INTRAGUILD PREDATION IS A MECHANISM OF DIVERGENT SELECTION IN THE THREESPINE STICKLEBACK

by

Sara Elizabeth Miller

B.A., University of Chicago, 2006

M.S. University of Missouri, St. Louis, 2010

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

August 2016

Abstract

Biotic interactions among species are thought to be important for the generation of phenotypic diversity. Intraguild predation is a common ecological interaction that occurs when a species preys upon another species with which it competes. This interaction is potentially a mechanism of divergence between intraguild prey populations, but it is unknown if cases of character shifts in intraguild prey are phenotypically plastic or an evolutionary response. I collected threespine stickleback (*Gasterosteus aculeatus*) from lakes with and without prickly sculpin (*Cottus asper*) and identified trait differences in armour and behaviour among populations in the wild. Differences in behavioural and morphological traits among freshwater populations persisted in a common garden, suggesting that adaptation to intraguild predation has a genetic basis. To date, the evolutionary effect that biotic selection has upon an organisms' genome remains largely unknown in natural populations. I used whole genome re-sequencing to investigate the extent of genetic differentiation between stickleback from populations with and without sculpin. The main axis of genetic variation in these populations is strongly associated with the presence or absence of sculpin. I identified the regions of the genome that have differentiated in parallel between lakes with and without sculpin, and measured the strength of this divergence. The presence or absence of sculpin corresponds to widespread differentiation that is unevenly distributed across the stickleback genome. Adaptation to intraguild predation may involve hundreds of genes with diverse functions. Observations of extensive phenotypic and genetic differentiation between stickleback from lakes with and without sculpin provide indirect evidence that sculpin are the cause of trait differences.

Pelvic morphology is one of the most conspicuously varying traits among freshwater stickleback populations. This variation has been hypothesized to be the result of predation by fish and/or insect predators. I conducted a selection experiment to test if sculpin were an agent of selection for pelvic spine length. The results were combined with other experimental selection studies and used in a meta-analysis. Fish predators are an agent of selection for longer pelvic spines, but the role of insect predators is still unclear. Intraguild predation is a mechanism of divergent selection in threespine stickleback.

Preface

A version of chapter 2 has been published as Miller SE, Metcalf D, Schluter D. (2015) Intraguild predation leads to genetically based character shifts in the threespine stickleback. *Evolution*, 69:3194-3203. I designed the experiment, collected field samples, gathered morphometric measurements, conducted behavioural tests, and wrote the paper. Daniel Metcalf assisted with field collections, animal care, and analysis of behavioural videos. Dolph Schluter helped with experimental design, statistical analysis, and writing of the paper.

Work for chapter 3 was conducted in collaboration with Dolph Schluter. I collected samples with assistance from various members of the Schluter lab. Daniel Bolnick, Jeffrey McKinnon, Sean Rogers, and Monica Yau generously provided additional stickleback specimens. Kevin Brix contributed the Na/Ca/Conductivity measurements used in table 3.1. I designed the experiment, prepared genomic libraries, created bioinformatics pipelines, and wrote the paper. Dolph Schluter contributed to the experimental design, bioinformatics methods, data analysis, and writing of the paper.

For Chapter 4, I designed and conduced the mesocosm experiment and meta-analysis and wrote the paper. Daniel Metcalf provided assistance with the set-up and monitoring of the mesocosm experiment. These new data were used in the meta-analysis along with studies referred to in Mirjam Barrueto's thesis (Barrueto 2009) and more recent publications. Tuomas Leinonen, Andrew MacColl, and Kenyon Mobley provided additional raw data that was incorporated into the meta-analysis. Dolph Schluter contributed to the

experimental design and analysis of the mesocosm experiment and meta-analysis and helped with writing the paper.

A version of chapter 5 has been published: Miller SE, Samuk KM, Rennison DJ. (2016) An experimental test of the effect of predation upon behaviour and trait correlations in the threespine stickleback. Biological Journal of the Linnean Society. The trout predation experiment was designed and run by Diana Rennison as part of her PhD thesis. I designed the behavioural experiment, conducted statistical analysis, and wrote the paper with input from the other authors. Behavioural data was collected and analysed by all of the authors.

Protocols requiring the use of live animals were approved by the UBC animal care committee (A07-0293, A11-0402). Permits for scientific collections were obtained from the British Columbia Ministry of Environment (NA-SU10-60714, NA-SU10-68002, NA-SU12-76311, NA-SU13-85151, NA-SU14-93473).

Table of Contents

Abstract ii

Preface iv

Table of Contents vi

List of Tables x

List of Figures xi

List of Abbreviations xiii

Acknowledgements xiv

Dedication xv

Chapter 1: Introduction 1

1.1 General Introduction 1

1.2 The Study System 2

1.3 Intraguild Predation and Threespine Stickleback 3

1.4 Summary of Studies 4

Chapter 2: Intraguild Predation Leads to Genetically Based Character Shifts in the Threespine Stickleback 7

2.1 Introduction 7

2.2 Materials and Methods 11

2.2.1 Study Populations and Sample Collection 11

2.2.2 Common Garden and Plasticity Experiment 12

2.2.3 Morphology 14

2.2.4 Stickleback Behaviour 16
2.2.5 Statistical Analysis 18
2.3 Results 18
2.3.1 Character Shifts in Wild-Caught Stickleback 18
2.3.2 Character Shifts Persisted in a Common Garden 19
2.3.3 Sculpin Exposure Induced Character Shifts in Marine Stickleback 20
2.3.4 Maternal Effects 21
2.4 Discussion 21
2.4.1 Trait Shifts in Response to Intraguild Predation 21
2.4.2 Trait Inducibility has been Lost in Freshwater Populations 23
Chapter 3: Intraguild Predation Leads to a Multitude of Genomic Changes but is Constrained by Genomic Architecture 39
3.1 Introduction 39
3.2 Materials and Methods 42
3.2.1 Sample Collection and Library Preparation 42
3.2.2 Bioinformatics Pipeline 44
3.2.3 Divergence Among Populations 45
3.2.4 Candidate Genes 48
3.3 Results 48
3.3.1 Sculpin Presence is not Correlated with Abiotic Environment 48
3.3.2 Genomic Divergence is Associated with Presence/Absence of Intraguild Predator 49

3.3.3 Genetic Differentiation is Extensive but Unevenly Distributed 49
3.3.4 Candidate Adaptive Genes 51
3.4 Discussion 51
Chapter 4: A Comparative Analysis of Experimental Selection on the Stickleback Pelvis 66
4.1 Introduction 66
4.2 Methods 71
4.2.1 Mesocosm Experiment 71
4.2.2 Comparison with Other Selection Studies 73
4.3 Results 75
4.3.1 Mesocosm Experiment 75
4.3.2 Meta-Analysis of Selection Studies 75
4.4 Discussion 76
Chapter 5: An Experimental Test of the Effect of Predation Upon Behaviour and Trait Correlations in Threespine Stickleback 92
5.1 Introduction 92
5.2 Methods 94
5.2.1 Experimental Design 94
5.2.2 Behavioural Assays 96
5.2.3 Armour Traits 98
5.2.4 Statistical Analysis 99
5.3 Results 99

5.4 Discussion 101

5.4.1 Stickleback Behaviour 101

5.4.2 Correlations Between Morphology and Behaviour 102

Chapter 6: Conclusion 112

6.1 Overview 112

6.2 Broader Implications 116

References 120

Appendices 135

Appendix A 135

Appendix B 136

List of Tables

Table 2.1: Wild caught stickleback trait measurements 36

Table 2.2: Trait loadings for principal component analysis of armour of wild stickleback ... 37

Table 2.3: Experimental stickleback trait measurements..... 38

Table 3.1: Abiotic traits for lakes 63

Table 3.2: Comparison of environmental variables between lake types..... 64

Table 3.3: Outlier windows and Hidden Markov Model results..... 65

Table 4.1: Experimental studies of piscivorous predators..... 88

Table 4.2: Experimental studies of insect predators 90

Table 5.1: Trait loadings for principal component analysis of armour of experimental stickleback..... 108

Table 5.2 Linear model of behavioural traits, armour principal component score, and treatment 109

Table 5.3: Behaviour and morphology trait values for each experimental pond 110

Table 5.4: Spearman's rank correlations between traits..... 111

List of Figures

Figure 2.1 : Map of sampling locations used in the chapter 26

Figure 2.2 : Schematic of crosses used in the common garden experiment..... 27

Figure 2.3 : Landmarks coordinates used for morphometrics 28

Figure 2.4 : The set-up for behavioral assays 29

Figure 2.5 : Armour PC1 and vertical position in the water column of wild populations of stickleback..... 31

Figure 2.6 : Armour PC1 for experimental stickleback in common garden 32

Figure 2.7 : Body shape axis 1 for experimental stickleback 33

Figure 2.8 : Position in the water column for experimental stickleback 34

Figure 2.9 : Time spent near the experimental shoal..... 35

Figure 3.1: Map of sampling locations of sequenced stickleback 56

Figure 3.2: Principal component analysis of abotic traits of lakes 57

Figure 3.3 : Principal component 1 of all SNPs..... 58

Figure 3.4 : Principal components 2 and 3 of all SNPs 59

Figure 3.5 : CS' and F_{ST} for 10,000 bp windows throughout the genome 60

Figure 3.6 : Genome-wide distribution of CS' score 61

Figure 3.7 : CS' score and hidden markov model for chromosome twelve 62

Figure 4.1 : Surviving threespine stickleback with clipped and unclipped pelvic spines..... 81

Figure 4.2 : Forest plot of the effect size for all fish predation experiments 82

Figure 4.3 : Forest plot of the effect size for all insect predation experiments 83

Figure 4.4 : Forest plot of the effect size for all fish predation studies 84

Figure 4.5 : Forest plot of the effect size for all insect predation studies. 85

Figure 4.6 : Effect size and standard error of experiments used in the meta-analysis 86

Figure 4.7 : Forest plot of the effect size of standard length for all predation experiments 87

Figure 5.1 : Reaction norms for behavioural traits between control and predation ponds 105

Figure 5.2 : Correlations between behaviour and armour traits. 106

List of Abbreviations

IGP	Intraguild Predation
PCA	Principal Component Analysis
CS’	Cluster Separation Score Metric
SNP	Single Nucleotide Polymorphism

Acknowledgements

I would like to thank my supervisor Dolph Schluter for his constant support and help throughout my PhD. He was instrumental in my growth as a scientist, writer, and thinker. I am also grateful to the valuable suggestions and advice of the members of my committee: Darren Irwin, Loren Rieseberg, and Eric Taylor.

The many members of the Schluter lab provided an amazing community in which to work. My dissertation would not have been possible without the pioneering work on the sculpin/no sculpin lake system by Travis Ingram and Richard Svanbäck. A special thanks to the legions of undergraduate (and graduate, and post doc) volunteers who helped me in the field and in the fish room. Seth Rudman answered all of my questions about the outside. Kieran Samuk answered all of my questions about computers. Kevin Brix gave me an appreciation for physiology. Thor Veen convinced me of the wonders of base R. Greg Owens, although only an honorary member of the lab taught me much about bioinformatics. Dan Metcalf was an invaluable research assistant and kept me from being eaten by bears. And a big thank you to Diana Rennison for all of the above and for your steadfast support, help, advice, and humour.

I am also appreciative to many members of the Biodiversity Building and the UBC community. In particular, Kira Delmore was my right hand for many years – I would not be here without you! And to the members of CDWMB, Erin Fenneman, Jessica Lu, Kaeli Johnson, Kate McGrath and Virginia Woloshen, thank you for helping me to keep my sanity.

Last but not least, to my Mom, thank you for your never-ending love and encouragement.

In memory of my father.

Chapter 1: Introduction

1.1 General Introduction

Species do not exist in isolation. Species interact with each other and these interactions can be an important cause of natural selection. Thompson (2013) has suggested that, "Evolutionary rates are just as much about the pace at which interactions among species evolve as they are about the rates of genome evolution of each species".

Interactions among species have been previously shown to lead to the evolution of divergent phenotypes. For example, it has been well established that interspecific competition for resources can lead to phenotypic divergence ("Character Displacement") as character shifts that allow species to use alternative resources will decrease competition and be favoured by natural selection (Schluter 2000b; Stuart and Losos 2013). Similarly, experimental studies have found that presence of predators can lead to the evolution of trait divergence (e.g. McPeek 1995; Vamosi 2002; Langerhans et al. 2007).

Intraguild predation is a widespread ecological interaction in which a predator is also a competitor with its prey species (Polis et al. 1989; Arim and Marquet 2004). Intraguild predation has also been predicted to lead to the evolution of trait divergence but this prediction has not been tested (Schluter 2000b; Nosil 2012).

My dissertation uses an interdisciplinary approach combining field measurements, modern genetic techniques, and selection experiments to explore the impact that biotic selection from a single species has on the evolution of another species. I focus primarily on

the evolution of trait divergence in the threespine stickleback (*Gasterosteus aculeatus*) in response to intraguild predation.

1.2 The Study System

The threespine stickleback is a small fish that is common in marine and freshwater habitats throughout much of the Northern Hemisphere. A striking characteristic of stickleback is the frequent parallel evolution among isolated freshwater populations in similar environments (McKinnon and Rundle 2002).

Lakes in southwestern British Columbia were created following the end of the last ice age, approximately 10,000 years ago, and were colonized by marine stickleback at that time (Bell and Foster 1994). In six of these lakes, a benthic and limnetic species of stickleback occur in sympatry in the same lake (Schluter and McPhail 1992; Gow et al. 2008). The remaining lakes contain only a single species of stickleback (McPhail 2007). Chapters 2-4 of thesis will focus on freshwater populations from lakes with a single species of threespine stickleback and chapter 5 will address populations containing two stickleback species.

An advantageous feature of lake stickleback is that the modern marine population is probably roughly equivalent to the original colonizing population. Comparisons between marine and freshwater stickleback can help us understand the direction of trait evolution in these populations. Furthermore, the Pacific Ocean marine population has a large population size and serves as a reservoir of standing genetic variation (Schluter and Conte 2009; Hohenlohe et al. 2010; Jones et al. 2012a). The recent origin of the freshwater populations means that little time has passed to allow for the origin and spread of new mutations and

as a result, standing genetic variation from the marine stickleback that colonized these populations is the likely source of most of the adaptive variation.

Lakes containing freshwater stickleback can vary in fish community composition (McPhail, 2007). Therefore, we can isolate the effect of intraguild predation on threespine stickleback by comparing stickleback between similar lakes that differ by the presence or absence of prickly sculpin (*Cottus asper*), an intraguild predator of freshwater stickleback.

Sculpin are bottom dwelling fish that live in the littoral zone and lack a swim bladder (McPhail 2007). Sculpin consume benthic invertebrates, but once sculpin reach 70mm, other species of fish, including threespine stickleback, become a component of their diet (McPhail 2007). Predation on stickleback therefore provides a dual reward for sculpin: the direct benefit of a meal, and the indirect benefit of reduced competition.

1.3 Intraguild Predation and Threespine Stickleback

Intraguild predation occurs when a predator kills and eats a species that is a potential competitor for shared resources (Polis et al. 1989). Intraguild predation is predicted to have a more complex effect on population dynamics than predation or competition alone (Polis et al. 1989; Holt and Polis 1997). Continual coexistence of intraguild predators (IG-predators) and intraguild prey (IG-prey) relies on several conditions: an IG-prey species should be a superior competitor on shared resources or should shift its niche in the presence of the IG-predators, and anti-predator defences may be important for IG-prey survival (Polis et al. 1989; Holt and Polis 1997; Daugherty et al. 2007; Kratina et al. 2010). As a result, intraguild predation may lead to changes in IG-prey phenotype for traits affecting competition and defence.

We predict that intraguild predation by prickly sculpin will cause evolutionary shifts in stickleback traits related to anti-predatory defence and foraging. The role of anti-predator defences has not been investigated in these populations. However, a previous study provided preliminary evidence that stickleback increased the proportion of zooplankton in their diet when sculpin are present. In a mesocosm experiment, a population of stickleback sympatric with sculpin consumed more zooplankton than stickleback from a population without sculpin (Ingram et al. 2012). In the wild, stickleback from lakes with sculpin have shifted to limnetic-like body shape compared to stickleback from lakes without sculpin (Ingram et al. 2012). A limnetic body shape has been correlated with increased feeding upon zooplankton in the open water (Willacker et al. 2010). However, these trait differences may be induced by the presence of sculpin and caused by phenotypic plasticity, not evolution.

I carried out a series of studies to test if intraguild predation has lead to the evolution of trait divergence between stickleback populations that occur with and without sculpin.

1.4 Summary of Studies

In chapter 2, I establish that stickleback from populations sympatric with sculpin have genetically based trait differences. I collected stickleback specimens from lakes with and without sculpin and measured defensive armour and behaviour in wild samples. To determine if differences among populations have a genetic basis or are induced by the presence of sculpin (phenotypic plasticity), I raised stickleback from lakes with and without sculpin, as well as marine stickleback, in a common garden in the lab. To examine if the

presence of sculpin induced trait changes, each stickleback family was split in half and raised in the presence or absence of sculpin. For stickleback reared in the common garden, I measured armour, body shape, and behaviour and compared these traits among half-families that were raised in the control or sculpin treatment.

Selection acts on phenotype, but a shift in phenotype is mediated through the evolution of genes that underlie those phenotypes. The effect that biotic selection has upon an organism's genome is largely unknown for wild populations. In Chapter 3, I used whole genome re-sequencing to investigate the extent of genetic differentiation between stickleback from populations with and without sculpin. I developed a genome scan metric (CS') to identify the regions of the genome that have differentiated in parallel between lakes with and without sculpin, and to quantify the strength of this divergence. I investigated the genome architecture of divergence between lakes with and without sculpin by calculating the number of genes and potentially the number of selective sweeps identified in regions that are differentiated among population types. I looked for overrepresentation of gene ontology (GO) terms for genes in outlier regions.

Chapter 4 tests if prickly sculpin are an agent of selection on the length of stickleback pelvic spines. In chapter 2, I discovered that stickleback populations sympatric with sculpin had longer pelvic spines than stickleback populations without sculpin. The stickleback pelvis has been hypothesized to be an anti-predator defence against piscivorous predators. Increased insect predation on stickleback with longer pelvic spines has been proposed as an alternative hypothesis for the variation in pelvic spine length. I carried out a mesocosm experiment to determine if sculpin preferentially consumed stickleback with shorter pelvic spines. I physically modified the length of the pelvic spines of stickleback

from two populations sympatric with sculpin and then compared the mortality rate of stickleback with clipped and unclipped pelvic spines experimentally in the presence of sculpin. To evaluate the predation hypotheses, I used a meta-analysis approach to combine the results of the mesocosm experiment with other experimental studies of selection on stickleback pelvic morphology from fish and insect predators.

Finally, in chapter 5 I extend my analysis to examine if biotic selection by another species is a mechanism of divergent selection on behaviour in benthic and limnetic stickleback. Limnetic stickleback primarily live in the open water and eat zooplankton, while benthic stickleback consume macroinvertebrates in the littoral zone (Schluter and McPhail, 1992). The two species have diverged in shoaling preference and preferred position in the water column (Larson, 1976; Vamosi and Schluter, 2002; Wark *et al.*, 2011). This behavioural divergence has been hypothesized to be the result of divergent selection driven in part by differential predation from coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) on limnetics in the open water. To experimentally test this hypothesis, benthic-limnetic hybrids families were split and raised in large experimental ponds in a predation treatment with trout, or in a control treatment without trout. I measured shoaling preference and preferred position of the stickleback hybrids in the water column using the behavioural tests developed in chapter 2.

Chapter 2: Intraguild Predation Leads to Genetically Based Character Shifts in the Threespine Stickleback[1]

2.1 Introduction

Interspecific resource competition can lead to increased phenotypic diversity as natural selection favours character shifts that decrease competition and promote the use of alternative resources (Schluter 2000a,b; Pfennig and Pfennig 2010; Stuart and Losos 2013). Other trophic interactions may also lead to divergence between closely related species (Schluter 2000b; 2003; MacColl 2011; Nosil 2012). Experimental studies have verified that divergence in traits in response to predation (Endler 1980; Vamosi 2002; Langerhans et al. 2007; Marchinko 2009) and parasitism (Hudson and Greenman 1998; Laine 2009) have a genetic basis. Intraguild predation has been predicted to increase phenotypic diversity between lineages (Schluter 2000b), but the evolution of character shifts in response to intraguild predation has not been tested.

Intraguild predation occurs when a predator is also a competitor of its prey species (Polis et al. 1989; Holt and Polis 1997; Hart 2002; Arim and Marquet 2004). Competition from intraguild predators (IG-predators) can shift the diet of intraguild prey (IG-prey) to include alternative food sources (Polis et al. 1989; Vance-Chalcraft et al. 2007; Ingram et al. 2012). Simultaneously, predation from IG-predators can result in increased anti-predator behaviour and defensive structures of IG-prey (Polis et al. 1989; Kratina et al. 2010;

[1] A version of this chapter has been published: Miller SE, Metcalf D, Schluter D. (2015) Intraguild predation leads to genetically based character shifts in the threespine stickleback. *Evolution*, 69:3194-3203.

Walzer and Schausberger 2013; Vanak et al. 2013), as well as behavioural shifts to alternative habitats to reduce predation on these same prey (Donadio and Buskirk 2006).

We investigated the evolution of character shifts in freshwater threespine stickleback *(Gasterosteus aculeatus)* in response to an intraguild predator. Freshwater populations formed when marine or anadromous (hereafter, "marine") stickleback became isolated in numerous lakes at the end of the last ice age, approximately 12,000 years ago. These populations adapted rapidly to freshwater in isolation from each other and from the marine environment (Bell and Foster 1994). A subset of these lakes was also colonized by prickly sculpin (*Cottus asper*) (Dennenmoser et al. 2015), a freshwater teleost fish and intraguild predator of the threespine stickleback (McPhail 2007). Sculpin grow to larger size than stickleback and consume juvenile and adult stickleback up to 60% of their body length (Reimchen 1994; McPhail 2007). Prickly sculpin are cryptic ambush predators of stickleback and they also eat benthic invertebrates (McPhail 2007).

Preliminary evidence indicates that intraguild predation has led to phenotypic changes in stickleback that decrease competition and/or predation from sculpin. In the wild, stickleback from lakes with sculpin show a shift to a limnetic-like body shape. In contrast, stickleback from lakes without sculpin are more benthic-like with a deeper body, a wider caudal-peduncle, and a posterior shift in the first dorsal spine (Ingram et al. 2012). Differences in stickleback body shape correlate with diet (Willacker et al. 2010). In a mesocosm experiment, stickleback from a population sympatric with sculpin had more zooplankton in their diet than stickleback from a population without sculpin, whose diet consisted of more benthic prey. When sculpin were experimentally added to mesocosms, stickleback from both populations increased the proportion of zooplankton consumed

(Ingram et al. 2012). The addition of sculpin also increased stickleback mortality and reduced growth rate, but to a lesser extent in the stickleback population sympatric with sculpin than stickleback from the sculpin-absent lake (Ingram et al. 2012), suggesting that they are less susceptible to predation. Differences between populations in other traits such as armour and behaviour are likely, but have not been measured. The presence of predators is often associated with greater defensive armour in stickleback (Reimchen 1994; Vamosi and Schluter 2004; Willacker et al. 2010; Leinonen et al. 2011; Lescak and von Hippel 2011; Lacasse and Aubin-Horth 2012), as well as differences in sociality and shoaling (Vamosi 2002; Bell and Sih 2007; Dingemanse et al. 2007; 2009; Lacasse and Aubin-Horth 2012). Longer spines increase the body diameter of the stickleback, making them more difficult for gape-limited predators to ingest (Hoogland et al. 1956) and lateral plates provide structural support for spines (Reimchen 1983). Increased zooplankton in the diet suggests greater use of the water column by stickleback from lakes with sculpin, which may decrease the rate of encounter (Lima and Dill 1990).

One approach to testing evolutionary character shifts in IG-prey is to ask whether putative cases fulfill criteria analogous to those routinely used to test for ecological character displacement (modified from Schluter and McPhail 1992): (1) Phenotypic differences have a genetic basis; (2) Differences are not due to chance; (3) Divergence should be the outcome of evolution rather than species sorting; (4) Shifts in phenotype reflect differences in resource use and/or predation risk; (5) Shifts are not the result of other environmental differences between sites with and without IG-predators; and (6) There is independent evidence that pre-shift IG-prey phenotypes compete with and suffer predation from the IG-predator.

Here we evaluate the first criterion. Character shifts in response to intraguild predation might be the result of either phenotypic plasticity or genetic change (West-Eberhard 2003). Plasticity can lead to rapid character shifts because the match of phenotype to environment occurs without waiting for the spread of adaptive alleles (West-Eberhard 2003; Schlichting and Pigliucci 1998). Adaptive phenotypic plasticity in IG-prey behaviour (Heithaus 2001; Janssen et al. 2007; Amarasekare 2008) or inducible anti-predator defences (Urbani and Ramos-Jiliberto 2010; Kratina et al. 2010; Nakazawa et al. 2010) have been shown to increase survival of IG-prey in theoretical models. Alternatively, genetic mapping studies based on crosses between marine and freshwater stickleback populations have found different QTL between populations inhabiting lakes with and without sculpin associated with body shape differences and armour components (Rogers et al. 2012), suggesting that many trait differences between the population types have at least a partial genetic basis. Distinguishing between phenotypic plasticity and genetic evolution is also important for predicting community dynamics (Cortez 2011; Yamamichi et al. 2011). However, experimental studies are required to test whether character shifts have a genetic basis (Scheiner 1993).

In this study, we describe character shifts in body armour, body shape, and behaviour among natural populations of stickleback that occur with and without prickly sculpin. Stickleback were raised in a common garden to determine the relative role of genetics and phenotypic plasticity in these shifts. We assessed the inducibility of these traits by rearing split families in the presence and absence of sculpin. We included marine stickleback in the experiment to determine if phenotypic plasticity was present in the form representing the ancestral state. If prickly sculpin have led to the evolution of character

shifts in stickleback, individuals raised in a common garden will replicate the phenotypes of the parental populations and the presence of sculpin will not induced trait shifts.

2.2 Materials and Methods

2.2.1 Study Populations and Sample Collection

Lake characteristics and information on fish community composition were obtained from Habitat Wizard (*www.env.gov.bc.ca/habwiz*) maintained by the British Columbia Ministry of Environment. We identified eight lakes (8.0-58.7 ha) in southwestern British Columbia with a simple fish community of threespine stickleback, coastal cutthroat trout (*Oncorhynchus clarkii clarkii*), and prickly sculpin (*Cottus asper*) and contrasted these populations with eight lakes (3.7-44.6 ha) containing only threespine stickleback and trout (Figure 2.1). Cutthroat trout are ubiquitous in lakes in this region. All lakes are in separate watersheds, ensuring no gene flow between populations. Lakes with and without sculpin did not differ in mean area (Mann-Whitney test, $U=18$, $P=0.16$), perimeter ($U=16$, $P=0.10$), mean depth ($U=25$, $P=0.77$), elevation ($U=34$, $P=0.88$), or distance to the ocean ($U=28$, $P=0.72$). The study populations also included "marine" stickleback from two geographically distinct populations. Modern marine (including anadromous) stickleback are thought to be phenotypically similar to the ancestral populations that initially colonized the freshwater lakes following the last ice age (Bell and Foster 1994). Marine stickleback have a diverse and largely uncharacterized predator community including several species of marine sculpin (McPhail 2007).

Adult stickleback were collected in May-June 2011 and 2012 using 10-15 baited minnow traps placed overnight along the shoreline of each lake and at the marine sites.

Specimens collected for morphological analysis from all populations (Table S1; n=7-26/population) were euthanized using buffered MS-222 (Argent Chemical Laboratories, Redmond, WA) and preserved in 95% ethanol. Some sites were sampled in subsequent years to increase sample size. Additional adult stickleback were collected for behavioural experiments in 2011, but a sufficient number of specimens was only available for seven lakes without sculpin, three lakes with sculpin and one marine population (n=12-27/population). Stickleback were transported to the aquatics facility at the University of British Columbia and allowed to acclimatize for one week prior to behavioural trials. In 2012, adult stickleback in reproductive condition were collected from three lakes with sculpin (Ambrose, Paq, and Rosseau), three lakes without sculpin (Trout, Cranby and Kirk) and from two marine populations (Oyster Bay and Little Campbell) for a common garden and plasticity experiment (Figure 2.2). Sculpin were collected from Paq Lake at this time and were transferred to the aquatics facility.

2.2.2 Common Garden and Plasticity Experiment

We raised stickleback in a common garden laboratory environment in the presence and absence of sculpin. We created four families from each population by artificially crossing pairs of wild-caught fish at the lakeside. Eggs were obtained by gently pressing on the sides of females and placed into lake water. Males were euthanized with an overdose of MS-222 and testes were dissected, minced, and added to the eggs. We made reciprocal F1 crosses between stickleback from a lake with sculpin (Paq) and a lake without sculpin (Trout) to test for maternal effects on phenotypes. Paq and Trout lake populations have divergent body shape (Ingram et al. 2012) but are less differentiated in armour (Table 2.1).

Four crosses used females from Paq Lake and males from Trout Lake and four crosses used females from Trout Lake and males from Paq Lake.

Fertilized eggs were transferred to the University of British Columbia within 24 hours. At that time, each clutch was split. Half the eggs were assigned to a sculpin treatment and half to a control treatment. Each 100L experimental tank was divided in the center with window screen and contained three kilograms of coarse limestone gravel and 1ppt sodium chloride. Each half clutch was added to one side while the other side was left empty. A low concentration of methylene blue was added to inhibit fungal growth. Tanks were kept at 16L:8D photoperiod. One Ambrose clutch, one Rosseau, and two F1 clutches did not hatch (Figure 2.2).

The development of induced defences may depend upon the timing of exposure to the stimulus (Harvell 1990). Limited evidence suggests that even stickleback embryos can change behaviour in response to cues from trout predation (Golub 2013). Because we were uncertain of the stage at which exposure to sculpin might lead to induced defences, we provided sculpin cues for the duration of the experiment, from fertilized eggs until nine months of age. To provide possible olfactory cues, daily we added a 50ml aliquot of water from a tank containing four adult sculpin to unhatched eggs in each sculpin treatment tank. This continued until stickleback hatched and fry were four weeks of age. Dechlorinated water was added to the control treatment during this time. At four weeks post-hatching, stickleback fry were too large to pass through the window screen dividing each tank and were gathered and moved to a random side of the tank. At that time, we reduced the number of fry to 20 per tank. In the case of half clutches with fewer than 20 fry we reduced the number of fry to an equal density in the control and sculpin treatment tanks.

In the sculpin treatment, a single adult sculpin was added to the other side of the tank. In the control treatment, an equal biomass (four fish) of adult stickleback was added. Adult stickleback were F2 hybrids between Paxton Lake benthic and limnetic stickleback that had been raised in the laboratory for an unrelated study. The window screen dividing each tank allowed experimental stickleback to receive constant visual and chemical cues from the sculpin or the adult stickleback.

Stickleback were fed hatched brine shrimp nauplii for the first four months, and then a mixture of brine shrimp and bloodworms for the remainder of the experiment. Adult stickleback in the control treatment were fed a 3:1 mixture of bloodworms and *Mysis* shrimp to satiation daily. Sculpin do not eat *Mysis* shrimp and were fed only bloodworms.

The experiment was stopped at 36 weeks post-hatching. Several adult control stickleback died during the experiment and were immediately replaced upon discovery. There were no sculpin mortalities. A Rosseau family was excluded from analysis after a sculpin jumped to the other side of the tank and consumed the experimental stickleback. The final sample size was 35 families in 70 tanks.

2.2.3 Morphology

Samples stored in 95% ethanol were rehydrated, fixed in 10% formalin, and stained with alizarin red to highlight bony characteristics following standard procedures (Peichel et al. 2001). We measured standard length, gape width, first and second dorsal spine length, pelvic spine length, pelvic girdle length, and lateral plate number on both wild-caught and experimental stickleback (Figure 2.3). Spine measurements were made on the left side of the fish using digital calipers. Lateral plates were counted under a dissecting microscope. All

armour traits were log(x+1) transformed to homogenize variance. Experimental stickleback smaller than 28mm were excluded from analysis because the development of lateral plates may be incomplete in smaller stickleback (Hagen 1973; Bell 2001; Rennison et al. 2015). All wild-caught stickleback were >28mm.

