix figure 1). Cooler sea surface temperatures can alter water currents and thus larval transport, food supply, and egg and larval development (Iversen and Danielssen 1984, Bradbury 2000). Therefore, the temporal data provide a more complete picture of the huge variation in size class structure seasonally, which is important for making informed conclusions. Moreover, consideration needs to be made for larger scale patterns, such as the global cycles of ENSO, on the year to year variation in abundance of these abundant reef fishes.

2.4.3 Fecundity

Reproductive output, such as examined here with nesting male triplefins, is a direct measure of fitness and presents a simple mechanism that would favour assortative mating among locally adapted phenotypes. The differences in the nest sizes, guarding male sizes, and the relationships between the two among habitats, provides further evidence of variation in habitat performance in *F. lapillum* and *F. varium*. Many fish species show variation in reproductive traits over environmental gradients, such as differences in fecundity and egg size (L’Abee-Lund and Hindar 1990). The linkage between habitat and mate choice has been identified as a powerful mechanism of speciation in parapatry or sympatry, and has been referred to as a ‘magic trait’, one that is subject to disruptive selection and simultaneously controls for non-random mating (Gavrilets 2004). Evidence that this linkage can lead to speciation has been demonstrated in examples of phytophagous insects (Berlocher and Feder 2002) and fish (Munday et al. 2004). There is prior evidence of sexual selection playing a role in diversification in at least some triplefin species. Mate choice has been shown in experimental situations for *Ruanoho* spp. (Wellenreuther et al. 2008a), where divergence in habitat use was demonstrated to automatically lead to differences in spatial breeding habitat. If the trait (i.e. the linkage between habitat and male reproductive output) in either *F. lapillum* or *F. varium* is under disruptive selection, then mating assortativeness should increase, and given sufficient time can lead to reproductive isolation (Beltman and Metz 2005).

The mean size of males guarding nests negatively correlated with wave exposure in *F. varium*. This suggests that there is more competition for nesting habitat at more sheltered sites in *F. varium*, *Forsterygion varium* nest on bedrock (Thompson 1986), and reef habitats at more exposed sites in the Hauraki Gulf are characteristically composed of hard rock such as greywacke, whereas more sheltered sites are largely softer sandstone (Edbrooke 2001). Furthermore, reefs are
more spatially compressed at sheltered sites due to shallower depths, and are less complex structurally (Shears and Babcock 2007), thus providing higher competition for space for *F. varium* in sheltered habitats. The mean size of males guarding nests varied among habitats in *F. varium* but not *F. lapillum*. *Forsterygion lapillum* is more of a generalist in habitat use (Wellenreuther et al. 2009), and has been shown to make use of a wider range of spawning substrata (Feary and Clements 2006), which could partially explain the wider size range in males guarding successfully nests.

Cobbled habitats were the most successful microhabitat for spawning for either species, in terms of sheer number of nests. For *F. lapillum* this is likely a density effect, as the highest densities of adult were found in COB microhabitats. Furthermore, for both species, the higher surface area of hard substrata available in these habitats provides more nesting substrata. Additionally, in EFD microhabitats, *F. varium* showed the strongest relationship between male size and nest size, suggesting a depth effect. This is consistent with habitat diversification along the depth axes in New Zealand triplefins (Wellenreuther et al. 2007). These results suggest that body size is important for mate choice in these triplefins. Body size has previously been shown to correlate positively with spawning success in *F. varium* (Thompson 1986), and play a role in mate choice in *Ruanoho* spp. (Wellenreuther et al. 2008a). There are numerous other examples of mate choice on body size (Lu and Bernatchez 1999, Schliewen et al. 2001, Jones et al. 2003, Knudsen et al. 2006), and this would appear to be a potent mechanism of disruptive selection in sympatric distributions. In showing habitat-fitness interactions with the nesting data, this suggests there is selection pressure on females choosing both larger males and for nesting in optimal spawning habitats. When nests end up in marginal or sub-optimal habitats (for example *F. varium* in MA microhabitats) the consequence is reduced fitness (i.e. lower reproductive output). Therefore, the results suggest mate choice is a potential mechanism of habitat diversification, and spawning differences driven by habitat have the potential to lead to assortative mating in *F. lapillum* and/or *F. varium*.

### 2.4.4 Conclusions

The data in this study show that the performance of each species is habitat-specific, thus providing strong selective pressure for species to develop preferences for such habitats at settlement. This was established by showing differences in proximate indicators of fitness (abundance and reproductive output) in relation to a broad habitat gradient (wave exposure) and on a more local level among microhabitats. Active habitat selection was further supported by the densities of 0+ cohorts, and the density of adult cohorts relative to the 0+ cohorts, further suggesting. This also
demonstrates the importance of recording abundance estimates by size/cohoot, and not just overall as in many studies. Together, these results suggest a basis for divergent selection in habitat use, and provide support that differentiation in habitat use is an important mechanism responsible for the ecological speciation seen in the New Zealand triplefin fish assemblage. Furthermore, the results suggest that the processes responsible for the adaptive radiation in New Zealand triplefins evident over evolutionary timescales (i.e. ecological speciation) are evident over ecological timescales (i.e. during the timeframe of this study), making it amenable to studying such processes.
Effects of wave exposure on growth in two triplefin species, *Forsterygion lapillum* and *F. varium*

3.1 Introduction

Quantification of age and growth are some of the most important biological variables, and the data provide the basis for a wide range of information including growth, mortality, longevity, age structure, and key life history transitions (Campana 2001). Furthermore, these variables are the core components for a variety of disciplines, from stock assessment to population ecology. As growth and age are fundamental measures of fitness, these data can also be used to answer questions in non-fishery or stock related disciplines, such as in the evolutionary ecology. Variation in demographic variables are likely a result of strong phenotype-environment interactions, subject to strong selection pressures. Are growth and longevity adaptive ecological processes driving the intraspecific variation that leads to adaptive radiations in fish groups?

Fishes are predominantly poikilotherms, and environmental variables can be expected to influence their demography, such as growth rates, longevity and body size (Atkinson 1994, Kingsolver and Huey 2008). The most obvious and well-studied environmental variable in this regard is temperature, and numerous studies on latitudinal gradients have generally shown temperature to positively correlate with growth, at least initially, and negatively correlate with life span (e.g. Meekan et al. 2001, Choat and Robertson 2002, Trip et al. 2008, Trip et al. 2014). Growth rates cannot be considered in isolation from body size, as size is a consequence of the rate and duration of earlier growth. Accordingly, larger body sizes can be attained by either growing faster or living longer.

Being small often means being vulnerable to predation (Arendt 1997), therefore attaining a larger body size is usually advantageous (Kingsolver and Huey 2008). Furthermore, being larger strongly influences an organisms’ capacity to obtain resources through a competitive advantage, or simply being bigger than potential prey. The maximum amount of energy reserves that can be stored also increases disproportionately with body size, so larger individuals have a lower risk of dying of starvation during periods of food shortage (Ludsin and DeVries 1997). The beneficial effects of greater relative size on survival are especially pronounced during the juvenile phases.
Rapid growth can also lead to increased reproductive success (Roff 1992, Stearns 1992). In many fish species, larger males have higher reproductive output, for instance by outcompeting other males (Breitburg 1987, Lindström 1988), or being preferred by females (Oliveira et al. 2000). Similarly, for females, fecundity is also widely shown to correlate positively with size, often related to egg mass production (e.g. Patten 1971).

A number of structures have been used to estimate age and growth in fishes, including fin spines and rays, vertebrae, scales, and bones (Campana 2001). Otoliths (ear bones) however, are by far the most widely used calcified structure, and the most reliable (Secor et al. 1995). Unlike other skeletal structures (e.g. vertebrae, scales), otolith growth is often decoupled from somatic growth (i.e. metabolically inert) (Francis et al. 1993, Campana 2001). Consequently, otolith growth is continuous throughout the entire lifespan, reflected in linear correlations between otolith dimensions (i.e. weight and length) and age, regardless of whether a species exhibits continuous or asymptotic growth (Boehlert 1985, Pawson 1990, Fletcher 1991, Francis and Campana 2004). Additionally, otoliths are relatively isolated from external environmental conditions inside the otic capsule (Campana 2001). Therefore, otoliths are a reliable tool for estimations of age in fishes, as they are not subject to the varying environmental influences that effect somatic growth.

The relationship between fish length and otolith dimensions can be used to confirm whether otolith growth is consistent with somatic growth or not (Campana 2001). Where strong isometric relationships exist between the otolith dimension (e.g. otolith radii) and fish length, it is likely to be consistent with somatic growth (Jones 1992). Again, this is often the case with fast-growing species (e.g. Morales-Nin and Aldebert 1997). In contrast, slow-growing species tend to have larger, heavier otoliths relative to their body length, as the otoliths continue to grow even when somatic growth slows or ceases, resulting in a decoupling of otolith and somatic growth (e.g. Secor and Dean 1989, Francis et al. 1993, Barber and Jenkins 2001). Moreover, this relationship may not remain constant for an individual’s lifetime, and can shift between life history stages, for instance following metamorphosis or settlement (Francis et al. 1993).

Age estimations in fishes can occur at two scales. Most fishes are thought to deposit successive layers of aragonite calcium carbonate on a daily basis (Campana 2001). However, a precaution is necessary, in that daily increments may not persist in otoliths of older fish (Pannella 1971, Campana and Neilson 1982). Consequently, daily ageing based on otolith microstructure tends to be aimed more at recruitment questions and studies of young fishes (Pannella 1971, Campana and Neilson 1982). Although it is assumed the presence of an endogenous circadian rhythm of daily increment formation is invoked in most fishes (Campana and Neilson 1985), verification is often recommended. Rings have been assumed to be daily in triplefins (Longenecker and Langston
2005, Plaza et al. 2012), although both of these studies failed to validate increments. The periodicity of increments have been verified for some triplefin species by the use of tetracycline hydrochloride in new recruits (Kohn and Clements 2011). *Forsterygion varium* was shown to deposit daily growth increments, however this was unable to be verified in *F. lapillum*; although, the sister species of *F. lapillum* (*F. nigripenne*) was validated (Kohn and Clements 2011). Therefore it would seem likely that daily rings are also deposited in *F. lapillum*, at least at the larval and early juvenile stages. Seasonal growth peaks also lead to increments clustering into annual bands, which are usually more reliably identified than daily increments. These annual increments provide information on age-at-capture, and the data are commonly used in the construction of population growth models (e.g. von Bertalanffy growth function). Annual ageing is therefore the most successfully used age determination in support of harvest calculations and population studies.

Despite the difference in time scales, daily and annual ageing estimates are governed by similar modes of analysis, and are both subject to the same sources of potential error. Firstly, not all calcified structures in fishes, including otoliths, form a complete growth sequence throughout the entire lifespan, nor do all axes on the given structure exhibit a complete growth record (Beamish 1979). Secondly, there is a strong degree of subjectivity in the reading of the increments (Campana 2001). With these caveats in mind, otolith increments still remain a great resource for age estimation in fishes.

There are several alternatives to age-based growth estimation in fishes, and these are useful when otolith increment analyses are not feasible. Length-frequency analysis is one of the simplest and oldest methods of estimating growth, and incorporates a variety of different length-based methods, all of which produce estimates of growth rate. Monitoring the progression of length-frequency modes through time is one of the simplest forms of analyses, and can be reliable, particularly in young, fast-growing fishes (Pauly and Morgan 1987, Campana 2001). This approach assumes that each identifiable peak on length-frequency analyses represents the modal length of a year-class or cohort, and where regular spatial data is available throughout the year, provides a valuable approach to estimations of growth rates (Francis and Francis 1992).

The aims of the current study are to use age and growth information of two species of triplefin, *Forsterygion lapillum* and *F. varium*, as a proxy for post-settlement performance across a wave exposure gradient in the Hauraki Gulf. We hypothesise that there will be intraspecific variation in demographic variables across this spatial scale, as New Zealand triplefins have been shown to diverge in habitat use (Wellenreuther et al. 2007). Specifically, the aims are to (i) test whether different locations differ in growth rate, (ii) determine maximum age for the two species, (iii) test...
whether different locations differ in maximum longevity, and (iv) test whether larger fish are older and/or grow faster.

### 3.2 Methods

#### 3.2.1 Sampling

Fish collection was undertaken within the Hauraki Gulf in northeastern New Zealand (fig. 3.1), a region that comprises an area of approximately 4000 km², and covers a wide range of exposures, depths and habitats, including both coastal and offshore locations. Sampling sites spanned a wave exposure gradient, based on fetch and wind/wave energy (see below and table 1 for details of exposure calculations), with replicate sites for sheltered, semi-exposed, exposed, and offshore (i.e. very-exposed) wave exposure groupings. Collection sites included Ti Point Wharf (TW), Jones Bay (Jo), Mathesons Bay (Ma), Nordic (No), Takatu Point (Ta), Mokohinau Islands (Mo), Great Barrier Island (GB). Note: there was no replicate site for exposed fish collections, as the replicate site for this in the wider study, and the only corresponding site, was in a no-take marine reserve (Waterfall Reef, Cape Rodney to Okakari Point marine reserve). All fish samples were collected between October 2013 and June 2015 in accordance with the University of Auckland Animal Ethics Committee approval 001047. Fishes were captured using handnets on SCUBA at an average depth of five metres (±3 m). Mindful of minimising the collection of animals, fishes were only collected with respect to the wave exposure groupings, and sampled equally across all habitats at each site (i.e. collected haphazardly with regard to habitat, as collecting specimens from different habitats within each wave exposure category would have required five times the number). Fishes were euthanised upon return to the boat or shore using an overdose of clove oil (active ingredient eugenol), and then immediately frozen.

#### 3.2.2 Wave exposure analysis

A combination of wave power data, fetch, and wind-derived exposure were used to verify the wave exposure groupings used in the present study. Empirical wave energy data derived from MetOcean Wavewatch (NOAA 2016) was used to calculate wave exposure. Wave power was chosen as the variable of interest, as it incorporates wave height and period. Spatial resolution from the grid cells of these data was not high enough to permit delineation between closely situated sites, such as along the Leigh coast. However, it did permit accurate wave power
comparisons between broader locations, such as the Leigh coast and the offshore islands (see appendix for graphic representation).

More precise levels of wave exposure at each site were approximated using an index of potential fetch, which is defined as the area of the sea surface over which waves are generated by wind with a constant direction and speed (Denny 2005). The exposure of each site was calculated as the total sum of the fetch based on defined settings, using the program ‘Fetch Effect Analysis’ (Villouta 2000). For each site location, each 10º on a compass rose a vector was projected a distance of 300 km, or until land is encountered, and all distances summed. The use of fetch as a proxy for exposure does not take into account differences in wave spectra among areas (Denny 1988), however, it does permit tentative conclusions to be made (Thomas 1986). Furthermore, in north-eastern New Zealand it is suitable, as the longest fetch and largest swells are from the east/northeast (Denny 2005).

To further verify the wave exposure groupings, wind-derived exposure calculations were performed (e.g. Burrows et al. 2008). Historical wind data was attained for a 10 year period (2005-2015) at a three hourly resolution for maximum wind speed and direction (NIWA 2016). These were recorded from wind stations closest to the representative sites (outer gulf coast=Leigh; offshore islands=Mokohinau Islands). Within each data set, maximum speed was averaged for each 10º vector, and multiplied by the corresponding vector for fetch calculations for each site. Subsequently, all 36 vector calculations were summed to get a site total (see table 3.1).
Figure 3.1. Map of sample sites in the outer Hauraki Gulf. Inset 1 shows sites on the coastal mainland: sheltered (Ti, Jo), semi-exposed (No, Ma), exposed (WR, Ta). Mo and GB are offshore islands (very exposed). Inset 2 shows Hauraki Gulf location in the North Island of New Zealand.
Table 3.1. Fetch and wave exposure calculations and coordinates for each sampling location.