To compare traits among stickleback of different sizes, all traits except lateral plates were size-adjusted to the mean standard length of the wild-caught samples (46.3 mm). For each trait, we fit a linear model with standard length as a covariate and population as a factor. All measurements were adjusted using the residuals from each regression (Vamosi 2002). The wild-caught samples were size-corrected separately from the common garden stickleback.

To minimize trait redundancy, stickleback armour variation was summarized with the first principal component (PC1) based on the correlation matrix between size corrected spine traits and lateral plates, separately for wild-caught and experimental stickleback. All armour traits had significant positive loadings on PC1, which accounted for 74.6% and 79.7% of the variance in wild stickleback and lab-raised stickleback (Table 2.2). Principal Component 1 was the only principal component with an eigenvalue greater than one therefore PC2-5 were not examined further.

We examined body shape in the experimental stickleback. The left side of each stickleback was photographed using a Nikon D300 camera. We placed 20 landmarks outlining the shape of the fish as well as the insertion points of spines and fins (Figure 2.3; Walker 1997; Ingram et al. 2012). Landmarks were digitized using tpsDig 2.16 software (Rohlf 2008) and were centered, scaled, and rotated using the *shapes* package (Dryden 2012) in the R 3.0 environment (R Core Team, 2014). We performed a linear discriminant

analysis (LDA) with the *MASS* package (Venables and Ripley 2010) to visualize shape differences among lakes. We used the tank (half-family) as our classification variable, and thus the LDA did not *a priori* differentiate between treatment or population type. An LDA was preferable to other types of multivariate methods such as a principal components analysis because it ignores trait combinations that vary only within populations (Tabachnick and Fidell 2012) and those resulting from measurement error or specimen bending. The first and second linear discriminant axes (LD1 and LD2) accounted for 34.3% and 15.9% of the observed variation in shape among half-families.

2.2.4 Stickleback Behaviour

We used a behavioural assay to measure position in the water column preferred by stickleback. Vertical position in the water column is a proxy for habitat use in guppies (Torres-Dowdall et al. 2012) and a lower position in the water column correlates with increased anxiety behaviour in zebrafish (*Danio rerio*, Egan et al. 2009; Cachat et al. 2010; Stewart et al. 2012). Limnetic stickleback from Paxton Lake prefer to be higher in the water column than benthic stickleback (Larson 1976).

Wild-caught stickleback in non-reproductive condition were transferred from their home tank to a holding basket next to the assay. Although Cachat et al. (2010) recommends a 1-hour recovery period, preliminary trials showed that a 15-minute acclimation period was sufficient. At the start of each trial, a focal fish was gently introduced to the top of an unfamiliar tank and was allowed to move freely for 330 seconds (Figure 2.4). The first 30 seconds of each trial were not analyzed, because the introduction of the stickleback into the tank often resulted in erratic movement. Trials were recorded

and videos were subsampled to 0.5 frames per second using VirtualDub (www.virtualdub.org). The x and y coordinate position of the focal fish was measured every two seconds using MtrackJ (Meijering et al. 2012) in ImageJ (Schneider et al. 2012). For each trial we calculated the mean vertical position and the total movement of the stickleback in pixels (distance traveled).

The water column height preference of the experimental stickleback was assayed at 28-31 weeks of age using ten stickleback chosen at random from each experimental tank. Tanks containing the same family were tested in the sculpin and control treatments sequentially in random order.

We further characterized the behaviour of experimental stickleback using a shoaling assay (Vamosi 2002; Kozak and Boughman 2008; Wark et al. 2011). A 100L tank was divided into two end compartments and one center arena using window screen (Figure 2.4). An experimental shoal of 10 unfamiliar stickleback was added to one end and two stickleback were added to the other end (Wark et al. 2011). A focal fish was introduced into the center arena and its distance to the stimulus shoal arena was used as a measure of shoaling preference. Shoaling assays were conducted two days after the water column preference assay using ten randomly chosen stickleback from each experimental tank. Stimulus stickleback were chosen from a stock of laboratory reared Priest Lake benthic stickleback. The stimulus population was selected because stickleback were similar in age and size to the experimental fish and were unrelated to all of the experimental populations. Experimental stickleback were moved to holding baskets near the shoaling assay for a fifteen-minute acclimation period. At the start of each trial, the focal stickleback was introduced into the center arena. Trials were recorded for 630 seconds and the first 30

seconds of each trial were not analyzed. The x and y coordinate position of the focal fish was calculated as described above. For each trial, we calculated the time spent within one body length of the stimulus shoal as well as the distance traveled. 463 shoaling videos were scored (Table 2.3).

2.2.5 Statistical Analysis

We tested for differences in mean trait values between wild-caught stickleback from lakes with sculpin and lakes without sculpin using linear models. Tests involving freshwater fish from the common garden used the tank mean as the unit of replication because each half-family was raised in the same tank. The experiment was analyzed using a linear mixed effects model with treatment (sculpin or control), population type (from a lake with or without sculpin) and their interaction as fixed factors and lake and family as random factors. Inducibility in the marine population was assessed in a separate analysis using a linear mixed effects model with treatment (sculpin or control) as a fixed factor and lake and family as random factors. Maternal effects were tested by comparing F1 crosses raised without sculpin using direction of cross as a fixed factor and family as a random factor.

2.3 Results

2.3.1 Character Shifts in Wild-Caught Stickleback

The presence of sculpin was associated with character shifts in armour and behaviour in wild populations of stickleback. Stickleback from lakes with sculpin had higher mean armour PC1 scores than stickleback from lakes without sculpin (Figure 2.5A; $F_{1,14}$= 33.9, P<0.001). All individual armour traits were greatest in stickleback from lakes with

sculpin (Table 2.3). There was no difference in gape width ($F_{1,14}$= 3.01, P=0.11) or standard length ($F_{1,14}$= 1.86, P=0.19) between lakes with and without sculpin.

Stickleback from lakes with sculpin also preferred a higher mean vertical position in the water column than stickleback from lakes without sculpin (Figure 2.5B; $F_{1,8}$= 8.0, P=0.02). Distance traveled was not different between stickleback from the two types of lakes ($F_{1,8}$= 0.12, P=0.73).

2.3.2 Character Shifts Persisted in a Common Garden

Common garden stickleback from lakes with sculpin had a higher mean armour PC1 score than populations from lakes without sculpin (Figure 2.6, filled circles; $F_{1,4}$= 12.5, P=0.047). Individual armour traits were similar between stickleback raised in the control treatment of the common garden and values of wild caught stickleback from the same lake (Table 2.4). Exposure to sculpin did not induce a detectable change in PC1 score (Figure 2.6, open circles; Treatment: $F_{1,19}$= 0.17, P=0.38; Treatment x Population Type: $F_{1,19}$= 0.41, P=0.53).

Stickleback from lakes with and without sculpin differed in mean body shape in the common garden (Figure 2.7, filled circles). Lakes with sculpin were significantly differentiated in both LD1 ($F_{1,4}$= 13.2, P=0.022) and LD2 ($F_{1,4}$= 31.1, P=0.005). Stickleback from lakes with sculpin had an anterior shift in first dorsal spine, decreased body depth, a narrower caudal-peduncle, larger eye diameter and a longer jaw. Exposure to sculpin did not induce a detectable difference in mean shape (LD1: $F_{1,19}$= 0.0, P=0.995; LD2: $F_{1,19}$= 0.26, P=0.62; all treatment x population type interactions were non-significant, P>0.1).

Common garden stickleback from lakes with and without sculpin also differed in behaviour. As we saw in wild-caught stickleback, lab-raised stickleback from lakes with sculpin preferred a higher mean position in the water column (Figure 2.8, filled circles; type: $F_{1,4}$= 16.1, P=0.016). Stickleback from lakes with and without sculpin traveled a similar distance during the trials (type: $F_{1,4}$= 0.8, P=0.41). In the shoaling assay, stickleback from lakes with sculpin spent less time near the stimulus shoal (decreased shoaling preference) than stickleback from lakes without sculpin (Figure 2.9, filled circles; $F_{1,4}$= 18.1, P=0.013). Population types traveled a similar distance during the shoaling assay ($F_{1,4}$= 0.9, P=0.39). Exposure to sculpin did not detectably alter any behaviour (water column position: $F_{1,17}$= 0.1, P=0.76; water column distance: $F_{1,17}$= 0.4, P=0.54; shoaling preference: $F_{1,16}$= 2.6, P=0.13; shoaling distance: $F_{1,16}$= 1.4, P=0.25; all treatment x population type interactions were non-significant, all P>0.1).

2.3.3 Sculpin Exposure Induced Character Shifts in Marine Stickleback

Phenotypic plasticity was observed in several traits in marine stickleback. Marine stickleback raised in the sculpin treatment had higher armour PC1 scores than those raised in the control treatment (Figure 2.6; $F_{1,7}$= 6.7, P=0.016). Adding the category "marine" as a population type to our previous analysis of experimental populations from lakes resulted in a significant treatment x population type interaction (PC1: $F_{1,27}$= 5.65, P=0.025), hinting that the marines are more plastic than the freshwater populations. Body shape did not differ between treatments (LD1: $F_{1,7}$= 0.1, P=0.81; LD2: $F_{1,7}$= 0.1, P=0.76). In the water column preference assay, marine stickleback from the sculpin treatment showed a marginal but non-significant increase in mean water column position (Figure 2.8; $F_{1,7}$= 4.5, P=0.07;

treatment x population type: $F_{1,25}$= 3.39, P=0.08), and in the shoaling assay, marines in the sculpin treatment had a decrease in shoaling tendency (Figure 2.9; $F_{1,5}$= 8.0, P=0.037) and a significant treatment x population type interaction ($F_{1,22}$= 6.77, P=0.016).

2.3.4 Maternal Effects

Armour traits in F1 hybrids between Trout Lake and Paq Lake stickleback were intermediate between the parental populations (Figure 2.6) and direction of cross did not affect trait value (PC1: $F_{1,4}$= 8.0, P=0.11). Similarly, overall body shape was intermediate between the parental populations, but F1 families with Trout Lake mothers (without sculpin) had a larger mean LD1 score (LD1: $F_{1,4}$= 9.5, P=0.037), than F1 families with Paq Lake mothers (with sculpin), indicating that maternal effects may impact body shape in these populations. There was no difference in LD2 ($F_{1,4}$= 1.4, P=0.3).

2.4 Discussion

2.4.1 Trait Shifts in Response to Intraguild Predation

The presence of an IG-predator, prickly sculpin, is associated with character shifts in multiple traits in the threespine stickleback, and the results herein indicate that these trait shifts have a genetic basis. Wild populations of stickleback sympatric with sculpin show parallel increases in armour morphology, prefer to be higher in the water column, and have been previously shown to differ in body shape (Ingram et al. 2012). These differences in armour, shape, and behaviour persisted in a common garden. To our knowledge, this system is the first confirmed case of genetically based character divergence associated with intraguild predation.

Competition, predation, or both might produce character shifts in response to intraguild predation and disentangling these interactions will be challenging. Piscivorous predators have previously been associated with longer spines and an increased number of lateral plates in stickleback (Hagen and Gilbertson 1972; Moodie 1972; Bell et al. 1993; Reimchen 1994; Reimchen and Nosil 2002; Baker et al. 2010; Leinonen et al. 2011, Lescak and von Hippel 2011). Increased armour in lakes with sculpin might be a response to increased predation, though number of lateral plates might also affect buoyancy (Myhre and Klepaker 2009) and drag (Walker 1997). Alternatively, it is possible that shifts in armour are the indirect outcome of a habitat shift between sculpin and stickleback. Prickly sculpin prefer the littoral zone of lakes where there is easy access to cover and benthic invertebrates (McPhail 2007). Sculpin may displace stickleback into the pelagic environment either by decreasing benthic resources, increasing the threat of predation, or both. Because coastal cutthroat trout are more prevalent in the open water (Reimchen 1994), longer spines might be an adaptation to increased predation from trout, rather than a direct response to predation by sculpin. A third, less plausible, hypothesis is that sculpin predation on benthic invertebrates indirectly relaxes selection for reduced spines. Juvenile stickleback are eaten by large aquatic insects and studies suggest that some insects capture stickleback by grabbing the spines (Reist 1980; Reimchen 1980; Marchinko 2009; although see Lescak et al. 2012 and Mobley et al. 2013). Spine length might represent a balance between selection for longer spines by gape-limited predators and selection for shorter spines via predation by aquatic insects upon juveniles stickleback (Reimchen 1980).

Similarly, trait shifts in behaviour could also be attributed to either competition or predation. We found that in the wild and in the lab, stickleback from lakes with sculpin

preferred to be higher in the water column. A position higher in the water column might lessen risk of predation from sculpin. We also observed a decreased shoaling preference in stickleback from lakes with sculpin and in marine stickleback reared in the sculpin treatment. Sculpin are ambush predators, therefore shoaling may not be an effective method for escaping sculpin predation. Alternatively, differences in water column and shoaling preference may be a response to changes in foraging behaviour caused by resource depletion by sculpin. The presence of sculpin has been demonstrated to induce a higher proportion of zooplankton in the stickleback diet (Ingram et al. 2012), and zooplankton is most abundant in the open water. Trait shifts in behaviour could also interact with shifts in morphological traits. For example, diet preference and body shape vary with lateral plate number (Bjaerke et al. 2010). Intraguild predation may independently select for trait shifts in behaviour and morphology, or changes in behaviour may have led to selection for changes in morphology (or vice versa). These alternatives underscore the challenge of elucidating the relative impacts of competition, predation, and their interactions in character shifts via intraguild predation.

2.4.2 Trait Inducibility has been Lost in Freshwater Populations

Phenotypic plasticity has been proposed as a possible explanation for trait shifts in IG-prey (Urbani and Ramos-Jiliberto 2010; Kratina et al. 2010; Nakazawa et al. 2010). Although adaptive plasticity has been reported in stickleback feeding morphology (Day et al. 1994; Day and McPhail 1996; Wund et al. 2008; Svanbäck and Schluter 2012) and body shape (Garduño-Paz et al. 2010; Svanbäck and Schluter 2012) we found no evidence for sculpin-induced plasticity in freshwater populations. However, marine stickleback reared in

the presence of sculpin exhibited slightly increased armour, an increase in preferred water column height, and a decrease in shoaling behaviour compared to the controls. To our knowledge, the increased armour in marine stickleback in the presence of sculpin is the first observation of induced structural defences in stickleback. Importantly, induced trait changes in the presence of sculpin were in the same direction as the trait shifts among freshwater stickleback populations with and without sculpin. Phenotypic plasticity in the ancestral colonizing population may have aided in the initial divergence between freshwater populations (Wund et al. 2008).

It should be noted that while stickleback in the sculpin treatment received lifelong visual and olfactory cues from sculpin, they were not exposed to predation. Stickleback in this treatment might not have recognized sculpin as a threat or constant exposure to sculpin may have resulted in habituation (Kelley and Magurran 2003). All behavioural assays were conducted without sculpin, and including sculpin cues during these assays might induce a change in behaviour.

This study provides evidence that intraguild predation leads to evolutionary divergence among stickleback populations (Schluter and McPhail 1992). Phenotypic differences between lakes with and without sculpin have a clear genetic basis. Character shifts have likely occurred in parallel across replicated populations, therefore these differences are not due to chance. Preliminary comparisons found no evidence of consistent environmental differences among lakes. However, the biotic and abiotic environment can influence species interactions and affect the structure of piscivorous communities (Jackson et al. 2001). To fully rule out the role of the environment in generating these evolutionary shifts will require further investigation of abiotic characteristics (e.g. pH, vegetation), and

the biotic community (e.g. aquatic insects, avian predators). Phenotypic differences between lakes with and without sculpin suggest that stickleback have evolved in response to competition and/or predation with sculpin.

Figure 2.1 : Map of sampling locations used in the chapter. Lakes 1-8 contain only stickleback, A-H indicates lakes that contain stickleback and sculpin. M1 and M2 are marine populations. The lakes are (1) Kirk, (2) Cranby, (3) Klein, (4) Trout, (5) Hoggan, (6) Bullocks, (7) Blackburn, (8) Stowell, (A) Cedar, (B) Ormond, (C) Pachena, (D) Rosseau, (E) Paq, (F) Ambrose, (G) North, (H) Brown, (M1) Little Campbell, (M2) Oyster Bay

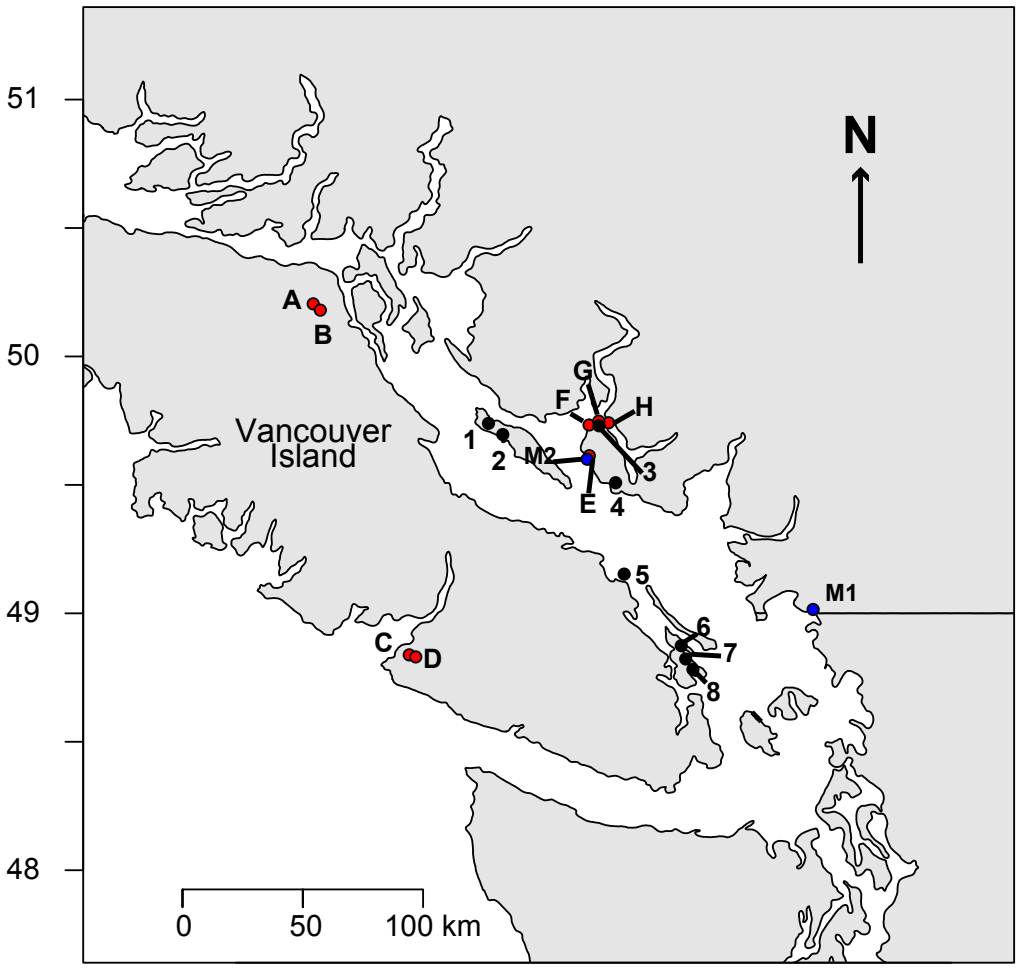


Figure 2.2 : Schematic of crosses used in the common garden experiment. Four crosses were created for each lake. One Ambrose clutch, one Rosseau, and two F1 clutches did not hatch. An additional family from Rosseau Lake was excluded when a sculpin consumed the experimental stickleback.

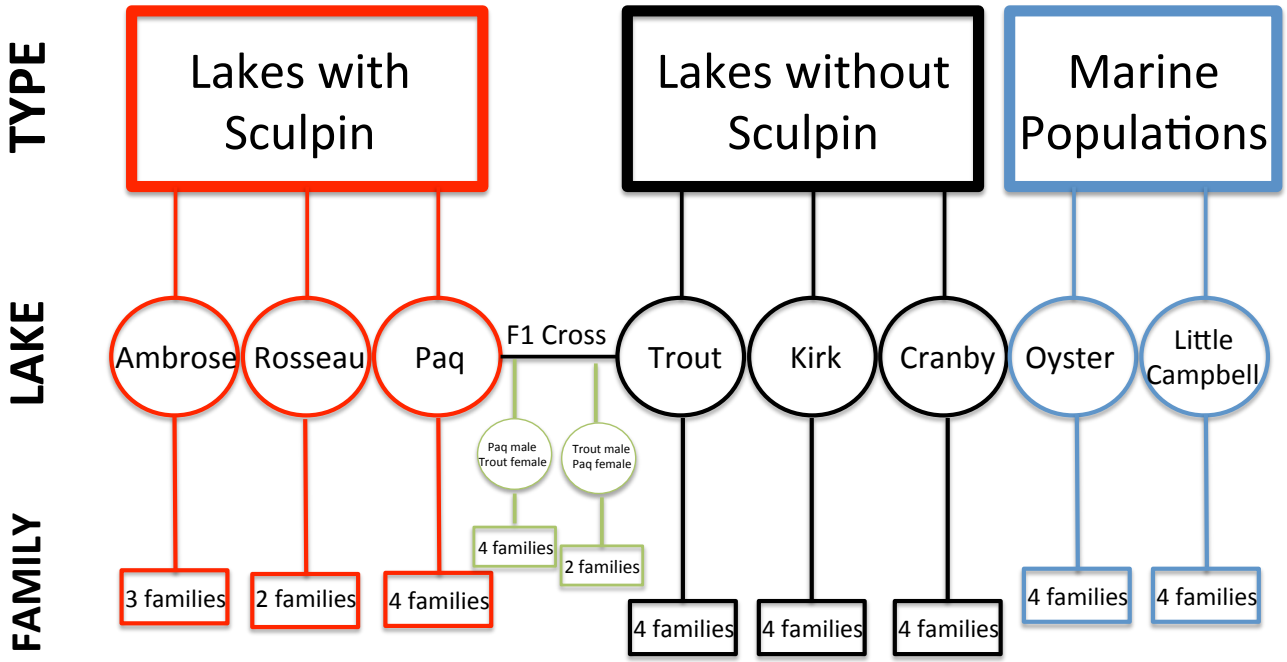


Figure 2.3 : Landmarks coordinates used for morphometrics Individual landmarks are indicated with numbers. Armour traits are abbreviated as follows: First dorsal spine (DS1), second dorsal spine (DS2), pelvic spine (PS), pelvic girdle (PG), and lateral plates (LP).

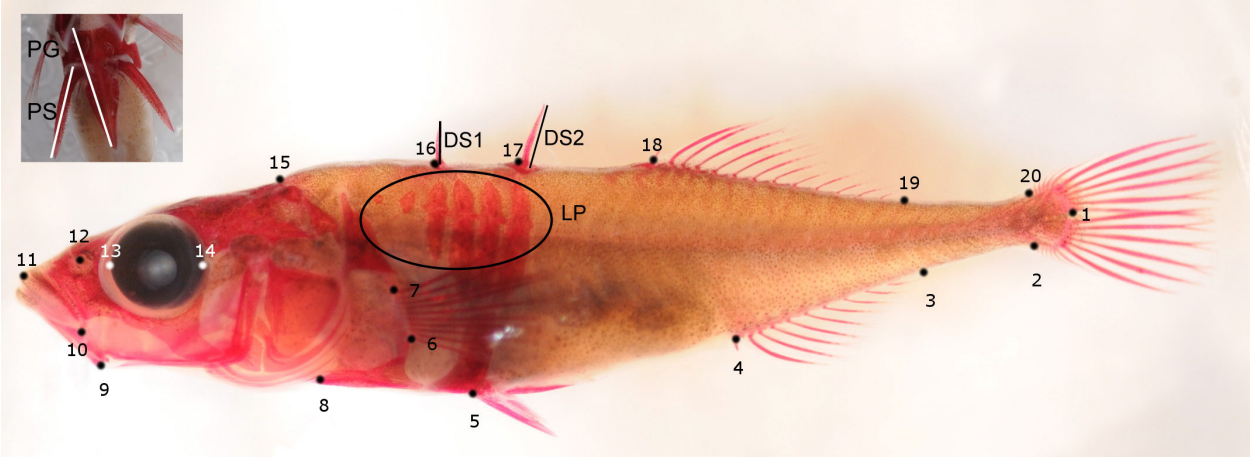
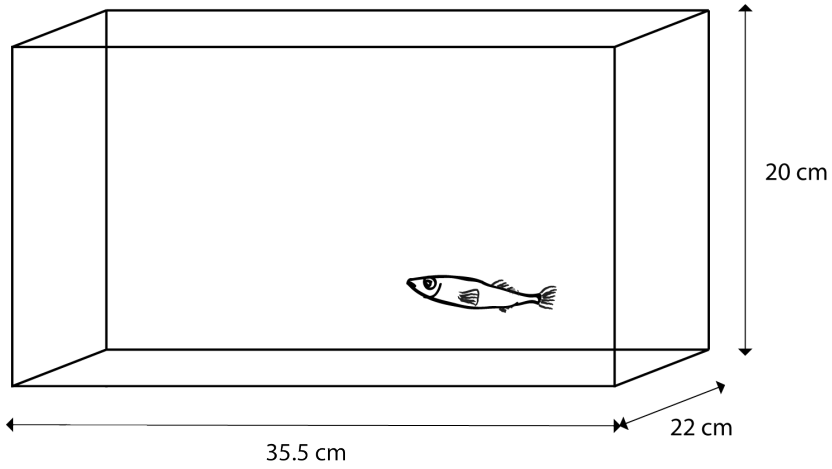


Figure 2.4 : The set-up for behavioral assays. (A) Water column preference assay tank. A focal stickleback is introduced into an unfamiliar 15L tank. Vertical position and distance traveled were measured. (B) Shoaling Assay Tank. A standard 100L aquarium tank was separated into a centre arena and two end compartments using window mesh (dotted outline). The tank was filled with 32cm of water. The back and sides of the assay tanks were covered with white paper to reduce external visual cues, and tanks were backlit to increase the contrast between the focal fish and the background. An experimental shoal with 10 stickleback was introduced into one end compartment and two stickleback were added to the other end compartment. A focal fish was introduced to the center arena and horizontal position and distance traveled were measured. All trials were recorded using wireless cameras (D-link DSC-932L).

A


20 cm
22 cm
35.5 cm

B

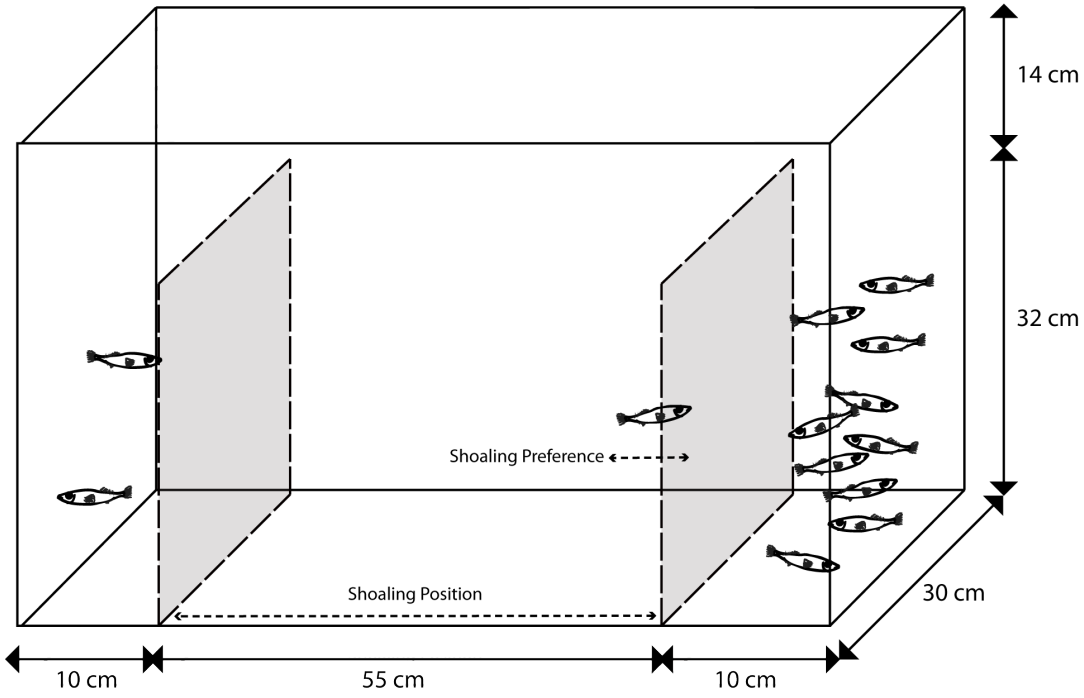

14 cm
32 cm
Shoaling Preference
Shoaling Position
30 cm
10 cm
55 cm
10 cm

Figure 2.5 : Character shifts in wild populations of stickleback. (A) Mean and standard error of armour PC1. Each point represents a single population. The solid horizontal lines give the means for each type of population. (B) Mean and standard error of vertical position in the water column (y-axis position) of wild caught stickleback measured in the lab in an unfamiliar tank. Each point is a single population. Horizontal lines give the means of each population type.

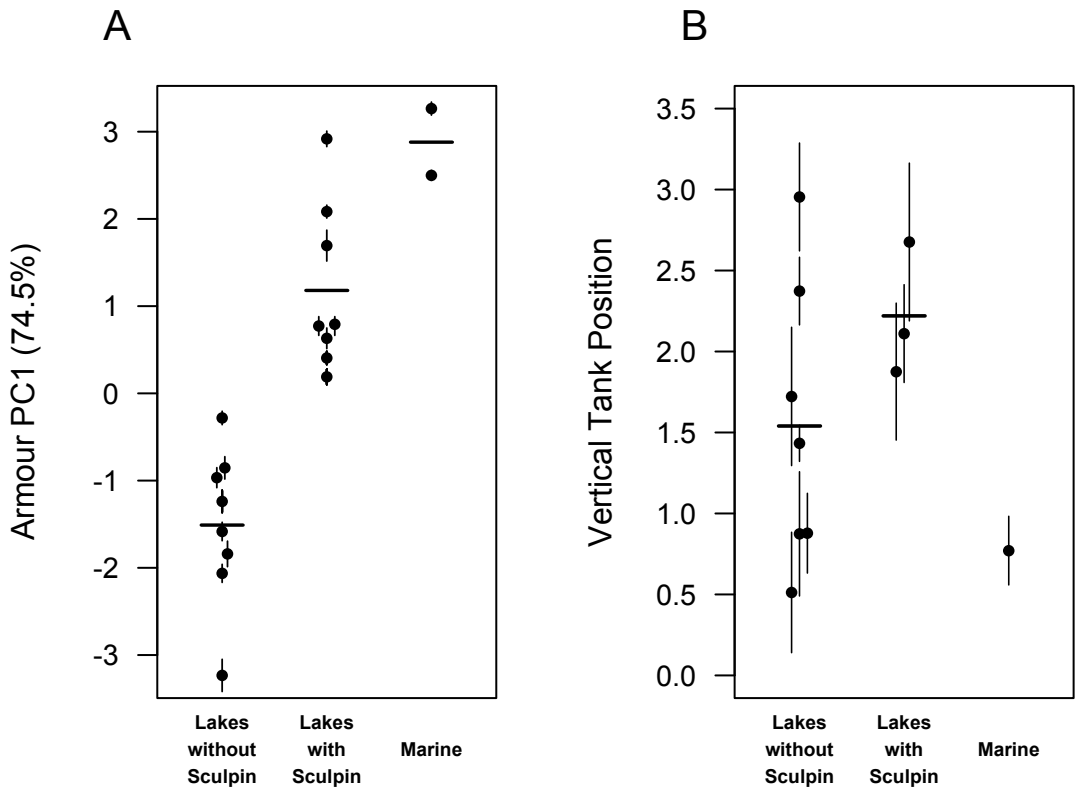


Figure 2.6 : Mean armour PC1 for experimental stickleback from each family raised in the control common garden (filled) and the sculpin treatment (open). The F1 is a cross between fish from Trout (sculpin absent) and Paq (sculpin present) Lakes. The father is first and the mother is second for F1 crosses. The mean and standard error of each lake and treatment is given on the left.