<table>
<thead>
<tr>
<th>Location</th>
<th>Exposure Grouping</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Sum of Fetch (km)</th>
<th>Max Wind*Fetch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti Point wharf</td>
<td>Sheltered</td>
<td>36°19’03.80”S</td>
<td>174°47’01.97”E</td>
<td>38</td>
<td>916</td>
</tr>
<tr>
<td>Jones Bay</td>
<td>Sheltered</td>
<td>36°22’49.59”S</td>
<td>174°47’04.1”E</td>
<td>415</td>
<td>11545</td>
</tr>
<tr>
<td>Mathesons Bay</td>
<td>Semi-exposed</td>
<td>36°18’13.87”S</td>
<td>174°47’01.97”E</td>
<td>1110</td>
<td>32266</td>
</tr>
<tr>
<td>Nordic</td>
<td>Semi-exposed</td>
<td>36°17’35.56”S</td>
<td>174°48’38.58”E</td>
<td>925</td>
<td>25890</td>
</tr>
<tr>
<td>Takatu Pt.</td>
<td>Exposed</td>
<td>36°22’02.95”S</td>
<td>174°52’07.61”E</td>
<td>2227</td>
<td>62376</td>
</tr>
<tr>
<td>Great Barrier Island</td>
<td>Offshore</td>
<td>36°03’11.89”S</td>
<td>175°24’10.98”E</td>
<td>4965</td>
<td>186925</td>
</tr>
<tr>
<td>Mokohinau (Edith)</td>
<td>Offshore</td>
<td>35°54’30.48”S</td>
<td>175°06’25.93”E</td>
<td>4674</td>
<td>174821</td>
</tr>
<tr>
<td>Mokohinau (Archway)</td>
<td>Offshore</td>
<td>35°54’41.43”S</td>
<td>175°06’08.78”E</td>
<td>700</td>
<td>26847</td>
</tr>
</tbody>
</table>

3.2.3 Otolith preparation and analyses

Frozen fish samples were thawed in the laboratory, and subsequently weighed and measured for standard length (SL) and total length (TL). Sagittal otoliths were extracted from each fish under a dissecting microscope, cleaned of endolymph tissue with ethanol and fine forceps, and stored dry in light-proof well boxes to prevent fading of marks (Hernaman et al. 2000). Before grinding, otoliths were weighed on a fine-scale balance, and measured along the longest axis (rostrum-postrostrum) using digital calipers. These data were used in relationships between otolith dimensions (length and weight) and fish total length, to determine whether the use of otolith increments is a good proxy for somatic growth. It is usual for other studies to calculate the regression relationship with otolith radius (e.g. Sim-Smith et al. 2012), however, the exact position of the core was not consistently attained. Consequently, otolith length (rostrum-postrostrum) was measured as the otolith length dimension.

Transverse sections were prepared for sagittal otoliths of both species. Sagittae were mounted on glass slides using preheated CRYSTAL BOND™ 509 clear mounting thermoplastic cement such that almost half the otolith protruded over the edge of the slide. Mounted sagittae were then sanded using sand paper (800 and 1,200 grit) at a right angle until the nucleus was exposed and then polished with lapping films (12 to 0.3 µm). Sagittae were then removed by heating the CRYSTAL BOND™ and remounted face down with the polished face facing the slide. Sanding and polishing was repeated as above to achieve a thin transverse section.
Attempts to read daily increment counts were made using a compound microscope (Leica DMR, up to 1000x), under transmitted light and using immersion oil (see fig. 3.2 for examples). We experienced extreme difficulty in attributing check marks as daily increments. Furthermore, where increment rings were present, they usually did not remain constant through the entire axis which was being read, which we attribute to the curvature in the otoliths (i.e. in order to see increments near the core region, the corresponding increments closer to the margin would be ground away). We are confident that several specimens were older than one year, based on size, date of collection, multiple annual rings present, and previous observational studies tracking individuals over time (Thompson 1979). Where we could estimate increments for much of the otolith, we never counted more than 183 increment rings. Therefore we were either not able to see all the increments, or they do not appear to be daily. Longenecker and Langston (2005) counted no more than 117 “daily” rings for a triplefin species, further raising doubts as to the periodicity and/or readability of daily increments in adult triplefins. Several other methods of otolith preparation were explored, including several transverse, sagittal and frontal sections of various thicknesses, all unsuccessful. Following this, otolith samples of both species were sent to a leading international otolith laboratory, of which samples were prepared and analysed. These were also unsuccessful, therefore subsequent analyses were completed with annual growth rings.

Figure 3.2. Example of transverse sagittal otolith section for a *F. lapillum* (27.2 mm TL) under transmitted light at A) 100x, and B) 250x.

The otolith sections prepared for daily ring counting were too thin to adjudge annual rings, as much of the broader check marks had been ground away. Therefore new sagittal otolith sections were prepared with considerably less grinding. Furthermore, reflected light was deemed easiest to see annual increments (see fig. 3.3). Digital images were taken of each otolith section using a
Leica compound microscope fitted with a Leica DC500 digital camera that was connected to Leica Application Suite (LAS) imaging software (Leica Microsystems, Wetzlar, Germany). Images were captured at $\times 100$–$600$ magnification and daily increments were counted from the core to the otolith edge along the longest axis. One annual ring was defined as one complete opaque and one complete translucent zone.

Unfortunately, due to only having 2-3 age classes in most samples, annual increments were unable to be used to in growth analyses (i.e. to estimate growth parameters for Von Bertalanffy growth functions). This is due to the lack of $x$-values (age classes) in calculations, and also the low precision. For example, an individual where one annual increment was counted could theoretically be anywhere between 365-730 days old. Attempts were made to improve precision by accounting for time of capture and approximate time of settlement, in combination with apportioning the opaque or translucent stage of the annual increment to assign fractions of years to individuals. Ultimately, these were unsuccessful. Although triplefins exhibit peak spawning periods, both species spawn over a reasonable proportion of the year – $F. \ varium$ and $F. \ lapillum$ are known to spawn for up to 8 and 11 months of the year, respectively (Wellenreuther and Clements 2007). Therefore, without daily increment analysis there is no way to be sure of an individual’s settlement date. Consequently, annual increment counts were utilised for maximum age only.

Figure 3.3. Examples of transverse sagittal otolith sections under reflected light for A) $F. \ lapillum$ (59.6 mm TL) and B) $F. \ varium$ (104.1 mm TL).
3.2.4 Validation of daily increments using tetracycline

Daily increment marks have previously been confirmed in New Zealand triplefins based on tetracycline injections (Kohn and Clements 2011). However, these were validated on recently settled recruits, not adult fishes, and *F. lapillum* otoliths were not clear enough to read (Kohn and Clements 2011). Therefore, attempts were made to validate daily increment formation for adult *F. lapillum* and *F. varium*. Ten individuals of each species were captured at Matheson Bay on October 31st 2012. Fishes were transported in 20 L sealed buckets containing seawater to the seawater facility at the University of Auckland’s Leigh Marine Laboratory, with travel time less than 30 minutes from capture. Aquaria consisted of 60 L glass tanks connected to a recirculating sea water system (ambient temperature 16°-18°C, salinity between 34 ‰ and 36 ‰). Prior to manipulation fish were acclimated for a period of 24 hours, maintained under standard husbandry conditions, and fed finely chopped mussel and pilchard for the duration of the experiment.

Injection with tetracycline hydrochloride 95% (SIGMA™) was conducted on ten individuals each from the two species (*F. lapillum* and *F. varium*). A solution of 5 mg tetracycline hydrochloride per 1 mg saline was prepared. Fish were anaesthetised with a clove oil/ethanol solution and then weighed. The tetracycline solution was then injected into the peritoneal cavity of the fish at a concentration of 50mg tetracycline hydrochloride per 1 kg of fish body weight (Hernaman et al. 2000).

Fishes were returned to a 12L:12D cycle, and were kept for an additional 16 days. Concluding this time period, fishes were euthanised using clove oil. Sagittal otoliths were extracted from the otic capsule of each fish under a dissecting microscope, cleaned of endolymph tissue with ethanol and fine forceps. The otoliths were then allowed to dry, and stored dry in a light proof container to prevent fading of the validation marks (Hernaman et al. 2000) until otolith preparation and analysis was performed.

Otolith preparation followed procedures above (section 3.2.3), however, otoliths were also examined here under ultraviolet light. The margin of the otoliths did fluoresce following the 16 days of holding post-tetracycline injections. However, the resolution of check marks was not clear enough to successfully see daily ring formations in the margin region (see fig. 3.4 for fluoresced examples).
3.2.5 Length-based growth estimates

Due to the unsuitable nature of daily increment formations in these otoliths, alternate methods of growth estimation were considered. As cohort data were available for both species at a reasonably high temporal resolution (length-frequency data were collected every two months for three years across the exposure gradient – see chapter 2), it was possible to produce estimates of growth rate using a length-based approach to growth. By using the identification of modes (cohorts/year classes), fisheries software tools can estimate growth parameters for use in growth functions, such as the von Bertalanffy growth function (eqn. 1) (Pauly and Morgan 1987, Fournier et al. 1990). A length-based approach to estimating growth rates is suitable for short-lived fishes that grow fast (Campana 2001), as is the case with triplefins. Here we utilise a modal progression analysis (MPA) using the FAO ICLARM Fisheries Assessment Tools software program software FiSAT II (an updated version of the ELEFAN software package), and used the well-defined length modes from length-frequency data to fit to a growth model (Gayanilo et al. 1996).

Modal class progression analysis refers to a methodology that infers growth from the apparent shift of the modes or means in a time series of length-frequency samples. Firstly size-frequency histograms were produced for both *F. lapillum* and *F. varium* for each sample date (18 bi-monthly samples over three years). Bhattacharya’s method was then used to decompose the composite length-frequency distributions into their components to identify means and modes. This involved visual identification and "linking" of the modes perceived to belong to the same age group (i.e. cohorts) identified for each sample period. Using the growth increments and size-at-(relative) age data resulting from the linking of modes these data can be used to estimate growth parameters. Lastly, Munro’s method was used to estimate the growth parameters length infinity ($L_\infty$) and growth rate ($K$) from these data by calculating the $Y:X$ ratios (eqn. 2) for all returns, using a range of trial values of $L_\infty$. The value of $L_\infty$ which yielded the lowest coefficient of variation (the ratio of
the standard deviation to the mean) of the ratios $Y:X$ was the estimate of asymptotic length (Munro 1982).

3.2.6 Data analysis

Analyses of total lengths for both species were tested using analyses of variance (ANOVA) on ranks, with pairwise comparisons using Dunn’s method. Linear regressions were used to calculate the relationship between otolith dimensions and fish total length. All growth parameters for the length-based growth were computed using modal progression analyses within the FiSAT II software, based on the equations below.

The conventional von Bertalanffy growth function (VGBF) which has been used to describe the adult phase of growth of many species of animals is as follows:

$$l_t = L_\infty \{1 - \exp[-K(t - t_0)]\} \quad (equation \ 1)$$

in which the length ($l$) at time $t$ is a function of the asymptotic length ($L_\infty$), the coefficient of growth ($K$), and the theoretical time of hatching of the animal ($t_0$).

Munro’s method follows rearrangements of the VGBF, and growth parameters are estimated using the equation:

$$K = \frac{\ln(L_\infty - L_t) - \ln(L_\infty - L_t + \Delta t - t)}{Y/X} \quad (equation \ 2)$$

where $L_t$ is length at tagging, and $L_{t + \Delta t}$ length at recapture (bi-monthly sample increments are treated analogous to mark-recapture increments). FiSAT II is unable to carry out significance tests on differences between models fitted to different data sets (i.e. between wave exposures). Size-at-age one was estimated from the growth functions.

3.3 Results

3.3.1 Length-based growth analyses

Length-frequency distributions for both triplefin species were sampled bi-monthly for the coastal wave exposure gradient (sheltered to exposed) over the course of three successive years (see chapter 2 for methods). Modes representing 0+ age class are well defined and separated from 1+
age classes. The distributions for age classes are approximately normally distributed, and these length-frequency data were successfully used in modal progression analyses (figs. 3.5–3.8).

Estimations from the von Bertalanffy growth function, based on the length-frequency data, showed that asymptotic length ($L_\infty$), growth rate coefficient ($K$), and size-at-age one year all positively correlated with wave exposure for *F. lapillum* (fig. 3.6, table 3.2). Fish length estimates for asymptotic length ranged from 53.6 mm at sheltered to 59.6 mm at exposed locations, and for age-at-year one from 49 mm at sheltered to 57 mm at exposed locations. Growth rate increased from 2.5 to 3.6 for *F. lapillum* from sheltered to exposed, respectively. *Forsterygion lapillum* are approximately 90-95% of maximum size after one year.

The pattern for *F. varium* was not as clear. However, estimates from the von Bertalanffy growth function for *F. varium* showed the same general trend as with *F. lapillum*, the fastest growth occurring at the most wave exposed populations ($K = 2.5$, size-at-age one year = 99 mm TL) (fig. 3.8, table 3.2). Furthermore, growth rate of exposed fish was more than double the more sheltered locations (sheltered and semi-exposed). There were some exceptions to the pattern, with both growth rates and age-at-year one at semi-exposed lower than at sheltered populations. Additionally, asymptotic length ($L_\infty$) was longest at semi-exposed (123.3 mm TL) and not for exposed (107.9 mm TL) (table 3.2). *Forsterygion varium* are approximately 70-80% of maximum size after one year.

Seasonal variation in growth was evident, determined from the length-frequency analyses. The period of highest growth (largest shift in modal size) for *F. lapillum* was an average of 5 mm/month, seen between October and December for sheltered populations, but December to April for semi-exposed and exposed. The slowest period of growth for semi-exposed and exposed populations of *F. lapillum* was between April and August, and February through August for sheltered populations (~2.5 mm/month). The period of highest growth for *F. varium* was between December and February for sheltered (5 mm/month) and exposed (10 mm/month) populations, however, semi-exposed populations exhibited reasonably constant growth throughout most of the year (~5 mm/month). The slowest period of growth for all populations of *F. varium* was the sampling period between June and August (0-2.5 mm/month).
Figure 3.5. Modal progression of *F. lapillum* populations at A) sheltered, B) semi-exposed, and C) exposed locations from October 2012 through August 2015.
Figure 3.6. von Bertalanffy growth functions for *F. lapillum* based on growth parameters estimated from the length-based growth (Munro’s method) at sheltered, semi-exposed and exposed locations.
Figure 3.7. Modal progression of *F. varium* populations at A) sheltered, B) semi-exposed, and C) exposed locations from October 2012 through August 2015.
Figure 3.8. von Bertalanffy growth functions for *F. varium* based on growth parameters estimated from the length-based growth (Munro’s method) at sheltered, semi-exposed and exposed locations.