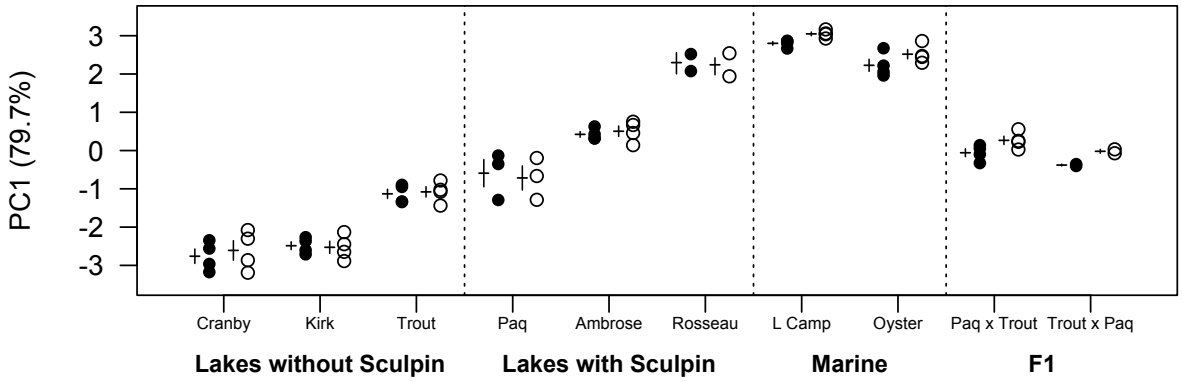


Figure 2.7 : Mean value of shape axis 1 from stickleback families reared in a common garden in a control treatment (filled) and a sculpin treatment (open). The F1 is a cross between fish from Trout (sculpin absent) and Paq (sculpin present) Lakes. For F1 crosses, the father's population is first and the mother's population is second. The mean and standard error of each lake and treatment is given on the left.

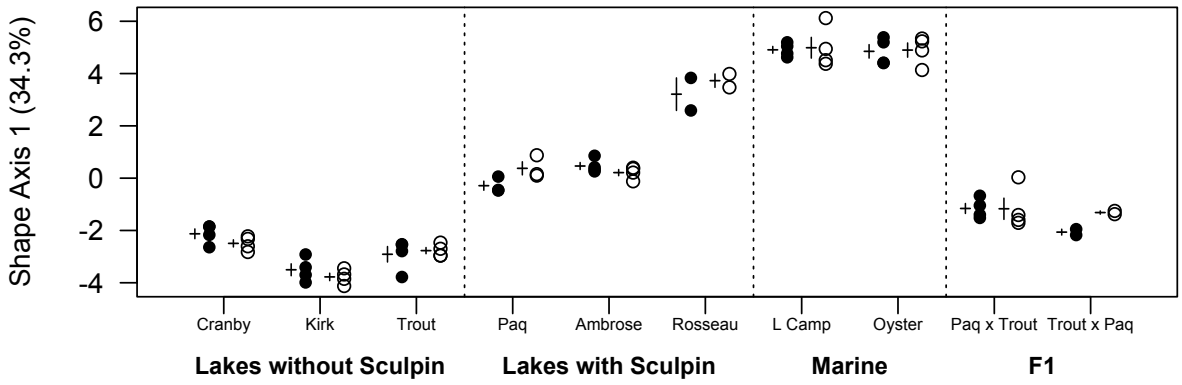


Figure 2.8 : Mean vertical position in the water column in an unfamiliar tank (y-axis position) of stickleback raised in a common garden. The control treatment is represented by closed symbols and the sculpin treatment is represented by open symbols. The mean and standard error of each lake and treatment is given on the left.

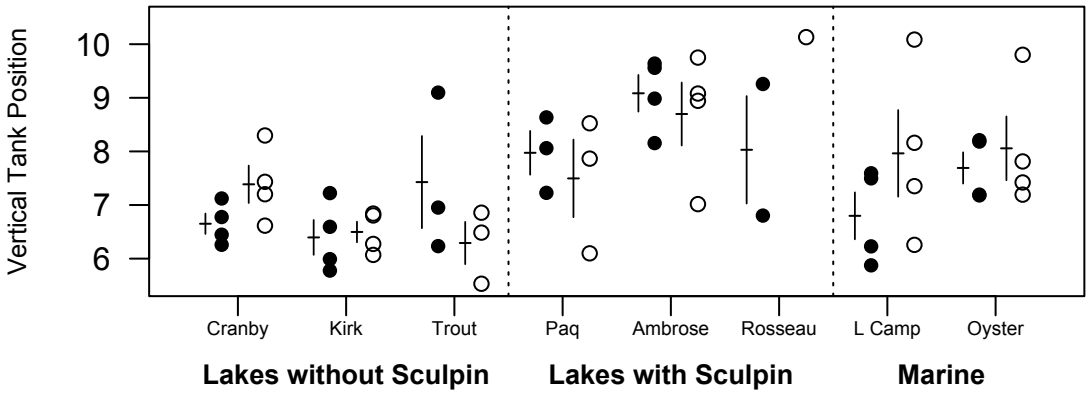


Figure 2.9 : Mean time spent near the shoal. The control treatment is represented by closed symbols and the sculpin treatment is represented by open symbols. The mean and standard error of each lake and treatment is given on the left.

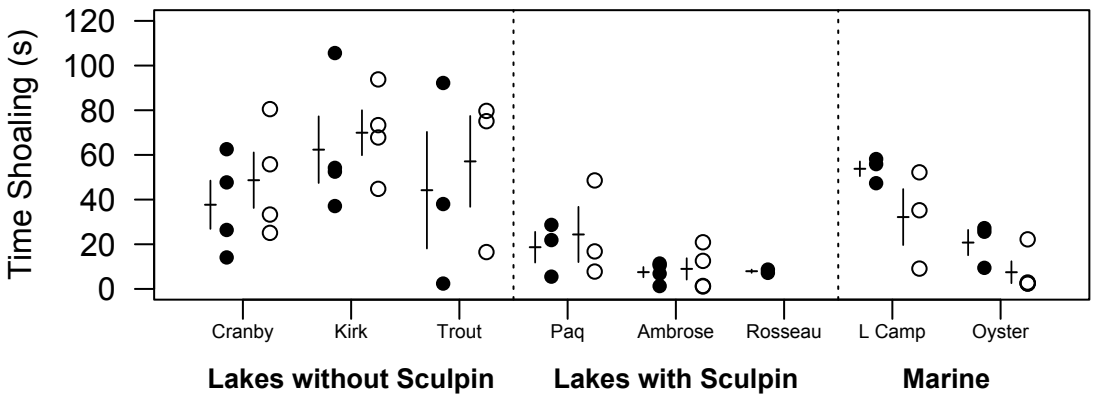


Table 2.1 Mean and standard error of traits measured in wild caught stickleback. First dorsal spine (DS1), second dorsal spine (DS2), pelvic spine (PS), pelvic girdle (PG), and lateral plates (LP). All spine traits have been size corrected.

Lake	Type	Year	Sample Size Armour	DS 1 (mm)	DS 2 (mm)	PS (mm)	PG (mm)	LP	Armor PC1	Sample Size Behaviour	Vertical position
Blackburn	No Sculpin	2011	20	2.2 ± 0.1	3.0 ± 0.1	3.4 ± 0.1	6.3 ± 0.2	3.7 ± 0.2	-1.84 ± 0.14	10	0.87 ± 0.38
Bullocks	No Sculpin	2011	26	0.8 ± 0.2	2.6 ± 0.1	2.8 ± 0.1	5.7 ± 0.1	3.3 ± 0.2	-3.23 ± 0.17	0	
Cranby	No Sculpin	2011	19	2.3 ± 0.1	3.1 ± 0.1	4.4 ± 0.1	1 ± 0.1	5.4 ± 0.2	-0.97 ± 0.1	12	1.72 ± 0.43
Hoggan	No Sculpin	2011	16	2.3 ± 0.1	3.0 ± 0.1	3.4 ± 0.1	6.3 ± 0.2	4.7 ± 0.15	-1.58 ± 0.1	11	0.51 ± 0.37
Kirk	No Sculpin	2011	10	2.8 ± 0.1	3.3 ± 0.1	3.7 ± 0.1	7.1 ± 0.2	3.1 ± 0.5	-1.24 ± 0.13	15	1.43 ± 0.11
Klein	No Sculpin	2011	20	2.4 ± 0.2	3.2 ± 0.1	4.0 ± 0.1	7.3 ± 0.1	5.8 ± 0.2	-0.85 ± 0.13	25	2.95 ± 0.33
Stowell	No Sculpin	2011	22	1.9 ± 0.1	2.6 ± 0.1	2.8 ± 0.1	6.7 ± 0.2	4.9 ± 0.2	-2.06 ± 0.1	19	0.88 ± 0.25
Trout	No Sculpin	2011	19	3.0 ± 0.1	3.8 ± 0.1	4.4 ± 0.1	7.7 ± 0.1	4.4 ± 0.2	-0.28 ± 0.08	22	2.37 ± 0.21
Ambrose	Sculpin	2011	19	3.3 ± 0.1	4.0 ± 0.1	5.1 ± 0.1	8.4 ± 0.1	6.1 ± 0.1	0.40 ± 0.07	24	2.11 ± 0.30
Brown	Sculpin	2011	7	3.2 ± 0.2	3.9 ± 0.05	4.7 ± 0.1	8.1 ± 0.1	6.4 ± 0.2	0.19 ± 0.09	0	
Cedar	Sculpin	2011	17	3.3 ± 0.2	4.2 ± 0.1	5.8 ± 0.1	8.7 ± 0.1	6.8 ± 0.3	0.77 ± 0.1	0	
North	Sculpin	2011	7	3.3 ± 0.4	4.2 ± 0.2	5.5 ± 0.2	8.9 ± 0.4	33.9 ± 0.3	1.69 ± 0.18	11	2.68 ± 0.49
Ormond	Sculpin	2012	25	4.8 ± 0.1	5.4 ± 0.1	7.1 ± 0.1	9.7 ± 0.1	6.6 ± 0.2	2.08 ± 0.07	0	
Pachena	Sculpin	2012	11	3.0 ± 0.1	3.9 ± 0.1	5.3 ± 0.1	9.4 ± 0.2	6.5 ± 0.2	0.63 ± 0.11	0	
Paq	Sculpin	2011	20	3.3 ± 0.1	4.1 ± 0.1	5.6 ± 0.1	9.1 ± 0.2	6.3 ± 0.2	0.79 ± 0.08	15	1.87 ± 0.42
Rosseau	Sculpin	2012	19	5.4 ± 0.2	6.0 ± 0.2	8.3 ± 0.2	11.1 ± 0.1	6.9 ± 0.2	2.92 ± 0.08	0	
L Camp	Marine	2012	11	4.8 ± 0.2	4.8 ± 0.1	8.0 ± 0.1	11.0 ± 0.1	33.6 ± 0.2	3.26 ± 0.08	0	
Oyster	Marine	2011	19	3.9 ± 0.1	4.9 ± 0.1	6.5 ± 0.1	10.1 ± 0.1	27.1 ± 0.5	2.50 ± 0.08	14	0.77 ± 0.21

Table 2.2: Principal component loadings for armour traits

	Wild-Caught Stickleback					Experimental Stickleback				
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
Trait										
Dorsal Spine 1	0.4342	0.3085	-0.8432	-0.0731	-0.0062	0.4483	0.2983	-0.5389	-0.6259	0.1669
Dorsal Spine 2	0.4702	0.2709	0.2974	0.5534	-0.5575	0.4446	0.4918	-0.0651	0.6865	0.2914
Pelvic Spine	0.4860	0.1598	0.2896	0.1534	0.7943	0.4830	0.0677	0.1522	0.0180	-0.8595
Pelvic Girdle	0.4706	-0.0256	0.3034	-0.7925	-0.2403	0.4514	-0.1303	0.7490	-0.2851	0.3701
Lateral Plates	0.3644	-0.8973	-0.1572	0.1919	-0.0221	0.4054	-0.8048	-0.3480	0.2352	0.1077

Table 2.3: Mean and standard error of traits measured in experimental stickleback in 'control' treatment. Sample size is number of families measured. First dorsal spine (DS1), second dorsal spine (DS2), pelvic spine (PS), pelvic girdle (PG), and lateral plates (LP). All spine traits have been size corrected.

Lake	Type	N	DS 1 (mm)	DS 2 (mm)	PS (mm)	PG (mm)	LP	Armor PC1
Cranby	No Sculpin	4	3.4 ± 0.1	4.1 ± 0.1	5.0 ± 0.1	8.6 ± 0.1	5.9 ± 0.3	14.1 ± 0.5
Kirk	No Sculpin	4	4.0 ± 0.02	4.6 ± 0.1	5.0 ± 0.1	7.9 ± 0.2	4.4 ± 0.3	15.3 ± 0.1
Trout	No Sculpin	4	4.5 ± 0.03	5.0 ± 0.1	6.2 ± 0.2	8.6 ± 0.1	4.9 ± 0.1	19.3 ± 0.5
Ambrose	Sculpin	3	4.5 ± 0.3	5.4 ± 0.3	6.3 ± 0.1	9.0 ± 0.1	6.7 ± 0.1	19.6 ± 0.8
Paq	Sculpin	4	4.1 ± 0.1	5.3 ± 0.1	7.3 ± 0.1	10.7 ± 0.1	7.1 ± 0.2	21.9 ± 0.3
Rosseau	Sculpin	2	6.1 ± 0.1	6.4 ± 0.1	8.8 ± 0.2	11.7 ± 0.2	7.9 ± 0.3	27.8 ± 0.7
L Camp	Marine	4	5.8 ± 0.1	6.0 ± 0.1	8.9 ± 0.2	11.7 ± 0.1	33.6 ± 0.2	27.4 ± 0.04
Oyster	Marine	4	5.6 ± 0.1	5.8 ± 0.1	8.5 ± 0.2	10.7 ± 0.1	31.8 ± 1.6	26.2 ± 0.4
Paq Male	F1	4	4.7 ± 0.1	5.2 ± 0.1	7.0 ± 0.04	9.8 ± 0.1	6.2 ± 0.1	21.3 ± 0.4
Trout Male	F1	2	4.5 ± 0.1	5.1 ± 0.1	6.7 ± 0.2	9.6 ± 0.02	6.4 ± 0.02	20.4 ± 0.2

Chapter 3: Intraguild Predation Leads to a Multitude of Genomic Changes but is Constrained by Genomic Architecture

3.1 Introduction

The evolution of a species is governed both by the abiotic environment and by biotic interactions with other species in the environment (Thompson 2013). Biotic natural selection has been shown to be an important mechanism for the generation of phenotypic diversity (Kingsolver et al. 2001; Rieseberg et al. 2002). Although selection acts on phenotypes, ultimately changes in phenotype are mediated through the evolution of genes. A full comprehension of how organisms adapt to each other therefore requires an understanding of the number, identity, distribution, effect size, and source of genes under selection. To date, the evolutionary effect of biotic selection upon an organism's genome remains largely unknown in natural populations.

This impact of biotic selection is especially interesting in the case of rapid adaptation. We identified multiple populations of threespine stickleback *(Gasterosteus aculeatus)* from similar lakes in southwestern British Columbia that differed mainly by the presence or absence of prickly sculpin (*Cottus asper*), an intraguild predator of stickleback. These populations originated approximately 10,000 years ago when marine stickleback from the Strait of Georgia colonized newly formed lakes following the melting of the glaciers at the end of the last ice age (McPhail 2007). As a result, stickleback in these lakes have independently adapted to new lakes, either with or without the same biotic agent of selection, over a short period of time. Comparing the genomes of stickleback from lakes

with sculpin and without sculpin would give insight into how the genes and genome of one species change in response to the presence of a single other species.

It is difficult to predict the number of genes that are under selection from a single biotic agent. Genetic studies of single traits under selection from other species often identify at least one gene with a large effect on fitness. For example, selection for cryptic coat colour by predators has caused the fixation of adaptive mutations affecting expression of the *Agouti* gene in deer mice living in soils of different colour (Linnen et al. 2009). In human populations, the *Duffy* blood group locus conferring resistance to malaria occurs at a high frequency in sub-Saharan Africa but is rare in regions without malaria (Hamblin and Di Rienzo 2000). However, these traits might not be representative of all those affected by biotic selection. Methods such as QTL mapping, used to identify allelic variants between populations or species with differing phenotypes, are biased towards detecting genes with large phenotypic effects and may underestimate the number of genes under selection (Rockman 2012).

We are only aware of two studies that have attempted to quantify genome-wide adaptation in one species due to another species. Bonhomme et al. (2015) used whole genome re-sequencing of inbred lines of a legume species to examine adaptation to root associated microorganisms. The authors identified 190 genes in 58 regions that had putatively undergone selective sweeps. Similarly, comparison of sequence divergence among four populations pairs of stick insects (*Timema cristinae*) living on different host plant species revealed 1000 SNPs that were F_{ST} outliers in all four population pairs (Soria-Carrasco et al. 2014). Together these results suggest that many genes may be responding

to biotic interactions. However, it is unclear if these results apply to other animal species or are typical of biotic selection in the wild.

Recent advancements in next generation sequencing are now making it possible to gain insight into the genomic architecture of adaptation by estimating the number and location of genes that have become differentiated in association with a selective agent, and potentially even the number of selective sweeps (Stapley et al. 2010). Studies of wild populations have primarily utilized reduced representation genome scans (e.g. Genotyping by Sequencing (GBS) or Restriction-site Associated DNA Sequencing (RADseq)). These methods produce greatly increased marker coverage compared to previous technologies. However, they only provide data for a limited portion of the genome and can introduce bias from loss of data at restriction cut sites or from PCR amplification (Andrews et al. 2016). Also problematic is that genetic differences not in linkage disequilibrium with markers will go undetected. A comprehensive understanding of the genes under selection requires whole genome re-sequencing to provide the increased precision needed to estimate the number and distribution of genes involved in biotic selection. The small genome size and high quality reference genome (Jones et al. 2012a) makes the threespine stickleback an ideal organism with which to answer these questions

Here we report the results of a genome-wide analysis examining the genetic basis of stickleback adaptation to the presence or absence of one other species, prickly sculpin, an intraguild predator. The presence of sculpin in lakes has been shown to be strongly associated with genetically based character differences in many stickleback traits including defensive armour, body shape, and behaviour (Miller et al. 2015). A major challenge to studying the genome-wide response to biotic selection in natural populations lies in

isolating the effect of a single agent of selection. Furthermore, demographic processes such as genetic drift or population bottlenecks change the frequency of non-adaptive neutral alleles and can produce false signatures of selection (Klopfstein 2005; Excoffier and Ray 2008). The unique natural history of these lakes allows us to overcome these challenges. By comparing multiple threespine stickleback populations of a similar age that have independently adapted to the presence/absence of sculpin, we can isolate the effect of an agent of selection in the wild, provided that the shifts are not caused by correlated factors. This project is the first of its kind to use whole genome re-sequencing to examine the evolutionary response of a single agent of selection on a vertebrate species in the wild.

3.2 Materials and Methods

3.2.1 Sample Collection and Library Preparation

Up to 25 adult threespine stickleback were collected during the breeding season in May-June 2012-2014 from each of eight freshwater lakes containing a fish community of threespine stickleback and coastal cutthroat trout (*Oncorhyncus clarkii clarkii*) and nine lakes containing threespine stickleback, cutthroat trout, and prickly sculpin. Cutthroat trout are found in virtually all lakes throughout southwest British Columbia. In some cases, lakes are connected via small streams to other lakes within the same watershed. However, all study lakes were in separate watersheds, ensuring that there is no contemporary gene flow between populations. Marine stickleback were collected from six localities (23 populations total, Figure 3.1). The Pacific Ocean marine population is thought to be largely undifferentiated with high gene flow (Jones et al. 2012a,b).

We tested if sculpin presence was correlated with environmental differences among lakes. We gathered information on the area, perimeter, maximum depth, mean depth, and pH of each lake from Habitat Wizard (*www.env.gov.bc.ca/habwiz*). We used Google maps (*www.maps.google.com*) to determine the elevation and shortest straight-line distance from the lake to the ocean. Water samples were collected from some lakes and sodium concentration (Na), calcium concentration (Ca) and conductivity were determined using a flame photometer (Table 3.1). Abiotic variables were log transformed. We performed a principal components analysis (PCA) of the correlation matrix for abiotic traits using the 'nipals' option in the pcaMethods package because this algorithm is capable of handing a small amount of missing data using a non-linear iterative partial least squares method (Stacklies et al. 2007).

Stickleback were euthanized with an overdose of MS-222 anaesthetic (Argent Chemical Laboratories, Redmond, WA) and stored in 95% ethanol. Samples were stained with alizarin red (Peichel et al. 2001) and the left side of each stickleback was photographed using a Nikon D300 camera. We placed 20 landmarks outlining the shape of the fish and the insertion points of spines and fins (Walker 1997; Ingram et al. 2012). Landmarks were digitized using tpsDig 2.16 software (Rohlf 2008) and were centered, scaled, and rotated using the *shapes* package in R (Dryden 2012). For each population, we did a PCA of morphological landmarks, and chose as a single representative fish from each population, that female fish closest to the centroid of PC1 and PC2. Due to sample limitations, a male stickleback was used for Paq (sculpin), Cedar (sculpin) and Black Lakes (non-sculpin).

This strategy of sequencing a single individual per lake was chosen to maximize the number of populations sampled rather than the number of individuals. When lakes were originally colonized, rapid population growth likely occurred coincident with adaptation. This can lead to false signatures of selection if neutral rare alleles in the founding population increase in frequency as a result of genetic drift (Klopfstein 2005).

Genomic DNA was extracted from a fin clip from the single fish from each population using a standard phenol/chloroform method. The DNA samples were standardized to 20ng/ul with a QuBit 2.0 fluorometer. Paired-end whole genome libraries were prepared for each fish using the Illumina TruSeq sample kit (Illumina, San Diego CA) and quantified using High-Sensitivity Bioanalyzer chips (Agilent Technologies, Inc.). Libraries were sequenced using Illumina HiSeq 2000 at the University of British Columbia and at Genome Quebec.

3.2.2 Bioinformatics Pipeline

Reads were aligned to the stickleback reference genome (gasAcu1 2006 assembly; Jones et al. 2012a) using the BWA aligner (version 0.7.6) (Li and Durbin 2009). Single Nucleotide Polymorphisms (SNPs) were identified using the UnifiedGenotyper tool in GATK (version 3.2.2) following the best practices recommendations for version 3.2.2 (DePristo et al. 2011; Van der Auwera et al. 2013). Picard (http://broadinstitute.github.io/picard) was used in conjunction with GATK to manipulate sequencing reads. Details of the bioinformatics pipeline used to generate SNPs are given in Appendix A. A BED file of the location of repeat regions was created using the RepeatMasker track in the USCS stickleback genome table browser (sticklebrowser.stanford.edu). Those SNPs with a

mapping quality score less than 100 (--minGQ), a mean read depth of less than 6 (--min-meanDP), or SNPs mapping to previously identified repeat regions were filtered with vcftools (version 0.1.11) (Danecek et al. 2011).

3.2.3 Divergence Among Populations

We used principal component analysis (PCA) on genotype values at SNPs to visualize the overall pattern of divergence in our populations. In each population, SNPs were given a numerical value relative to the reference sequence (e.g. REF/REF = 0, ALT/ALT = 1; REF/ALT = 0.5). Missing values were filled in using the average value of that SNP across all populations. The PCA of the covariance matrix was calculated for all SNPs using the 'svd' option in the pcaMethods package (Stacklies et al. 2007).

To identify the regions of the genome that have differentiated in parallel between lakes with and without sculpin, and to measure the strength of this divergence, fish were grouped into lakes with sculpin and lakes without sculpin. We calculated F_{ST} between these groups in 10,000 bp sliding widows with a step size of 5,000 bp. F_{ST} was calculated using the Weir and Cockerham formula (Weir and Cockerham 1984). However, because there is no gene flow between lakes with sculpin (or lakes without sculpin), these groups are not true subpopulations. Therefore F_{ST} may not be the appropriate measure of genetic differentiation for these populations.

We generated a modified cluster separation score (CSS) between fish from different lake types (groups) (Jones et al. 2012a) in windows across the genome. The CSS metric distinguishes between highly divergent regions of the genome for isolated populations adapting to the same ecological conditions. We modify this method by using principal

components analysis rather than multi-dimensional scaling (MDS) and weight the score by sequence coverage. This method preserves covariance of the data and is less computationally taxing than CSS. To do this, the genome was analyzed within 10,000 bp sliding windows with step size of 5,000 bp. A PCA was conducted for each window. We retained the first two principal components in each window and then measured the amount of divergence by calculating the distance between the scores for all pairs of individual fish from different lake types, adjusting for the average distance between pairs of fish within groups. The formula used is:

$$\text{CS}' = \frac{\sum_{i=1}^{s}\sum_{j=1}^{n} Di,j}{(sn)} - \left(\frac{1}{s+n}\right)\left(\frac{\sum_{i=1}^{s-1} Di,i+1}{\frac{(s-1)}{2}} + \frac{\sum_{j=1}^{n-1} Dj,j+1}{\frac{(n-1)}{2}}\right)$$

D is the Euclidean distance in the first two principal component axes between a pair of fish, *i* and *j* are individual fish from different groups, and *s* and *n* are the sample sizes of each group. To control for variation in sequence coverage, we divided CS' by the number of sequenced bases within a window (both variant and invariant sites). Windows containing less than 500 bp or containing fewer SNPs than the total number of fish were dropped. Higher CS' values indicate greater divergence between groups. A negative value is possible and signifies that the average pairwise distance between fish in different lake types is less than the pairwise distance between fish of the same lake type.

We assessed the statistical significance of CS' values using permutation tests. Within each window, we randomly redistributed the individual fish to the two groups, keeping the number of fish in each group the same, 10,000 times and calculated a CS' score each time. We generated a P-value by calculating the proportion of times in which the value obtained from the permutated data exceeded the CS' score calculated from the real data. Windows

were considered outliers based on a P-value threshold defined by false a discovery rate (FDR) of 0.05 ($P<0.001$). The FDR threshold was determined using the 'fdrtool' package. A $\mathbf{X}^2$ goodness of fit test was performed to test if outlier windows were evenly distributed among chromosomes, adjusting for chromosome size.

The boundaries of divergent genomic regions between lakes with and without sculpin may be larger than 10,000 bp. Matching this prediction, we often found that neighbouring windows were identified as outliers. To define the boundaries of divergent regions we used a two state Hidden Markov Model (HMM) of log CS' scores using default parameter values in the R package depmixS4 (Visser and Speekenbrink 2010).

The CS' metric gives a conservative estimate of the regions under selection because only the regions that differentiate sculpin from non-sculpin stickleback repeatedly across multiple independent populations will be identified. Because CS' measures differences in DNA sequence, our approach also requires that virtually the same alleles are involved in adaptation to sculpin presence/absence across lakes. Standing genetic variation is common in natural populations (Barrett and Schluter 2008). Reuse of standing genetic variation has been shown to be important in the repeated evolution of freshwater stickleback (Jones et al. 2012a). For example, reduction in lateral plates in freshwater stickleback occurs from the reuse of the Ectodysplasin (EDA) 'low' allele present as standing genetic variation in the colonizing marine stickleback populations (Colosimo et al. 2005, Jones et al. 2012a).

3.2.4 Candidate Genes

To identify the genes that are divergent between lakes with and without sculpin we looked at the number and identity of genes within outlier windows. We used the biomaRt package (Durinck et al. 2009) to identify genes that occur within the window. We then looked for enrichment of gene ontology (GO) terms within outlier windows using GOwinda (Kofler and Schlotterer 2012). Portions of the genome are not sequenced with Illumina whole genome re-sequencing because of limitations of this technology. GOwinda controls for sequence coverage by comparing outlier SNPs to the total SNPs from which sequence is available. GOwinda also uses a permutation approach to control for bias in gene length. We generated a GO annotation file by obtaining zebrafish GO annotations from FuncAssociate 2 (Berriz et al. 2009) and then matched stickleback genes to the zebrafish orthologs using biomaRt. We ran GOwinda twice, first using SNPs only within outlier windows and then including SNPs 2000-bp upstream or downstream of outlier windows to account for nearby regulatory SNPs.

3.3 Results

3.3.1 Sculpin Presence is not Correlated with Abiotic Environment

There were no consistent environmental differences between lakes with and without sculpin for individual variables and lakes overlapped broadly in their abiotic traits along PC1 ($U=28$, $P=0.48$) and PC2 ($U=36$, $P=1.0$) (Table 3.2, Figure 3.2).

3.3.2 Genomic Divergence is Associated with Presence/Absence of Intraguild Predator

We generated a total dataset of 5.7 million filtered SNPs from 23 populations and performed a PCA using all SNPs. The first principal component (PC1) explained 14.7% of the variation and separated individuals from lakes with sculpin from individuals from lakes without sculpin ($F_{2,20}$=52.9, P<1e-8) (Figure 3.3A). Stickleback sympatric with sculpin had genome PC1 values that were more similar to the marine stickleback than stickleback from sculpin-absent lakes. This finding suggests that stickleback from lakes with sculpin share more marine genotypes at SNPs having high trait loadings on PC1. We performed a second PCA using only the individuals from lake populations (reduced dataset of 4.6 million SNPs). The first PC of this second PCA similarly distinguished lakes with and without sculpin ($F_{1,15}$=26.3, P=0.0001) and explained 11.6% of the variation (Figure 3.3B). The main axis of genetic variation in these populations is strongly associated with the presence/absence of sculpin. For PC2 to PC22 populations are somewhat differentiated by geography but this signal is not strong (Figure 3.4). For example, PC2 separates some but not all of the lakes from Vancouver Island from all other lakes, while PC3 distinguishes some of the populations from the Sunshine Coast.

3.3.3 Genetic Differentiation is Extensive but Unevenly Distributed

To identify genomic regions that have strongly differentiated in parallel between stickleback from lakes with and without sculpin, we calculated a cluster separation score CS' between stickleback from the two lakes types. Across all sliding windows, CS' is strongly positively correlated with F_{ST} (r=0.9) (Figure 3.5). Sufficient data were available for 88,711

sliding windows genome wide. Overall, 1473 windows were identified as outliers, accounting for 1.7% of sampled windows. Each outlier window had an average of 0.83 genes (sd=0.8), and individual genes often spanned multiple outlier windows. Combined, outlier windows contained more than 500 genes (Appendix B).

We compared the distribution of outlier windows with the expected distribution based upon the number of windows per chromosome for which sufficient sequence was available (at least 500 informative bases). Outlier windows were not randomly distributed throughout the genome (X^2 =2392, df=21, P< $2.2e^{-16}$). For example, large portions of chromosomes 4, 7, and 12 showed elevated divergence between lakes with and without sculpin, while other regions such as chromosomes 14 and 15 were undifferentiated between lake types (Figure 3.6).

The number of genes or windows differentiating populations does not count the number of selective sweeps because a selective sweep will likely encompass multiple outlier windows. Although lakes are isolated from each other, the same marine population presumably originally colonized the lakes, potentially bringing similar standing variation each time. If these populations experienced selection upon the same standing genetic variation present in the colonizing marine fish, then we should be able to detect the regions that have undergone selective sweeps repeatedly in independent lakes as one or more adjacent outlier windows. A hidden Markov model (HMM) was implemented to define the boundaries of these outlier regions. The HMM estimates the location of state shifts between divergent regions and regions having little or no divergence (Visser and Speekenbrink 2010). This model collapsed the 1,473 outlier windows into 164 distinct outlier regions

across the genome (Figure 3.7, Table 3.2). The median width of regions in the 'selected' state containing an outlier window was 130,000 bp.

3.3.4 Candidate Adaptive Genes

Within the outlier windows are many putative candidate genes for phenotypic differences between stickleback from lakes with and without sculpin. Several of the genes found within outlier windows have known roles in zebrafish development and might correspond to the phenotypic differences observed among populations of stickleback from lakes with and without sculpin. Such genes include *SATB2*, which is involved in the development of the vertebrate jaw (Fish et al. 2011), *PDLIM7*, a gene necessary for pectoral fin development (Camarata et al. 2010), *GIGYF1*, a modifier of IGF-I signalling (Giovannone et al. 2003), and *KCTD12*, a gene that may influence zebrafish thigmotaxis behaviour (Lee et al. 2014).

Nevertheless, there was no significant enrichment of any GO terms associated with SNPs within outlier regions or with SNPs within 2000 bp up or downstream of outlier windows. We have no evidence that intraguild predation preferentially leads to selection on genes associated with a particular cellular component, molecular function, or biological process.

3.4 Discussion

The presence/absence of prickly sculpin, an intraguild predator of stickleback has resulted in widespread but unevenly distributed divergence across the threespine stickleback genome. Although the freshwater populations have been isolated from each

other for only 10,000 years, we observed extensive parallel differentiation in more than 1.7% of the measured genome between populations of stickleback from lakes with and without sculpin. Our methodology only identifies outlier regions that have diverged in parallel between lakes with and without sculpin. Genes that have diverged in only one lake will go undetected therefore these results are a conservative estimate of the proportion of the genome implicated in adaptation to the presence or absence of sculpin. The presence/absence of a single biotic agent had a rapid and profound effect on genomic divergence in stickleback.