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Sheltered</th>
<th>Semi-exposed</th>
<th>Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. lapillum</em></td>
<td>Estimate of L&lt;sub&gt;∞&lt;/sub&gt; (asymptotic growth)</td>
<td>53.63 mm</td>
<td>59.47 mm</td>
<td>59.57 mm</td>
</tr>
<tr>
<td></td>
<td>Estimate of K (growth coefficient)</td>
<td>2.502</td>
<td>2.849</td>
<td>3.579</td>
</tr>
<tr>
<td></td>
<td>Estimate of length at 1 year</td>
<td>49 mm</td>
<td>55 mm</td>
<td>57 mm</td>
</tr>
<tr>
<td><em>F. varium</em></td>
<td>Estimate of L&lt;sub&gt;∞&lt;/sub&gt;</td>
<td>120.07 mm</td>
<td>123.20 mm</td>
<td>107.86 mm</td>
</tr>
<tr>
<td></td>
<td>Estimate of K</td>
<td>1.397</td>
<td>1.104</td>
<td>2.548</td>
</tr>
<tr>
<td></td>
<td>Estimate of length at 1 year</td>
<td>90 mm</td>
<td>83 mm</td>
<td>99 mm</td>
</tr>
</tbody>
</table>

### 3.3.2 Otolith analyses
3.3.2.1 Fish size

Total length for *F. lapillum* collected for the otolith analyses positively correlated with wave exposure (H$_3 = 117.712$, $P < 0.001$), with pairwise comparisons showing all wave exposure locations significantly different from each other ($P < 0.05$), except exposed vs offshore (fig. 3.9 A). *Forsterygion varium* collected for otolith analyses also showed the same positive correlation (H$_3 = 10.556$, $P = 0.014$), with pairwise comparisons showing sheltered fish significantly smaller than all other locations ($P < 0.05$) (fig. 3.9 B).

![Figure 3.9. Boxplots of fish length for A) F. lapillum, and B) F. varium for samples collected at different exposure locations in the Hauraki Gulf. Dashed lines represent sample means.](image)

3.3.2.2 Relationships between otolith growth and somatic growth

Otolith length showed a strong positive correlation with TL for both *F. lapillum* (fig 3.10 A, r$^2 = 0.84$, F$_{1,295} = 1504$, $P < 0.001$) and *F. varium* (fig. 3.10 B, r$^2 = 0.90$, F$_{1,257} = 2202$, $P < 0.001$). The relationship between otolith weight and TL was also strongly positively correlated for both *F. lapillum* (fig 3.10 C, r$^2 = 0.77$, F$_{1,302} = 988$, $P < 0.001$) and *F. varium* (fig. 3.10 D, r$^2 = 0.89$, F$_{1,267} = 2108$, $P < 0.001$). The strong relationships between otolith dimensions and TL in both species indicates that otolith growth is a good proxy for somatic growth rates, thereby validating the use of them in this study to compare growth between populations and species. These results are also consistent with otolith/fish length relationships for *F. lapillum* from other studies (Smith 2009, Kohn 2011).
3.3.2.3 Annual otolith counts

The maximum age for *F. lapillum* was three years old (fig. 3.11 A). Maximum age of *F. lapillum* positively correlated with wave exposure, where the two most sheltered locations (sheltered and semi-exposed) had a maximum age of two years old, and the two most exposed locations (exposed and offshore) were three years old (fig. 3.11 A). Similarly, maximum age can be described in *F. varium* as positively correlating with exposure, albeit less rigorously. The oldest *F. varium* analysed via annual otolith counts was four years, found at an exposed location (fig. 3.11 B). No sheltered *F. varium* individual was estimated at older than two years old (fig. 3.11 B). Size-at-age one estimated from the annual otolith counts was approximately 45-55 mm for *F. lapillum*, and 80-90 mm for *F. varium*. More specifically, the minimum sizes-at-age one year were 43.6 and 73.8 mm TL for *F. lapillum* and *F. varium*, respectively for (figs. 3.11 A, B).
Figure 3.11. Otolith annual increment counts for A) *F. lapillum*, and B) *F. varium* across a wave exposure gradient in the outer Hauraki Gulf. von Bertalanffy growth functions estimated from length-based growth (figs. 3.6 & 3.8) are overlaid for comparison (i.e. growth curves here are not created from the annual otolith counts). No offshore growth curves were estimated due to insufficient bi-monthly sampling in the length-based growth.
3.4 Discussion

3.4.1 Growth rate and size

Chapter three provides evidence that habitat influences intraspecific growth rates, maximum size and longevity. Growth rate in particular serves as a good proxy for post-settlement performance, and faster growth provides support for the fitness differences seen in chapter two. There are obvious fitness advantages to growing faster, as smaller fish are more vulnerable to predation, and so faster growth should decrease mortality. Furthermore larger size affords competitive advantages by being more successful at attaining resources and mates (as body size is important for mate choice). It was hypothesised in chapter three that there would be variation in growth across the wave exposure gradient, based on the fact that there were known differences in habitat use across this gradient (Wellenreuther et al. 2007).

There was direct evidence that bigger fish grew faster: growth rate positively correlated with wave exposure and length-at-age one year was greatest for both species at exposed locations. The differences in growth rate between wave exposure locations correlates to differences in resources. *Forsterygion lapillum* and *F. varium* are both generalist carnivores, preying upon small, mobile benthic invertebrates (Feary et al. 2009). However, the highest prey item by volume for both species are gammarid amphipods (Feary et al. 2009). Gammaridean amphipod density has been shown to strongly correlate with wave exposure, with amphipod densities up to 20 times higher at exposed sites relative to sheltered sites (Fenwick 1976).

Growth has previously been assessed in New Zealand triplefin larvae. Research on *F. lapillum* larvae in the Wellington region has shown higher quality larvae (including faster larval growth) in harbour (i.e. sheltered) versus open coast (i.e. exposed) larval populations (Shima and Swearer 2009a). Furthermore, the coastal larvae showed carry-over effects from their dispersal histories into recruitment and adulthood (Shima and Swearer 2009b, 2010, Shima et al. 2015). Shima and Swearer (2009a) attributed the higher quality coastal larvae to both more larval food and predators, thereby maximising growth and condition of larvae and selectively removing underperforming individuals. Conversely, in northern New Zealand, there were no pre-settlement differences in growth rates between four locations (including harbour and open coast sites) for *F. varium* (Kohn 2011). Furthermore, in the region of our study system (Hauraki Gulf and offshore islands), there is a higher diversity and abundance of mesozooplankton (larval food) coastally than offshore (Zeldis and Willis 2015), consistent with the Wellington region. Together, the positive correlation between growth and wave exposure in our study, in conjunction with a negative correlation between larval food supply and our wave exposure locations (Zeldis and Willis 2015), and no pre-settlement differences found in *F. varium* (Kohn 2011), suggest that the differences we
observed in growth and longevity are predominantly caused by post-settlement factors. We argue that the differences between our study system and the Wellington region are due to the unique geographical setting and associated hydrography of the Wellington Harbour embayment and Cook Strait.

There was seasonal variation in growth, with the most growth occurring in spring and summer, and lowest growth during winter. There are two main explanations for these growth patterns. The first is again related to resource availability, with the period of peak triplefin growth coinciding with the period of peak density of their prey. As mentioned above the highest diet category by volume for both *F. lapillum* and *F. varium* are gammarid amphipods (Feary et al. 2009). Seasonal abundances of amphipods in temperate locations have been shown to generally track the irradiance of light and primary productivity, with peak abundances during spring through late summer (Mukai 1971, Edgar 1983, Imada and Kikuchi 1984, Takeuchi et al. 1990). More specifically, studies from the Hauraki Gulf have shown gammarid amphipod densities to peak during summer in coralline turf (Choat and Kingett 1982) and *Carpophyllum* spp. habitats (Taylor 1998). However, there were no clear seasonal patterns in epifaunal amphipod abundance in *Ecklonia radiata* (Taylor 1998).

The second explanation for the observed patterns in triplefin growth relates to their spawning. The lowest growth (during winter) coincides with the peak spawning periods of these two species (Wellenreuther and Clements 2007). During this period, large amounts of energy are directed towards gonad production and spawning behaviour. *Forsterygion lapillum* experienced more sustained lower growth periods (up to half of the year) than *F. varium*. Although spawning peaks around August for both species, this can be explained by spawning being more protracted for *F. lapillum* (up to 11 months) than *F. varium* (up to 8 months) (Wellenreuther and Clements 2007). That growth increases immediately after peak spawning (August) further suggests decreased growth is linked to the spawning cycles in these two species.

Estimations from the length-based analysis show the growth curves of both species to have an asymptote. We suggest this apparent asymptote can be explained by the very short lifespan of these species. As lifespan does not extend much beyond the decline in growth rate, the asymptote is caused by senescence, and not by determinate growth, i.e. ~80% of maximum size is not reached in the first ~15 % of lifespan (sensu Choat and Robertson 2002). Furthermore, the growth curves were estimated from the length-based data, in which the cohorts were split into new recruits (0+) and adults (>1). This was due to fish growing so fast that it was unrealistic to separate 1+ and 2+ cohorts (e.g. *F. lapillum* are ~90-95% maximum size after one year). Therefore, growth is tracked accurately over the first year, but after that (of which the majority of
individuals don’t live past 2 years anyhow) cohorts are grouped together, resulting in exaggerated asymptotes. The difference between species (in percent of maximum size attained at year one) can be explained by *F. varium* living up to a year longer than *F. lapillum*.

### 3.4.2 Longevity

Maximum age was estimated as three and four years in *F. lapillum* and *F. varium*, respectively. The present study is the first to identify maximum age of a New Zealand triplefin at four years old, although it is suggested that in colder southern waters, longevity could be greater (Stewart and Clements 2015). Only three *F. lapillum* were estimated as three years old, and only one *F. varium* estimated as four, however, samples sizes were relatively low. Future research on these species would benefit from higher sample sizes, and more samples at the largest end of populations. Nevertheless, the estimates of maximum age provided here provide a starting point for future work.

Data suggests there was a positive correlation between maximum age and wave exposure for both species. This was particularly evident for *F. lapillum*. For *F. varium* it was more obvious that sheltered individuals did not live as long as fish at more exposed locations. Resolution of age was relatively low with only three to four age classes. Initial plans were to attain age and growth from daily increments, thereby providing a much greater level of resolution of length-at-age estimates. Nevertheless, the maximum age did provide evidence of general trends between broad scale habitats even at the level of annual increment resolution. As *F. lapillum* and *F. varium* at more exposed locations are larger (fig. 3.9 & chapter 2), this suggests that bigger fish – in addition to growing faster (see above) – are older.

Predation can be regarded as a strong factor in the differences seen between longevity at different wave exposure locations. Firstly, differences in growth between each location (see above) means that by attaining a larger size quicker, individuals spend less time in the vulnerable juvenile/early adult period when they are susceptible to predation. This is particularly important as adult triplefins (the most abundant reef fishes in New Zealand) are known to cannibalise juvenile triplefins (pers. obs., Montgomery 2003). Therefore reaching a size bigger than adult triplefin gape width is paramount to survival and consequently increased longevity. Secondly, more exposed habitats in the Hauraki Gulf are more complex (Shears and Babcock 2007). Structurally complex habitats can reduce predation rates by providing prey refuges (Anderson 1984, Savino and Stein 1989, Christensen and Persson 1993, Hixon and Beets 1993), including refuge in turbulence (Illich and Kotrschal 1990). Furthermore, predatory fishes have been found to be less
efficient foragers in more complex (Savino and Stein 1982, Anderson 1984, Persson and Eklov 1995) and turbulent environments (Landry et al. 1995).

### 3.4.3 Implications of faster growth and increased longevity

The results from this study demonstrate differences in growth rate and longevity across broad spatial scales. All else being equal, individuals at more exposed locations have a direct fitness advantage. By growing faster, this means that more exposed populations approach their first winter, and thus spawning season, in a more desirable situation. Thompson (1986) suggested that very few *F. varium* less than ~80 mm in length were successful in spawning. Considerably more *F. varium* at exposed populations, relative to other locations, attain ~80 mm by their first winter (see fig. 3.8), thereby having a direct performance advantage by increasing their chances at obtaining and defending a territory and attracting mates. Similarly, increased longevity can result in higher reproductive output. If an individual at a certain location can live an extra year than in other populations (see fig. 3.11), then they have an extra spawning season to produce offspring. Furthermore, as egg production is exponentially proportional to fish size (Hixon et al. 2014; and references therein), then the larger fish at more exposed locations will have considerably higher fecundity per spawning event, and thus transferring more of their genes onto the next generation.

The differences in demographic variables provide evidence that *F. lapillum* and *F. varium* have higher performance at more wave exposed habitats, thus providing a potential for selection. As there is no spatial segregation between populations (i.e. populations of *F. lapillum* are sympatric in the Hauraki Gulf – Rabone et al. 2015), then intraspecific interactions can lead to divergence in habitat use. That is, frequency-dependent selection can generate disruptive selection if there are alternate phenotypes, as there will be fitness consequences for phenotypes that match the habitats conferring higher fitness (i.e. more wave exposed sites) versus those that do not.

### 3.4.4 Conclusions and future directions

This chapter revealed that both maximum age and growth rates for *F. lapillum* and *F. varium* positively associated with wave exposure in the Hauraki Gulf. We provide evidence that larger fish are larger due to a combination of living longer and growing faster. Ideally, we would have higher resolution age data (i.e. daily increments) to more precisely compare length-at-age and maximum age. Nevertheless, these data still provide strong evidence of variations in growth and longevity. Furthermore, the short lifespan and fast growth rates estimated for these two triplefin species verify the use of a length-based approach to assessing growth, which is generally regarded
as only being suitable for fast-growing short-lived fishes. Future work would benefit from assessing early growth rates. As daily otolith increments have been successfully assessed in larval and juvenile triplefins (Shima and Swearer 2009b, Kohn 2011, Kohn and Clements 2011), a targeted collection during early summer (~3-4 month individuals) would permit comparing early growth rates across broad spatial scales using the more precise daily otolith increments.

The demographic work provided some interesting insights into the distribution and abundance data in this system. The differences here provide evidence of intraspecific variation in demography in each species, and infers differences in post-settlement performance driven by broad scale habitat variables. We suggest these differences could be due to the availability of resources and the sheltered afforded by the complex environment at more exposed locations. By demonstrating intraspecific differences in proximate mechanisms (i.e. demography) in both species, we further inform our understanding of the adaptive habitat diversification responsible for generating the diverse New Zealand triplefin assemblage.
Chapter 4

Phenotypic variation correlates with wave exposure in the temperate reef fish, *Forsterygion lapillum*

4.1 Introduction

Phenotypic variation between populations plays a central role in the early stages of evolutionary divergence (Schluter 2000). The environmental control of phenotypic expression, combined with variation in the environment, may result in populations being distinguishable from one another in morphology, behaviour, physiology and/or life history (Morrissey 2011). However, the specific mechanisms generating this phenotypic variation remain unclear (Langerhans 2008). The study of morphological variation in fish populations in relation to environmental gradients is therefore important in evaluating the nature of divergent natural selection in fish radiations.

Environmental heterogeneity across a species’ range may exert local selective pressures that act to maximise individual fitness within specific environments (Kawecki and Ebert 2004). Aquatic habitats are such environments, where physical conditions can change markedly across the geographical range of a species. For many fishes, water flow regimes impose demanding challenges to performing ecologically-important activities such as feeding and predator avoidance. Sustained increases in water flow can induce changes in the body form of fish, and the effects of water action on morphology in fishes are well documented, with phenotypic differences often generated across water velocity gradients (Langerhans 2008: see references therein, Binning and Roche 2015). One of the primary examples of environmentally-induced change are those related to locomotion (Vogel 1994, Webb 1994, Domenici 2003). Fishes that lead benthic lives however, are generally considered poor swimmers, and adapt hydrodynamically to water flow regimes in different ways to maintain their position on the substratum (Webb et al. 2010). In areas of high flow environments, this often result in changes in body shapes that best reduce drag (e.g. Walker 1997, Langerhans et al. 2003). These primarily drag-reduction and energy-saving strategies are seen in fishes such as sculpins (e.g. Witkowski 1979, Kerfoot and Schaefer 2006, Bogdanov 2007). Sculpins and similarly shaped benthic fishes are described as cottiform in shape (Webb 1994) (i.e. small benthic fishes that rest on their pectoral fin rays), and triplefins also fit this general description.
A diversity of fin shapes exist in fishes (Domenici 2003). In particular, pectoral fin size and shapes are largely determined by function and habitat, and play an important role in interactions with water movement (Webb 1994), being central to swimming performance and efficiency across a wide range of fishes (Walker and Westneat 2002b, Blake 2004). Pectoral fins vary from drag-based rowing with rounded fins to lift-based flapping with tapered fins (Blake 1981, Vogel 1994, Walker and Westneat 2002b, Fulton et al. 2013). Furthermore, fin shape differences have been observed both among species (Fulton et al. 2001, Kane and Higham 2012, Aguilar-Medrano et al. 2013, Binning and Roche 2015, Colombo et al. 2016) and within species (Fulton et al. 2013, Binning et al. 2014, Natsumeda et al. 2014), underlining a similar pattern of morphological variation observed at the family and species levels. Triplefins have well developed pectoral fins (Stewart and Clements 2015), and to the authors knowledge, their specific form and function has not been previously examined in detail; though the functional role of pectoral fins in other benthic-associated fishes is to hold position in strong water flows fishes (e.g. Webb 1989, Wilga and Lauder 2001, Taft et al. 2008, Kane and Higham 2012). As natural selection is predicted to favour different morphologies under alternative flow conditions, it can consequently drive major patterns of phenotypic variation in fishes (Langerhans 2008).

Body size and shape are phenotypic traits that are easily observed (Zelditch et al. 2012), and variation in both can be obvious (McKinnon and Rundle 2002). The analysis of morphometric characters has been widely accepted and used by fish biologists as a way to differentiate amongst different species and amongst populations within a species (e.g. Fulton et al. 2005, Gomes and Monteiro 2008, Kerschbaumer et al. 2014, Natsumeda et al. 2014). The focus of the present study investigates these morphological traits in the common triplefin, *Forsterygion lapillum* (F. Tripterygiidae). *Forsterygion lapillum* is one of the most abundant species on coastal reef habitats in New Zealand (Feary and Clements 2006, Wellenreuther et al. 2007, Smith et al. 2013) inhabiting a wide range of habitats from sheltered harbours to exposed rocky reefs. This species thus encounters considerable variation in depth, exposure and co-occurring biota. However, given the small territory and extreme philopatry in this small demersal fish, environmental variables that individuals are exposed to vary little following settlement. Analysis of habitat variables confirmed that *F. lapillum* is the most generalist species of triplefin studied in New Zealand (Wellenreuther et al. 2009). Van Valen’s (1965) “niche variation hypothesis” suggests that populations with broader niches (i.e. generalist populations) should be phenotypically more variable than those with narrower niches (i.e. specialist populations). This has been shown empirically across a wide range of taxa, from plants (Sultan et al. 2009) to fishes (Svanback and Schluter 2012). Additionally, DNA analyses show there to be a lack of genetic structure, and thus extensive gene flow in *F. lapillum* across the Hauraki Gulf (Rabone et al. 2015).
The aim of the present study was to investigate phenotypic variation in *F. lapillum* across spatial scales that we know to have no genetic structure in this species. More specifically, we tested the relationship between wave exposure and morphology in *F. lapillum* populations throughout the Hauraki Gulf, using both traditional linear morphological measurements and a geometric morphometric approach. Based on previous literature on benthic-associated fishes, we hypothesise that a more robust *F. lapillum* body form (i.e. larger in the anterior region) will be evident at more wave exposed sites. Additionally, we aimed to quantify variation in size and shape of pectoral fins. We hypothesise that pectoral fin morphology will be strongly correlated with wave exposure, as pectoral fins are very important in hydrodynamics and locomotion in many fish groups (Webb 1994). Understanding phenotype-environment relationships in this generalist triplefin in the light of literature information will improve our understanding of the processes generating intraspecific variation in this fish species, and ecological speciation in general.

### 4.2 Methods

#### 4.2.1 Sampling

Sampling was undertaken within the Hauraki Gulf in northeastern New Zealand (Fig. 4.1), a region that comprises an area of approximately 4000 km², and covers a wide range of exposures, depths and habitats, including both coastal and offshore locations. Sampling sites spanned a wave exposure gradient, based on fetch and wind/wave energy (see below), with replicate sites for sheltered, semi-exposed, exposed to offshore (i.e. very-exposed) exposure groupings. See table 1 for details of exposure calculations.

All fish samples were collected in accordance with the University of Auckland Animal Ethics Committee approval 001047. Fish were captured using handnets on SCUBA or using baitcatchers at an average of five metres depth, with no fish collected more three metres shallower or deeper. Fish were euthanised upon return to the boat or wharf using an overdose of clove oil (active ingredient eugenol), and then immediately frozen. Samples were subsequently thawed in the laboratory before all measurements and digital photographs were taken. All fish collected were adults.
Figure 4.1. Map of sample sites in the Hauraki Gulf. Inset 1 shows sites on the coastal mainland: sheltered (Tw, Jo), semi-exposed (No, Ma, Wa), exposed (Ta). Mo and GB are offshore islands (very exposed). Inset 2 shows Hauraki Gulf location in the North Island of New Zealand.

4.2.2 Wave exposure analysis

A combination of wave power data, fetch, and wind-derived exposure were used to verify the wave exposure groupings used in the present study. Empirical wave energy data derived from MetOcean Wavewatch (NOAA 2016) was used to calculate wave exposure. Wave power was chosen as the variable of interest, as it incorporates wave height and period. Spatial resolution from the grid cells of these data was not high enough to permit delineation between closely
situated sites, such as along the Leigh coast. However, it did permit accurate wave power comparisons between broader locations, such as the Leigh coast and the offshore islands (see appendix for graphic representation).

More precise levels of wave exposure at each site were approximated using an index of potential fetch, which is defined as the area of the sea surface over which waves are generated by wind with a constant direction and speed (Denny 2005). The exposure of each site was calculated as the total sum of the fetch based on defined settings, using the program ‘Fetch Effect Analysis’ (Villouta 2000). For each site location, each 10º on a compass rose a vector was projected a distance of 300 km, or until land is encountered, and all distances summed. The use of fetch as a proxy for exposure does not take into account differences in wave spectra among areas (Denny 1988), however, it does permit tentative conclusions to be made (Thomas 1986). Furthermore, in north-eastern New Zealand it is suitable, as the longest fetch and largest swells are from the east/northeast (Denny 2005).

To further verify the wave exposure groupings, wind-derived exposure calculations were performed (e.g. Burrows et al. 2008). Historical wind data was attained for a 10 year period (2005-2015) at a three hourly resolution for maximum wind speed and direction (NIWA 2016). These were recorded from wind stations closest to the representative sites (outer gulf coast=Leigh; inner gulf=Whangaparaoa; offshore islands=Mokohinau Islands). Within each data set, maximum speed was averaged for each 10º vector, and multiplied by the corresponding vector for fetch calculations for each site. Subsequently, all 36 vector calculations were summed to get a site total (see table 4.1).
Table 4.1. Fetch and wave exposure calculations and coordinates for each sampling site.

<table>
<thead>
<tr>
<th>Location</th>
<th>Exposure Grouping</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Sum of Fetch (km)</th>
<th>Max Wind*Fetch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ti Point wharf</td>
<td>Sheltered</td>
<td>36°19'03.80&quot;S</td>
<td>174°47'01.97&quot;E</td>
<td>38</td>
<td>916</td>
</tr>
<tr>
<td>Jones Bay</td>
<td>Sheltered</td>
<td>36°22'49.59&quot;S</td>
<td>174°47'04.1&quot;E</td>
<td>415</td>
<td>11545</td>
</tr>
<tr>
<td>Mathesons Bay</td>
<td>Semi-exposed</td>
<td>36°18'13.87&quot;S</td>
<td>174°47'01.97&quot;E</td>
<td>1110</td>
<td>32266</td>
</tr>
<tr>
<td>Nordic</td>
<td>Semi-exposed</td>
<td>36°17'35.56&quot;S</td>
<td>174°48'38.58&quot;E</td>
<td>925</td>
<td>25890</td>
</tr>
<tr>
<td>Waiheke</td>
<td>Semi-exposed</td>
<td>36°46'34.55&quot;S</td>
<td>175°02'04.26&quot;E</td>
<td>1675</td>
<td>36881</td>
</tr>
<tr>
<td>Takatu Pt.</td>
<td>Exposed</td>
<td>36°22'02.95&quot;S</td>
<td>174°52'07.61&quot;E</td>
<td>2227</td>
<td>62376</td>
</tr>
<tr>
<td>Great Barrier Island</td>
<td>Offshore</td>
<td>36°03'11.89&quot;S</td>
<td>175°24'10.98&quot;E</td>
<td>4965</td>
<td>186925</td>
</tr>
<tr>
<td>Mokohinau (Edith)</td>
<td>Offshore</td>
<td>35°54'30.48&quot;S</td>
<td>175°06'25.93&quot;E</td>
<td>4674</td>
<td>174821</td>
</tr>
<tr>
<td>Mokohinau (Archway)</td>
<td>Offshore</td>
<td>35°54'41.43&quot;S</td>
<td>175°06'08.78&quot;E</td>
<td>700</td>
<td>26847</td>
</tr>
</tbody>
</table>

4.2.3 Body shape morphometrics

Fish for body shape and morphology were collected between January and June 2008 from a previous study (Croq 2009) at locations corresponding to three of these wave exposure levels (sheltered, semi-exposed and offshore). All fish collected were adults, and are assumed to represent the adult shape although not necessarily maximum size. Collection sites include Ti Point Wharf (TW), Jones Bay (Jo), Mathesons Bay (Ma), Waiheke Island (Wa), Mokohinau Islands (Mo), Great Barrier Island (GB). Note: these samples were also the same that DNA analyses were performed on, showing a lack of genetic structure in Hauraki Gulf *F. lapillum* (Rabone et al. 2015). Sex was attributed to individuals where possible, by presence of gonads in the peritoneal cavity.

4.2.3.1 Linear head and body measurements

Twelve different morphological measures of *F. lapillum* were taken on 370 individuals. Linear measurements were made using Vernier calipers between two homologous points on the fish (Fig 2a). Length measurements included: total length (TL), eye diameter (ED), interorbital width (IOW), head length (HL), maximum body depth (BD), pre-pectoral length (PPL), pre-dorsal length (PDL), gape width (GW), gape length (GL), post-anal length (PAL), caudal peduncle depth
(CPD), and caudal fin length (CFL). Regions of interest were chosen with respect to foraging traits and habitat characteristics described in the literature for other fish species (Fisher 1998, Blake 2004, Langerhans et al. 2004, Riopel et al. 2008). All measurements (except total length) were divided by total length to standardise for fish size. Non-parametric analyses of variance (Kruskall-Wallis one way ANOVA tests) were used to test for statistical differences of each measurement across the exposure gradient. Pairwise comparisons using Dunn’s method was employed where significant interactions occurred.

4.2.3.2 Geometric morphometrics

Morphometric analyses were performed on 304 *Forsterygion lapillum* individuals. Digital images were taken of the left lateral side of each individual on a dark background, with a scale included beside the fish. Any fish that could not be positioned flat and on a straight axis was not used in the analyses. The external body shape of each fish was outlined by digitizing 15 homologous landmarks (fig. 4.2), plus two landmarks on the scale bar, using the software program TpsDig2 (Rohlf 2008). Landmarks were then superimposed to remove differences due to orientation, position and size (Sheets 2004). To arrive at the best biological description, we used two different superimposition methods to interpret shape, both Partial Procrustes superimpositions (hereafter Procrustes superimposition) and Bookstein coordinates. Procrustes superimposition involves calculating the centroid of each configuration, and then making this centroid the centre of the coordinate system (Webster and Sheets 2010). Bookstein coordinates uses fixed baseline endpoints (landmarks 1 to 7 were chosen, as they are longest natural axis that passes through the centroid of the fish) and centres the coordinates from them, losing the variance from those two landmarks in the process. Both sets of results were in general agreement, however, Bookstein coordinates were easier to interpret, therefore subsequent analyses were undertaken using these superimpositions. All subsequent geometric morphometric analyses were conducted using the IMP software series (Sheets 2004), unless otherwise stated. After superimposition, the data were converted into principal warps using the thin-plate spline (TPS) model (Bookstein 1991). Thin-plate spline files were translated into the IMP format by using CoordGen6, and subsequently loaded into CVAGen6 for canonical variates analysis (CVA). These variables could then be used in conventional multivariate analyses because they possess the same number of variables as degrees of freedom (Zelditch et al. 2012). To test for allometric scaling, linear regressions on shape (landmarks) versus size (log centroid size) was run using the software program TpsRegr (Rohlf 2008). Results indicated very weak allometry, as size only explained 1-3% of variation in shape for the different populations, therefore multivariate analyses were continued without
correction for size. Canonical variates analysis (CVA) is a multi-axis discriminant function and an ordination method for depicting differences visually, and for testing the ability of variables to correctly predict group (e.g. population or species) differences based on measurements (Zelditch et al. 2012). Rather than a set of loadings along length or width variables, as is commonly produced when using traditional morphometrics based on length or width measurements, the mean body shapes and deformation grids were produced (Yee et al. 2011). Deformation grids and landmark displacement vectors based on the thin-plate spline model were used to visualize the regions of greatest discrimination for each exposure group. Following this, multivariate analysis of variance (MANOVA) was conducted on the mean shapes. Shape-based assignments (Bartlett’s assignment test) were performed in CVAGen6 (Sheets 2004), calculating the mean Mahalanobis’ distances between each exposure group, to determine the probability that each specimen is closer to the mean of the group that it was assigned a priori, than that of another group. Lastly, each exposure group was tested directly against each other using Hotelling’s T2 test on the Bookstein coordinates (table 4.2). Similarly, Hotelling’s T2 test was also used to compare differences in shape using Bookstein coordinates between both sexes in each exposure location. The percentage of individuals correctly assigned to their respective groups for males and females was also calculated using a Bartlett’s assignment test based on Mahalanobis’ distance.
4.2.4 Pectoral fin morphology

Fish for pectoral fin morphology were collected between September 2013 and March 2014 from all four exposure groupings along the wave exposure gradient (these are the same samples collected for ageing work – chapter 3). Collection sites included Ti Point Wharf (TW), Jones Bay (Jo), Mathesons Bay (Ma), Nordic (No), Takatu Point (Ta), Mokohinau Islands (Mo), Great Barrier Island (GB). Notes: due to weather restrictions on different sampling trips, fish were collected from a different location for pectoral fin morphology (Archway Bay – westerly facing) compared with the fish for body morphometrics and morphology (Edith Passage – easterly facing). There was also no replicate site for exposed fish collections, as the replicate site for this
in the wider study, and the only corresponding site, was in a no-take marine reserve (Waterfall Reef, Cape Rodney to Okakari Point marine reserve).