More than 500 genes were identified in outlier windows, suggesting that the presence/absence of the intraguild predator has resulted in selection on a large number of genes. Outlier windows in the present study were identified by parallel evolution and are unlikely to be caused by neutral evolutionary processes. It is generally considered that lake populations evolved independently of each other after colonization by the common marine ancestor therefore changes in genetic variation caused by neutral evolutionary processes (e.g. population bottlenecks) would be unique to each lake.

Genome scans do not provide information on the effect sizes that candidate genes have on the phenotype or fitness of an organism. Thus we cannot quantify the number of genes of large and small effect. However, the large number of candidate genes identified in outlier windows is consistent with polygenic adaptation of many alleles of small effect on fitness.

Why is the presence/absence of a single biotic agent correlated with differentiation of so many genes? There are several possible explanations. First, intraguild predation is associated with character shifts involving many traits including body shape, defensive

armour, diet, and behaviour (Ingram et al. 2012; Miller et al. 2015). These traits are likely to have a polygenic basis and QTL studies of some of these same traits in stickleback often find them mapping to multiple genomic regions (e.g. Rogers et al. 2012). Selection upon many traits may necessitate change in many genes. Second, although prickly sculpin are a single agent of selection, and represent the only consistently observed difference between the two types of lakes, intraguild predation may lead indirectly to multifarious selection upon stickleback. Sculpin directly select on stickleback phenotypes and they may also result in downstream effects by changing how stickleback interact with other components of the lake ecosystem. For example, the absence of sculpin may allow stickleback to colonize the shallow benthic environment, and because coastal cutthroat trout live primarily in the open water (Reimchen 1994), this habitat shift would change the stickleback diet and indirectly decrease predation from trout. Finally, outlier regions occur more often on chromosomes with large regions of low recombination (Roesti et al. 2013). Linkage disequilibrium is increased in regions of low recombination (Hartl and Clark, 1997). As a result, neutral or even deleterious alleles can hitchhike along with linked genes under selection, which could lead to an overestimate of the number of 'selected' genes (Excoffier and Ray, 2008), although not of the number of selected sweeps.

Within the list of outlier genes, we identified candidate genes for phenotypic traits previously found to differ among populations with and without sculpin (see chapter 2), such as fin position, mouth shape, and behaviour. Our genome scan was also able to identify candidate genes for many other phenotypes that may also be under selection, including immune function, brain development, and muscle structure (Appendix B). Interestingly, our analysis found no significant enrichment of GO terms in outlier windows. Adding (or

subtracting) a single species from the environment leads to selection on many genes but does not preferentially cause selection on genes with a certain function.

Outlier windows were clustered in the genome. Other genome scan studies have found that heterogeneous genomic divergence – variation in genetic differentiation across the genome – is common (e.g. Nosil et al. 2009; Lawniczak et al. 2010; Delmore et al. 2015). Clustered architectures are predicted when differentiation occurs with gene flow because nearby co-adapted loci are less likely to be broken up by recombination than neutral loci (Yeaman 2013). During the colonization phase, lakes would have probably experienced gene flow with stickleback in the marine environment. However, this period was most likely short, and subsequently, lakes would have diverged in allopatry. Heterogeneous genomic divergence may instead occur as a by-product of constraints caused by underlying features of the genome such as variation in recombination rate (Cruickshank and Hahn 2014). Further investigation is necessary to understand the mechanism causing heterogeneous genomic divergence among these populations.

Other whole genome resequencing studies examining genomic divergence between contrasting environments have reported fewer genes under selection. Jones et al. (2012a) examined the genetic basis of adaptation of marine stickleback to freshwater environment and found that 0.18 – 0.26% of the genome was differentiated between replicate populations of marine and freshwater stickleback. A study looking at parallel adaptation to hypoxic conditions at high altitude in 6 breeds of dog reported genomic differentiation at 28 regions containing 141 candidate genes (Gou et al. 2014). Adaptation of *Arabidopsis lyrata* to serpentine soils found significant allele frequency differences in only 96 of the 8.4 million SNPs identified (Turner et al. 2010). Although quantitative analysis of the genomic

architecture of selection between biotic and abiotic agents will require more studies, preliminarily evidence suggests that biotic selection is associated with higher genomic divergence.

Our findings have several implications. First, biotic selection affects many genes. The presence or absence of sculpin, a single species, appears to have led to differentiation in 1.7% of the genome. Yet sculpin are not the only biotic agent of selection in lakes. If the amount of differentiation is typical, a large percentage of the genome may be under selection as a consequence of interactions with other species. Second, adaptation to other species is not necessarily slow. A change to a new optimum can occur quickly when the initial genetic variance in the population is large (Stephan 2016). Lastly, stickleback from populations sympatric with sculpin retained more marine genetic variants. This strongly suggests that it was release from selection by sculpin that was the cause of genetic divergence between population types. Consequently, biotic selection appears to have had a profound effect upon the stickleback genome.

Figure 3.1 : Locations of populations sampled. Lakes with sculpin are in red: (A) Cedar, (B) Ormond, (C) Ambrose, (D) North, (E) Brown, (F) Paq, (G) Rosseau, (H) Pachena. Lakes without sculpin in in green: (1) Tom, (2) Cranby, (3) Kirk, (4) Klein, (5) Trout, (6) Hoggan, (7) Bullock, (8) Stowell, (9) Black. Marine populations are in blue: (M1) Seyward Estuary, (M2) Oyster Lagoon, (M3) Little Campbell River, (M4) Salmon River, (M5) West Creek, (M6) Bamfield.

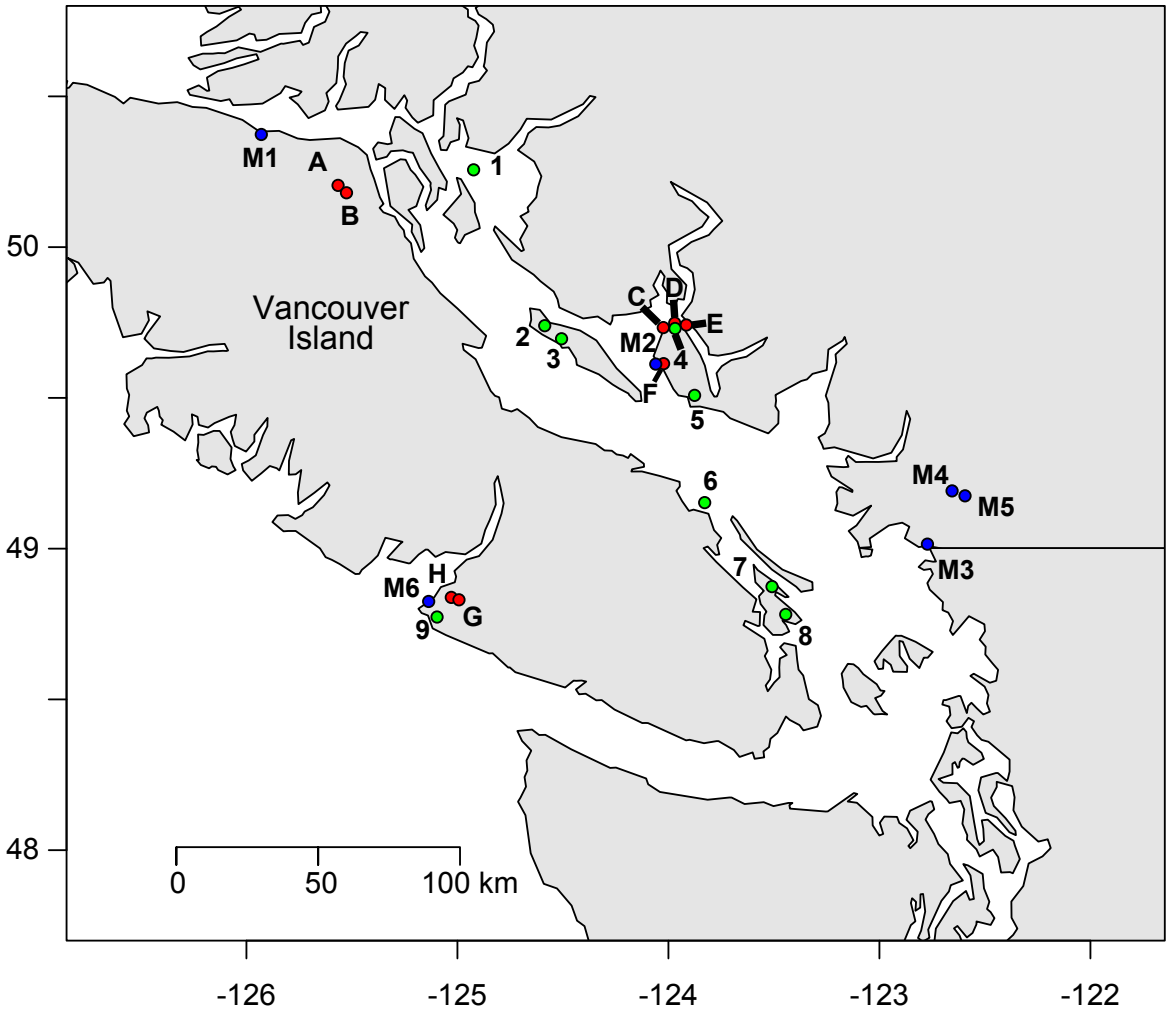


Figure 3.2 : Principal component analysis (PCA) of abiotic traits of lakes with sculpin (red) and lakes without sculpin (black). Abiotic trait values are given in Table 3.1.

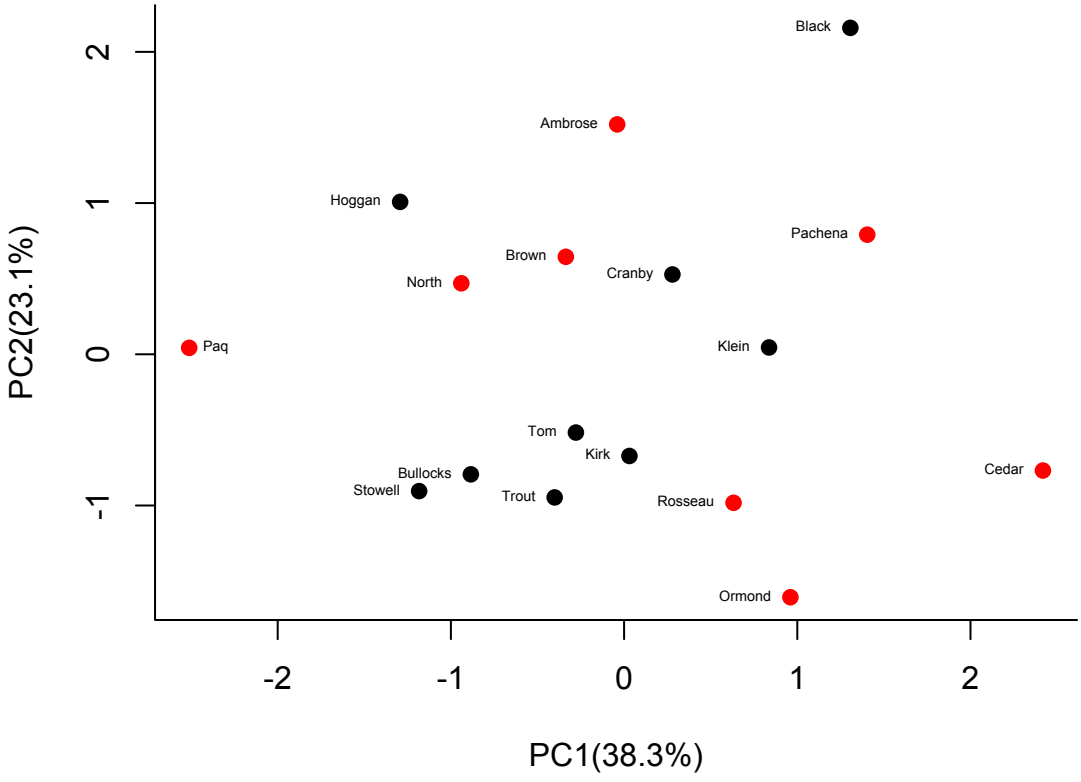


Figure 3.3 : Principal component analysis of all SNPs from (A) all populations and from (B) only freshwater populations. Only the first principal component (PC1) is shown. Each point is a single individual from a population. Lakes with and without sculpin are separated from each other along PC1.

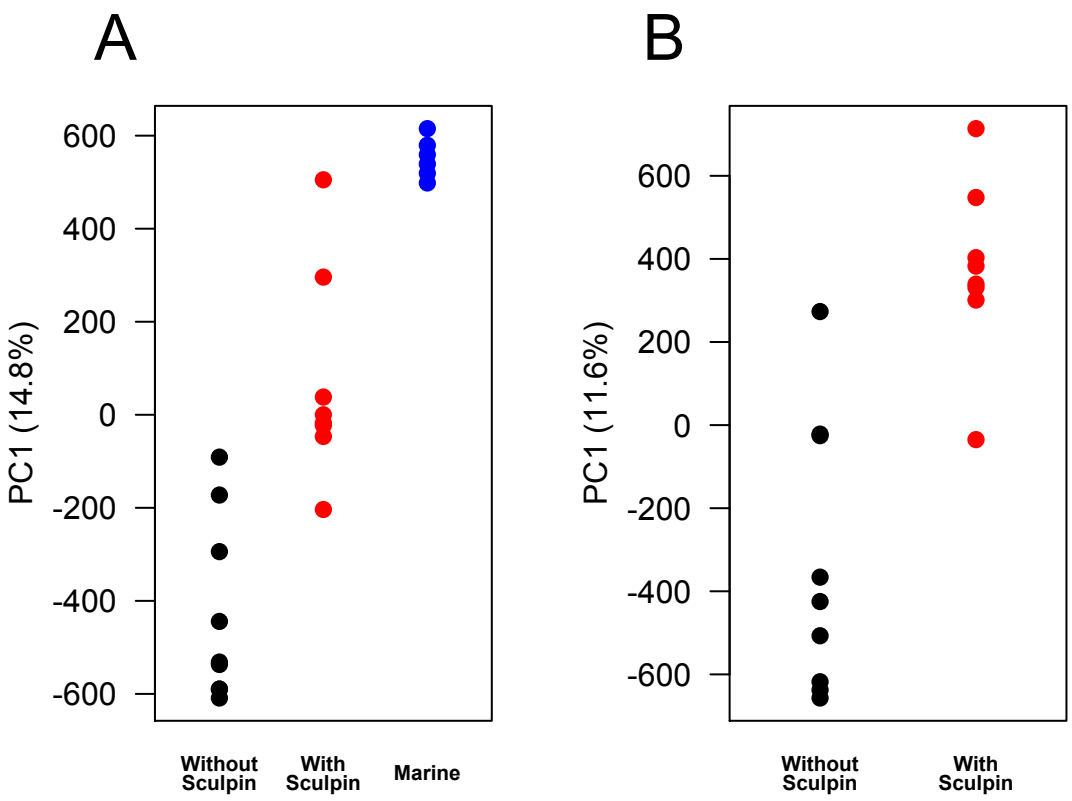


Figure 3.4 : Plot of principal components 2 and 3 from the principal component analysis of all SNPs from all populations presented in figure 3.3A. Each point is a single individual from a population. Lakes with sculpin are in red, lakes without sculpin are in black, and marine populations are in blue.

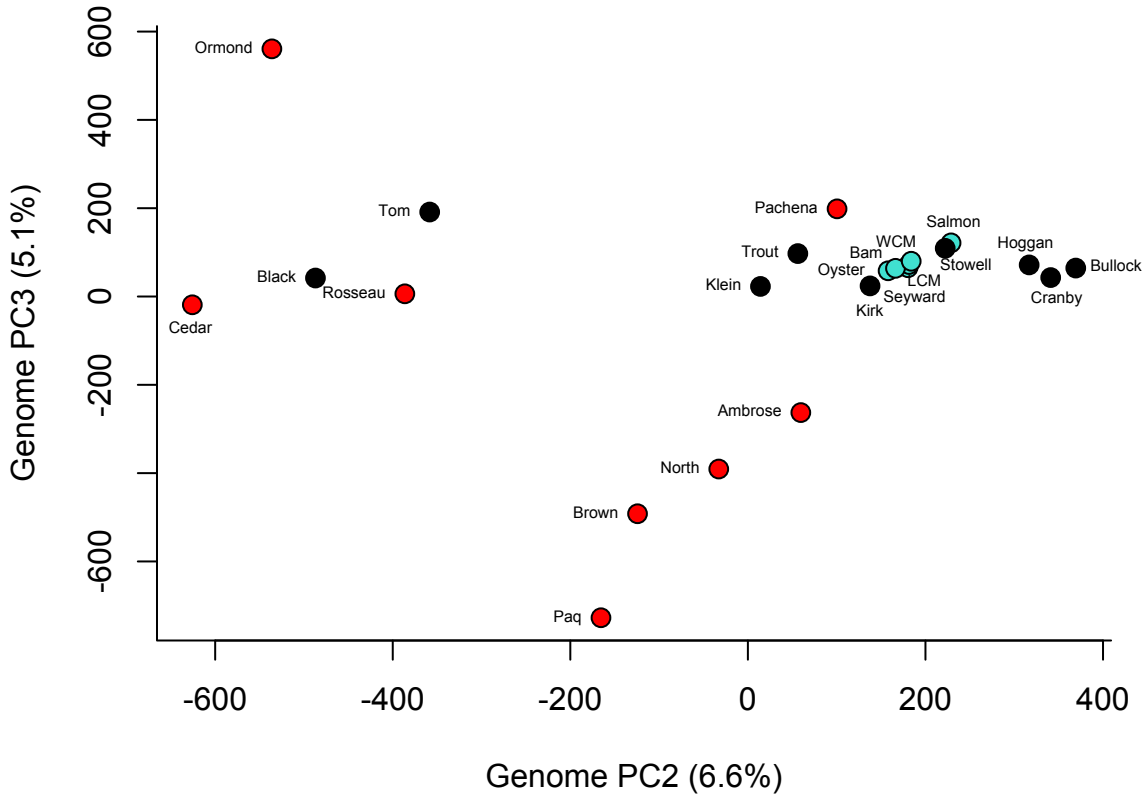


Figure 3.5 : Plot of CS' and F_{ST} for 10,000 bp windows throughout the genome. Points in red are outlier windows for CS' score. F_{ST} was calculated by comparing allele frequency between lakes with sculpin and lakes without sculpin.

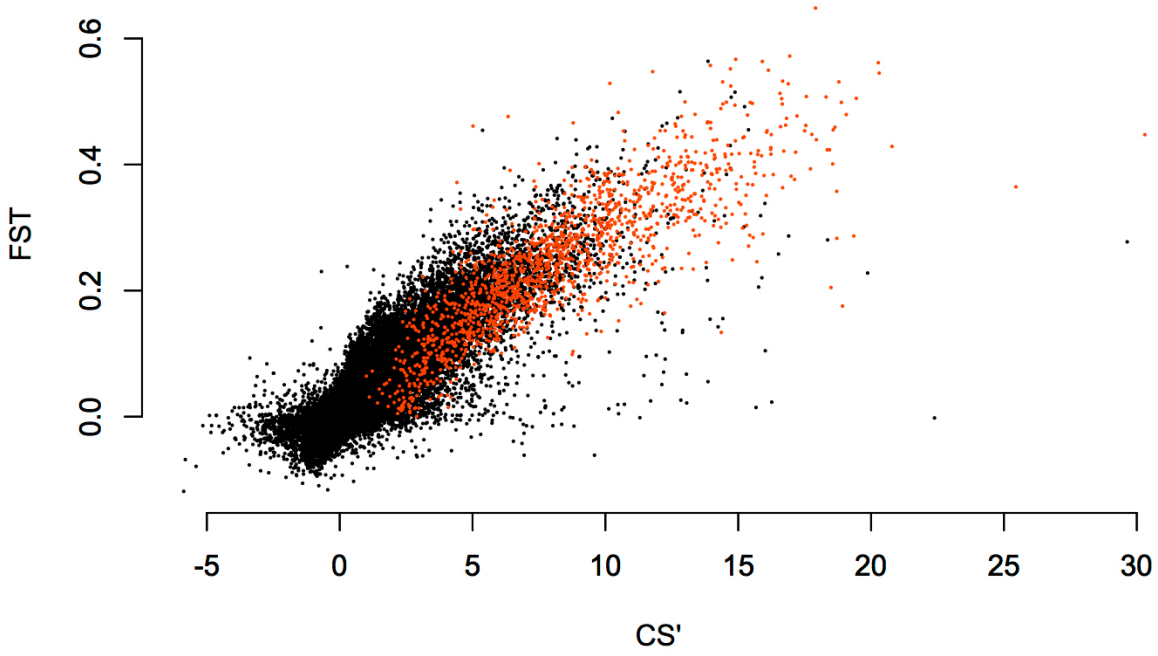


Figure 3.6 : Genome-wide distribution of CS' score. Points have been averaged over ten windows of 10,000 bp. Stickleback populations from lakes with and without sculpin are highly differentiated at many sites across the genome. All chromosomes are plotted on the same scale.

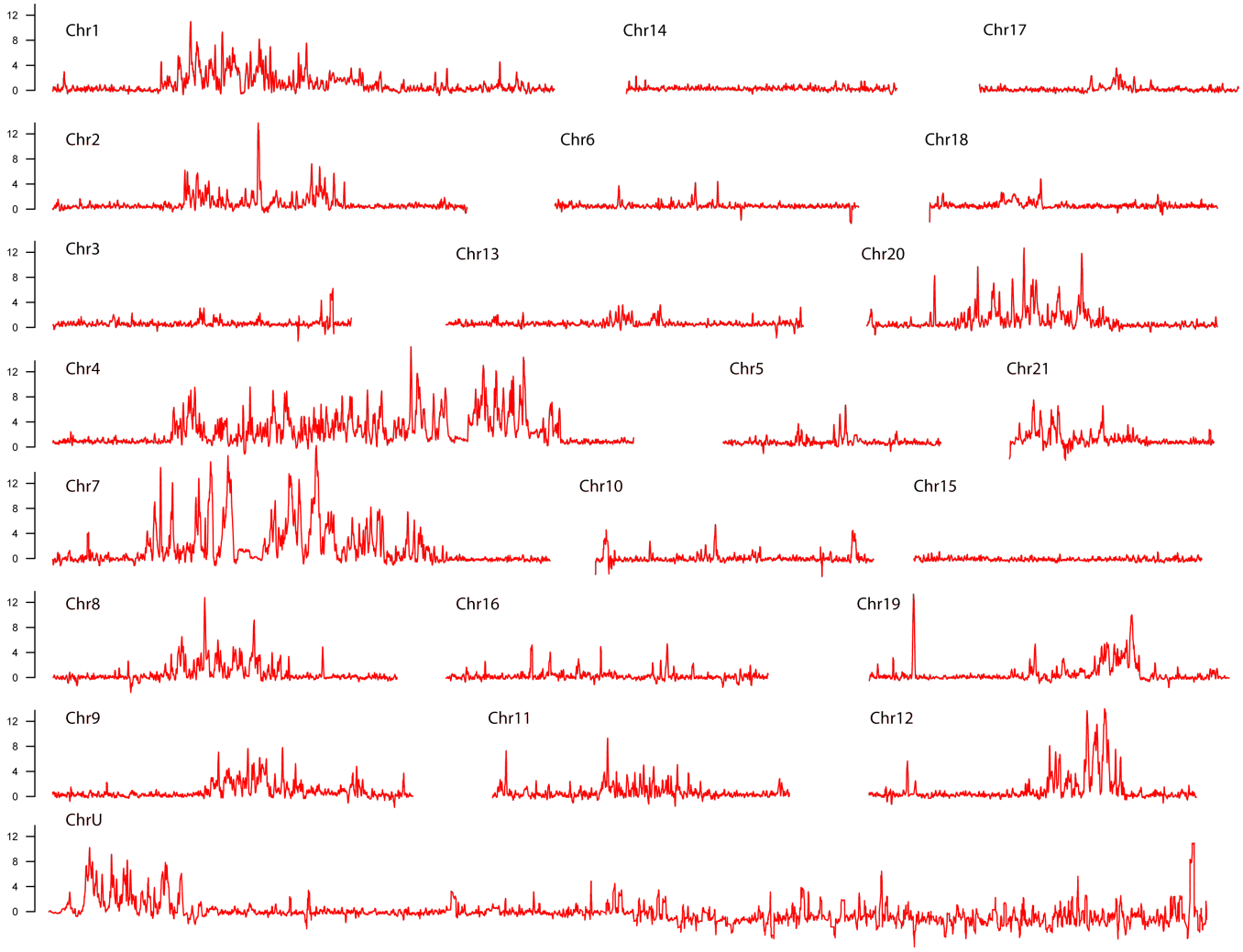


Figure 3.7 : CS' score between lakes with and without sculpin for chromosome twelve. Outlier windows are indicated in red. State changes in the hidden markov model are shown in blue. Adjacent outlier windows are frequently grouped next to each other and are likely part of the same selective sweep.

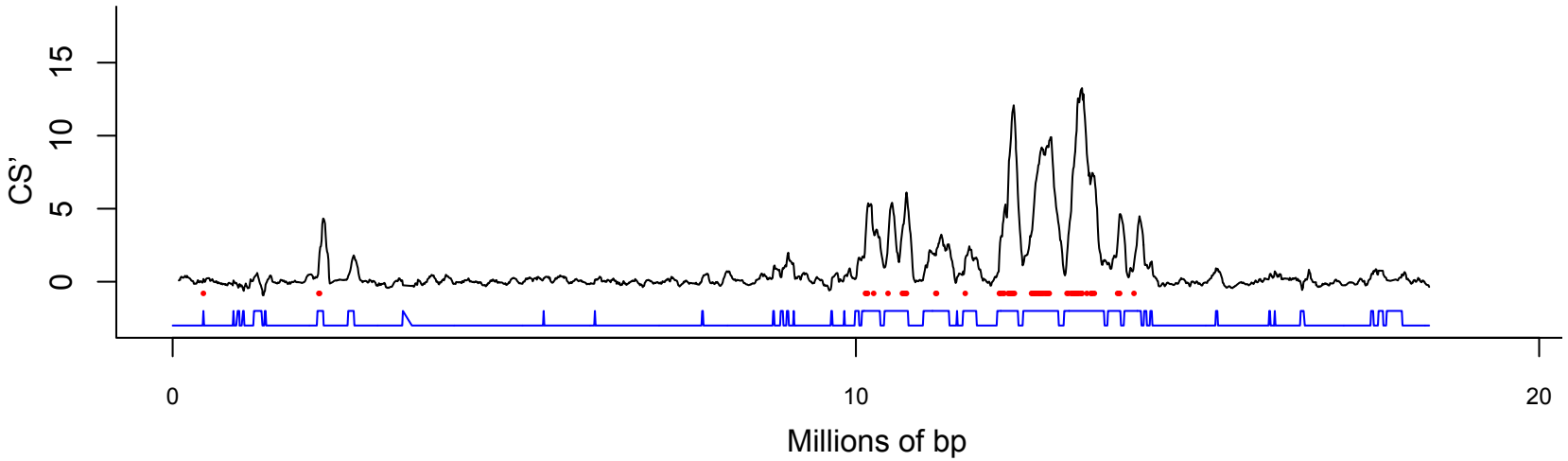


Table 3.1 : Abiotic traits measured from lakes with and without sculpin. Area, perimeter, max depth, mean depth, and pH were obtained from HabitatWizard. Elevation and the distance from the lake to the nearest ocean (To Sea) were calculated using google maps.

Lake	Type	Area	Perimeter	Max Depth	Mean Depth	Elevation	To Sea	pH	Na	Ca	Conductivity
Black	no sculpin	130	8400	27.2	11.1	111	1350	6.7			
Bullocks	no sculpin	9.4	1300	7	4	33	3310	8.5			
Cranby	no sculpin	44.6	3280	12.3	3.2	69	2530	7.5			
Hoggan	no sculpin	19.66	2219	NA	3	63	310				
Kirk	no sculpin	8.3	1372	20	8.3	121	2543				
Klein	no sculpin	13.5	2650	42	12	135	2660	6.8	55	246	0.0736
Stowell	no sculpin	5.64	983	7.5	4.6	77	1400	7.1			
Tom	no sculpin	17	2600	5	1.7	198	1689	6.4			
Trout	no sculpin	7.56	1308	17.4	5.8	157	2420	7.1	210	459	0.0677
Ambrose	sculpin	29.8	3200	33	13.3	56	940	5.6	138	173	0.0309
Brown	sculpin	18.764	1796	17.6	3.5	49	1120	6.8	108	38	0.0369
Cedar	sculpin	31	4900	20	3	204	11800	6.7	86	40	0.357
North	sculpin	12.79	1737	16.46	10.06	45	1040	7.1	115	329	0.0488
Ormond	sculpin	8	1700	NA	NA	227	10140	6.4			
Pachena	sculpin	58.68	4389	26.2	10.7	88	4730	6.7			
Paq	sculpin	12.14	1785	4.3	2.2	12	880		320	449	0.1583
Rosseau	sculpin	13.1	2120	11	5.8	137	6405	6.6			

Table 3.2 : Mann-Whitney test results from environmental variables between lakes with and without sculpin.

Traits	U	P
Perimeter	29	0.54
Max Depth	26	0.82
Mean Depth	29	0.83
Elevation	40	0.74
Distance to Ocean	32	0.74
pH	38	0.09
Sodium [Na]	4	0.86
Calcium [Ca]	8	0.38
Conductivity	6	0.86
Abiotic PC1	28	0.48
Abiotic PC2	36	1.0

Table 3.3 : Genome-wide distribution of windows identified as outliers by permutation test (Outlier Windows) and regions that both contain outlier windows and were identified by the hidden Markov Model (HMM).

Chromosome	Outlier Windows	HMM
1	104	17
2	42	11
3	4	3
4	305	29
5	4	2
6	4	3
7	436	22
8	70	9
9	61	12
10	3	3
11	27	11
12	156	11
13	7	4
14	1	1
15	7	3
16	16	6
17	4	2
18	13	2
19	23	3
20	109	7
21	16	3
Un	59	NA
Total	1471	164

Chapter 4: A Comparative Analysis of Experimental Selection on the Stickleback Pelvis

4.1 Introduction

Natural selection leads to changes in trait distribution when agents of selection cause differential fitness among individuals with different phenotypes (Endler 1986). Measurements of selection in the wild have now become commonplace (Kingsolver et al. 2001). However, identifying the mechanisms of selection presents a greater challenge because to determine the cause of natural selection requires demonstrating a link between the agent of selection, differential fitness, and a change in trait distribution.

Observational studies of natural selection in the wild are correlational and provide indirect evidence that the putative agent of selection is the cause of changes in trait distribution (Wade and Kalisz 1990; Schluter 2009). Natural selection may instead be the result of variation in the environment or the presence of another agent of selection. Experimental studies of selection are a powerful tool for identifying agents of selection. Researchers can manipulate the trait of interest to measure selection in isolation. However, selection experiments are notoriously difficult to perform. Experimental selection studies often lack the sample size to accurately estimate the effect size, resulting in wide confidence intervals for the estimate of selection on the trait of interest. Measurements of selection in the wild find that selection on morphological traits is typically weak to moderate (Hoekstra et al. 2001; Kingsolver et al. 2001) and conducting experimental studies with sufficient replicates to detect weak selection can be prohibitive for logistical reasons.

Combining results from multiple experimental studies of the same agent of selection might offer a solution to these difficulties by producing an aggregate estimate of selection that is more precise than any individual study (Arnqvist and Wooster 1995; Hersch and Phillips 2004; MacColl 2011). Meta-analysis of multiple studies has been used to estimate an effect size for diverse traits including *Daphnia* response to predator kairomones (Riessen 1999), and local adaptation in plants (Leimu and Fischer 2008).

Threespine stickleback (*Gasterosteus aculeatus*) from isolated populations display a wide range of phenotypic variation for many traits (Bell and Foster 1994). Importantly, stickleback inhabiting similar environments frequently show parallel changes in the same traits, suggesting that trait divergence is caused by ecological differences among environments (e.g. Kaeuffer et al. 2012; McKinnon and Rundle, 2002). This link between phenotypic variation and environmental variation has made the stickleback a model organism for investigating the mechanisms of selection driving these parallel trait changes.