Pectoral fin morphology was analysed for 250 *F. lapillum* individuals. Digital images were taken of the amputated left pectoral fin of each individual spread to full extension, with a scale included beside the fish. Any fin that was damaged or could not be positioned flat was removed from the analysis. All images were analysed using ImageJ software (version 1.46r), generating measurements of the leading edge and total fin area for each photographed fin. Area was standardised for fish size by dividing the area by the total length of the individual. Aspect ratio was then calculated as the leading edge squared, divided by the standardised total area. Kruskall-Wallis one way ANOVA on ranks tests were used to test for differences of pectoral fin size, and one way ANOVA tests for pectoral fin aspect ratio across the exposure gradient. Pairwise comparisons using Dunn’s and Holm-Sidak methods were employed respectively where significant interactions occurred.

### 4.3 Results

#### 4.3.1 Head and body morphology

Total length of *F. lapillum* differed significantly between sites (\(H_3 = 90.29, P < 0.001\)), with median total fish lengths of 45.64 mm, 48.7 mm, 52.02 mm and 50.5 mm for sheltered, semi-exposed, exposed and offshore populations, respectively (fig. 4.3). Pairwise comparisons showed all groups to differ significantly from each other (all \(P < 0.05\)), except exposed vs offshore. The largest recorded fish was 66.17 mm total length from the Mokohinau Islands (offshore), and the largest size range of adult fish collected was 31.55 mm, also offshore (fig. 4.3).

The areas of greatest phenotypic differentiation, along with overall length, were in the eye and head region (figs. 4.4 A, C). Eye size and inter-orbital width were greater at sheltered sites (\(H_2 = 53.11, P < 0.001\) and \(H_2 = 62.71, P < 0.001\), respectively). Pre-dorsal length was negatively correlated with wave exposure (\(H_2 = 178.39, P < 0.001\)), and both head length (\(H_2 = 94.29, P < 0.001\)) and pre-pectoral length (\(H_2 = 64.89, P < 0.001\)) were shorter offshore compared to other sites. Body depth was greater at offshore sites compared to other sites (\(H_2 = 26.99, P < 0.001\)). No differences were found for gape width (\(H_2 = 9.39, P = 0.06\)) or gape length (\(H_2 = 1.59, P = 0.451\); fig 4.4 C) between sites varying in wave exposure. The caudal peduncle was deeper in *F. lapillum* from sheltered sites than either semi-exposed or offshore sites (\(H_2 = 10.08, P = 0.006\); fig. 4.4 D). Semi-exposed fish were shorter in post-anal length than those from both sheltered and offshore
sites ($H_2 = 22.13, P < 0.001$). There was no difference in caudal fin length ($H_2 = 5.75, P = 0.064$) between sites varying in wave exposure.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Total Length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheltered</td>
<td>20</td>
</tr>
<tr>
<td>Semi-exposed</td>
<td>30</td>
</tr>
<tr>
<td>Exposed</td>
<td>40</td>
</tr>
<tr>
<td>Offshore</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>70</td>
</tr>
</tbody>
</table>

Figure 4.3. Boxplot of total length of adult *F. lapillum* from sites differing in wave exposure.
Figure 4.4. Mean standardised morphological measurements of the A) eye region, B) mouth region, C) head region, and D) tail region of *F. lapillum* by exposure. Bars represent standard errors of the mean. Abbreviations: total length (TL), eye diameter (ED), interorbital width (IOW), gape width (GW), gape length (GL), maximum body depth (BD), head length (HL), pre-pectoral length (PPL), pre-dorsal length (PDL), post-anal length (PAL), caudal peduncle depth (CPD), and caudal fin length (CFL).
4.3.2 Pectoral fin morphology

Pectoral fin size of *F. lapillum* differed significantly across the wave exposure gradient ($H_3 = 26.74, P < 0.001$; fig. 4.5 A). Fin size was positively correlated with wave exposure for coastal sites, with exposed fish having larger fins than both sheltered and semi-exposed sites ($P < 0.01$ and $P < 0.05$, respectively). Fish from offshore sites, however, had the smallest fin size of all sites. The mean aspect ratio of the pectoral fin negatively correlated with wave exposure ($F_{1,161} = 16.068, P < 0.001$) (fig. 4.5 B). Aspect ratio differed across all wave exposures sites ($P < 0.05$), except sheltered and semi-exposed sites ($P = 0.12$).

![Graph A: Fin Size](image)

![Graph B: Aspect Ratio](image)

**Figure 4.5.** Mean measurements of pectoral fin morphology for *F. lapillum* across an exposure gradient. (A) Fin size, and (B) aspect ratio. Bars represent standard errors of the mean.
4.3.3 Geometric morphometric analyses

Morphometric analyses based on the Bookstein coordinates of the landmarks showed that body shape varied significantly among wave exposure groups, and that the morphometric data were congruent with the univariate morphology measurements. Each exposure group formed distinct clusters in a canonical variate analysis (CVA) along the first two axes (fig. 4.6 A). Differentiation in body shape as captured by CV axes is depicted visually using deformation grids with vector displacement of landmarks (fig. 4.6 B, C). Mahalanobis’ distance-based test (Bartlett’s test) run on the canonical variates showed that assignment of specimens to the correct groups was accurate 79.2, 71.2, and 90.3 percent of the time for sheltered, semi-exposed and offshore exposure groups, respectively. Subsequently, pairwise comparisons using Hotelling’s $T^2$ test on Bookstein coordinates revealed significant differences in body shape between each wave exposure group, i.e. sheltered vs semi-exposed ($F_{30, 163} = 6.24, P < 0.001$), sheltered vs offshore ($F_{30, 209} = 18.08, P < 0.001$), and semi-exposed vs offshore ($F_{30, 153} = 20.18, P < 0.001$). Multivariate analysis of variance (MANOVA) showed that the first axis was the most meaningful ($\lambda = 0.24, \chi^2_{52} = 387.05, P < 0.001$, eigenvalue = 1.68), with this axis depicting significant variation in body depth and the head region (fig. 4.6 B). The second axis was also significant ($\lambda = 0.65, \chi^2_{25} = 119.28, P < 0.001$ eigenvalue = 0.55), and was primarily related to body length and the eye region (fig. 4.6 C).

Figure 4 shows thin-plate spline deformation grids comparing the mean group shapes of fish from exposed and sheltered sites. Fish from exposed sites were longer overall, had a deeper body, and a more anteriorly-positioned pectoral fin base (fig. 4.7 A). Fish from sheltered sites had larger eyes set further apart, as well as a longer head region in general (fig. 4.7 B). Relative differences in pectoral fin shape and size are depicted also.
Figure 4.6. A) Canonical variate analysis (CVA) of *F. lapillum* body shape landmarks from sites differing in wave exposure: sheltered (triangles), semi-exposed (crosses), and offshore (stars) locations. Deformation grids with relative displacement vectors for each landmark, visualise the areas of greatest body shape change captured by B) CVA axis 1, and C) axis 2.
Figure 4.7. Thin-plate spline visualisation of mean shape differences between *F. lapillum* from A) offshore and B) sheltered sites. All differences are linearly extrapolated by a factor of 3.
There was also clear evidence of sexual dimorphism across the wave exposure gradient. Multivariate analysis of variance comparing males versus females and exposure yielded four significant canonical variate axes (table 4.2). These axes described differences in most of the main morphological characters, including body depth, head region, body length, eyes and fin position, with males being generally larger overall. Subsequent pairwise comparisons using Hotelling’s $T^2$ tests showed that males and females differed in body shape at both offshore ($F_{30, 84} = 3.523, P < 0.001$) and sheltered sites ($F_{30, 94} = 2.4, P < 0.001$), although not at semi-exposed sites ($F_{30, 37} = 1.43, P = 0.148$). Bartlett’s test for distance-based assignment indicated that all fish were correctly assigned to their respective groups between 60 and 80 percent of the time, except for females from semi-exposed locations, of which 23% were correctly assigned.

**Table 4.2. MANOVA results for canonical variate analysis comparing males vs females and exposure location.**

<table>
<thead>
<tr>
<th>Axis</th>
<th>df</th>
<th>$\lambda$</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>Eigenvalues</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVA 1</td>
<td>130</td>
<td>0.1292</td>
<td>552.6171</td>
<td>&lt; 0.001</td>
<td>1.80094</td>
</tr>
<tr>
<td>CVA 2</td>
<td>100</td>
<td>0.3618</td>
<td>274.5295</td>
<td>&lt; 0.001</td>
<td>0.650015</td>
</tr>
<tr>
<td>CVA 3</td>
<td>72</td>
<td>0.5969</td>
<td>139.3178</td>
<td>&lt; 0.001</td>
<td>0.312904</td>
</tr>
<tr>
<td>CVA 4</td>
<td>46</td>
<td>0.7837</td>
<td>65.8126</td>
<td>0.029</td>
<td>0.165028</td>
</tr>
</tbody>
</table>

### 4.4 Discussion

Chapter four demonstrated that morphological variation in *F. lapillum* was strongly correlated with wave exposure, with greatest differences were seen in body length, head and eye size, and pectoral fin shape and size, in addition to sexual dimorphism. Local adaptation to environmental parameters has been shown extensively in the threespine stickleback complex and other freshwater fishes (e.g. McKinnon and Rundle 2002, Langerhans et al. 2003, Reimchen and Nosil 2006, Martin and Wainwright 2011). The fact that this variation manifests repetitively over different spatial scales (e.g. the Jones Bay-Takatu Point gradient and the onshore-offshore gradient) indicates that wave exposure is a very strong factor in driving local adaptation.

There are biophysical reasons to think that the morphological variation in *F. lapillum* is functionally significant, and if this is the case then this would have fitness consequences. The morphological divergence between sites varying in wave exposure, in conjunction with the lack of genetic structure across these populations (Rabone et al. 2015), largely suggests a plastic phenotypic response in this species to the physical force of water movement. Further work
addresses the evolutionary implications of this phenotypic variation in greater detail (chapter 5). Here we explore the biomechanical basis for variations in phenotype in this species in response to the oscillatory flows produced by wave energy.

4.4.1 Functional implications in changes in body morphology

Mean adult length of *F. lapillum* correlated positively with wave exposure. Population differences in body length are often thought to reflect maximum life span, as longer-living fish would ultimately attain larger sizes (Reimchen and Nosil 2006). From ageing research on this species (chapter 3), we know that *F. lapillum* grows significantly faster at more wave exposed sites, and the oldest fish were also found at exposed and offshore sites. Therefore, differences in body length can be, at least partly, explained by these demographic differences. However, substantial theoretical and empirical evidence has shown that the survival of fish is not exclusively related to size (e.g. Pepin et al. 1992, Cowan et al. 1996), and thus body shape is also very important.

Offshore *F. lapillum* were deeper bodied and more robust anteriorly compared to fish from other sites. Locating the centre of mass further forward is thought to improve hydrodynamics in higher water velocities (Arnold et al. 1991, Webb 1994). However, most examples in fishes with an anterior centre of mass are cottiform and usually live in fast-flowing streams and rivers, or the shallow intertidal, where they experience very high water flow. *Forsterygion lapillum* lives predominantly in the shallow subtidal, where the water flow is more turbulent due to the combined effects of wind, wave and swell, in conjunction with diverse topography, but probably less extreme than the unidirectional flow encountered in most fluvial systems.

Locomotory behaviour can vary within fish functional groups, and triplefin behaviour differs from many cottiform fishes. For example, sculpins are largely sit-and-wait predators (Norton 1991), whereas most triplefins (including *F. lapillum*) are more active predators (Feary et al. 2009). As a corollary, most triplefins do not conform to a solely drag-reduction behaviour like most other cottiform-shaped fishes, and frequently swim in short bursts just above the substratum while chasing prey, and also while interacting with other fishes in antagonistic displays (Thompson and Jones 1983; pers. obs.). A more robust anterior body form can enhance stability while swimming (Domenici 2003), and Videler (1993) suggests that fishes from deeper more structurally complex habitats would exhibit deeper bodies. This difference in habitat combined with more active behaviour could explain the differences seen in head and body morphology relative to that seen in cottiforms and other small benthic fishes. The present study could have benefitted from more width based measurements, to address the hydrodynamically relevant body fineness in more detail. Locomotory behaviour is therefore of importance with regard to body morphology, and this
relationship with the environment is key to understanding intraspecific differences seen across the wave exposure gradient. Since head and body morphology reflect the composite of several functional systems (i.e. feeding, locomotion, sensory physiology etc.), adaptation of these regions may be less flexible to changes in flow demand (Kane and Higham 2012). Consequently, the intraspecific differences seen in this study may reflect functional responses to water flow while also reflecting responses to a host of other functional demands, and these potentially competing fitness peaks can complicate correlations with water flow. As a result, parts of the body that have fewer functional roles (e.g. pectoral fins) may provide better tests of hypotheses for morphological differentiation.

4.4.2 Functional implications to changes in pectoral fin morphology

The pectoral fins in triplefins are well developed (Stewart and Clements 2015), suggesting an important functional role. Size and shape of pectoral fins in *F. lapillum* strongly correlated with wave exposure. Fish from sheltered sites had small and high aspect ratio pectoral fins (more tapered), with fin size becoming larger and aspect ratio lower (broader) as wave exposure increased. Such a strong fin morphology-wave exposure relationship suggests that variation in fin morphology is directly related to the oscillatory water movement. An exception to the pattern was pectoral fin area in offshore fish, which we attribute to the aspect and thus fetch of the Archway Bay site at the Mokohinau Islands. The orientation of this location is such that it would receive the lift but not the full energy of oceanic swells, and this may be a factor in the smaller fin sizes at this site.

The primary function of pectoral fins across many taxa is for propulsion (Blake 1981, Webb 1994), and labriform locomotion is the most ubiquitously studied form of paired median fin-based propulsion (Walker and Westneat 2002a, b). The morphology of pectoral fins in this swimming mode has been shown to correlate with wave exposure gradients, with higher aspect ratio usually seen in fishes that inhabit more exposed, wave-swept habitats, where they are associated with higher swimming speeds (Fulton et al. 2005). As described in the previous section, most triplefins are intermediate in swimming mode between the free swimming bentho-pelagic fishes and the bottom-clingers such as cottiforms. Triplefins do not exhibit sustained swimming behaviour, lacking a swim-bladder to aid in buoyancy control (Clements 2003), however, they do swim in bursts not far from the substratum (Francis 2013; pers. obs.). Consequently, the pattern of pectoral fin size and shape in *F. lapillum* associated with wave exposure differs from that described in other studies of wave exposure-driven pectoral fin morphology (e.g Bellwood et al. 2002, Fulton et al. 2005, Fulton et al. 2013). Notothenoids are benthic-associated fishes with enlarged pectoral
fins that more closely resemble triplefins in body form and subtidal habitat. They are primarily stationary benthic fishes lacking a swimbladder, and their pectoral fins are thought to contribute to the acceleration of fast start-gait propulsion (Montgomery and Macdonald 1984). However, they use sculling pectoral fins rather than caudal propulsion for these rapid escapes, and have a largely labriform swimming mode (Montgomery and Macdonald 1984).

Benthic-associated fishes are often characterised as having low lift but relatively high drag, and offset the drag by increasing friction, such as with extensive pectoral fins capable of grasping the substratum and/or creation of negative lift (Webb 1994). *Forsterygion lapillum* lacks both the fin ray hooks seen in many families such as scorpionfishes, hawkfishes and blennies (Brandsatter et al. 1990), and the suction mechanisms found in clingfishes (Kleinteich et al. 2014). Nor is there any distinct regionalisation in pectoral fins, such as those found in gurnards (Finger 1982) or certain sculpins (Taft et al. 2008). Cottiform-shaped fishes use body and caudal fin propulsion for a fast start-gait swimming mode, rather than sustained swimming (Webb 1994), and pectoral fins are thus more adapted for position holding (Webb 2006). Since lift increases with the square of fluid speed across a hydrofoil, increased negative lift can be generated by higher water flow (Webb 1994), and can be improved by changes in fin morphology, fin position and behaviour. Positioning pectoral fins at a negative angle, and thus using water flow to create downward force, is a common behaviour seen in a variety of fish groups from benthic sharks (Wilga and Lauder 2001) to teleosts (Webb 1989, Webb et al. 1996, Coombs et al. 2007, Taft et al. 2008, Carlson and Lauder 2010).

We argue that the locomotory mode of triplefins is intermediate between the caudal fin swimming mode of cottiforms and the sculling mode of labriforms. This is a consequence of triplefins, which lack swimbladders, spending a fair proportion of time resting on the substratum, yet still remaining active in sustained bursts. It is feasible that the pectoral fin could act in a dual role, thus creating negative lift when stationary, and aiding stability and locomotion when actively swimming in the water column. The contribution of each function (negative lift or stability/propulsion) could be dependent on the degree of turbulence, as for example sculpins’ pectoral fin function changes from primarily gripping in low-flow species, to a more equal dependence on gripping and lift generating in high-flow species (Kane and Higham 2012). This is consistent with the variation in pectoral fin morphology we see here in relation to wave exposure. Further research to address the apparent trade-off in triplefin pectoral fin performance and function is worth considering.
4.4.3 Eye region

*Forsterygion lapillum* from more exposed habitats had larger eyes that were set closer together (i.e. more dorsally positioned with smaller interorbital distance). Eye morphology can differ markedly between fishes living in different water flow regimes (Bogdanov 2007, Haas et al. 2015), although the mechanism for this remains elusive. Length-weight relationships (ordinary least-squares linear regression) in both the present study (data not shown here) and others (Taylor and Willis 1998, Croq 2009) indicate that *F. lapillum* exhibits isometric growth. Differences in eye size and interorbital distance should thus correlate positively with body size. This is likely the case for *F. lapillum* across the wave exposure gradient in the Hauraki Gulf, where larger eyes are simply a consequence of difference in head and body depth.

4.4.4 Sexual dimorphism

We found significant sexual dimorphism in offshore and sheltered *F. lapillum* populations, with males being longer overall and larger in the head region. This is most likely related to the strong site fidelity seen in triplefins (Thompson 1983, Clements 2003, Shima et al. 2012), and the disparity of spawning roles. Males are highly territorial, especially during the spawning season where they defend territories, while females move about in search of mates (Thompson 1983, Clements 2003). Larger, more robust males would have an advantage in intraspecific male competition and nest defence during the spawning season. Larger males would also fare better in interspecific competition, as is shown for the variable triplefin *F. varium*, which engages in antagonistic displays with the labrid, *Pseudolabrus celidotus* (Thompson and Jones 1983). These interactions are size-specific, and large *F. varium* successfully defend their territory against wrasses nearly twice their size (Thompson and Jones 1983).

Sexual dimorphism seen in this study could also be driven by female mate choice, especially as males prepare a nest site and intensively display to attract females to spawn. Selection generally favours female mate choice of larger males, as this is a good indicator of health, access to territory and resources, and also higher stores of energy and sperm for spawning (Howard et al. 1998). Furthermore, male spawning success was positively correlated with body size in other small benthic nesting fishes with very similar reproductive strategies (Thompson 1986, Cote and Hunte 1989). Females of some fish species even delay spawning in the presence of smaller males, waiting for larger ones to show up (Foote 1989). It is likely that the sexual dimorphism is initially driven by sexual selection, and subsequently male size differences are exaggerated by the effects of wave exposure.
4.4.5 Conclusions

In conclusion, *F. lapillum* displays morphological differentiation in body size and shape, and in pectoral fin morphology, all of which strongly correlated with wave exposure. Larger, more robust bodies, with larger, broader pectoral fins were found at more exposed sites, and we attribute this as direct responses to differences in water movement, related to both locomotion and position holding. Future research could investigate the apparent trade-off between performance and function in morphology, with particular respect to fins. The sexual dimorphism seen within and between locations is most likely related to the reproductive behaviour of this species, where males actively guard nests, and large size is probably advantageous for nest defence and mate attraction. The lack of genetic structure in *F. lapillum* in the Hauraki Gulf (Rabone et al. 2015) suggests that the variation in phenotype seen in the present study is likely due to developmental plasticity. Laboratory rearing studies are required to test this hypothesis (see chapter 5).

Consistent phenotype-habitat associations often occur repeatedly across parallel environmental settings, such as the post-glacial lake, limnetic-benthic systems (e.g. sticklebacks and *Lepomis* sunfishes). Are the abiotic effects of the exposure gradient studied here in the Hauraki Gulf such an association, and does the pattern of eco-morphological variation in response to this abiotic variable apply to other triplefins? Examinations of other species are necessary to answer this. However, the current study supports the prediction that this generalist species is amenable to being phenotypically variable (sensu Van Valen 1965), and the environment-phenotype correlations seen here may represent an initial intraspecific stage of the interspecific habitat diversification that generated the New Zealand triplefin assemblage as a whole.
Chapter 5

Phenotypic plasticity evident across a small spatial scale in the temperate marine reef fish *Forsterygion lapillum*

5.1 Introduction

Adaptation to different ecological environments via divergent natural selection can generate phenotypic variation, culminating in genetic differences between populations (Nosil 2012). An alternative to heritable adaptive variation is the evolution of phenotypic plasticity (West-Eberhard 1989). Rather than generating genetic divergence, variable and divergent environments may result in the evolution of generalised and highly plastic populations in which individuals can express a range of phenotypes according to their surrounding environment (Nosil 2012). Much of the time however, phenotypic plasticity probably reflects a combination of genetic and plastic influences, which may interact in a variety of complex ways (West-Eberhard 1989).

Empirical evidence of phenotypic plasticity has continued to accumulate, and has been shown to exist in a wide spectrum of taxonomic groups, including plants (Schlichting 1986, Sultan 1987), insects (McLeod 1968, Lee 1984, Zera 2004, Whitman and Ananthakrishnan 2009), and amphibians (Pfennig and Murphy 2002, Guayasamin et al. 2015). Furthermore, morphological plasticity is prevalent in freshwater fishes, in particular from northern hemisphere post-glacial lake habitats (Robinson and Parsons 2002). A common feature in these lakes is the repeated divergence of alternate morphologies into benthic (littoral) and pelagic (limnetic) habitats. The most well studied of these is the threespine stickleback (*Gasterosteus aculeatus*) species complex (McKinnon and Rundle 2002). In pelagic habitats, sticklebacks demonstrate greater diet-induced morphological plasticity than benthic morphs (Day et al. 1994). *Lepomis* sunfishes also exhibit alternate plastic phenotypes in these two adjacent habitats, with morphological differences seen in both trophic- and habitat-related traits (Yavno and Fox 2014). Similarly, perch (*Perca fluviatilis*) are highly plastic in response to habitat and feeding mode, and the plastic responses are so sensitive that the morphology is rapidly reversible (Olsson and Eklov 2005). The prodigious radiations seen in cichlids are further examples of phenotypic plasticity in freshwater fishes, where diet-induced morphological plasticity is regularly seen in this family, being particularly prevalent in the head, jaw and mouth (e.g. Meyer 1987, Witte et al. 1990, Stauffer and van Snik Gray 2004).
The resistant force of water can exert local selective pressures over small spatial scales, and several morphologically plastic examples in response to different flow regimes are evident. For example, Atlantic salmon (*Salmo salar m. sebago*) reared in high and low flow stream environments became more robust in high flow, and brown trout (*Salmo trutta m. lacustris*) became more streamlined in faster flows (Pakkasmaa and Piironen 2001). Freshwater sculpins demonstrate intraspecific plastic differences of general body form, such as decreased body size, head and body depths and increased caudal peduncle depth in populations exposed to high flow relative to those in low flow (Kerfoot and Schaefer 2006, Bogdanov 2007).

It is argued that plasticity is less prevalent in marine species due to the more homogenous and stable environment, where high gene flow is often linked with constraining adaptive genetic divergence. This is also applicable for large bodies of water (e.g. lakes relative to streams). For example, plasticity is less prevalent in marine sticklebacks relative to freshwater sticklebacks (Svanback and Schluter 2012), lake sticklebacks relative to stream sticklebacks (Sharpe et al. 2008), and oceanic silversides relative to estuarine silversides (Brown et al. 2012). Accordingly, there are few examples of phenotypic plasticity in marine fishes. One of the first known examples is the Antarctic notothenioid *Trematomus newnesi*, which exhibits plasticity in regions of the head and mouth, and in body colour (Eastman and DeVries 1997, Barrera-Oro et al. 2012). Although the causal explanation for the basis of this plasticity is not fully understood, it is suggested that large mouth morph is more benthic-associated than the typical semi-pelagic morph (Eastman and DeVries 1997). The damselfish *Abudefduf saxatilis* exhibits plastic responses in the presence of gene flow, with the position of the pectoral fins and morphological differentiation in body and head shape correlated to environmental variables along the Mexican coast (Pineros et al. 2015). Marine sculpins show considerable disparity in body and pectoral fin morphology in relation to water flow, and although not specifically tested, it is suggested that plasticity is responsible for much of the variation, and is an area of ongoing study (Kane and Higham 2012). Very few marine studies have explicitly addressed whether the phenotype-environment relationship is due to adaptation or alternative evolutionary processes (Knope and Scales 2013). Disentangling the relative contributions of genetic and environmental effects to phenotypic variation is thus an important goal in developing our understanding of the drivers that both promote and maintain diversity in marine systems.

Chapter 4 showed that *F. lapillum* from different locations (i.e. with regards to wave exposure) were distinguishable from one another in morphology. This observed morphological variation in *F. lapillum* across a wave exposure gradient could stem from genetically-based differences, or either adaptive or non-adaptive phenotypic plasticity. Phenotypic plasticity may be favoured in generalist populations if it increases niche width (Van Valen 1965), and the study species, *F.*
lapillum, is considered the most generalist species of triplefin in New Zealand (Wellenreuther et al. 2009). Triplefins exhibit almost no post-settlement dispersal, being highly philopatric, and occupying the same small territory throughout their lives (Thompson 1983, Clements 2003, Subedar 2009, Shima et al. 2012). Furthermore, there is a lack of genetic structure in the F. lapillum populations (Rabone et al. 2015) that were shown to exhibit considerable morphological variation in chapter 4. Together, this suggests that the morphological variation in wild F. lapillum is largely plastic in nature, but this hypothesis remains to be tested.

The aims of this chapter are to use reciprocal common garden experiments to test phenotype-environment interactions in F. lapillum. The main hypothesis under test is that the mechanism for the environmentally-induced morphological variation seen in the wild fish (chapter 4) is phenotypic plasticity. If plasticity is responsible, we expect that two sets of new recruits of F. lapillum collected from different sites will not be morphologically divergent when raised in identical conditions. Conversely, we expect that new recruits of F. lapillum all caught from the same site and raised in distinct water movement regimes will exhibit morphological differentiation. Furthermore, we hypothesise that artificial water movement in the laboratory will induce a similar range of morphological responses to those seen across the wave exposure gradient in the wild.

5.2 Methods

5.2.1 Fish collection

All fish collected for experiments were new recruits, defined as having settling within the previous two weeks by their small size (≤ 30 mm) (Connell and Jones 1991, McDermott and Shima 2006, Wellenreuther and Clements 2008, Mensink and Shima 2014) and their lack of fully developed pigmentation (Connell and Jones 1991). Fish were captured using handnets on SCUBA in 5-6 m depth. Captured fish were transferred into 20 L buckets and transported to the Leigh Marine Laboratory. Digital photographs of collected fish were taken in a flat-bottomed, rectangle shaped fish bin, with a ruler for scale, to allow measurement of average size on day of capture using photographic software (ImageJ version 1.46r). All fish samples were collected in accordance with the University of Auckland Animal Ethics Committee approval 001047.
5.2.2 Common garden experiment

Recruit fish for the common garden experiment were collected on November 4th 2014 at two different sites – Jones Bay (36°22'49.12"S, 174°49'01.19"E) and Takatu Point (36°22'02.95"S, 174°52'07.61"E). These sites are on the Tawharanui Peninsula, located in the northern Hauraki Gulf, New Zealand (fig. 5.1). They were selected as representatives of each end of the coastal wave exposure gradient (see exposure calculations below), with Jones Bay considered sheltered habitat and Takatu Point exposed, and wild populations at these two sites were phenotypically distinct (chapter 4).

*Forsterygion lapillum* recruits were raised in six separate identical 32 L flow-through aquaria, with three aquaria each containing sheltered or exposed fish. Aquaria were filled with cobble substrate and all aquaria were randomly assorted with respect to location in the tank room. All aquaria were fed equal amounts of the same food daily; either prepared ground aquaculture meal or finely chopped green mussels and pilchards. Recruits were raised for seven months, and subsequently euthanized after this duration for morphological analyses. The presence of gonads in the peritoneal cavity indicated that the majority of individuals had reached maturity during this timeframe. This is consistent with wild *F. lapillum*, where fish are found to be mature within approximately six months, or accordingly during their first winter (Doak 2003, Francis 2013). There was an 18 % mortality rate over the course of the experiment, equally distributed between aquaria (i.e. source locations).

5.2.3 Water movement experiment

Newly settled *F. lapillum* collected for the water movement experiment were all collected on October 28th 2014 during one dive from the same site (Jones Bay). Fish were split evenly into eight 60 L aquaria. Each aquarium was replenished by flow-through seawater, although distribution of water differed between treatments. The first treatment (four aquaria) consisted of low flow water delivered through a hose (hereafter low energy). For the second treatment (four aquaria), water was delivered through a hose at the same flow rate into a 20 L bucket situated above the aquarium. The bucket was fitted with a ball float connected to a rubber plug. When the bucket filled, the plug was opened via the ball float, and the contents of the bucket emptied rapidly (2-3 seconds) through a wide bore (4 inch) PVC pipe, creating extremely turbulent, non-laminar water movement in the aquarium (hereafter high energy). All other variables between treatments were identical. Substratum was limited to halved flat-bottomed cinder blocks, and thus fish could not seek refuge from water movement in any areas of the aquaria. All aquaria were fed
equal amounts daily of the same food; either prepared ground aquaculture meal or finely chopped green mussels and pilchards. Recruits were also raised for seven months, and subsequently euthanized for morphology analysis. There was a 17% mortality rate over the course of the experiment, equally distributed between treatments.