One of the most conspicuous differences among stickleback populations is variation in pelvic morphology (Bell and Foster 1994). The stickleback pelvis is a bony structure consisting of a pelvic girdle and two hinged pelvic spines (Bell 1988). When extended, the pelvic spines brace against the pelvic girdle making them lock open (Reimchen 1983). Complete loss of the pelvic structure has occurred independently in multiple populations of threespine stickleback, ninespine stickleback (*Pungitius pungitius*), and brook stickleback (*Culaea inconstans*) (Nelson 1969; Nelson and Atton 1971; Klepaker et al. 2013). Pelvic loss has a genetic basis and loss of pelvic morphology has evolved multiple times in *Gasterosteus* (Kingsolver et al. 2001; Shapiro et al. 2004; Chan et al. 2010) and in *Pungitius* (Bell and Foster 1994; Shapiro et al. 2009; Shikano et al. 2013). Variation in

pelvic morphology can occur in the same lake both within (Bell 1988; Lescak et al. 2013) and between stickleback species (Reimchen 1983; McPhail 1992) and can be stable over multiple generations (Lescak et al. 2013) or can vary among stickleback of different size classes (Reimchen and Nosil 2002).

Predation has been hypothesized as the driver of variation in pelvic morphology. Pelvic spines are predicted to be an anti-predator defence against gape-limited piscivorous predators. It is theorized that spines help stickleback escape from predatory fish by piercing the mouth parts of predators and/or by increasing the effective diameter of the stickleback, thereby making it more difficult for the stickleback to be swallowed (Hoogland et al. 1956; Hagen and Gilbertson 1972). Several lines of observational evidence support the hypothesis that longer pelvic spines provide protection from fish predators. In laboratory feeding trials, pike (*Esox lucius*) preferentially consumed de-spined stickleback (Hoogland et al. 1956). In the wild, stickleback in the stomach contents of trout (*Oncorhynchus clarkii*) have shorter spines than stickleback collected using seine nets (Moodie 1972). Lastly, an increase in the abundance of predators or in the number of piscivorous predator species in the wild is repeatedly correlated with longer pelvic spines in stickleback and an increase in other armour traits (Moodie 1972; Vamosi 2003; Marchinko 2009; Miller et al. 2015). Combined, this evidence suggests that natural selection from fish predators increases pelvic armour.

Predation on juvenile stickleback by large aquatic insects has been hypothesized as the agent of selection for the reduction or loss of pelvic armour in many freshwater populations (Hoogland et al. 1956; Hagen and Gilbertson 1972; Reimchen 1980). Dragonfly nymphs (*Aeshna* sp.) can eat 1-2 juvenile stickleback per day (Hoogland et al. 1956; Reimchen 1980). Aquatic insects do not occur in the marine environment, and stickleback

from freshwater populations typically have reduced pelvic armour compared with the marine form (Klepaker et al. 2013). Two mechanisms have been proposed to explain why a reduction in the number and size of pelvic spines may provide a selective advantage against insect predation. Spines may provide a convenient "handhold" for insect predators to capture and hold on to stickleback (Reimchen 1980). Consequently, stickleback with shorter or absent spines will be able to avoid capture more easily. An alternative mechanism hypothesizes that individual stickleback with more armour might grow more slowly because investment in armour traits requires resources that would otherwise be used for growth (Marchinko and Schluter, 2007). Increased armour might thus prolong the length of time during which juvenile stickleback are small in size and most vulnerable to insect predation.

Several experimental studies have tested the role of predators as agents of selection on pelvic spines, but these experiments have produced somewhat inconsistent results. In some experiments, predatory fish more readily consume stickleback with shorter pelvic spines (e.g. Reist 1980; Lescak and Hippel 2011) while other experiments show no significant differences in fitness between stickleback with different pelvic morphology (e.g. Reist 1980; MacColl and Chapman 2011). Similarly, insect predators preferentially consume stickleback with longer pelvic spines in some experiments (Reist 1979; Marchinko 2009), while other experiments report non-significant estimates of selection on pelvic morphology (e.g. Lescak et al. 2012; Zeller et al. 2012; Mobley et al. 2013). In all cases, experimental estimates of selection on pelvic morphology are based on small sample sizes and have wide confidence intervals, indicating that estimates of the effect size are highly uncertain.

In this paper we address the causes of selection on stickleback pelvic spine length with an experiment and a meta-analysis. Our experiment focuses on stickleback from lakes

that contain prickly sculpin (*Cottus asper)*, an intraguild predator that eats stickleback and competes with stickleback for benthic resources. Stickleback sympatric with sculpin consistently have longer pelvic spines than stickleback from lakes in which prickly sculpin are absent (Miller et al. 2015). Variation in pelvic spine length among populations has a genetic basis (Rogers et al. 2012; Miller et al. 2015). A previous mesocosm experiment found higher mortality from sculpin predation on stickleback from lakes without sculpin (Ingram et al. 2011). However, stickleback sympatric with sculpin also exhibit genetically based difference in behaviour which may be important for escaping sculpin predation (Ingram et al. 2012; Miller et al. 2015), therefore the decrease in mortality cannot be directly attributed to longer pelvic spines. Furthermore, the presence of sculpin could also be correlated with another agent of selection for longer spines. For example, sculpin may displace stickleback from the benthic habitat into the open water where predation by coastal cutthroat trout is the agent of selection for longer pelvic spines. Therefore, experimental manipulation of the putative agent of selection is necessary to test the mechanism causing the association between pelvic spine length and fitness.

We tested if prickly sculpin are an agent of selection on stickleback pelvic spines by isolating the effect of spines as an anti-predator defence against sculpin in a mesocosm experiment. We physically modified the length of the pelvic spines of stickleback from two populations sympatric with sculpin and then compared the mortality rate of stickleback with clipped and unclipped pelvic spines experimentally in the presence of sculpin. The results of our mesocosm experiment were combined with previously published experimental selection studies to address the problem of low power and wide confidence intervals of the effect of

pelvic armour. We used a meta-analysis approach to determine the magnitude and direction of selection on pelvic morphology by both fish and insect predators.

4.2 Methods

4.2.1 Mesocosm Experiment

Experimental mesocosms were established in 20 plastic 1136L cattle tanks 1m deep by 2 m wide. Mesocosms were filled with water and seeded with benthic mud and zooplankton collected from nearby experimental ponds. To stimulate primary production, 0.05 g KH_2PO_4 and 1.0 g KNO_3 was added to each mesocosm. A 25 cm diameter open-ended cylinder constructed from stiff black 7 mm plastic mesh was attached to the side of each cattle tank and suspended 0.5 m above the bottom to provide shade and a refuge from predation. Mesocosms were allowed to settle for two weeks prior to the addition of fish.

Adult stickleback were collected from Paq Lake and Ambrose Lake and sculpin were collected from Paq Lake using minnow traps and by dipnet. Fish were transported to 100L holding tanks in the aquatic facility at the University of British Columbia and allowed to recover for several days. Paq and Ambrose are in separate watersheds in the Sechelt Peninsula. Both lakes contain a simple fish community composed of threespine stickleback, prickly sculpin, and coastal cutthroat trout.

To create variation in the length of the pelvic spine, stickleback were briefly anesthetised in MS-222 (1g/L) and pelvic spines were clipped to 2.5mm (the average length of stickleback pelvic spines from lakes without sculpin (Miller et al. 2015)). Control stickleback were anaesthetized and handled in a similar manner but pelvic spines were not

modified. Stickleback were returned to the 100L tanks for 24 hours of observation. There was no mortality following spine clipping.

The standard length of each stickleback was measured prior to introduction (36.5-60.6 mm). Four size-matched clipped and unclipped stickleback were added to each mesocosm (eight total). Paq Lake stickleback were used for 10 mesocosms and Ambrose Lake stickleback were used for the remaining 10 mesocosms. Following the first set of trials, sufficient Paq lake stickleback were available for six additional trials (N=26 trials total). A single sculpin (95-105mm) was added to each mesocosm two days after the stickleback introduction. A visual survey of the number of stickleback in each mesocosm was conducted daily. Dead stickleback that did not show evidence of sculpin predation were replaced with a similar-sized individual having the same pelvic phenotype. A trial was considered to be complete when half of the stickleback were consumed. At that time, the sculpin was removed and remaining stickleback were collected. We carefully examined each stickleback for signs of injury and recorded standard length and pelvic phenotype. Over the course of the experiment, visibility in mesocosms decreased. As a result, several trials were stopped when greater or fewer than four stickleback remained. To ensure that all surviving stickleback were collected, each mesocosms was trapped with minnow traps for 48 hours.

Paired t-tests were performed separately for Paq and Ambrose Lake mesocosms to compare the frequency and size of surviving clipped and unclipped stickleback using trial as the replicate. The log odds ratio was calculated for each trial and then a summary log odds ratio was estimated using the Peto method (Borenstein et al, 2009). A positive log odds ratio indicates stickleback with unclipped spines were more likely to survive, and a negative log odds ratio indicates increased survival of stickleback with clipped spines.

4.2.2 Comparison with Other Selection Studies

We conducted a meta-analysis by searching the literature for experimental studies measuring selection on stickleback pelvic morphology from insectivorous or piscivorous predators. Variation in pelvic morphology could be naturally occurring variation, physical modifications, or F2 or backcross hybrids between populations having divergent phenotypes. Studies were only included in the meta-analysis if sufficient information was available to allow us to calculate the standard error of the effect size, which also required multiple independent trials. Using these criteria, we excluded Ziuganov and Zotin (1995) because the study had a single uncontrolled experimental replicate. We were also forced to leave out Reimchen (1980) because results from multiple replicates were pooled, which loses all information on the variance between trial outcomes, and the original data was no longer available. Although Reist (1979, 1980) presented pooled data across replicates, the results for most trials were available in Reist (1978). When data for individual trials was not available, we contacted the authors of the original study. Leinonen *et al.* (2011), McColl et al. (2011), and Mobley et al. (2013) generously provided raw data for individual trials.

We used standardized mean difference in trait values between treatments, *d* (predation – control), as our measure of effect size. For studies reporting a continuous measure of pelvic spine length or standard length, *d* was calculated using the formula for independent groups (Borenstein et al, 2009). This metric is similar to the standardized selection differential (*i*) (equation 6.1 in Endler, 1986) except that *d* uses the pooled standard deviation across groups, whereas *i* uses the standard deviation from only the control treatment. The values of the two measures were always similar. For studies

measuring selection on the presence/absence of the pelvic structure, either with experimental manipulation or using natural existing variation, a log odds ratio was calculated from the proportion of survivors with and without pelvic spines/girdles in the two treatments for each trial. Then, an overall summary log odds ratio was calculated for each experiment using the Peto method (Borenstein et al, 2009). Summary log odds ratios were converted to *d* to facilitate comparisons across studies (Hasselblad and Hedges 1995).

Experimental design, target population, and stickleback species varied among studies, therefore the summary effect for the meta-analysis was calculated using a random effect model separately for insect and piscivorous predators. Effect sizes were weighted using the inverse of the sampling variance of the experiment (Borenstein et al, 2009). The random effects model assumes that the true effect size may vary from study to study (Borenstein et al, 2009). The summary effect is therefore an estimate of the mean distribution of the true effect size of pelvic morphology on the probability of survival. To minimize bias from the inclusion of multiple experiments from a single study, we calculated a second summary effect for each predator type using a single estimate for each study. A fixed effects meta-analysis was used to estimate the summary effect for each study. As before, a random effect model was then used to calculate an overall summary effect across studies. Standard length was reported in fewer studies. A summary effect for length was similarly calculated with a random effect model separately for insect and piscivorous predators. All summary effects were calculated using the 'meta' package (Schwarzer 2015) in the R statistical environment (R Development Core Team, 2015).

4.3 Results

4.3.1 Mesocosm Experiment

Trials took 15-50 days to reach 50% stickleback mortality. None of the surviving stickleback showed evidence of wounds from unsuccessful predation attempts. We found no significant difference in survival of stickleback with clipped and unclipped pelvic spines (Figure 4.1; Paq: t=0.75, df=15, P=0.47; Ambrose: t=0, df=9, P=1). The summary log odds ratio for all trials was 0.118 (95% CI: -0.358, 0.594) representing an 11.1% increase in survival probability for stickleback with unclipped pelvic spines, but this result was not significant and the confidence intervals were wide. Results were similar when comparing each lake individually (Paq Lake: 0.189, 95% CI: -0.414, 0.793; Ambrose Lake: 0.00, 95% CI: -0.775, 0.775). Surviving clipped and unclipped stickleback did not differ in standard length (Paq: t=-0.09, df=15, P=0.93; Ambrose: t=-1.45, df=9, P=0.18). There was no difference in mean standard length at the start of the experiment compared to mean standard length of the survivors (Paq: t=-1.19, df=15, P=0.25; Ambrose: t=-0.48, df=9, P=0.65).

4.3.2 Meta-analysis of Selection Studies

We identified 25 published and unpublished experiments that met our criteria. Combined, these experiments represented 213 independent trials measuring selection on pelvic morphology in the presence of fish or insect predators. Studies included three species of stickleback, four species of insect predators, and four species of fish predators. Most experiments were conducted by adding stickleback with variation in pelvic morphology to a mesocosm containing a predator. Discrete variation in pelvic morphology was generated by

experimentally manipulating pelvic spine length, or by using study populations with naturally occurring variation in pelvic spine presence/absence. Several studies used F2 or backcrosses to create continuous variation in pelvic morphology. Standard length data was available for three fish predation experiments and five insect predation experiments. Details and effect sizes for all studies are reported in Tables 4.1 and 4.2.

For fish predation experiments, longer pelvic spines increased survival (Figure 4.2), with a mean effect size of 0.13 (95% CI: 0.02, 0.23; P=0.02). This is equivalent to an increase in the mean pelvic spine length by 0.13 of a standard deviation in the presence of fish predators. Insect predation favoured slightly shorter pelvic spines, with a mean effect size of -0.05 (95% CI: -0.28, 0.17; P=0.65), but this result was not significant (Figure 4.3). Using a single estimate for each study, the summary mean effect was 0.14 (95% CI: 0.004, 0.28; P=0.04) for fish predation experiments (Figure 4.4) and 0.04 (95% CI: -0.19, 0.28; P=0.71) for insect predation experiments (Figure 4.5). Insect predation experiments had a larger variance in effect size than fish predation experiments (Figure 4.6).

Fish predators had no effect on standard length (Figure 4.7). The summary mean effect fish predation was -0.02 (95% CI: -0.83, 0.78; P=0.96). Insect predators preferentially consumed smaller fish (Figure 4.7), with a summary mean effect of 0.27 (95% CI: -0.14, 0.67; P=0.20), but this effect was not significant.

4.4 Discussion

Pelvic morphology is a highly variable trait among and often within stickleback populations. The mechanisms of selection that produce this trait variation are still uncertain. Stickleback in lakes with sculpin have longer pelvic spines than stickleback from lakes

without sculpin (Miller et al. 2015). To test if prickly sculpin are an agent of selection on stickleback pelvic spines, we experimentally modified the length of pelvic spines and measured differential mortality between stickleback with clipped and unmodified pelvic spines. We observed an 11% increase in the probability of survival for stickleback with unclipped pelvic spines. However, the confidence intervals for this estimate overlapped with zero and this effect was not statistically significant. From this data alone, we were unable to conclude that prickly sculpin preferentially consumed stickleback with shortened pelvic spines.

There are four possible reasons why our experiment failed to detect selection on pelvic spine length from prickly sculpin predation. (1) Selection on stickleback pelvic spines is caused by other factors and sculpin are not an agent of selection on this trait. For example, lakes with sculpin also contain coastal cutthroat trout and a variety of avian predators, which may be an alternative agent of selection favouring increased pelvic spines (although lakes without sculpin also contain these predators) (Miller et al. 2015). (2) A challenge of selection experiments is choosing the correct size class of both the agent of selection and target of selection (Endler 1986). Natural selection may favour greater pelvic morphology but only when stickleback and/or sculpin are at a different size class than that used in the experiment. For example, none of the surviving stickleback in this experiment had defensive wounds, in contrast to a similar experiment by Lescak and von Hippel (2011) that identified wounds caused by the predator (trout) in 40% of trials. The current experiment used adult sculpin near the upper limit of the size range of sculpin in Paq Lake (personal observation), whereas smaller sculpin might be gape limited and thus select for greater armour. (3) Manipulations of spines were not effective because the spines were not

scaled to body size. (4) Natural selection indeed favoured longer pelvic spines but we were unable to detect an effect because our experiment was underpowered (type II error). We observed a trend towards increased survival of stickleback with unclipped pelvic spines, but as in most other experiments of this kind (Figure 4.2), this result was not statistically significant and confidence intervals for treatment effects were large.

Partly to overcome the lower power of individual studies, we compiled a meta-analysis of experimental studies of selection on pelvic morphology from insect and fish predators. We found that fish predators indeed selected for longer pelvic spines, with a summary effect size of 0.13 units of a standard deviation. The effect sizes were similar when experiments were combined into a single estimate for each study. If we assume that pelvic spine length has a heritability of 0.38 (Leinonen et al. 2011b), using the 95% confidence interval of our estimate of effect size, we predict that the mean pelvic spine length would increase by one standard deviation in 12 – 132 generations. This value represents a small to moderate effect on fitness and is comparable to 0.14, the mean absolute value for linear selection differentials from the Kingsolver selection dataset (Kingsolver et al. 2001). On the basis of our meta-analysis, and in agreement with observational studies, we conclude that fish predators are an agent of selection favouring increased pelvic spines.

In contrast, it is still unclear if insect predators are an agent of direct selection on the length of stickleback pelvic spines. The summary effect size indicated a very small increase in survival for stickleback with shorter pelvic spines. However, the large confidence interval for this estimate ranges from -0.28 to 0.17 preventing us from ruling out either selection for increased or decreased pelvic morphology by insects. Although Reimchen's

(1980) hypothesis has been frequently cited, there is as of yet no convincing evidence in support of insect predators selecting for smaller spines by the "handhold" mechanism.

Studies included in the meta-analysis measured selection at a range of body size in multiple stickleback species for several species of insect predator. This variation in methodology may obscure the effect of insect predation. For example, Lescak et al. (2013) observed that dragonfly naiads preferred to eat stickleback with pelvic armour when the fish were smaller than the dragonfly but preferred stickleback without pelvic armour when the fish was larger then the dragonfly. The summary effect of insect predation revealed that insects preferred to eat smaller stickleback, but this effect was not significant.

This leaves open the question of what is the selective mechanism underlying loss or reduction of the pelvis in many stickleback populations. Armour reduction may occur as a by-product of selection on another trait. Reduced pelvic armour has been proposed to increase buoyancy (Myhre and Klepaker 2009) and manoeuvrability (Reimchen 2000). One hypothesis is that indirect selection against pelvic spines occurs because investment in armour is costly in energy and materials thereby limiting minerals such as calcium or phosphorus available for growth (Giles, 1983). Small juvenile stickleback are eaten by insects therefore a slower growth rate increases the length of time that juveniles are vulnerable to insect predation. Direct selection for increased growth rate could lead to indirect selection for decreased armour. However, support for the ion limitation hypothesis is mixed. Bell (1993) compared the frequency of pelvic reduction with calcium concentration for 179 Alaskan lakes. When native piscivorous predators were present, none of the lakes had a reduction in pelvic structure. When predatory fish were absent from the lakes, pelvic reduction was associated with low calcium concentrations. However, pelvic reduction has

been observed in Canadian lakes with high concentrations of calcium (Klepaker at al. 2013). Marchinko and Schluter (2007) raised stickleback with differing numbers of lateral plates, another type of bony armour, in freshwater and saltwater. Supporting the ion limitation hypothesis, in freshwater, stickleback with more lateral plates grew more slowly compared to stickleback with fewer lateral plates. However, a later study by Rollins et al. (2014) did not detect higher growth rate of stickleback with decreased pelvic armour in the lab or in the wild, suggesting that ion limitation may vary among traits or that the effect was too small to detect in that study.

We find support for the hypothesis that fish predation selects for longer pelvic spines in stickleback. The magnitude of this selection was small to moderate. At this time we are unable to support or reject the hypothesis that insect predators favour shorter pelvic spines.

Figure 4.1 : Number of surviving threespine stickleback with the clipped and unclipped pelvic spine treatment at the end of the mesocosm experiment. Lines connect stickleback from a single mesocosm replicate. Each trial started with four fish of each phenotype.

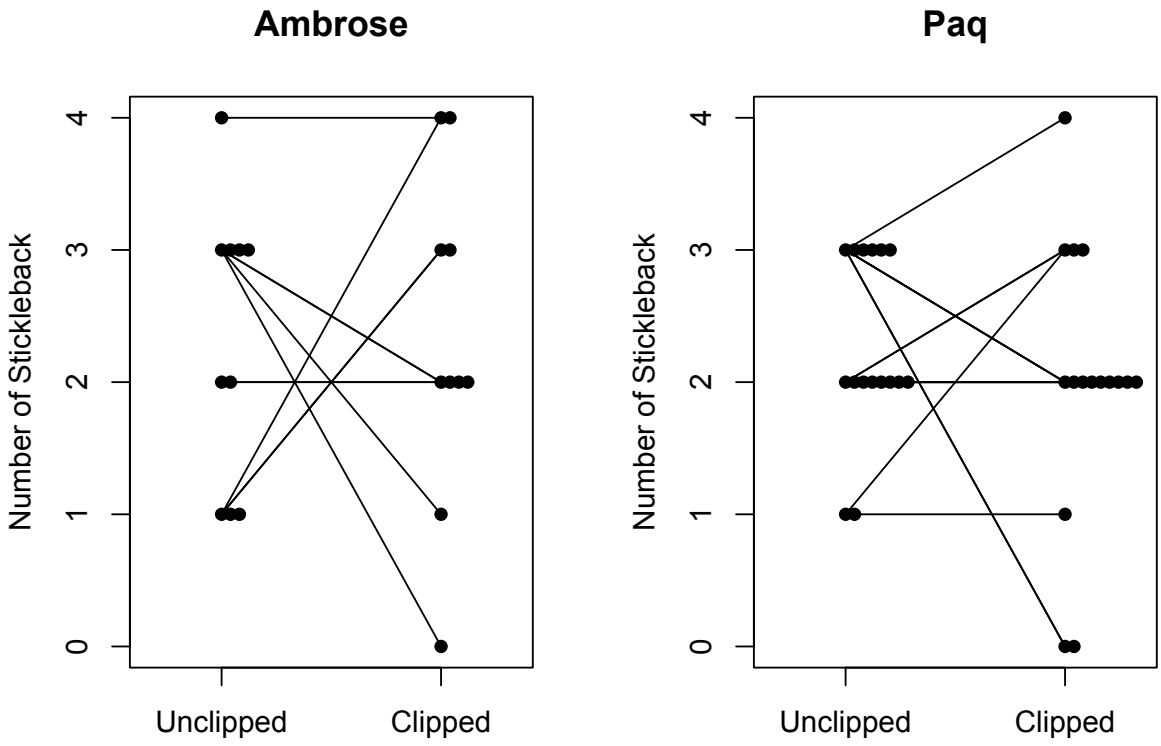


Figure 4.2 : Forest plot of the effect size for all fish predation experiments using multiple estimates from each study. The grey box indicates the mean of the effect size (d) for each experiment and the lines give the 95% confidence interval of the effect. The weighted mean was calculated using a random effects model. W is the weight of the study in the model. The weighted mean is indicated with the dotted line and the 95% confidence interval of this estimate is contained within the red triangle.

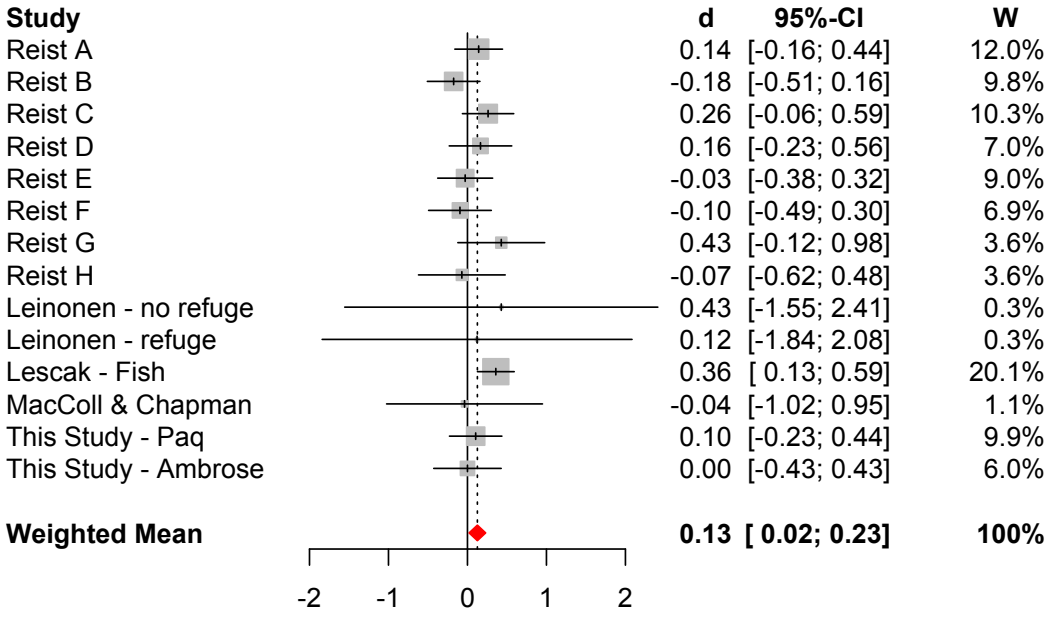


Figure 4.3 : Forest plot of the effect size for all insect predation experiments using multiple estimates from each study. The grey box indicates the mean of the effect size (d) for each experiment and the lines give the 95% confidence interval of the effect. The weighted mean was calculated using a random effects model. W is the weight of the study in the model. The weighted mean is indicated with the dotted line and the 95% confidence interval of this estimate is contained within the red triangle.

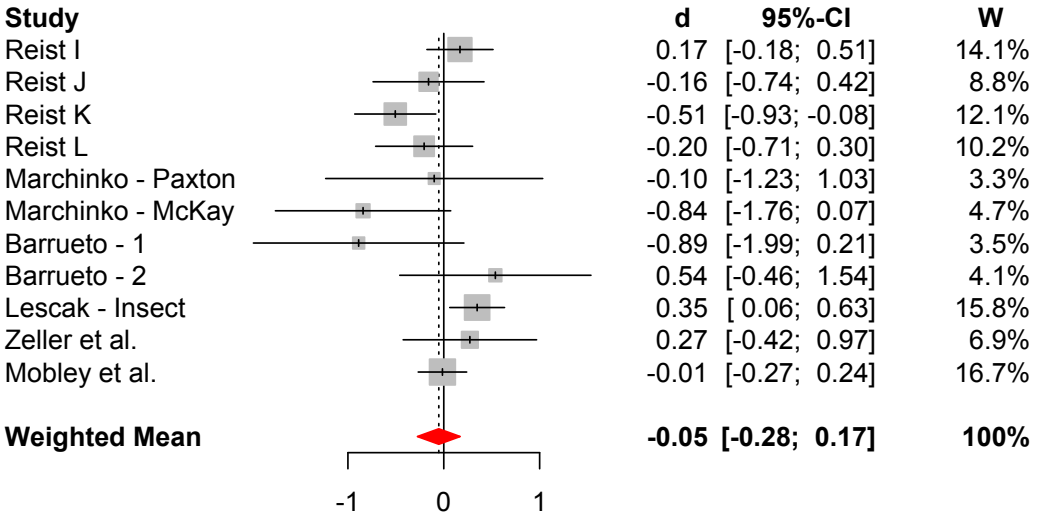


Figure 4.4 : Forest plot of the effect size for all fish predation studies with a single estimate from each study. The grey box indicates the mean of the effect size (d) for each study and the lines give the 95% confidence interval of the effect. For studies containing multiple experiments, the mean effect size of each study was calculated using a fixed effects model. The weighted mean for all studies was calculated using the inverse variance method with a random effects mode. W is the weight of the study in the model. The weighted mean effect is indicated with the dotted line and the 95% confidence interval of this estimate is contained within the red triangle.

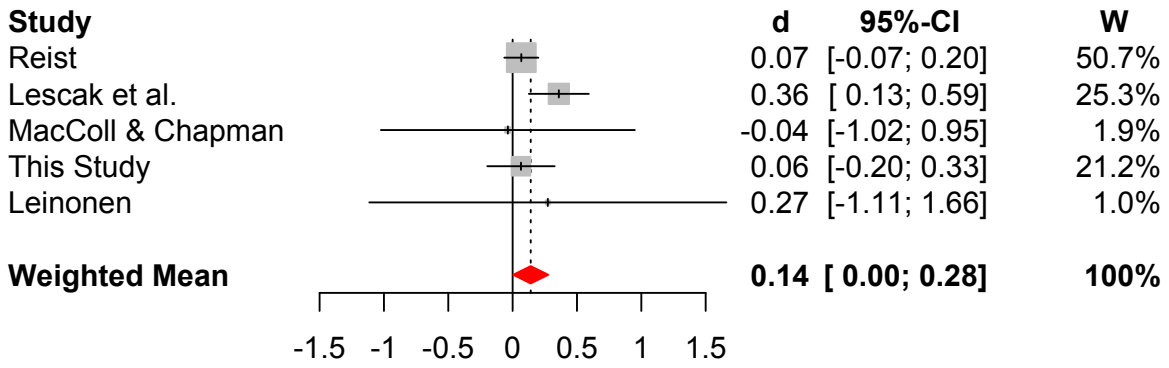


Figure 4.5 : Forest plot of the effect size for all insect predation studies with a single estimate from each study. The grey box indicates the mean of the effect size (d) for each study and the lines give the 95% confidence interval of the effect. For studies containing multiple experiments, the mean effect size of each study was calculated using a fixed effects model. The weighted mean for all studies was calculated using the inverse variance method with a random effects mode. W is the weight of the study in the model. The weighted mean effect is indicated with the dotted line and the 95% confidence interval of this estimate is contained within the red triangle.

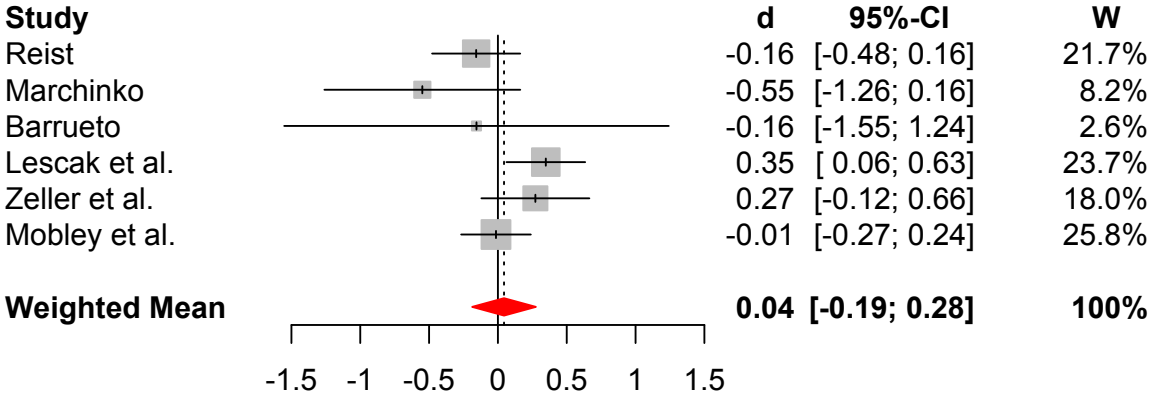


Figure 4.6 : The effect size and standard error of (A) fish predation experiments and (B) insect predation experiments used in the meta-analysis.