Figure 5.1. Map of the Hauraki Gulf, New Zealand, with the sample sites inset: Jones Bay (sheltered) and Takatu Point (exposed).
5.2.4 Wave exposure analysis

A combination of wave power data, fetch, and wind-derived exposure were used to verify the wave exposure groupings used in the present study. Empirical wave energy data derived from MetOcean Wavewatch (NOAA 2016) was used to calculate wave exposure. Wave power was chosen as the variable of interest, as it incorporates wave height and period. Spatial resolution from the grid cells permitted delineation between the two sites, where sites significantly differed from each other in wave power (Mann-Whitney U statistic = 3002, T = 46320, \( P < 0.001 \)), and are graphically represented in appendix (see appendix for graphic representation).

More precise levels of wave exposure at each site were approximated using an index of potential fetch, which is defined as the area of the sea surface over which waves are generated by wind with a constant direction and speed (Denny 2005). The exposure of each site was calculated as the total sum of the fetch based on defined settings, using the program ‘Fetch Effect Analysis’ (Villouta 2000). For each site location, each 10º on a compass rose a vector was projected a distance of 300 km, or until land is encountered, and all distances summed. The use of fetch as a proxy for exposure does not take into account differences in wave spectra among areas (Denny 1988), however, it does permit tentative conclusions to be made (Thomas 1986). Furthermore, in north-eastern New Zealand it is suitable, as the longest fetch and largest swells are from the east/northeast (Denny 2005).

To further verify the wave exposure groupings, wind-derived exposure calculations were performed (e.g. Burrows et al. 2008). Historical wind data was attained for a 10 year period (2005-2015) recorded at the Leigh wind station (station 1340), at a three hourly resolution for maximum wind speed and direction (NIWA 2016). Within each data set, maximum speed was averaged for each 10º vector, and multiplied by the corresponding vector for fetch calculations for each site. Subsequently, all 36 vector calculations were summed to get a site total. Summed wind-derived exposure calculations for Jones Bay and Takatu Point were 11,545 and 62,376, respectively.

5.2.5 Linear body measurements

Twelve different independent morphological measures of *F. lapillum* were taken on fish from the common garden experiment (sheltered \( n = 19 \) and exposed \( n = 17 \)) and the water movement experiment (low flow \( n = 37 \) and dump bucket \( n = 36 \)). Linear measurements were made using Vernier calipers between two homologous points on the fish. Length measurements included: total length (TL), eye diameter (ED), inter-orbital width (IOW), head length (HL), maximum body
depth (BD), pre-pectoral length (PPL), pre-dorsal length (PDL), gape width (GW), gape length (GL), post-anal length (PAL), caudal peduncle depth (CPD), and caudal fin length (CFL). All measurements (except total length) were expressed as ratios of total length to correct for differences in fish size. Morphological regions of interest were chosen with respect to foraging traits and habitat characteristics described in the literature for other fish species (Fisher 1998, Blake 2004, Langerhans et al. 2004, Riopel et al. 2008). Two-tailed t-tests were used to compare differences between treatments for each empirical measurement.

5.2.6 Pectoral fin morphology

Pectoral fins play an important role in interactions with water movement (Webb 1994) and fish swimming efficiency (Blake 2004), and pectoral fin morphology in wild *F. lapillum* populations positively correlated with wave exposure (chapter 4). Pectoral fin morphology was analysed for all *F. lapillum* individuals from both experiments. Digital images were taken of the amputated left pectoral fin of each individual spread to full extension, with a scale included beside the fin. Any fin that was damaged or could not be positioned flat was removed from the analysis. All images were analysed using ImageJ software to obtain measurements of the length of the leading edge of the fin and total fin area. Area was standardised for fish size by dividing the area by the total length of the individual. Aspect ratio was then calculated as the leading edge squared, divided by this standardised total area. Two-tailed t-tests and Mann-Whitney rank sums tests were used to compare differences between treatments in pectoral fin morphology for the common garden and water motion experiments.

5.2.7 Geometric morphometrics

Morphometric shape analyses were performed for all *F. lapillum* individuals from both experiments. Fish were not fed on day of sampling to eliminate any effect of variation in stomach fullness on shape. Digital images were taken of the left lateral side of each individual on a dark background, using an 18.0 megapixel camera, with a scale included beside the fish. Where necessary, small pins were used to extend fins and spines. Any fish that could not be positioned flat and on a straight axis were not used in the analyses. The external body shape of each fish was outlined by digitizing 15 discrete homologous anatomical landmarks (fig. 5.2), plus two landmarks on the scale bar, using the software program TpsDig2 (Rohlf 2008). Landmarks were then superimposed to remove differences due to orientation, position and size (Sheets 2004). To arrive at the best biological description, we used two different superimposition methods to
interpret shape, Partial Procrustes superimpositions (hereafter Procrustes superimposition) and Bookstein coordinates. Procrustes superimposition involves calculating the centroid of each configuration, and then making this centroid the centre of the coordinate system (Webster and Sheets 2010). Bookstein coordinates uses fixed baseline endpoints (landmarks 1 to 7 were chosen, as they are longest natural axis that passes through the centroid of the fish) to centre the coordinates, losing the variance from those two landmarks in the process. The results of the two methods (i.e. Procrustes and Bookstein) were consistent, therefore we chose to conduct all analyses using Procrustes superimposition, which is generally considered to be more statistically reliable (Sheets 2004). All subsequent geometric morphometric analyses were conducted using the IMP software series (Sheets 2004), unless otherwise stated. After superimposition, data were converted into principal warps using the thin-plate spline (TPS) model (Bookstein 1991). Thin-plate spline files were translated into the IMP format using CoordGen6, and subsequently loaded into CVAGen6 for canonical variate analysis (CVA). Canonical variate analysis is a multi-axis discriminant function and ordination method for depicting differences visually, and for testing the ability of variables to correctly predict group (e.g. population or species) differences based on measurements (Zelditch et al. 2012). Rather than a set of loadings along length or width variables, as is commonly produced when using traditional morphometrics based on length or width measurements, mean body shapes and deformation grids were produced (Yee et al. 2011). Deformation grids and landmark displacement vectors based on the thin-plate spline model were used to visualize the regions of greatest discrimination for each exposure group. Following this, multivariate analysis of variance (MANOVA) was conducted on the mean shapes. Classification of groups per experiment (treatments) was made using jackknifed grouping from a CVA-distance based method, where one known specimen is left out at a time, and assigned using the CVA axes. The jackknife rate is a better estimate of the performance of the method than the re-substitution rate found by analysing the same specimens used to create the discriminant function (Solow 1990). Lastly, TwoGroups was used to statistically test the between-treatment difference in mean shape based on Procrustes distance, using the parametric Goodall’s F-test with 100 bootstraps. This is a means of graphically depicting the differences in average landmark distribution for each group (treatment) by plotting simultaneous superimpositions.
5.3 Results

5.3.1 Common garden experiment

There was no difference in mean total length of *F. lapillum* recruits at the start of the common garden experiment (*t*<sub>23</sub> = 0.330; *P* = 0.744), with fish collected from sheltered and exposed sites having an average total length (± SEM) of 24.6 mm (± 0.40 mm) and 24.4 mm (± 0.53 mm), respectively (fig. 5.3). Upon completion of the experiment, there were still no differences between the treatments (*t*<sub>34</sub> = 0.158; *P* = 0.876), with average total lengths of 40.7 mm (± 1.03 mm) and 40.4 mm (± 1.24 mm) for sheltered and exposed treatments, respectively (fig. 5.3). Upon
completion of the common garden experiment, mean pectoral fin sizes did not significantly differ between treatments ($t_{34} = 1.199; P = 0.239$) (fig. 5.4 A), and there was no difference in fin shape (aspect ratio) between treatments ($t_{34} = 1.210; P = 0.235$) (fig. 5.4 B). There were also no differences (t-tests, all $P < 0.05$) between any of the linear body measurements between treatments for the common garden experiment (fig. 5.5).

Figure 5.3. Boxplot showing the total lengths of *F. lapillum* at the beginning and conclusion of the common garden experiment, depicting recruits collected from sheltered and exposed sites. Dashed lines represent the sample mean.

Figure 5.4. Mean measurements of A) fin size and B) aspect ratio for pectoral fin morphology of *F. lapillum* at the conclusion of the common garden experiment for sheltered and exposed treatments. Bars represent standard errors of the mean.
5.3.2 Water movement experiment

At the start of the water movement experiment, mean total fish length (± SEM) was 24.4 mm (± 0.52 mm). There was no difference in mean total length of *F. lapillum* after the experiment was completed (\(t_{71} = 0.215; P = 0.830\)), with average total length for fish from the low energy and high energy treatments being 36.7 mm (± 0.73 mm) and 36.5 mm (± 0.85 mm), respectively (fig. 5.6). Upon completion of the water movement experiment, mean pectoral fin sizes did not differ significantly between treatments (\(T_{32, 41} = 1226; P = 0.645\)) (fig. 5.7 A), and there was no difference in fin shape (aspect ratio) between treatments (\(T_{32, 41} = 1086; 41; P = 0.278\)) (fig. 5.7 B). There were no differences (t-tests, all \(P > 0.05\)) between any of the linear body measurements between treatments for the water movement experiment, except for pre-dorsal length (\(t_{71} = 2.744; P = 0.008\)) and caudal peduncle depth (\(t_{71} = 2.150; P = 0.035\)) (fig. 5.8).
Figure 5.6. Boxplot describing the total lengths of *F. lapillum* at the conclusion of the water movement tank experiment. Dashed lines represent the sample mean.

Figure 5.7. Mean measurements of A) fin size and B) aspect ratio for the pectoral fin morphology of *F. lapillum* from the different treatments of the water movement tank experiment. Bars represent standard errors of the mean.
Chapter 5 – Phenotypic Plasticity

Figure 5.8. Mean morphological measurements of the eye, mouth, head and tail regions of *F. lapillum* from the different treatments of the water movement experiment. Low flow (black); dump bucket (grey). Bars represent the standard errors of the mean. Abbreviations: total length (TL), eye diameter (ED), inter-orbital width (IOW), gape width (GW), gape length (GL), maximum body depth (BD), head length (HD), pre-pectoral length (PPL), pre-dorsal length (PDL), post-anal length (PAL), caudal peduncle depth (CPD), and caudal fin length (CFL).

5.3.3 Geometric morphometrics

The CVA axes plot of all four treatments (fig. 5.9) showed a strong separation in the shapes of *F. lapillum* between experiments (three distinct canonical variates, axis 1: eigenvalue = 2.33; Wilks lambda = 0.0984; $\chi^2_{78} = 208.73$; $P < 0.001$; axis 2: eigenvalue = 0.95; Wilks lambda = 0.3277; $\chi^2_{50} = 100.42$; $P < 0.0001$; axis 3: eigenvalue = 0.56; Wilks lambda = 0.6405; $\chi^2_{24} = 40.10$; $P < 0.05$). Canonical variate analysis for the common garden experiment showed no significant separation in overall fish body shape between treatments (eigenvalue = 4.154; $P = 0.168$). Conversely, CVA results for the water movement experiment showed a significant separation in fish body shape between treatments (one distinct canonical variate, axis 1: eigenvalue = 1.66; Wilks lambda = 0.3398; $\chi^2_{26} = 59.37$; $P < 0.001$). A MANOVA of body shape for each experiment was not significant for the common garden experiment ($P = 0.119$) and significant for the water movement
experiment \((P < 0.05)\), although grouping explained as a percent of the total was similar for the two experiments and error variance was high in both groups (table 5.1).

![Canonical variate analysis](image)

**Figure 5.9.** Canonical variate analysis depicting all four treatments from both experiments using Procrustes superimposition on landmarks: sheltered (red), exposed (pink), low energy (blue), high energy (black).

**Table 5.1.** Results of MANOVA on fish body shape for the common garden experiment (sheltered vs exposed) and the water movement experiment (low energy vs high energy).

<table>
<thead>
<tr>
<th></th>
<th>Common Garden</th>
<th>Water Movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>(F)</td>
<td>2.0203</td>
<td>2.0801</td>
</tr>
<tr>
<td>(df)</td>
<td>1, 33</td>
<td>1, 69</td>
</tr>
<tr>
<td>(P)</td>
<td>0.119</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total SS</td>
<td>0.028375</td>
<td>0.05841</td>
</tr>
<tr>
<td>Explained SS</td>
<td>0.001637</td>
<td>0.001734</td>
</tr>
<tr>
<td>Unexplained SS</td>
<td>0.026738</td>
<td>0.056677</td>
</tr>
<tr>
<td>% explained</td>
<td>33.78</td>
<td>35.07</td>
</tr>
</tbody>
</table>
Chapter 5 – Phenotypic Plasticity

The deformation grids produced by the analysis (fig. 5.10) depicts the shape changes for the first CVA axis between sheltered and exposed fish for the common garden experiment (fig 5.10a), and between low energy and high energy fish for the water movement experiment (fig. 5.10b). The grid shows that for the common garden experiment the head was the most differentiated, but overall there was no significant difference in mean body shape, as can be seen in the large overlap in the scatterplot (fig. 5.9). The deformation grid for the water movement experiment shows the head region also as the region of greatest differentiation, and there was a clear shift in shape direction antero-dorsally between low energy and high energy.

Jackknife groupings from the CVA distance based method showed correct assignment rate was poor for the common garden experiment, with 38.8 % correct and significant. It was much higher for the water movement experiment, with 65.0 % correct and significant. For the common garden experiment, although there were slight differences in some of the landmarks around the head region, the two treatments did not differ significantly in shape (fig. 5.11 A) ($F_{26, 884} = 1.43; P = 0.24$). Conversely, the two treatments for the water movement experiment did differ significantly in shape (fig. 5.11 B) ($F_{26, 1768} = 3.31; P < 0.01$).

Figure 5.10. Deformation grid with vectors of displacement for fish shape along the first CVA axis for A) the common garden experiment, and B) the water movement experiment.
5.4 Discussion

Chapter five showed that the morphological variation seen in chapter four is largely plastic. Plasticity is a trait (i.e. a property of a genotype), and like other traits is subject to evolution by natural selection (Richards et al. 2006). More specifically, phenotypic plasticity refers to the potential of specific traits of a genotype to respond to different environments (Richards et al 2006). Theory predicts that divergence may arise prior to any genetic segregation as a result of environmentally induced phenotypic plasticity at the individual level (West-Eberhard 1989, West-Eberhard 2003). Where the traits involved are of high functional significance, the presence of different forms creates the circumstances in which genetic divergence is favoured (Wimberger 1994, Smith and Skúlason 1996).

Body shape and pectoral fin morphology are undoubtedly important functional traits in *F. lapillum*, involved in locomotion and position holding. Furthermore, behavioural specialisations in particular are thought to facilitate morphological divergence (West-Eberhard 1989), and in the

Figure 5.11. Average landmark configurations of specimens from the two experiments, depicting mean shape for A) common garden experiment treatments: sheltered (asterisks, black outline), exposed (triangles, blue outline), and B) water movement experiment: low energy (asterisks, black outline), high energy (triangles, blue outline).
The water movement experiment was the reciprocal of the common garden experiment, as *F. lapillum* recruits were collected from the same location and raised in distinct conditions of water movement. This experiment produced limited morphological differences between treatments, with only two of the twelve linear body measurements differing between treatments. However, geometric morphometric analyses did reveal significant differences in body shape between treatments. The direction of shape change in the experiments (i.e. body becomes larger/more robust anterio-dorsally with increasing water energy) is consistent with that seen in wild populations of *F. lapillum*, even if not significant across all measurements. This confirms that there is a consistent phenotypic response to water energy in this species, and provides evidence that water movement elicits responses in certain morphological traits. Consistent phenotypic differences between tank experiments and the wild populations have been demonstrated in other fishes, for example sticklebacks (Baker 1984) and damselfishes (Binning et al. 2015). If morphology were largely heritable, we would expect no differences between treatments in morphology at the conclusion of the experiment. Therefore, the direction of shape change in combination with the common garden experiment provides evidence that morphological variation in *F. lapillum* is environmentally-induced.

It is possible that that a more extended experimental duration may have yielded significant change to more of the measured parameters. Time constraints limited us to a seven month duration. However, we expected this to be sufficient, as this species is both fast growing (i.e. mature after ~six months, and ~90-95% maximum size after one year) and short-lived (i.e. most of the source populations live only two years) – see chapter 3. Interestingly, *F. lapillum* grew less in captivity than in wild populations, suggesting that food was possibly limiting in comparison to wild populations. However, the fish at the completion of the experiments were mature individuals and approximately 70-80% of the maximum size of wild adults, representing a significant proportion of the species size range and lifespan. Furthermore, other common garden experiments that successfully tested similar hypotheses of morphological variation between divergent systems were
shorter in duration, e.g. ~55 days (Langerhans et al. 2004), 80 days (Yavno and Fox 2013, 2014) and 100 days (Fischer-Rousseau et al. 2010).

The high energy water movement treatment did not produce the variation in pectoral fin morphology seen between different exposure regimes in the wild. This was unexpected, as pectoral fins play an important role in eco-morphological responses to water movement in other fishes (Fulton et al. 2001, Kane and Higham 2012, Binning and Roche 2015), and a strong relationship between pectoral fin morphology and wave exposure relationship was seen in wild populations of *F. lapillum*. This remains unclear, but there is the potential that the plasticity in pectoral fins develops later in fish growth (i.e. beyond the seven months of experimental duration employed here), or that the water movement conditions in the dump tank setup may have differed from those that trigger plasticity in the trait in the wild.

In conclusion, the results of the water movement experiment and the common-garden experiment generated a pattern of morphological variation that, while not as extreme as that across the natural exposure gradient, nevertheless is consistent with the trend of morphological differences seen in the wild. The results from this study thus suggest that the morphological variation seen in wild populations (i.e. chapter 4) of *F. lapillum* in the Hauraki Gulf is largely environmentally-induced, and thus morphological plasticity is evident over a remarkably small spatial scale, and within an area of very high gene flow. Consistent phenotype-habitat associations often occur repeatedly across parallel environmental settings, such as post-glacial lake, limnetic-benthic systems (e.g. sticklebacks and *Lepomis* sunfishes). Are the abiotic effects of the exposure gradient studied here in the Hauraki Gulf such an association, and does the pattern of eco-morphological variation in response to this abiotic variable apply to other marine taxa? Tests such as the present study that document evidence of morphological variation and plasticity are essential to further understanding the basis and implications of such processes. Furthermore, this is one of the few studies on phenotypic plasticity in marine fishes or in marine systems in general, and improves our understanding of the drivers that promote and maintain diversity in these systems.
Chapter 6

General Discussion

6.1 Ecological speciation on contemporary timescales

The high diversity in the New Zealand triplefin fish clade is thought to be largely the result of ecological speciation via habitat diversification (Wellenreuther et al. 2007), but the relationships between habitat variables and fitness in individual species had not previously been examined. This thesis examined how variation in the environment can generate fitness differences that have potential evolutionary consequences, with a view to determine whether processes that are hypothesized to have taken place over evolutionary timescales (i.e. ecological speciation) are evident over ecological timescales. Furthermore, the overall goal was to identify mechanisms that may have driven habitat diversification in the New Zealand triplefin radiation. This thesis thus informs the field of ecological speciation in general, and as few field studies have examined this process in marine fishes, empirical studies of this nature on marine fishes are important in working towards an understanding of the drivers that promote and maintain diversity in the sea.

Trait utility is one of Schluter’s four criteria that define an adaptive radiation, and is the focus of much of this thesis. One feasible way to examine trait utility is to look at habitat performance, and this was examined in two different contexts: in terms of direct fitness measures, and based on engineering considerations of phenotype, and we need both to inform the other. Fecundity is arguably the most direct measure of fitness, as the quantity of offspring carried to the next generation is fitness. Additionally, abundance is largely a result of successful offspring settling in a given habitat (and suggested here to be as a result of active habitat selection), and constitutes a reasonably direct, quantifiable measure of fitness. Furthermore, growth, maximum size and longevity are also all undoubtedly indicators of fitness. Together, the differences in these traits that were found among habitats all provide evidence that certain population of triplefins are performing better in a given habitat. Consequently, these fitness advantages can have evolutionary outcomes, potentially leading to adaptation. Whilst data on the performance or fitness advantages of traits such as these indicate how well and individual or population is doing, we also need to know how this performance comes about.

An “engineer’s criterion of good design” (Gould 1976) is a term describing the suitability of a certain trait for a given environment. If a trait is superior a priori then fundamentally the
individual possessing that certain morphological, physiological or behavioural trait should have a
greater chance of survival, and subsequently preserving this trait by succeeding to reproduce. This
aspect of habitat performance was examined in the form of morphology, an aspect not previously
documented in the New Zealand triplefin fauna. The growing literature on fish form and function
provides a framework for which body type is suited to which environment. Subsequently, by
examining the differences in morphology of *F. lapillum* in this study we get an indication of
whether these phenotypes are expected to perform better or worse in certain environments.
Mapping these morphological differences onto the fitness performance parameters from previous
chapters (i.e. abundance, growth, fecundity etc.) shows the relationships between phenotypic
variation and fitness. This is why it is insightful to examine habitat performance from both
aspects.

It would seem counterintuitive that in populations with high gene flow that any difference, such as
morphological traits, would become fixed. However, theory predicts that where there is complete
gene flow across a spatial area – particularly in combination with an environmental gradient –
strong frequency-dependent selection can lead to speciation due to competition for limiting
resources (Doebeli and Dieckmann 2003). Wave exposure was thus identified as the predominant
factor associated with driving selection pressures in this system, and constitutes an environmental
gradient. There was consistent evidence of this environmental factor generating disruptive
selection across several of the variables examined, demonstrated by the variation in performance
of all the measured ecological traits. High ecological contact (i.e. gene flow) is important in these
situations, as other individuals are part of the environment that determines the fitness of a given
individual (Dieckmann et al. 2004). Furthermore, such frequency-dependent selection operating in
sympatry is thought to drive speciation much more rapidly than in allopatric scenarios. A good
example of this is the sympatric *Cyprinodon* pupfish clade in San Salvador Island, which is
diverging up to 51 times faster than allopatric *Cyprinodon* populations for certain trophic traits
(Martin and Wainwright 2011).

The two main habitat variables in this study (wave exposure and microhabitat) are likely to be
spatially correlated (i.e. non-randomly distributed) due to the shape of the coastline, geological
makeup and topography, and aspect relative to the open ocean. Examples of this are evident in the
scarcity of cobbled habitats at sheltered locations relative to other locations, and the east-facing
coastline of Great Barrier Island, where reefs are exposed to the same levels of wave energy for
several kilometres. If certain environments are correlated, then it would be expected that >50% of
genes will come from populations in the environment that are clustered together more
(Richardson et al. 2014). These pre-adapted phenotypes will then facilitate adaptation, assuming
that they are compatible with local genes (Richardson et al. 2014). As said above, gene flow will actually facilitate rather than impede population differentiation (Bolnick and Otto 2013). Thus clustering of environments is analogous to active habitat selection in that the non-random distribution of individuals, when matched (i.e. certain phenotypes) with habitats conferring higher fitness, can result in fine-scale population differentiation. Therefore any environments that are clustered, and certainly the evidence for active habitat selection, offers the potential for microgeographic adaptation to occur in triplefins (*sensu* Richardson et al. 2014).

Variation in nature is ubiquitous and often random in direction, creating raw material, and “evolution by natural selection is no more than a tracking of these changing environments by differential preservation of organisms better designed to live in them” (Gould 1976 pp 28). In addition, in this sympatric system, phenotypic plasticity provides raw material upon which natural selection can act. As theory predicts that divergence may arise prior to any genetic segregation as a result of environmentally induced phenotypic plasticity at the individual level (West-Eberhard 1989, West-Eberhard 2003), there is the potential for divergence to be occurring on a contemporary timeframe in *F. lapillum*. At a minimum the plasticity seen here suggests that it may have been an important factor in the diversification that generated this group of fishes.

### 6.2 Future directions

Hindsight reveals a few things that could have improved the study. The failure to quantify daily increments in adult triplefins meant I was unable to achieve the level of resolution hoped for in the growth and size at age comparisons. While the length-based approach using modal progression analysis proved a valuable alternative, a targeted collection of juvenile individuals up to around 3-4 months rather than collecting all ages with the emphasis on adults. Daily increment analysis has been estimated in early juvenile triplefins (Kohn and Clements 2011). Early growth in the first few months after settlement is essential to an individual’s fitness, and this would provide insight into patterns of variation in habitat performance.

More work would have to be undertaken in order to more comprehensively understand the input of active settlement vs early mortality on density distributions. This would involve an intense sampling effort, although it would be practically impossible to capture the whole settlement event. However, a targeted sampling regime immediately following settlement could be done such as daily UVC’s for say 3-4 weeks in just a couple of sites at the opposite end of the wave exposure gradient. Regardless, there are several lines of evidence that suggest active habitat selection is
largely responsible for shaping triplefin density differences (see chapter 2 introduction and discussion), therefore, this additional intensive sampling is probably unnecessary.

Research on the interplay between morphological and behavioural evolution during the process of ecotypic differentiation is limited. Not only can behaviour influence the ecological conditions to which animals are exposed, but the morphology of an animal can constrain its behavioural capabilities and can limit the ecological conditions to which it is exposed (e.g. Weislo 1989). Consequently, behaviour and morphology may often evolve in concert, producing integrated character combinations that function well in local environmental contexts (Brodie 1989, Foster et al. 1992). This thesis touched on this interplay with the size of males and their successful enticement of mates and subsequent guarding of nests, and also suggested that behavioural posturing, in conjunction with fin and body morphology, might be important component of dealing with water velocity. Further behavioural research has the potential to prove insightful on this evolutionary system.

Measuring fecundity with clutch size and male guarding size is the most direct measure of fitness in this study. Doing paternity studies on recruits would be more direct, as you could get lots of eggs in one habitat but no larval survival. However, the nest photo analysis was the best that could be done given the constraints of the project.

While the results of this study provide evidence for phenotypic plasticity in this species, the complete genetic architecture is unclear, and further testing is required to elucidate whether the plasticity is adaptive or not, and how sensitive to developmental perturbation are the traits involved. For a start, longer experimental durations (e.g. ~2 years) would potentially emphasise the differences in seen in morphology. Additionally, it would be informative to test whether the morphological plasticity is reversible or a one-way process? This would involve rearing fish through to maturity, such as in chapter 4, but then swapping the individuals into the opposite environments and continuing to rear them. Lastly, it would be interesting to see if phenotypic plasticity is a conserved trait, and whether there is still evidence of it in the sister species of *F. lapillum, F. nigripinne*. The latter is considerably more specialised in habitat use than *F. lapillum*.

Energetic stores can be used as a proxy for fitness (e.g. Robinson et al. 1993). For example, during multi-day storm events, opportunities to forage in these shallow water triplefin species would be severely diminished. Energy could be conserved by having a more streamlined body (Marshall et al. 1999), which would thus likely contribute to increased overall fitness. Therefore information on the relationships between phenotypic variation and energy reserves could be informative.
6.3 Conclusions

This thesis has demonstrated intraspecific variation in various measures of fitness and habitat performance, and as shown by the consistent differences in ecological traits, the main environmental factors associated with driving selection pressures were wave exposure and microhabitat type. These results inform our understanding of niche diversification in triplefins by identifying characters under selection. Mapping phenotypic variation onto performance traits, both in terms of engineering considerations of phenotype and in terms of fitness, informs our understanding of the processes involved in the ecological speciation of this group of fishes. Plasticity was shown to be the mechanism for the observed morphological variation in *F. lapillum*. Phenotypic plasticity theoretically has the potential to generate disruptive selection, as the plastic morphologies will better suit their respective environments, thus providing the raw material upon which natural selection can act. Together, this suggests a basis for divergent selection in habitat use, and supports the hypothesis that differentiation in habitat use is an important mechanism responsible for the ecological speciation seen in the New Zealand triplefin fish assemblage.
References

List of References


References


References


References


References


Pepin, P., Shears, T.H. and Delafontaine, Y. (1992). Significance of body size to the interaction between a larval fish (Mallotus villosus) and a vertebrate predator (Gasterosteus aculeatus). Marine Ecology Progress Series 81: 1-12.


125


References


Appendix

Appendix figure 1. Sea surface temperature from Leigh marine laboratory monitoring, and seasonal density of four species of triplefins (all age classes).
Appendix figure 2. Fifteen year time series of mean monthly wave power for outer Hauraki Gulf coastal vs offshore sites.

Appendix figure 3. Mean monthly wave power, averaged over fifteen years (1998-2012) for outer Hauraki Gulf coastal vs offshore sites.
Appendix table 1. Bonferroni’s pairwise comparisons for wave exposure, microhabitat and year for *Forsterygion lapillum* density from the general linear model. Significant interactions are highlighted.

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Based on observed means. The error term is mean square (error) = 0.368.
* The mean difference is significant at the 0.05 level.
Appendix table 2. Bonferroni’s pairwise comparisons for wave exposure, microhabitat and year for *Forsterygion varium* density from the general linear model. Significant interactions are highlighted.

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Based on observed means. The error term is mean square (error) = 0.127.

* The mean difference is significant at the 0.05 level.
Appendix table 3. Bonferroni’s pairwise comparisons for wave exposure, microhabitat and year for *Ruanoho whero* density from the general linear model. Significant interactions are highlighted.

<table>
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Based on observed means. The error term is mean square (error) = 0.049.

* The mean difference is significant at the 0.05 level.
Appendix table 4. Bonferroni’s pairwise comparisons for wave exposure, microhabitat and year for *Notoclinops segmentatus* density from the general linear model. Significant interactions are highlighted.

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Based on observed means. The error term is mean square (error) = 0.055.

* The mean difference is significant at the 0.05 level.
Appendix figure 4. Seasonal density of all individuals for three successive years of A) *F. lapillum*, C) *F. varium*, C) *R. whero*, and D) *N. segmentatus* across a wave exposure gradient in the Hauraki Gulf. Shaded bars indicate peak spawning periods.
Appendix figure 5. Seasonal density (fish per m²) of all individuals for three successive years of A) *F. lapillum*, B) *F. varium*, C) *R. whero*, and D) *N. segmentatus* in different habitats. EFD = *Ecklonia* forest deep; EFS = *Ecklonia* forest shallow; MA = mixed algae; STA = shallow turfing algae; COB = cobbles.
Appendix figure 6. Fifteen year time series of mean monthly wave power for Jones Bay vs Takatu Point.

Appendix figure 7. Mean monthly wave power, averaged over fifteen years (1998-2012) for Jones Bay vs Takatu Point.
Appendix figure 8. Relationship between male length and nest size for A) *F. lapillum* and B) *F. varium* at different wave exposure locations.
Appendix figure 9. Relationship between male length and nest size for *F. varium* in different habitats.