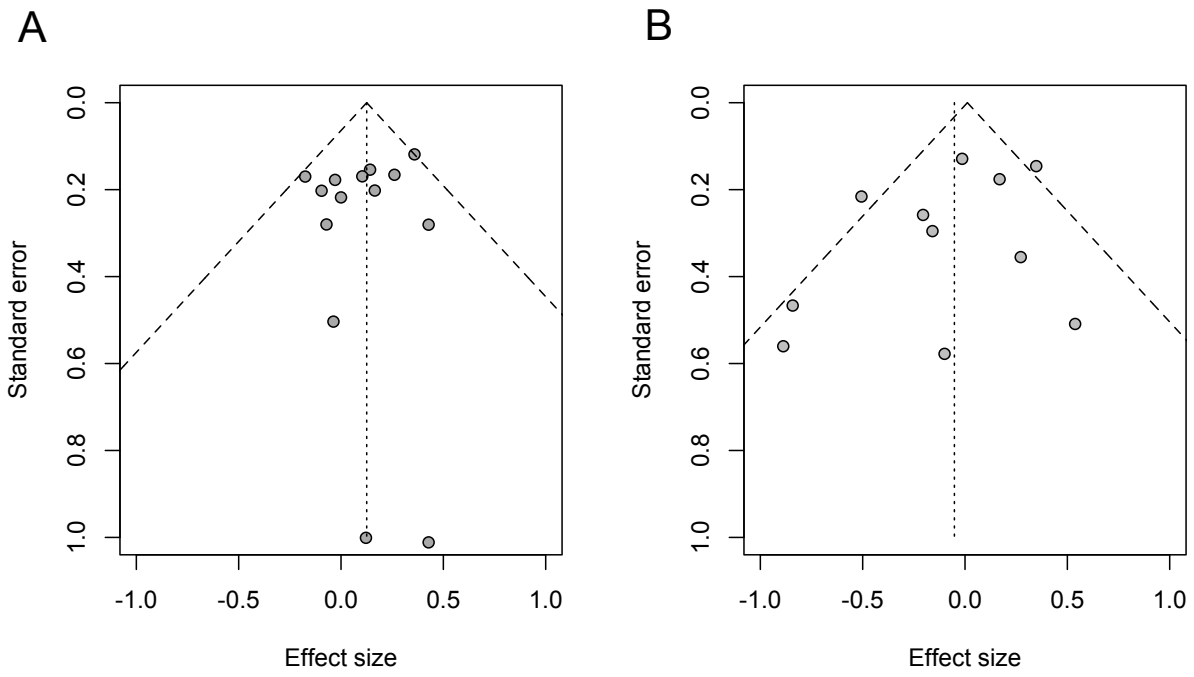


Figure 4.7 : Forest plot of the effect size of standard length for all predation experiments. A random effects model was run for fish and insect predation studies. The grey box indicates the mean of the effect size (d) for each experiment and the lines give the 95% confidence interval of the effect. The weighted mean was calculated using a random effects model. W is the weight of the study in the model. The weighted mean is indicated with the dotted line and the 95% confidence interval of this estimate is contained within the red triangle.

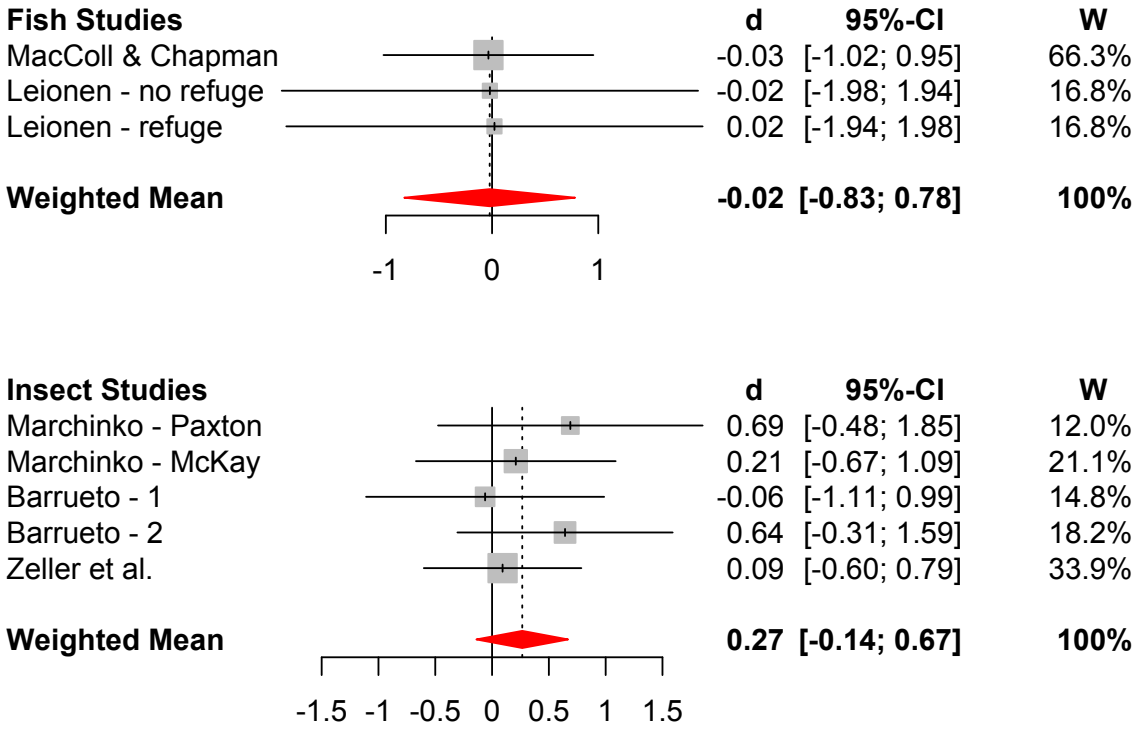


Table 4.1 : Experimental studies of selection on stickleback pelvic morphology by piscivorous predators. Variation in the length of pelvic spines (source of variation) was obtained from populations with naturally occurring variation, by creating crosses between populations with divergent pelvic spine lengths, or by experimentally modifying spines. All effect sizes were converted to the standardized mean difference (d). Significant effect sizes are given in bold. Trait abbreviations are as follows: PG: pelvic girdle, PS: pelvic spine, ST: spine triangle (triangle formed by pelvic and dorsal spines), SL: standard length. Unreplicated experiments were excluded from the meta-analysis (#).

Author	Year	Experiment	Species	Source	Population	Predator	Traits	Size (mm)	Trials	Fish	logodds	95% CI	d	d 95% CI
Reist	1980	A	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Esox lucius*	PS	20-29.9	7	205	0.259	(-0.291, 0.808)	0.142	(-0.160, 0.446)
		B	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Esox lucius*	PS	30-39.9	6	170	-0.318	(-0.921, 0.286)	-0.175	(-0.508, 0.157)
		C	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Esox lucius*	PS	40-49.9	6	171	0.473	(-0.117, 1.063)	0.261	(-0.064, 0.586)
		D	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Esox lucius*	PS	20-29.9	4	115	0.300	(-0.420, 1.020)	0.165	(-0.231, 0.562)
		E	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Esox lucius*	PS	30-39.9	5	150	-0.052	(-0.684, 0.581)	-0.029	(-0.377, 0.320)
		F	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Esox lucius*	PS	40-49.9	4	115	-0.172	(-0.893, 0.548)	-0.095	(-0.492, 0.302)
		G	*Culea inconstans*	modified wild caught	Wakomao Lake, AB	*Esox lucius*	PS	30-39.9	2	60	0.777	(-0.221, 1.77)	0.428	(-0.122, 0.978)
		H	*Culea inconstans*	modified wild caught	Wakomao Lake, AB	*Esox lucius*	PS	40-49.9	2	60	-0.129	(-1.123, 0.867)	-0.071	(-0.620, 0.478)
Ziuganov & Zotin	1995	fish	*Pungitius pungitius*	wild caught	Levin Navolok, Russia	*Perca fluviatilis*	PG	51-62	1 #	200	1.800		0.993	
Lescak *et al.*	2011		*Gasterosteus aculeatus*	wild caught	Wallace Lake, AK	*Oncorhynchus mykiss*	PG	37-45	26	260	0.650	**(0.228, 1.073)**	0.359	**(0.126, 0.592)**
Leionen *et al.*	2011		*Gasterosteus aculeatus*	half-sib crosses	Baltic Sea	*Esox lucius*	PS	6 months	2	325			0.175	(-1.789, 2.140)
	2011		*Gasterosteus aculeatus*	half-sib crosses	Baltic Sea	*Esox lucius*	PS	6 months	2	325			0.173	(-1.790, 2.137)
MacColl & Chapman	2011		*Gasterosteus aculeatus*	F2	Marine x Hoggan, BC	*Cottus asper*	PS, SL	33.2-43.3	16	160			-0.037	(-1.025, 0.950)
This study		Paq	*Gasterosteus aculeatus*	modified wild caught	Paq Lake, BC	*Cottus asper*	PS, SL	36-51	16	128	0.189	(-0.414, 0.793)	0.104	(-0.228,0.437)
		Ambrose	*Gasterosteus aculeatus*	modified wild caught	Ambrose Lake, BC	*Cottus asper*	PS, SL	41-63	10	80	0.00	(-0.775, 0.775)	0.00	(-0.427, 0.427)

Table 4.2 : Experimental studies of selection on stickleback pelvic morphology by insect predators. Variation in the length of pelvic spines (source of variation) was obtained from populations with naturally occurring variation, by creating crosses between populations with divergent pelvic spine lengths, or by experimentally modifying spines. All effect sizes were converted to the standardized mean difference (d). Significant effect sizes are given in bold. Trait abbreviations are as follows: PG: pelvic girdle, PS: pelvic spine, SL: standard length. One study estimated stickleback length from mean values (^). Experiments were excluded from the meta-analysis when results from multiple trials were presented as pooled data (*), or when experiments were unreplicated (#).

Author	Year	Experiment	Species	Source	Population	Predator	Traits	Size (mm)	Trials	Fish	logodds	95% CI	d	d 95% CI
Reist	1979	I	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Lethocerus americanus*	PS	20-29.9	11	149	0.306	(-0.320, 0.931)	0.169	(-0.176, 0.513)
		J	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Lethocerus americanus*	PS	30-39.9	4	53	-0.288	(-1.339, 0.764)	-0.159	(-0.738, 0.421)
		K	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Dysticus spp.*	PS	20-29.9	7	98	-0.918	**(-1.684, -0.151)**	-0.506	**(-0.929, -0.083)**
		L	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Dysticus spp.*	PS	20-29.9	5	69	-0.371	(-1.289, 0.547)	-0.205	(-0.711, 0.301)
		M	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Dysticus spp.*	PS	30-39.9	1#	14	0.575		0.317	
		N	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Aeshna spp.*	PS	20-29.9	1#	11	2.99		1.646	
		O	*Culea inconstans*	wild caught	Wakomao Lake, AB	*Aeshna spp.*	PS	20-29.9	7*	91	-0.308		-0.170	
Reimchen	1980		*Gasterosteus aculeatus*	wild caught	Boulton Lake, BC	*Aeshna spp.*	PG	15-25	7*	408	-0.159		-0.088	
Ziuganov & Zotin	1995	insect	*Pungitius pungitius*	wild caught	Levin Navolok, Russia	*Odonata spp. Dysticus spp.*	PG	51-62	1 #	200	-1.520		-0.838	

Author	Year	Experiment	Species	Source	Population	Predator	Traits	Size (mm)	Trials	Fish	logodds	95% CI	d	d 95% CI
Marchinko	2009	Paxton	*Gasterosteus aculeatus*	F2 hybrids	Paxton x Marine	*Aeshna spp. Notonecta spp.*	PS, SL	10-18	6	477			-0.100	(-1.232, 1.032)
		McKay	*Gasterosteus aculeatus*	F2 hybrids	McKay x Marine	*Aeshna spp. Notonecta spp.*	PS, SL	10-23	10	767			-0.842	(-1.757, 0.073)
Barrueto	2009	1	*Gasterosteus aculeatus*	modified lab raised	Salmon River	*Notonecta spp.*	PS, SL	11-22	7	423			-0.888	(-1.986, 0.210)
		2	*Gasterosteus aculeatus*	backcrosses	Paxton Lake, BC	*Notonecta spp.*	PG, SL	9-18	8	573			0.538	(-0.460, 1.536)
Lescak *et al.*	2012		*Gasterosteus aculeatus*	wild caught	Wallace Lake, AK	*Aeshna spp.*	PG	23-57	11	220	0.630	**(0.112, 1.150)**	0.348	**(0.062, 0.634)**
Zeller *et al.*	2012		*Gasterosteus aculeatus*	wild caught	Bern, Switzerland	*Aeshna spp.*	PS, SL	adults	16	960			0.272	(-0.424, 0.968)
Mobley *et al.*	2013		*Pungitius pungitius*	modified wild caught	Bothnian Bay, Sweden	*Aeshna spp.*	PS	29.9-35.1^	20	200	-0.026	(-0.484, 0.432)	-0.014	(-0.267, 0.238)

Chapter 5: An Experimental Test of the Effect of Predation Upon Behaviour and Trait Correlations in Threespine Stickleback[1]

5.1 Introduction

Ecological speciation occurs when reproductive isolation evolves as a consequence of divergent natural selection between contrasting environments (Schluter 2009; Nosil 2012). While there are many examples of ecological speciation in nature, our understanding of the underlying mechanisms remains incomplete (Rundle and Nosil 2005; Nosil 2012). Divergent selection can occur in response to differences in resource availability and as a result of biotic interactions such as predation, competition, or intraguild predation (Schluter 2000; 2009; Miller et al. 2015). Experimental studies have shown that differential predation can lead to the evolution of divergent morphological traits (e.g. Jiggens et al. 2001; Vamosi and Schluter 2002; Rundle et al. 2003; Nosil and Crespi 2006; Langerhans et al. 2007; Diabaté et al. 2008; Marchinko 2009; Svanbäck and Eklöv 2011). However, less attention has been given to the role of divergent selection in the evolution of behavioural diversity.

Benthic and limnetic threespine stickleback (*Gasterosteus aculeatus sp.*) are a classic example of ecological speciation. The two species have evolved in sympatry in five lakes in coastal British Columbia (Schluter and McPhail, 1992). The species differ in many morphological and behavioural traits. Relative to benthics, limnetics have longer spines and more lateral plates (McPhail 1984; Vamosi 2002). Nesting males show habitat isolation

[1] A version of this chapter has been published: Miller SE, Samuk KM, Rennison D. (2016). An experimental test of the effect of predation upon behaviour and trait correlations in threespine stickleback. *Biological Journal of the Linnean Society*

(Southcott et al. 2013). Limnetics have an increased shoaling preference (Vamosi and Schluter 2002; Wark et al. 2011), and are generally higher in the water column (Larson 1976). In comparison, benthics are more often solitary (Vamosi and Schluter 2002; Odling-Smee et al. 2008; Wark et al. 2011), and prefer to be lower in the water column (Larson 1976). Limnetics primarily eat zooplankton in the open water while benthics consume macroinvertebrates in the littoral zone (Schluter and McPhail 1992). In the open water, limnetics encounter coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) more frequently (Reimchen 1994). Consequently, many of the phenotypic differences between the species are thought to be the result of differential predation on limnetics by trout (Vamosi and Schluter 2002).

Indirect evidence from observational or comparative studies is insufficient to determine if a trait is the target of divergent selection (Schluter 2009). The presence of aquatic predators can co-vary with environmental factors (*e.g*. abiotic conditions, food resources) (Jackson et al. 2001). Controlled experiments manipulating the presence/absence of predators are necessary to confirm that trait shifts are caused by divergent selection from predation. Comparing trait shifts between species is further problematic because species have fixed differences in many traits. As a result, it is difficult to separate the trait(s) that are the target of divergent selection from those traits that are genetically linked but not under direct selection. Predation may also lead to selection for correlations between advantageous combinations of behaviour and defence morphology (Sinervo and Svensson 2002; Murren 2012). Creating advanced generation crosses between species with divergent phenotypes can create trait combinations not normally seen in the

wild. When such crosses are combined with predator exposure, it is possible to test if predation is responsible for changes in traits and trait correlations.

We experimentally tested the hypothesis that differences in behaviour between benthic and limnetic stickleback are the result of divergent selection from coastal cutthroat trout predation. Benthic-limnetic hybrid families were introduced into large, naturalistic experimental ponds in the presence/absence of trout predation. Experimental stickleback reproduced annually in the ponds and underwent two generations of differential selection prior to measurement in behavioural assays. We measured two putative anti-predator behaviours, which have been previously shown to differ between the two species - preferred position in the water column and shoaling preference (Larson 1976; Vamosi 2002; Kozak and Boughman 2008; Wark *et al.* 2011). Behaviours that differ consistently between control and predation ponds can be interpreted to arise in response to trout predation. We then tested for correlations between behaviour and defensive armour, and compared the strength of these correlations between treatments. If trout predation selects for combinations of behaviour and defensive armour, trait correlation will be greater in the predation treatment.

5.2 Methods

5.2.1 Experimental Design

In May 2011, four F1 crosses were made between wild-caught benthic females and limnetic males from Paxton Lake, Texada Island. The F1 crosses were reared in 300L tanks in the laboratory without predators for one year until adulthood. In May 2012, adult stickleback were collected from First Lake, an advanced generation hybrid population. First

Lake is a small shallow lake on Texada Island that was founded in 1981 with Paxton Lake benthic x limnetic F1 stickleback (McPhail 1993). We consider this population to be a single family of ~F29 benthic-limnetic hybrids at the time of sampling. The First Lake population was included in the study because the greater number of recombination events this population has undergone affords us the opportunity to investigate the effect of linkage on adaptation.

In May 2012, the five hybrid families (Four F1s and one First Lake) were introduced in a split plot design to pairs of semi-natural ponds (n=21-31 individuals/pond; 10 ponds total) at the University of British Columbia's experimental pond facilities. Each paired pond contained a single family. Stickleback bred in all experimental ponds creating F2s or ~F30s (First Lake ponds) in the summer of 2012. In the summer of 2013, the F2/F30 stickleback bred to form a F3/F31 generation. All behavioural assays were conducted on adult stickleback from the 2013 (F3/F31) cohort.

The experimental ponds are 25m x 15m with a shallow littoral area and a 6m deep open water region. These ponds contain a natural assemblage of food resources and contain invertebrate and avian predators. For each set of paired ponds, one pond was randomly assigned to a predation treatment and the other pond to a control treatment. Adult coastal cutthroat trout were collected from Placid Lake in the Malcolm Knapp Research Forest located 50 km east of Vancouver, BC. Two trout were added to each predation pond in September 2012. The trout died in the summer of 2013 and were replaced with three new trout in September 2013.

5.2.2 Behavioural Assays

Behavioural assays were conducted from November 8-14, 2013, in tanks adjacent to the experimental ponds. Twelve randomly chosen stickleback were collected from each pond with unbaited minnow traps (n=120 total). Paired ponds were tested sequentially, alternating between treatments. Sticklebacks were transferred in a bucket from the pond to the behavioural assay area for a 15-minute acclimation period prior to the start of the behavioural trials. At that time, each stickleback was placed into an individual mesh basket inside a larger aquarium so that we could follow the behaviour of individuals across assays. Behavioural tests were conducted in the following order: stickleback were tested in the novel tank test, returned to the holding basket for 15 minutes, and then tested in the shoaling assay.

The novel tank diving test measures stickleback movement and position in a new tank. Vertical position in the water column of a tank has been used as a proxy for habitat usage in guppies (*Poecilia*) and stickleback (Larson 1976; Torres-Dowdall et al. 2012; Miller et al. 2015). In zebrafish, anxiety (e.g. following exposure to alarm pheromones) leads to a reduction in exploration and a lower position in a tank (Egan et al. 2009; Cachat et al. 2010; Stewart et al. 2012). During the trial, a focal fish was gently introduced to the top centre of an empty unfamiliar 35.5 cm x 22 cm x 20 cm tank and allowed to move freely for 630 seconds. All assays were recorded with wireless D-Link DCS-930L webcams (DLink Corporation, Taiwan). We excluded the first 30 seconds of each assay as the introduction of a stickleback often resulted in erratic movement (Miller et al. 2015). Videos were subsampled to 0.5 frames per second using VirtualDub software (www.virtualdub.org). The MtrackJ plugin (Meijering et al. 2012) in ImageJ (Schneider et al. 2012) was used to

measure the x and y coordinates of the focal fish every 2 seconds. We calculated the mean vertical position of the focal fish, the latency to enter the upper half of the tank, and the distance that the focal fish travelled during the assay.

The second assay assesses shoaling preference by measuring the time that the focal stickleback spends near a stimulus shoal (Vamosi 2002; Kozak and Boughman 2008; Wark et al. 2011). Assay tanks were 75 cm x 30 cm x 46 cm with two 10 cm end compartments on either side of the tank that were separated from a large centre arena with window screen (Figure 2.4). Ten stimulus stickleback (shoal) were added to one end compartment and two stimulus stickleback (distractor) were added to the other end compartment (Wark et al. 2011). The stimulus sticklebacks were limnetic stickleback from Priest Lake reared at the experimental pond facility. This population was unrelated and unfamiliar to the experimental stickleback and was chosen because individuals have a high shoaling tendency (Wark et al. 2011) and were similar in size to the experimental stickleback. At the start of the shoaling assay, the focal stickleback was gently introduced into the centre arena and was allowed to move for 630 seconds. We measured the x and y coordinates of the focal fish every 2 seconds following the method used in the novel tank test. We used two metrics to assess shoaling behaviour: the mean horizontal position in the tank (shoaling position), and the time that the focal fish spends within one body length of the experimental shoal (shoaling preference).

As a result of camera error, two trials were not analysed. Following Wark et al. (2011), we excluded trials in which the focal fish did not move during the trial (novel tank n=10; shoaling n=12). In total, 110 novel tank trials and 108 shoaling trials were measured.

5.2.3 Armour Traits

Immediately following the shoaling assay, stickleback were euthanized in MS-222 and fixed in 10% formalin. Specimens were later stained with alizarin red to highlight bony structures following established protocols (Peichel et al. 2001). On the left side of each stained specimen we measured the length of length of the first and second dorsal spines, pelvic spine, pelvic girdle, the number of lateral plates and standard length. Specimens lacking an armour component were assigned a value of zero. Lateral plate number and standard length were not significantly correlated. All other armour traits were positively correlated with standard length and were size corrected to the average length (43.82 mm) using the equation $Y_i = X_i - \beta(L_i - \bar{\mathrm{L}})$. Where Y_i is the size-adjusted trait, X_i is the original trait, β is the regression coefficient of the original trait values on standard length, L_i is the standard length of the individual and $\bar{\mathrm{L}}$ is the average length (Vamosi 2002). For second dorsal spine, pond had a significant effect on β and thus this trait was size corrected independently for each pond (pond did not have a significant effect for other traits). A principal component analysis (PCA) of the correlation matrix of size-corrected armour traits was used to visualize the overall defensive armour of each stickleback. The first principal component (PC1) accounted for 40.9% of the variation in stickleback armour and primarily describes the pelvic spine and pelvic girdle (Table 5.1). The second principal component (PC2) accounted for 25.8% of the variation and describes the length of the first and second dorsal spine.

5.2.4 Statistical Analysis

A linear mixed effects model was used to test if performance in behavioural assays differed between treatments and if armour traits affected these behaviours. Principal component score, treatment, and population (Paxton Lake or First Lake) were fixed factors. Pond and family were random factors. Population was not a significant covariate and was dropped from the final model.

All traits were not normally distributed. Therefore, Spearman's rank correlations were used to evaluate the correlations between armour and behavioural measurements. Confidence intervals for trait correlations were calculated by bootstrapping (1000 replicates) with RVAideMemoire (Hervé 2014). For traits with significant correlations, we compared the magnitude of the correlations between treatments using the Wilcoxon signed-rank test on Spearman rank correlations calculated separately for each pond. All statistical analysis were conducted in R (version 3.1) (R Core Team, 2014)

5.3 Results

The presence of trout did not have a measurable effect upon stickleback behaviour (Table 5.2; Figure 5.1). Predation and control ponds did not differ in vertical position in the water column, the latency to enter the upper half of the tank, or distance travelled during the novel tank assay. Fish from all ponds spent more time shoaling than the random expectation, regardless of treatment (one sample t-test: $t=9.29$, $P<0.0001$, $df=10$). In the shoaling assay, we observed a trend of increased time spent with the shoal (shoaling preference) in the control ponds for four of the five families (Treatment: $F_{1,4}=3.24$,

P=0.15), and focal fish from control ponds travelled more during the assay (Figure 5.2; Treatment: $F_{1,4}$ =5.69, P=0.08), although these results were not significant.

We observed variation in armour traits among experimental families (Table 5.3). The first PC differentiated stickleback with robust pelvic armour (limnetic-like) and stickleback with reduced pelvic armour (benthic-like), while PC2 separated individuals with longer dorsal spines (limnetic-like) from those with reduced dorsal spines (benthic-like). Predation and control ponds did not differ in PC1 (Treatment: $F_{1,4}$=0.43, P=0.55), PC2 (Treatment: $F_{1,4}$=2.5, P=0.18), or standard length (Treatment: $F_{1,4}$=0.19, P=0.69).

There was a positive correlation between PC1 score and mean vertical position during the novel tank test (Figure 5.2A; Spearman's rank correlation coefficient, ρ= 0.261, P=0.006, 95% CI: 0.068-0.442). Individuals with increased pelvic armour preferred a higher vertical position in the water column (PC1: $F_{4,97}$=4.10, P=0.045). There was a negative correlation between PC2 and distance travelled during the novel tank test (Figure 5.2C; ρ= -0.260, P=0.006, 95% CI: -0.428 , -0.071). Scores along PC2 and distance travelled during the shoaling assay were not correlated with each other (Table 5.4), but there was a significant Treatment x PC2 interaction ($F_{1,95}$=4.52, P=0.04). One individual had an extreme value for PC2; however, the correlation between these traits remained significant when this point was removed (without point, ρ= -0.245, P=0.01). Behaviour was not correlated with standard length (Table 5.4). All other armour and behaviour correlations were non-significant (Table 5.2, Table 5.4).

Trout predation did not change the strength of the correlations between PC1 and water column position (Figure 5.2B; Wilcoxon signed-rank test, z=9, n=5, P=0.812), or PC2 and distance travelled during the water column assay (Figure 5.2D; z=5, n=5, P=0.625).

5.4 Discussion

Divergent selection from trout predation has been hypothesized to be an important driver of behavioural differences between benthic and limnetic stickleback (Larson 1976; Vamosi 2002; Vamosi and Schluter 2004; Wark *et al.* 2011). To test this hypothesis, we reared families of benthic-limnetic hybrids in natural-like experimental ponds in the presence or absence of trout predation. Contrary to predictions, there was no significant difference in behaviour between predation and control ponds. Instead, armour morphology was a stronger predictor of behaviour than trout predation.

5.4.1 Stickleback Behaviour

Preferred position in the water column did not differ between predation and control ponds. Stickleback in predation ponds had a decreased shoaling preference, but this result was non-significant. If differences in benthic and limnetic behaviour are not caused by divergent selection from trout predation, then behavioural differences may be the result of selection from other factors that differ between the benthic and limnetic habitats. For example, benthics forage for invertebrates in the littoral zone, while limnetics eat zooplankton near the surface of the water (Larson 1976; Odling-Smee et al. 2008). Therefore differences in water column preference may be caused by divergence in diet and/or foraging behaviour between the two species. Similarly, limnetics are frequently observed in large aggregations (Larson 1976) and have a stronger shoaling preference than benthics (Vamosi 2002; Kozak and Boughman 2008; Wark et al. 2011). The differences in shoaling behaviour in the lakes may be due to differences in the structural complexity and

amount of open space between the two environments (Odling-Smee et al. 2008) rather than a consequence of increased trout predation. A shift in resource or habitat use could also have driven changes in shoaling preference. Compared to control ponds, predation ponds had a decrease in population density and a shift in diet towards benthic resources (Rudman et al. 2016). Selection for benthic-like trophic characteristics may have led to a decrease in shoaling preference. Trout predation may have also led to non-consumptive changes in behaviour by reducing competition and increasing intimidation in the open water environment (Preisser et al. 2005). Our findings suggest that differential predation alone is unlikely to explain the differences in shoaling behaviour and water column preference observed in the wild.

The experimental ponds provide an improvement over behavioural studies conducted in mesocosms or in the laboratory because experimental subjects can be manipulated in a natural environment. However, the paired design limited the statistical power of this experiment to detect small differences in behaviour between treatments. Additionally, behaviours were assayed at a single end point; therefore, if paired ponds did not start at the same trait value this would decrease our ability to detect a treatment effect.

5.4.2 Correlations Between Morphology and Behaviour

The likelihood that an individual escapes a predation event may be determined by an interaction between behavioural and morphological traits (e.g. Brodie 1992; Dewitt 1999; Buskirk and McCollum 2000; Relyea 2001). We found a correlation between behavioural traits and bony armour. Armour PC1 (increased pelvic armour) was associated with a higher position in the water column and armour PC2 (longer dorsal spines) was

associated with increased movement during the water column assay. These correlations may be underestimated because behavioural traits have high variance and any measurement error can decrease the correlation between traits (Whitlock and Schluter 2014). As a result, correlations between these traits in the wild are likely greater than reported in this study. Functionally these associations match the greater pelvic armour and preference for a higher water column position found in limnetics (Larson 1976). A previous study by Grand (2000) found that within benthic stickleback that those individuals with reduced pelvic armour were less bold than individuals with increased pelvic armour.

The observed correlations between armour morphology and behaviour could result from genetic linkage or pleiotropy (Schlosser and Wagner 2004). Several inferences can be made regarding the possible genetic basis of the correlations. Recombination events in advanced generation hybrids should uncouple many traits that were genetically linked in limnetics and benthics. Yet three generations of recombination were insufficient to break up the association between armour and behaviour in the F3 families and >30 generations of recombination in First Lake ponds did not decrease the correlation. The maintenance of these correlations in spite of genome-wide recombination indicates that genetic linkage or pleiotropy underlies these associations.

Prior studies in stickleback support a role for linkage or pleiotropy between behaviour and morphology. Lateral plate number and body orientation during schooling have been genetically mapped to the same chromosomal segment (Greenwood et al. 2013). A single gene (*Ectodysplasin*) in this low recombination region has been previously shown to have pleiotropic effects upon lateral plate development, neuromast position, schooling behaviour, and salinity preference (Barrett et al. 2009; Wark and Peichel 2009;

Wark et al. 2012; Mills et al. 2014). A recent study has also uncovered a correlation between anti-predator behaviour and pigmentation in juvenile stickleback (Kim and Velando 2015), suggesting that these correlations may be more widespread then previously appreciated.

When certain trait combinations are preferentially favoured, natural selection may directly or indirectly lead to an increase in the correlation between these traits (Sinervo and Svensson 2002; Murren 2012). While we describe a correlation between multiple armour and behavioural traits, the strength of these correlations did not differ between treatments. Therefore we were unable to support the hypothesis that trout predation is the causal mechanism for the associations. However, the lack of change in correlation between treatments could be a consequence of the limited power of our experiment, or insufficient variation in correlation for selection to act upon. Trout may have also played an important role during the historical divergence between benthic and limnetic stickleback. Therefore, while trout predation may not be the proximate cause for the correlation between defence morphology and behaviour, it cannot be ruled out as the ultimate cause for this association. Future work examining the genetic basis of these traits will be required to elucidate the role of pleiotropy and linkage in behaviour and armour morphology in stickleback.

Figure 5.1 : Mean value for behavioural traits between control and predation ponds presented as reaction norms. The standard error is given for each pond. Each family is represented with a separate colour with the First Lake family given in red.

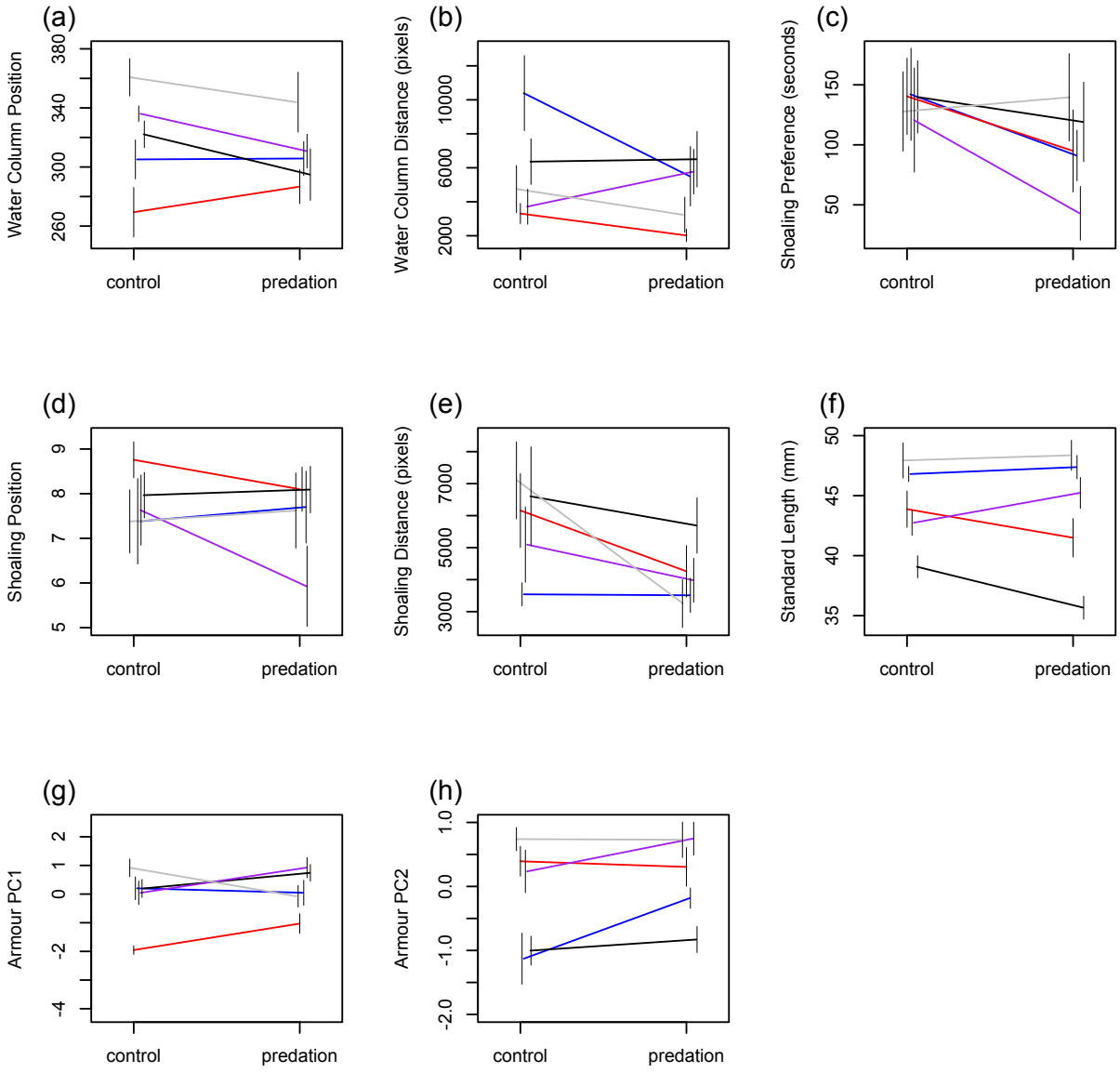


Figure 5.2 : (A) Association between the mean position in the water column and armour PC1 with linear regression line. Trait variation in PC1 (lateral plates and pelvic spines) is shown in red along the x-axis. Each point is an individual from either a predation (filled symbols) or control (open symbols) pond. (B) Spearman's correlation coefficient between armour PC1 and mean vertical position in the water column for each pond. Paired ponds are connected with a line. The F3 families are circles and the family from First Lake is a square. (C) Association between armour PC2 and distance travelled during the water column assay with linear regression line. Trait variation in PC2 (first and second dorsal spines) is shown in red along the x-axis. Individuals from predation ponds are indicated with filled symbols and individuals from control ponds are shown with open symbols. (D) Spearman's correlation coefficient between armour PC2 and distance in the water column assay. Paired ponds are connected with a line. The F3 families are circles and the First Lake family is a square.

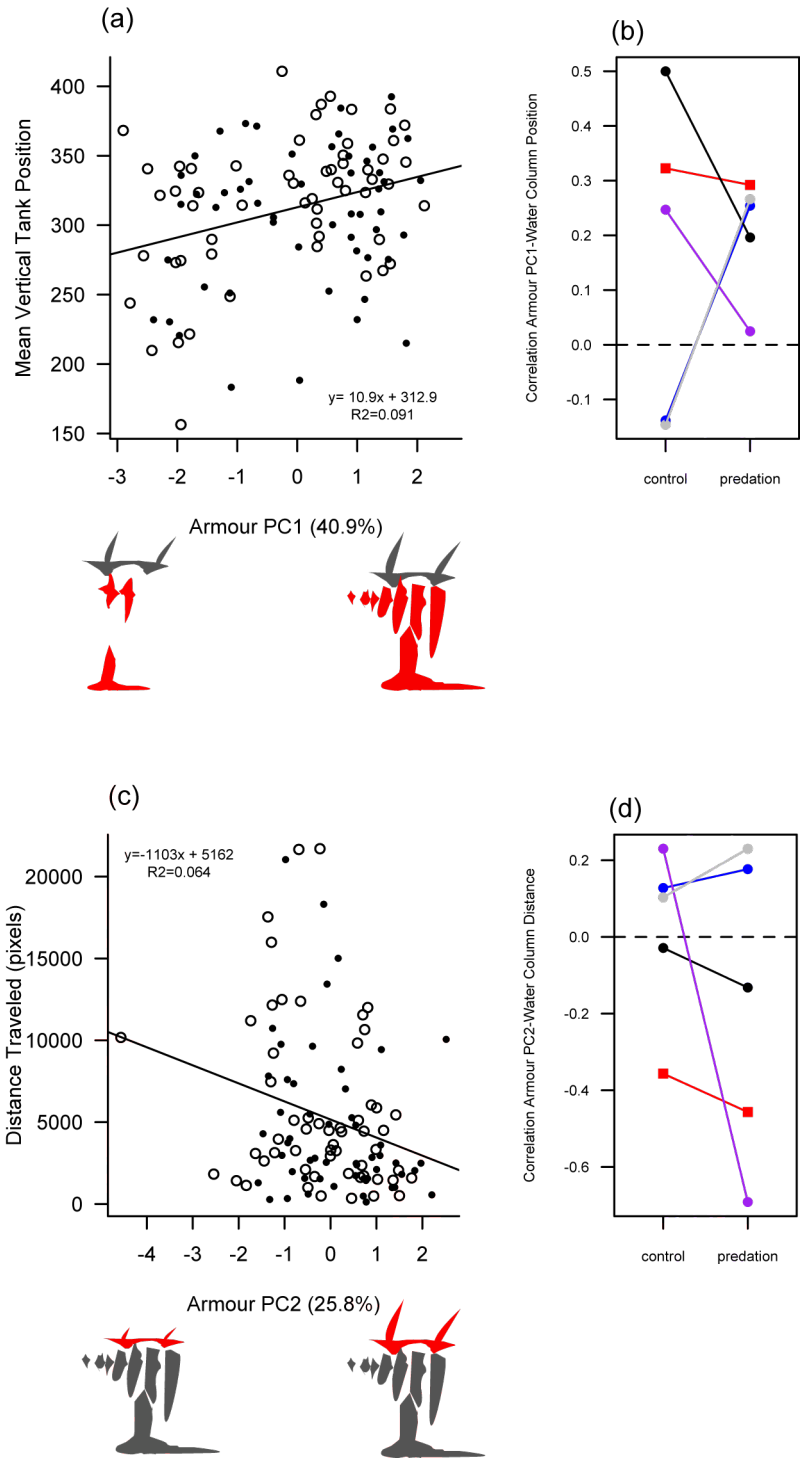

(a)
Mean Vertical Tank Position
400
350
300
250
200
150
y= 10.9x + 312.9
R2=0.091
-3
-2
-1
0
1
2
Armour PC1 (40.9%)
(b)
Correlation Armour PC1-Water Column Position
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
control
predation
(c)
Distance Traveled (pixels)
20000
15000
10000
5000
0
y=-1103x + 5162
R2=0.064
-4
-3
-2
-1
0
1
2
Armour PC2 (25.8%)
(d)
Correlation Armour PC2-Water Column Distance
0.2
0.0
-0.2
-0.4
-0.6
control
predation

Table 5.1 Trait Loadings from the principal component analysis (PCA).

Trait	PC1	PC2
Dorsal Spine 1	0.111	0.716
Dorsal Spine 2	0.195	0.659
Pelvic Spine	0.641	-0.114
Pelvic Girdle	0.632	-0.196
Lateral Plates	0.372	-0.031

Table 5.2 Results of the linear mixed effects model of behavioural traits, armour principal component score, and treatment. The 95% confidence intervals are given for each effect. Significant associations are in bold.

	Treatment				**PC1**				**Treatment x PC1**			
Novel Tank Test	df	F	P	95% CI	df	F	P	95% CI	df	F	P	95% CI
Mean Vertical Position	1,4	1.42	0.30	-29.4, 4.7	1,97	4.10	**0.05**	-0.8, 17.3	1,97	0.14	0.71	-15.0, 10.2
Latency to upper tank	1,4	1.12	0.35	-37.3, 77.5	1,97	0.17	0.68	-6.5, 35.6	1,97	2.69	0.10	-55.1, 5.2
Distance traveled	1,4	0.98	0.38	-4026, 1808	1,97	0.78	0.38	-886, 1027	1,97	0.61	0.44	-820, 1888
Shoaling Assay												
Mean horizontal position	1,4	0.71	0.45	-1.5, 0.77	1,95	0.04	0.83	-0.5, 0.4	1,95	0.03	0.86	-0.7, 0.6
Shoaling preference	1,4	3.24	0.15	-84.0, 11.9	1,95	0.24	0.62	-23.7, 16	1,95	0.00	0.98	-28.9, 29.7
Distance traveled	1,4	5.69	0.08	-3266, 252	1,95	0.00	0.94	-784, 511	1,95	0.52	0.47	-571, 1226

	Treatment				**PC2**				**Treatment x PC2**			
Novel Tank Test	df	F	P	95% CI	df	F	P	95% CI	df	F	P	95% CI
Mean Vertical Position	1,4	1.33	0.31	-34.2, 15.7	1,97	0.49	0.49	-16.4, 6.0	1,97	0.38	0.54	-11.0, 20.8
Latency to upper tank	1,4	0.85	0.41	-44.8, 86.6	1,97	0.02	0.88	-39.6, 12.5	1,97	3.6	0.06	-1.9, 75.6
Distance traveled	1,4	0.90	0.40	-1838, 1585	1,97	1.69	0.20	-1696, 609	1,97	0.02	0.88	-1838, 1585
Shoaling Assay												
Mean horizontal position	1,4	0.75	0.44	-1.2, 0.6	1,95	1.78	0.19	-0.6, 0.5	1,95	2.3	0.13	-1.4, 0.2
Shoaling preference	1,4	3.27	0.15	-82.7, 12.8	1,95	0.81	0.37	-38.0, 11.1	1,95	0.39	0.54	-26.1, 49.9
Distance traveled	1,4	5.88	0.07	-3269, 189	1,95	0.09	0.77	-176, 1378	1,95	4.52	**0.04**	-2380, -82

Table 5.3 Measured trait values for each pond. First dorsal spine (DS1), second dorsal spine (DS2), pelvic spine (PS), and pelvic girdle (PG) are size corrected. Stickleback are hybrids between benthics and limentics from Paxton Lake. The population from Paxton Lake are from the F3 generation and the population from First Lake are from the F31 generation. The mean and standard error are given for each trait.

Population	Family	Treatment	N	DS 1 (mm)	DS 2 (mm)	PS (mm)	PG (mm)	LP	Length (mm)	Armour PC1	Armour PC2
Paxton	D1	control	12	1.8 ± 0.3	2.2 ± 0.3	2.3 ± 0.4	5.3 ± 0.8	3.9 ± 0.3	46.8 ± 0.6	0.20 ± 0.4	-1.13 ± 0.4
Paxton	D1	predation	11	1.7 ± 0.3	3.0 ± 0.1	2.0 ± 0.5	4.6 ± 0.9	3.5 ± 0.3	47.4 ± 1.0	0.05 ± 0.44	-0.18 ± 0.16
First Lake	D2-1	control	12	1.9 ± 0.3	3.1 ± 0.1	0.2 ± 0.2	0.7 ± 0.3	1.8 ± 0.4	43.9 ± 1.5	-1.95 ± 0.15	0.39 ± 0.23
First Lake	D2-1	predation	12	1.6 ± 0.4	3.2 ± 0.1	1.0 ± 0.5	2.5 ± 0.8	2.3 ± 0.4	41.5 ± 1.6	-1.03 ± 0.34	0.30 ± 0.30
Paxton	D2-2	control	12	0.6 ± 0.3	3.0 ± 0.1	2.2 ± 0.4	4.9 ± 0.8	3.8 ± 0.2	39.1 ± 0.9	0.20 ± 0.31	-1.00 ± 0.23
Paxton	D2-2	predation	12	0.5 ± 0.3	2.9 ± 0.3	3.0 ± 0.3	5.6 ± 0.5	3.8 ± 0.2	35.7 ± 1.0	0.74 ± 0.29	-0.83 ± 0.20
Paxton	D3-1	control	11	2.1 ± 0.4	3.4 ± 0.1	2.1 ± 0.5	4.6 ± 0.7	3.3 ± 0.4	43.0 ± 1.1	0.04 ± 0.41	0.23 ± 0.33
Paxton	D3-1	predation	12	2.7 ± 0.3	3.4 ± 0.1	2.9 ± 0.5	4.4 ± 0.8	4.6 ± 0.2	45.2 ± 1.3	0.92 ± 0.37	0.75 ± 0.27
Paxton	D3-2	control	12	2.4 ± 0.3	3.2 ± 0.3	2.8 ± 0.4	5.4 ± 0.6	3.8 ± 0.3	47.9 ± 1.5	0.92 ± 0.31	0.74 ± 0.18
Paxton	D3-2	predation	12	2.2 ± 0.3	3.5 ± 0.1	2.0 ± 0.4	4.3 ± 0.8	2.7 ± 0.4	48.4 ± 1.3	-0.08 ± 0.38	0.73 ± 0.28

Table 5.4 Spearman's rank correlations between behavioural traits and morphological traits. Correlations were calculated between behavioural traits, and armour PC1, armour PC2, and standard length. The 95% confidence intervals (95% CI) were calculated for each correlation by bootstrapping. All significant correlations are in bold.

PC1			
	Rho	**P**	**95% CI**
Water column position	0.261	**0.006**	0.068, 0.442
Water column latency	0.213	0.086	-0.032, 0.423
Distance water column assay	0.169	0.078	-0.028, 0.353
Shoaling preference	-0.013	0.890	-0.202, 0.179
Shoaling position	-0.110	0.261	-0.295, 0.085
Distance shoaling	0.011	0.909	-0.167, 0.183

PC2			
	Rho	**P**	**95% CI**
Water column position	-0.104	0.283	-0.090, 0.283
Water column latency	-0.108	0.387	-0.103, 0.334
Distance water column assay	0.260	**0.006**	-0.428, -0.071
Shoaling preference	0.088	0.367	-0.283, 0.127
Shoaling position	0.094	0.333	-0.280, 0.112
Distance shoaling	-0.003	0.978	-0.202, 0.205

Standard Length			
	Rho	**P**	**95% CI**
Water column position	0.147	0.128	-0.051, 0.342
Water column latency	0.131	0.294	-0.090, 0.359
Distance water column assay	0.029	0.764	-0.167, 0.208
Shoaling preference	-0.065	0.506	-0.242, 0.124
Shoaling position	-0.094	0.337	-0.265, 0.090
Distance shoaling	-0.026	0.787	-0.225, 0.148

Chapter 6: Conclusion

6.1 Overview

Organisms experience selection from both the abiotic and the biotic environments. As a result, interactions among species can be a mechanism of evolution by natural selection within a species (Thompson 2013). My dissertation has attempted to quantify the impact that biotic selection from a single species has had on the evolution of another species. I focus primarily on the evolution of trait divergence in the threespine stickleback in response to intraguild predation.

In chapter 2, I established that the presence of an intraguild predator, prickly sculpin, is associated with character shifts in multiple traits in the threespine stickleback. Wild populations of stickleback sympatric with sculpin were previously shown to differ in body shape (Ingram et al. 2012). I demonstrated that compared to stickleback from lakes without sculpin, populations of stickleback from lakes with sculpin show parallel increases in armour morphology and prefer to be higher in the water column in laboratory tests. These differences in armour, shape, and behaviour persisted when stickleback crosses from these populations were raised in a common garden, suggesting that trait differences have a genetic basis. I then examined if experimental exposure to sculpin induced trait changes. I found limited phenotypic plasticity in marine stickleback in response to sculpin exposure, but I did not observe an induced response in the freshwater stickleback. Behavioural and morphological trait differences between freshwater populations with and without sculpin thus have a genetic basis and suggest an evolutionary response to intraguild predation.

These findings are the first confirmed case of genetically based character divergence associated with intraguild predation.

The evolution of phenotypes is tied to the genes that underlie the phenotypes. In chapter 3, I investigated the genomic architecture of divergence between stickleback from lakes with and without sculpin. The effect of biotic selection upon the entire genome of an organism is largely unknown in natural populations. Therefore this study provides the first description of the genome-wide response of a vertebrate species to a single biotic agent in the wild. I used whole genome re-sequencing of eight populations from lakes without sculpin, nine populations from lakes with sculpin, and six marine populations. I found that genetic variation in these populations is strongly associated with the presence or absence of sculpin. Using a genome scan metric modified from Jones et al. (2012a), I identified regions of the genome that have differentiated in parallel between lakes with and without sculpin. I find that intraguild predation is associated with extensive genomic differentiation. Outlier windows between lakes with and without sculpin contain more than 500 genes with diverse functions. Genes identified in outlier windows contain many candidate genes for further study. However, I did not find overrepresentation of any GO terms for genes in outlier regions.

Outlier windows were distributed unevenly across the genome. Some chromosomes had a large number of outlier windows while other chromosomes were underrepresented. The number of genes or windows differentiating populations does not count the number of selective sweeps because a selective sweep will likely encompass multiple outlier windows. I used a hidden Markov model to estimate the location of state shifts between divergent regions and regions having little or no divergence to estimate the number of selective

sweeps. This model collapsed the 1473 outlier windows into 164 distinct outlier regions across the genome. This smaller number of outlier regions still represents a large portion of the genome that is differentiated.

Observing trait differences between populations with and without sculpin is insufficient evidence to conclude that sculpin are the agent of selection leading to the evolution of those traits (Wade and Kalisz 1990). The presence of sculpin could be merely correlated with another mechanism of selection (e.g. decreased time in benthic habitat, see Chapter 4). For example, stickleback from lakes with sculpin have pelvic spines that are on average 2.3mm longer than stickleback from lakes without sculpin (Chapter 2). Longer pelvic spine have been hypothesized to be a defence against fish predators because spines are sharp and increase the effective diameter of the stickleback making it more difficult to ingest (Hoogland et al. 1956; Hagen and Gilbertson 1972). In contrast, shorter pelvic spines may provide a selective advantage against insect predators (Reimchen 1980). In chapter 4, I used a mesocosm experiment to test if prickly sculpin are an agent of selection on stickleback pelvic spine length. Using clippers, I physically shortened stickleback pelvic spines of individuals from two populations of stickleback sympatric with sculpin and compared the mortality rates of stickleback with clipped and unclipped pelvic spines in the presence of sculpin. I observed an increase in the probability of survival of stickleback with unclipped pelvic spines, but this effect had wide confidence intervals and was not statistically significant. From the mesocosm experiment I was unable to conclude whether prickly sculpin preferentially consumed stickleback with shortened pelvic spines.

I combined the results of my mesocosm experiment with other experimental studies of selection on stickleback pelvic morphology from fish or insect predators. I used a meta-

analysis approach to evaluate if fish predators or insect predators were agents of selection on pelvic spine length. From this analysis I concluded that fish predators indeed favoured longer pelvic spines by preferentially eating shorter-spined fish. The summary effect size of fish predators on the length of pelvic spines, 0.13 units of standard deviation, was comparable to other measures of selection in the wild (Kingsolver et al. 2001). While this effect size is considered small to moderate, if consistent over multiple generations, and assuming a heritability of 0.3, it is sufficient to change the mean length of pelvic spines by one standard deviation in less than 100 generations. The summary effect of insect predators on stickleback pelvic spine length had wide confidence intervals and overlapped with zero. Therefore I was unable to rule out selection for increased or decreased pelvic morphology by insect predators. Many experimental measures of selection have small sample sizes with a low number of replicates, resulting in wide confidence intervals for the estimate of selection (Hersch and Phillips 2004). As this study demonstrates, combing results from multiple experimental studies can produced an aggregate estimation of selection that may be more precise than any single study (Hersch and Phillips 2004; MacColl 2011).

In my final chapter, I examined if biotic selection from trout is a mechanism of divergent selection on stickleback behaviour. Benthic and limnetic stickleback occur in sympatry in multiple lakes (Schluter and McPhail, 1992) and have diverged in behavioural traits and quantity of armour (Larson, 1976; Vamosi and Schluter, 2002; Wark *et al.*, 2011). Behavioural divergence between these stickleback species has been hypothesized to be the result of divergent selection driven in part by increased predation from coastal cutthroat trout on limnetics in the open water. However, differences in behavioural traits between

benthic and limnetic stickleback could be the consequence of another mechanism of selection. For example, divergence in diet between the species may lead to different foraging strategies and consequently result in differences in preference in the water column. In a selection experiment, I tested if the presence or absence of coastal cutthroat trout predation was correlated with differences in behaviour. Split families of benthic-limnetic F1 hybrids were reared for three generations in large experimental ponds in the presence or absence of trout predation. I compared shoaling preference and preferred position in the water column of stickleback taken from the control and predation treatment ponds. Stickleback behaviour did not differ appreciably among treatments and estimates of the effect of trout on behavioural traits had wide confidence intervals. Therefore it is possible that either trout are not an agent of selection on benthic and limnetic behavioural differences, or that the study lacked the power to detect differences in behaviour between treatments.

6.2 Broader Implications

The work described in my thesis has several implications. First, intraguild predation is an important mechanism of divergence. Intraguild predation is a taxonomically widespread species interaction (Polis et al. 1989; Arim and Marquet, 2004). Furthermore, intraguild predation may be more common then previously reported, as many examples of character shifts currently attributed to competition may instead be the result of intraguild predation. For example, a decrease in range overlap between large mammalian carnivores with similar carnassial tooth length is frequently cited as an example of character displacement (Davies et al. 2007). However, interspecific killings occur more often between

carnivore species with similar diets (Donadio and Buskirk 2006) and in regions with the most resources (Vanak et al. 2013). As a consequence, shifting to an alternative habitat decreases competition and predation (Donadio and Buskirk 2006) and may be an adaptive response to either (although see Pfennig and Pfennig 2010).

Phenotypic plasticity in the ancestral marine colonizing population may have aided in the initial adaptation to freshwater populations (Wund et al. 2008), but subsequent trait divergence among lakes with and without sculpin is due to genetic differences. Phenotypic differences between populations in different environments can be caused by evolution or by phenotypic plasticity – the ability of a organism to modify its phenotype in response to environmental change (West-Eberhard 2003). In a common garden, I found that the presence of sculpin induced slightly increased armour, an increase in preferred water column height, and a decrease in shoaling behaviour in marine stickleback. Importantly, induced trait changes in the presence of sculpin were in the same direction as the trait shifts among freshwater stickleback populations with and without sculpin. In contrast, I found no evidence for sculpin-induced plasticity in stickleback from freshwater populations reared in the presence of sculpin. Instead, trait differences between stickleback populations from lakes with and without sculpin had a genetic basis. The role of phenotypic plasticity in driving diversification has been the subject of recent debate (Pfennig et al. 2010). The advantage of phenotypic plasticity, particularly in the case of rapid evolution, is that upon exposure to a novel environment, phenotypes can arise quickly without the need for the mutation and spread of adaptive alleles (West-Eberhard 2003). However, phenotypic plasticity can also be costly to maintain (Dewitt et al. 1998). For trait divergence among

stickleback populations with and without sculpin, the role of phenotypic plasticity was likely limited.

My study suggests that the addition or removal of a single biotic agent can have a profound effect on genomic divergence in stickleback. More than 500 genes were differentiated between lakes with and without sculpin. Furthermore, the number of differentiated genes is likely an underestimate as my methodology only identifies genes in outlier regions that have diverged in parallel between lakes with and without sculpin. Intraguild predation was predicted to cause evolution of the genes for foraging and anti-predatory defence traits. I identified genes in outlier windows that are potential candidates for these traits. In addition, outlier windows contain genes for other phenotypes that may differ between stickleback from lakes with and without sculpin. These phenotypes, such as differences in brain development or immunity, would not have been easily detected by traditional morphological studies. Together, the presence or absence of sculpin has an effect on numerous genes and phenotypes.

It will be necessary to examine the genome-wide response to biotic selection in other organisms to determine if these findings are typical. However, there are methodological constraints that researchers should consider when designing these studies. Previous studies of the genetic basis of selection have relied on a candidate gene approach in which the researcher selects genes to study *a priori* (Nadeau and Jiggins 2010) or reduced representation genome scans (e.g. Hohenlohe et al. 2010). These approaches have severe limitations. Candidate gene studies are constrained by both the number of divergent phenotypes identified as well as the researcher's ability to identify putative candidate genes for those phenotypes. These approaches do not adequately address

quantitative or polygenic traits (Akey 2009). Additionally, reduced representation genome scans are unable to detect selection on genes that are not linked to markers. Therefore, by not examining the entire genome, these methods will fail to detect many genes thereby underestimating the number of genes under selection. An understanding of the genomic consequences of biotic selection will require more whole genome sequencing studies.

These findings have ramifications for the role of biotic interactions in the evolution of species. While comparisons are preliminary, more genes may be under selection from biotic interactions than from abiotic agents of selection. When a species adapts to another species, they are co-evolving with each other (Thompson 2013). Therefore adaptation to an abiotic agent is likely to be a stationary adaptive peak, but adaptation to another species may involve a constantly moving target. The multifarious selection hypothesis of speciation predicts that speciation is more likely the more traits that are under selection (Nosil et al. 2009). Consequently, biotic interactions may be an important mechanism for speciation.

References

Akey, J. M. 2009. Constructing genomic maps of positive selection in humans: Where do we go from here? Genome Research 19:711–722.

Amarasekare, P. 2008. Coexistence of intraguild predators and prey in resource-rich environments. Ecology 89:2786–2797.

Andrews, K. R., J. M. Good, M. R. Miller, G. Luikart, and P. A. Hohenlohe. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics 17:81–92.

Arim, M., and P. A. Marquet. 2004. Intraguild predation: a widespread interaction related to species biology. Ecology Letters 7:557–564.

Baker, J. A., M. A. Wund, R. Y. Chock, L. Ackein, R. Elsemore, and S. A. Foster. 2010. Predation history and vulnerability: Conservation of the stickleback adaptive radiation. Biology Conservation 143:1184–1192.

Barrett, R. D. H., and D. Schluter. 2008. Adaptation from standing genetic variation. Trends in Ecology & Evolution 23:38–44.

Barrett, R. D. H., S. M. Rogers, and D. Schluter. 2008. Natural selection on a major armor gene in threespine stickleback. Science 322:255–257.

Barrett, R.D.H., T.H. Vines, J.S. Bystriansky, and P.M. Schulte. 2009. Should I stay or should I go? The Ectodysplasin locus is associated with behavioural differences in threespine stickleback. Biology Letters 5:788–791.

Barrueto, M. 2009. Adaptive significance of pelvic girdle loss in threespine stickleback. (M.S. Thesis). University of British Columbia, Vancouver, BC.

Bell, M. A. 1988. Stickleback fishes: Bridging the gap between population biology and paleobiology. Trends in Ecology & Evolution 3:320–324.

Bell, A. M., and A. Sih. 2007. Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). Ecology Letters 10:828–834.

Bell, M. A. 2001. Lateral plate evolution in the threespine stickleback: getting nowhere fast. Genetica 112:445–461.

Bell, M. A., and S. A. Foster, eds. 1994. The Evolutionary biology of the threespine stickleback. Oxford University Press, Oxford, UK.

Bell, M. A., G. Orti, J. A. Walker, and J. P. Koenings. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. Evolution 47:906–914.

Bertram, B. C. R. 1978. Living in groups: predators and prey. Pp 64-96 in J.R. Krebs & N.B. Davies, eds. Behavioral ecology. Blackwell, Oxford.

Berriz, G. F., J. E. Beaver, C. Cenik, M. Tasan, and F. P. Roth. 2009. Next generation software for functional trend analysis. Bioinformatics 25:3043–3044.

Bjaerke, O., K. Ostbye, H. M. Lampe, and L. A. Vøllestad. 2010. Covariation in shape and foraging behaviour in lateral plate morphs in the three-spined stickleback. Ecology of Freshwater Fish 19:249–256.

Bonhomme, M., S. Boitard, H. San Clemente, B. Dumas, N. Young, and C. Jacquet. 2015. Genomic Signature of Selective Sweeps Illuminates Adaptation of Medicago truncatulato Root-Associated Microorganisms. Molecular Biology and Evolution 32:2097–2110.

Brodie, E.D. III. 1992. Correlational selection for color pattern and antipredator behavior in the garter snake Thamnophis ordinoides. Evolution 1284–1298.

Buskirk, V. 2000. Functional mechanisms of an inducible defence in tadpoles: morphology and behaviour influence mortality risk from predation. Journal of Evolutionary Biology 13:336–347.

Cachat, J., A. Stewart, L. Grossman, S. Gaikwad, F. Kadri, K. M. Chung, N. Wu, K. Wong, S. Roy, C. Suciu, J. Goodspeed, M. Elegante, B. Bartels, S. Elkhayat, D. Tien, J. Tan, A. Denmark, T. Gilder, E. Kyzar, J. DiLeo, K. Frank, K. Chang, E. Utterback, P. Hart, and A. V. Kalueff. 2010. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nature Protocols 5:1786–1799.

Camarata, T., D. Snyder, T. Schwend, J. Klosowiak, B. Holtrup, and H.-G. Simon. 2010. Pdlim7 is required for maintenance of the mesenchymal/epidermal Fgf signaling feedback loop during zebrafish pectoral fin development. BMC Developmental Biology 2010 10:104.

Chan, Y. F., M. E. Marks, F. C. Jones, G. Villarreal, M. D. Shapiro, S. D. Brady, A. M. Southwick, D. M. Absher, J. Grimwood, J. Schmutz, R. M. Myers, D. Petrov, B. Jónsson, D. Schluter, M. A. Bell, and D. M. Kingsley. 2010. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. Science 327:302–305.

Colosimo, P. F., K. E. Hosemann, S. Balabhadra, G. Villarreal, M. Dickson, J. Grimwood, J. Schmutz, R. M. Myers, D. Schluter, and D. M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles. Science 307:1928–1933.

Cortez, M. H. 2011. Comparing the qualitatively different effects rapidly evolving and rapidly induced defences have on predator-prey interactions. Ecology Letters 14:202–209.

Cruickshank, T. E., and M. W. Hahn. 2014. Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. Molecular Ecology 23:3133–3157.

Danecek, P., A. Auton, G. R. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, R. Durbin, 1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. Bioinformatics 27:2156–2158.

Daugherty, M. P., J. P. Harmon, and C. J. Briggs. 2007. Trophic supplements to intraguild predation. Oikos 116:662–677.

Davies, T., S. Meiri, T. G. Barraclough, and J. L. Gittleman. 2007. Species co-existence and character divergence across carnivores. Ecology Letters 10:146–152.

Day, T., and J. D. McPhail. 1996. The effect of behavioral and morphological plasticity on foraging efficiency in the threespine stickleback (*Gasterosteus sp*). Oecologia 108:380–388.

Day, T., J. Pritchard, and D. Schluter. 1994. Ecology and genetics of phenotypic plasticity: A comparison of two sticklebacks. Evolution 48:1723–1734.

Delmore, K. E., S. Hübner, N. C. Kane, R. Schuster, R. L. Andrew, F. Câmara, R. Guigó, and D. E. Irwin. 2015. Genomic analysis of a migratory divide reveals candidate genes for migration and implicates selective sweeps in generating islands of differentiation. Molecular Ecology 24:1873–1888.

Dennenmoser, S., A. W. Nolte, S. Vamosi, and S. M. Rogers. 2015. Phylogeography of the prickly sculpin (Cottus asper) in north-western North America reveals parallel phenotypic evolution across multiple coastal-inland colonizations. Journal of Biogeograpy 42:1626-1638.

DePristo, M. A., E. Banks, R. Poplin, K. V. Garimella, J. R. Maguire, C. Hartl, A. A. Philippakis, G. del Angel, M. A. Rivas, M. Hanna, A. McKenna, T. J. Fennell, A. M. Kernytsky, A. Y. Sivachenko, K. Cibulskis, S. B. Gabriel, D. Altshuler, and M. J. Daly. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nature Genetics 43:491–498.

Dewitt, T. J., A. Sih, and D. S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution 13:77–81.

Dewitt, T., A. Sih, and J. Hucko. 1999. Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. Animal Behaviour 58: 397–407.

Diabaté, A., R.K. Dabiré, K. Heidenberger, J. Crawford, W.O. Lamp, L.E. Culler, and T. Lehmann. 2008. Evidence for divergent selection between the molecular forms of Anopheles gambiae: role of predation. BMC Evolutionary Biology 8:5.

Dingemanse, N. J., F. Van der Plas, J. Wright, D. Réale, M. Schrama, D. A. Roff, E. Van der Zee, and I. Barber. 2009. Individual experience and evolutionary history of predation affect expression of heritable variation in fish personality and morphology. Proceedings of the Royal Society of London B 276:1285–1293.

Dingemanse, N. J., J. Wright, A. J. N. Kazem, D. K. Thomas, R. Hickling, and N. Dawnay. 2007. Behavioral syndromes differ predictably between 12 populations of three-spined stickleback. Journal of Animal Ecology 76:1128–1138.

Donadio, E., and S. W. Buskirk. 2006. Diet, Morphology, and Interspecific killing in carnivora. American Naturalist 167:524–536.

Dryden, I.L. 2012. Package 'shapes': Statistical Shape Analysis, v.1.1-3 Available at http://CRAN.R-project.org/package=shapes. (CRAN: Comprehensive R Archive Network, 2009).

Durinck, S., P. T. Spellman, E. Birney, and W. Huber. 2009. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. Nature Protocols 4:1184–1191.

Egan, R. J., C. L. Bergner, P. C. Hart, J. M. Cachat, P. R. Canavello, M. F. Elegante, S. I. Elkhayat, B. K. Bartels, A. K. Tien, D. H. Tien, S. Mohnot, E. Beeson, E. Glasgow, H. Amri, Z. Zukowska, and A. V. Kalueff. 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behavioural Brain Research 205:38–44.

Elmer, K. R., and A. Meyer. 2011. Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends in Ecology & Evolution 26:298–306.

Endler, J. A. 1980. Natural selection on color patterns in Poecilia reticulata. Evolution 34:76–91.

Endler, J. A. 1986. Natural Selection in the Wild. Princeton University Press.

Excoffier, L., and N. Ray. 2008. Surfing during population expansions promotes genetic revolutions and structuration. Trends in Ecology & Evolution 23:347–351.

Fish, J. L., B. Villmoare, K. Köbernick, C. Compagnucci, O. Britanova, V. Tarabykin, and M. J. Depew. 2011. Satb2, modularity, and the evolvability of the vertebrate jaw. Evolution and Development 13:549–564.

Garduño-Paz, M. V., S. Couderc, and C. E. Adams. 2010. Habitat complexity modulates phenotype expression through developmental plasticity in the threespine stickleback. Biological Journal of the Linnean Society 100:407–413.

Giovannone, B., E. Lee, L. Laviola, F. Giorgino, K. A. Cleveland, and R. J. Smith. 2003. Two Novel Proteins That Are Linked to Insulin-like Growth Factor (IGF-I) Receptors by the Grb10

Adapter and Modulate IGF-I Signaling. Journal of Biological Chemistry 278:31564–31573.

Golub, J. L. 2013. Embryonic learning in threespine stickleback (*Gasterosteus aculeatus*) (Ph.D. Thesis). Clark University, Worcester, MA.

Gou, X., Wang, Z., Li, N., Qiu, F., Xu, Z., Yan, D., Yang, S., Jia, J., Kong, X., Wei, Z. and S. Lu 2014. Whole-genome sequencing of six dog breeds from continuous altitudes reveals adaptation to high-altitude hypoxia. Genome research 24:1308-1315.

Gow, J.L., S.M. Rogers, M. Jackson, and D. Schluter. 2008. Ecological predictions lead to the discovery of a benthic-limnetic sympatric species pair of threespine stickleback in Little Quarry Lake, British Columbia. Canadian Journal of Zoology 86:564-571.

Grand, T.C. 2000. Risk-Taking by Threespine Stickleback (*Gasterosteus aculeatus*) Pelvic Phenotypes: Does Morphology Predict Behaviour? Behaviour 137:889–906.

Greenwood, A.K., A.R. Wark, K. Yoshida, and C.L. Peichel. 2013. Genetic and Neural Modularity Underlie the Evolution of Schooling Behavior in Threespine Sticklebacks. Current Biology 23:1884–1888.

Hagen, D. W. 1973. Inheritance of numbers of lateral plates and gill rakers in Gasterosteus aculeatus. Heredity 30:303–312.

Hagen, D. W., and L. G. Gilbertson. 1972. Geographic variation and environmental selection in *Gasterosteus aculeatus L.* in the Pacific Northwest, America. Evolution 26:32–51.

Hamblin, M. T., and A. Di Rienzo. 2000. Detection of the Signature of Natural Selection in Humans: Evidence from the Duffy Blood Group Locus. The American Journal of Human Genetics 66:1669-1679.

Hart, D. R. 2002. Intraguild predation, invertebrate predators, and trophic cascades in lake food webs. Journal of Theoretical Biology 218:111–128.

Hasselblad, V., and L. V. Hedges. 1995. Meta-analysis of screening and diagnostic tests. Psychological Bulletin 117:167–178. American Psychological Association.

Hartl, Daniel L., Andrew G. Clark, and A. G. Clark. 1997. Principles of population genetics. Vol. 116. Sunderland: Sinauer associates.

Harvell, C. D. 1990. The ecology and evolution of inducible defenses. Quarterly Review of Biology 65:323–340.

Heithaus, M. R. 2001. Habitat selection by predators and prey in communities with asymmetrical intraguild predation. Oikos 92:542–554.

Hepher, B. 1988. Nutrition of Pond Fishes. Cambridge University Press.

Hersch, E. I., and P.C. Phillips. 2004. Power and potential bias in field studies of natural selection. Evolution 58:479–485.

Hervé, M. 2014. RVAideMemoire: Diverse Basic Statistical and Graphical Functions, Version 0.9-52. Available at https://cran.r-project.org/package=RVAideMemoire
Hohenlohe, P. A., S. Bassham, P. D. Etter, N. Stiffler, E. A. Johnson, and W. A. Cresko. 2010. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. PLoS Genet 6:e1000862.

Holt, R. D., and G. A. Polis. 1997. A theoretical framework for intraguild predation. American Naturalist 149:745–764.

Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli, and J. G. Kingsolver. 2001. Strength and tempo of directional selection in the wild. Proceedings of the National Academy of Sciences 98:9157–9160.

Hoogland, R., D. Morris, and N. Tinbergen. 1956. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defence against predators (Perca and Esox). Behavior 10:205–236.

Hudson, P., and J. Greenman. 1998. Competition mediated by parasites: biological and theoretical progress. Trends in Ecology & Evolution 13:387–390.

Ingram, T., R. Svanbäck, N. J. B. Kraft, P. Kratina, L. Southcott, and D. Schluter. 2012. Intraguild predation drives evolutionary niche shift in threespine stickleback. Evolution 66:1819-1832.

Jackson, D. A., P. R. Peres-Neto, and J.D. Olden. 2001. What controls who is where in freshwater fish communities - the roles of biotic, abiotic, and spatial factors. Canadian Journal of Fisheries and Aquatic Sciences 58:157-170.

Janssen, A., M. W. Sabelis, S. Magalhães, M. Montserrat, and T. van der Hammen. 2007. Habitat structure affects intraguild predation. Ecology 88:2713-2719.

Jiggins, C.D., R.E. Naisbit, R.L. Coe, and J. Mallet. 2001. Reproductive isolation caused by colour pattern mimicry. Nature 411:302–305.

Jones, F. C., M. G. Grabherr, Y. F. Chan, P. Russell, E. Mauceli, J. Johnson, R. Swofford, M. Pirun, M. C. Zody, S. White, E. Birney, S. Searle, J. Schmutz, J. Grimwood, M. C. Dickson, R. M. Myers, C. T. Miller, B. R. Summers, A. K. Knecht, S. D. Brady, H. Zhang, A. A. Pollen, T. Howes, C. Amemiya, J. Baldwin, T. Bloom, D. B. Jaffe, R. Nicol, J. Wilkinson, E. S. Lander, F. Di Palma, K. Lindblad-Toh, and D. M. Kingsley. 2012a. The genomic basis of adaptive evolution in threespine sticklebacks. Nature 484:55–61.

Jones, F. C., Y. F. Chan, J. Schmutz, J. Grimwood, S. D. Brady, A. M. Southwick, D. M. Absher, R. M. Myers, T. E. Reimchen, B. E. Deagle, D. Schluter, and D. M. Kingsley. 2012b. A Genome-wide SNP Genotyping Array Reveals Patterns of Global and Repeated Species-Pair Divergence in Sticklebacks. Current Biology. 22:83–90.

Kaeuffer, R., Peichel, C. L., Bolnick, D. I., and A.P. Hendry. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. Evolution 66:402-418.

Kelley, J. L., & Magurran, A. E. 2003. Learned predator recognition and antipredator responses in fishes. Fish & Fisheries 4:216-226.

Kim, S.Y., and A. Velando. 2015. Phenotypic integration between antipredator behavior and camouflage pattern in juvenile sticklebacks. Evolution 69:830–838.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. American Naturalist 157:245–261.

Klepaker, T., K. Østbye, and M. A. Bell. 2013. Regressive evolution of the pelvic complex in stickleback fishes: a study of convergent evolution. Evolutionary Ecology Research 15:413–435.

Klopfstein, S. 2005. The Fate of Mutations Surfing on the Wave of a Range Expansion. Molecular Biology and Evolution 23:482–490.

Kofler, R., and C. Schlotterer. 2012. Gowinda: unbiased analysis of gene set enrichment for genome-wide association studies. Bioinformatics 28:2084–2085.

Kozak, G. M., and J. W. Boughman. 2008. Experience influences shoal member preference in a species pair of sticklebacks. Behavioral Ecology 19:667–676.

Kozak, G.M., and J. W. Boughman. 2012. Plastic responses to parents and predators lead to divergent shoaling behaviour in sticklebacks. Journal of Evolutionary Biology 25:759–769.

Kratina, P., E. Hammill, and B. R. Anholt. 2010. Stronger inducible defences enhance persistence of intraguild prey. Journal of Animal Ecology 79:993–999.

Lacasse, J., and N. Aubin-Horth. 2012. A test of the coupling of predator defense morphology and behavior variation in two threespine stickleback populations. Current Zoology 58:53–65.

Lahti, D. C., N. A. Johnson, B. C. Ajie, S. P. Otto, A. P. Hendry, D. T. Blumstein, R. G. Coss, K. Donohue, and S. A. Foster. 2009. Relaxed selection in the wild. Trends in Ecology & Evolution 24:487–496.

Laine, A. L. 2009. Role of coevolution in generating biological diversity: spatially divergent selection trajectories. Journal of Experimental Botany 60:2957–2970.

Langerhans, R. B., M. E. Gifford, and E. O. Joseph. 2007. Ecological speciation in Gambusia fishes. Evolution 61:2056-2074.

Larson, G. L. 1976. Social behavior and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake. Canadian Journal of Zoology 54:107-121.

Lawniczak, M. K. N., S. J. Emrich, A. K. Holloway, A. P. Regier, M. Olson, B. White, S. Redmond, L. Fulton, E. Appelbaum, J. Godfrey, C. Farmer, A. Chinwalla, S.-P. Yang, P. Minx, J. Nelson, K. Kyung, B. P. Walenz, E. Garcia-Hernandez, M. Aguiar, L. D. Viswanathan, Y.-H. Rogers, R. L. Strausberg, C. A. Saski, D. Lawson, F. H. Collins, F. C. Kafatos, G. K. Christophides, S. W. Clifton, E. F. Kirkness, and N. J. Besansky. 2010. Widespread divergence between incipient Anopheles gambiae species revealed by whole genome sequences. Science 330:512–514.

Leinonen, T., G. Herczeg, J. M. Cano, and J. Merilä. 2011. Predation-imposed selection on threespine stickleback (*Gasterosteus aculeatus*) morphology: a test of the refuge use hypothesis. Evolution 65:2916–2926.

Lescak, E. A., and F. von Hippel. 2011. Selective predation of threespine stickleback by rainbow trout. Ecology of Freshwater Fish 20:308–314.

Lescak, E. A., F. von Hippel, B. K. Lohman, and M. L. Sherbick. 2012. Predation of threespine stickleback by dragonfly naiads. Ecology of Freshwater Fish 21:581–587.

Lescak, E. A., F. von Hippel, R. R. Bernhardt, and M. A. Bell. 2013. Pelvic girdle reduction and asymmetry in threespine stickleback from Wallace Lake, Alaska. Evolutionary Ecology Research 15:155–170.

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760.

Linnen, C. R., E. P. Kingsley, J. D. Jensen, and H. E. Hoekstra. 2009. On the origin and spread of an adaptive allele in deer mice. Science 325:1095–1098.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68:619–640.

MacColl, A. D. C. 2011. The ecological causes of evolution. Trends in Ecology & Evolution 26:514–522.

Maccoll, A., and S. M. Chapman. 2011. A benthic predatory fish does not cause selection on

armour traits in three-spined stickleback *Gasterosteus aculeatus* (*Gasterosteiformes*: *Gasterosteidae*). Biological Journal of the Linnean Society 104:877-885.

Marchinko, K. B. 2009. Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. Evolution 63:127–138.

Marchinko, K. B., and D. Schluter. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. Evolution 61:1084–1090.

McPeek, M. A. 1995. Morphological evolution mediated by behavior in the damselflies of two communities. Evolution:749-769.

McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (Gasterosteus): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Canadian Journal of Zoology 62:1402-1408.

McPhail, J.D. 1993. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): origin of the species pairs. Canadian Journal of Zoology 71:515-523.

McPhail, J. D. 2007. The freshwater fishes of British Columbia. Univ of Alberta Press.

Meijering, E., O. Dzyubachyk, and I. Smal. 2012. Methods for cell and particle tracking. Methods in Enzymology 504:183-200.

Mills, M.G, A.K. Greenwood, and C.L. Peichel. 2014. Pleiotropic effects of a single gene on skeletal development and sensory system patterning in sticklebacks. EvoDevo 5: 1-10.

Miller, S.E., D.M. Metcalf, and D. Schluter. Intraguild predation leads to genetically based character shifts in the threespine stickleback. Evolution 69:3194-3203.

Mobley, K. B., Ruiz, R. C., Johansson, F., Englund, G., & Bokma, F. 2013. No evidence that stickleback spines directly increase risk of predation by an invertebrate predator. Evolutionary Ecology Research 15:189-198.

Moodie, G. E. E. 1972. Predation, natural selection and adaptation in an unusual threespine stickleback. Heredity 28:155-167.

Murren, C.J. 2012. The Integrated Phenotype. Integrative and Comparative Biology 52:64–76.

Myhre, F., and T. Klepaker. 2009. Body armour and lateral-plate reduction in freshwater three-spined stickleback *Gasterosteus aculeatus*: adaptations to a different buoyancy regime? Journal of Fish Biology 75:2062-2074.

Nadeau, N. J., and C. D. Jiggins. 2010. A golden age for evolutionary genetics? Genomic studies of adaptation in natural populations. Trends in Genetics 26:484–492.

Nakazawa, T., T. Miki, and T. Namba. 2010. Influence of predator-specific defense adaptation on intraguild predation. Oikos 119:418–427.

Nelson, J. S. 1969. Geographic Variation in the Brook Stickleback, Culaea inconstans, and Notes on Nomenclature and Distribution. Journal of the Fisheries Board of Canada. NRC Research Press Ottawa, Canada.

Nelson, J. S., and F. M. Atton. 1971. Geographic and morphological variation in the presence and absence of the pelvic skeleton in the brook stickleback, Culaea inconstans (Kirtland), in Alberta and Saskatchewan. Canadian Journal of Zoology 49:343-352.

Nosil, P. 2012. Ecological Speciation. Oxford University Press, Oxford, UK.

Nosil, P., and B.J. Crespi. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. Proceedings of the National Academy of Sciences 103:9090–9095.

Nosil, P., D. J. Funk, and D. Ortiz-Barrientos. 2009. Divergent selection and heterogeneous genomic divergence. Molecular Ecology 18:375–402.

Odling-Smee, L.C., J.W. Boughman, and V.A. Braithwaite. 2008. Sympatric species of threespine stickleback differ in their performance in a spatial learning task. Behavioral Ecology and Sociobiology 62:1935–1945.

Peichel, C. L., K. S. Nereng, K. A. Ohgi, B. L. E. Cole, P. F. Colosimo, C. A. Buerkle, D. Schluter, and D. M. Kingsley. 2001. The genetic architecture of divergence between threespine stickleback species. Nature 414:901–905.

Pfennig, D. W., and K. S. Pfennig. 2010. Character displacement and the origins of diversity. American Naturalist 176:S26–S44.

Pfennig, D. W., M. A. Wund, E. C. Snell-Rood, T. Cruickshank, C. D. Schlichting, and A. P. Moczek. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends in Ecology and Evolution 25:459–467.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology & Systematics 20:297–330.

Preisser, E.L., D.I. Bolnick, and M.F. Benard. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86:501–509.

Purcell, S., B. Neale, K. Todd-Brown, and L. Thomas. 2007. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. The American Journal of Human Genetics 81:559-575.

Reimchen, T. 1994. Predators and morphological evolution in threespine stickleback. Pp 240-276 in M.A. Bell and S.A. Foster, eds. The evolutionary biology of the threespine stickleback. Oxford Univ. Press, Oxford, U.K.

R Core Team 2015. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria.

Reimchen, T. E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? Canadian Journal of Zoology 58:1232-1244.

Reimchen, T. E. 1983. Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). Evolution:931–946.

Reimchen, T. 1994. Predators and morphological evolution in threespine stickleback. Pp 240-273 in M.A. Bell and S.A. Foster, eds. The evolutionary biology of the threespine stickleback. Oxford Univ. Press, Oxford, U.K.

Reimchen, T. E. 2000. Predator handling failures of lateral plate morphs in *Gasterosteus aculeatus*: functional implications for the ancestral plate condition. Behaviour 137:1081-1096.

Reimchen, T. E., and P. Nosil. 2002. Temporal variation in divergent selection on spine number in threespine stickleback. Evolution 56:2472–2483.

Reist, J. D. 1980. Selective Predation Upon Pelvic Phenotypes of Brook Stickleback, Culaea inconstans, by Northern Pike, Esox lucius. Canadian Journal of Zoology 58:1245–1252.

Relyea, R.A. 2001. Morphological and Behavioral Plasticity of Larval Anurans in Response to Different Predators. Ecology 82:523–540.

Rennison, D. J., K. Heilbron, R. D. H. Barrett, and D. Schluter. 2015. Discriminating selection on lateral plate phenotype and its underlying gene, Ectodysplasin, in threespine stickleback. American Naturalist 185:150–156.

Rieseberg, L. H., A. Widmer, and A. M. Arntz. 2002. Directional selection is the primary cause of phenotypic diversification. Proceedings of the National Academy of Sciences 99:12242-12245.

Roesti, M., D. Moser and D. Berner. 2013. Recombination in the threespine stickleback genome – patterns and consequences. Molecular Ecology 22:3014-3027.

Rockman, M. V. 2012. The QTN program and the alleles that matter for evolution: all that's gold does not glitter. Evolution 66:1–17.

Rogers, S. M., P. Tamkee, B. Summers, S. Balabahadra, M. Marks, D. M. Kingsley, and D. Schluter. 2012. Genetic signature of adaptive peak shift in threespine stickleback. Evolution 66:2439–2450.

Rohlf, F. 2008. tpsDig2, digitize landmarks and outlines, version 2.12. Department of Ecology and Evolution, State Univer. of New York at Stony Brook.

Rudman S., J. Heavyside, D.J. Rennison, and D. Schluter. 2016. Piscivore addition causes a trophic cascade within and across ecosystem boundaries. Oikos. In press.

Rundle, H.D., and P. Nosil. 2005. Ecological speciation. Ecology Letters 8:336-352.

Rundle H.D., S. Vamosi, and D. Schluter. 2003. Experimental test of predation's effect on divergent selection during character displacement in sticklebacks. Proceedings of the National Academy of Sciences 100:14943–14948.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology & Systematics 24:35–68.

Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer Associates Incorporated.

Schluter, D. 2000a. Ecological Character Displacement in Adaptive Radiation. American Naturalist 156:S4–S16.

Schluter, D. 2000b. The ecology of adaptive radiation. Oxford University Press, Oxford, UK.

Schluter, D. 2003. Frequency dependent natural selection during character displacement in sticklebacks. Evolution 57:1142–1150.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. Science 323:737–741.

Schluter, D., and G. L. Conte. 2009. Genetics and ecological speciation. Proceedings of the National Academy of Sciences 106:9955–9962.

Schluter, D., and J. D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140:85–108.

Schlosser, G, and G.P. Wagner. 2004. Modularity in Development and Evolution. Chicago, IL: University of Chicago Press.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature methods 9:671-675.

Schwarzer, G. 2015. meta: General Package for Meta-Analysis. R package version 4.3-1. http://CRAN.R-project.org/package=meta

Shapiro, M. D., B. R. Summers, S. Balabhadra, J. T. Aldenhoven, A. L. Miller, C. B. Cunningham, M. A. Bell, and D. M. Kingsley. 2009. The Genetic Architecture of Skeletal Convergence and Sex Determination in Ninespine Sticklebacks. Current Biology 19:1140–1145.

Shapiro, M. D., M. E. Marks, C. L. Peichel, B. K. Blackman, K. S. Nereng, B. Jónsson, D. Schluter, and D. M. Kingsley. 2004. Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. Nature 428:717–723.

Shikano, T., V. N. Laine, G. Herczeg, J. Vilkki, and J. Merila. 2013. Genetic Architecture of Parallel Pelvic Reduction in Ninespine Sticklebacks. G3 3:1833–1842.

Sinervo, B., and E. Svensson. 2002. Correlational selection and the evolution of genomic architecture. Heredity 89:329–338.

Soria-Carrasco, V., Z. Gompert, A. A. Comeault, T. E. Farkas, T. L. Parchman, J. S. Johnston, C. A. Buerkle, J. L. Feder, J. Bast, T. Schwander, S. P. Egan, B. J. Crespi, and P. Nosil. 2014. Stick Insect Genomes Reveal Natural Selection's Role in Parallel Speciation. Science 344:738–742.

Southcott, L., L. Nagel, T. Hatfield, and D. Schluter. 2013. Weak habitat isolation in a threespine stickleback (*Gasterosteus spp.*) species pair. Biological Journal of the Linnean Society 110:466-476.

Stacklies, W., H. Redestig, M. Scholz, D. Walther, and J. Selbig. 2007. pcaMethods a bioconductor package providing PCA methods for incomplete data. Bioinformatics 23:1164–1167.

Stapley, J., J. Reger, P. G. D. Feulner, C. Smadja, J. Galindo, R. Ekblom, C. Bennison, A. D. Ball, A. P. Beckerman, and J. Slate. 2010. Adaptation genomics: the next generation. Trends in Ecology & Evolution 25:705–712.

Stephan, W. 2016. Signatures of positive selection: from selective sweeps at individual loci to subtle allele frequency changes in polygenic adaptation. Molecular Ecology 25:79–88.

Stewart, A., S. Gaikwad, E. Kyzar, J. Green, A. Roth, and A. V. Kalueff. 2012. Modeling anxiety using adult zebrafish: A conceptual review. Neuropharmacology 62:135–143.

Stuart, Y. E., and J. B. Losos. 2013. Ecological character displacement: glass half full or half empty? Trends in Ecology & Evolution 28:402–408.

Svanbäck, R., and P. Eklöv. 2011. Catch me if you can-predation affects divergence in a polyphenic species. Evolution 65:3515–3526.

Svanbäck, R., and D. Schluter. 2012. Niche specialization influences adaptive phenotypic plasticity in the threespine stickleback. American Naturalist 180:50–59.

Tabachnick, B. G., and L. S. Fidell. 2012. Using Multivariate Statistics. Pearson Higher Ed.

Thompson, J. N. 2013. Relentless Evolution. University of Chicago Press.

Torres-Dowdall, J., C. A. Handelsman, D. N. Reznick, and C. K. Ghalambor. 2012. Local adaptation and the evolution of phenotypic plasticity in Trinidadian guppies (*Poecilia reticulata*). Evolution 66:3432–3443.

Turner, T. L., E. C. Bourne, E. J. Von Wettberg, and T. T. Hu. 2010. Population resequencing reveals local adaptation of Arabidopsis lyrata to serpentine soils. Nature Genetics 42:260-263.

Urbani, P., and R. Ramos-Jiliberto. 2010. Adaptive prey behavior and the dynamics of intraguild predation systems. Ecological Modeling. 221:2628–2633.

Vamosi, S. M. 2002. Predation sharpens the adaptive peaks: survival trade-offs in sympatric sticklebacks. Annales Zoologici Fennici. 39:237–248.

Vamosi, S. 2003. The presence of other fish species affects speciation in threespine sticklebacks. Evolutionary Ecology Research 5:717–730.

Vamosi, S. M., and D. Schluter. 2002. Impacts of trout predation on fitness of sympatric sticklebacks and their hybrids. Proceedings of the Royal Society B: Biological Sciences 269:923–930.

Vamosi, S. M., and D. Schluter. 2004. Character shifts in the defensive armor of sympatric sticklebacks. Evolution 58:376–385.

Van der Auwera, G. A., M. O. Carneiro, C. Hartl, R. Poplin, G. del Angel, A. Levy-Moonshine, T. Jordan, K. Shakir, D. Roazen, J. Thibault, E. Banks, K. V. Garimella, D. Altshuler, S. Gabriel, and M. A. DePristo. 2013. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Current Protocols in Bioinformatics 11-10.

Vanak, A. T., D. Fortin, M. Thaker, M. Ogden, C. Owen, S. Greatwood, and R. Slotow. 2013. Moving to stay in place: behavioral mechanisms for coexistence of African large carnivores. Ecology 94:2619–2631.

Vance-Chalcraft, H. D., J. A. Rosenheim, J. R. Vonesh, C. W. Osenberg, and A. Sih. 2007. The influence of intraguild predation on prey suppression and prey release: a meta-analysis. Ecology 88:2689–2696.

Venables, W. N., and B. D. Ripley. 2010. Modern Applied Statistics with S. Springer Verlag.

Visser, I., and M. Speekenbrink. 2010. depmixS4: an R package for hidden Markov models. Journal of Statistical Software 36:1-21.

Wade, M. J., and S. Kalisz. 1990. The Causes of Natural Selection. Evolution 44:1947.

Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus L. (Gasterosteidae*) body shape. Biological Journal of the Linnean Society 61:3–50.

Walzer, A., and P. Schausberger. 2013. Phenotypic plasticity in anti-intraguild predator strategies: mite larvae adjust their behaviors according to vulnerability and predation risk. Experimental and Applied Acarology 60:95-115.

Wark, A.R., and C.L. Peichel. 2009. Lateral line diversity among ecologically divergent threespine stickleback populations. Journal of Experimental Biology 213: 108–117.

Wark, A.R., M.G. Mills, L.H. Dang, Y.F. Chan, F.C. Jones, S.D. Brady, D.M. Absher, J. Grimwood, J. Schmutz, R.M. Myers, and D.M. Kingsley. 2012. Genetic architecture of variation in the lateral line sensory system of threespine sticklebacks. G3 2:1047–1056.

Wark, A. R., B. J. Wark, T. J. Lageson, and C. L. Peichel. 2011. Novel methods for discriminating behavioral differences between stickleback individuals and populations in a laboratory shoaling assay. Behavioral Ecology and Sociobiology 65:1147–1157.

Weir, B. S., and C. C.Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution:1358-1370.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford University Press.

Willacker, J. J., F. A. von Hippel, P. R. Wilton, and K. M. Walton. 2010. Classification of threespine stickleback along the benthic-limnetic axis. Biological Journal of the Linnean Society 101:595–608.

Wund, M. A., J. A. Baker, B. Clancy, J. L. Golub, and S. A. Foster. 2008. A Test of the "Flexible Stem" Model of Evolution: Ancestral Plasticity, Genetic Accommodation, and Morphological Divergence in the Threespine Stickleback Radiation. American Naturalist 172:449–462.

Yamamichi, M., T. Yoshida, and A. Sasaki. 2011. Comparing the effects of rapid evolution and phenotypic plasticity on predator-prey dynamics. American Naturalist 178:287–304.

Appendices

Appendix A

```
#!/bin/sh

#  thesis_pipeline.sh
#
#
#

lake='lake_name'
project='sculpin'
bwa='/filelocation/bwa-0.7.6a' #version 0.7.6
picard='/filelocation/picard-tools-1.97' #version 1.97
GATK='/filelocation/GATK3' #version 3.2.2
ref='/filelocation/gasAcu1.fa' #reference genome Broad Instituted 2006 Assembly


# find the suffix array (SA) coordinates of good hits of each individual
bwa aln -q20 $ref $lake_R1.fastq > $lake_R1.sai
bwa aln -q20 $ref $lake_R2.fastq > $lake_R2.sai

# read and generate alignments in the SAM format
bwa sampe $ref $lake_R1.sai $lake_R2.sai $lake_R1.fastq $lake_R2.fastq >
    $lake.q20.sam

# add read groups, sort sam file, produce bam and bai files using Picard's
    AddOrReplaceReadGroups
java -Djava.io.tmpdir=/tmp -jar $picard/AddOrReplaceReadGroups.jar RGID=$lake
    RGLB=$lake RGSM=$lake RGPL=ILLUMINA RGPU=$project I=$lake.q20.sam O=$lake.bam
    SORT_ORDER=coordinate CREATE_INDEX=TRUE VALIDATION_STRINGENCY=LENIENT

# create list of targets for realignment;
# intervals file must end in ".intervals" or other approved extension
java -Xmx2g -jar $GATK/GenomeAnalysisTK.jar -I $lake.bam -T
    RealignerTargetCreator -R $ref -o $lake.temp.intervals

# run the realigner using list of realignment targets.
# default LOD is 5.0 but lower recommended for low coverage
java -Xmx4g -Djava.io.tmpdir=/tmp -jar $GATK/GenomeAnalysisTK.jar -I $lake.bam -R
    $ref -T IndelRealigner
-targetIntervals $lake.temp.intervals -o $lake.realigned.bam -LOD 0.4

# mark pcr duplicates
java -Djava.io.tmpdir=/tmp -jar $picard/MarkDuplicates.jar I=$lake.realigned.bam
    O=$lake.realigned.mkdup.bam M=$lake.realigned.markdup.metrics
    REMOVE_DUPLICATES=FALSE VALIDATION_STRINGENCY=LENIENT

# make an index file of marked duplicate bam file
java -Djava.io.tmpdir=/tmp -jar $picard/BuildBamIndex.jar I=
    $lake.realigned.mkdup.bam O=$lake.realigned.mkdup.bai
    VALIDATION_STRINGENCY=LENIENT

# call SNPs with GATK, includes invariant sites
java -jar /Linux/GATK3/GenomeAnalysisTK.jar -R $ref -T UnifiedGenotyper -l INFO -
    I $lake.realigned.mkdup.bam -o $lake.invariant.vcf --output_mode
    EMIT_ALL_CONFIDENT_SITES --genotype_likelihoods_model BOTH
```

Appendix B

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
GRM5	ENSGACG00000007968	groupI	6315506
ctsc	ENSGACG00000007985	groupI	6368016
ZFP36	ENSGACG00000008351	groupI	7086897
	ENSGACG00000008353	groupI	7087527
cpd	ENSGACG00000008673	groupI	7617542
gosr1	ENSGACG00000008685	groupI	7633993
abhd15	ENSGACG00000008738	groupI	7711424
pde3a	ENSGACG00000008742	groupI	7742778
slco1c1	ENSGACG00000008749	groupI	7779850
dus4l	ENSGACG00000008772	groupI	7799466
MNT	ENSGACG00000008790	groupI	7804379
ELN	ENSGACG00000008825	groupI	7895088
phc2b	ENSGACG00000009625	groupI	9505875
ppp1r37	ENSGACG00000009629	groupI	9508614
mrpl28	ENSGACG00000009641	groupI	9544230
relb	ENSGACG00000009656	groupI	9552408
snrpa	ENSGACG00000009838	groupI	9799920
FLJ41131	ENSGACG00000009856	groupI	9803940
itpkcb	ENSGACG00000009859	groupI	9807301
	ENSGACG00000009863	groupI	9816673
gfm1	ENSGACG00000009864	groupI	9818367
neu4	ENSGACG00000009879	groupI	9829619
wdr53	ENSGACG00000009884	groupI	9833731
cux2	ENSGACG00000009915	groupI	9866672
alcam	ENSGACG00000010444	groupI	10881353
igsf9b	ENSGACG00000010671	groupI	11124934
paf1	ENSGACG00000010818	groupI	11597447
TAOK1	ENSGACG00000010833	groupI	11607936
tsr1	ENSGACG00000010843	groupI	11619039
synrg	ENSGACG00000011112	groupI	12185852
DUSP18	ENSGACG00000011118	groupI	12219197
tada2	ENSGACG00000011119	groupI	12229512
nbeab	ENSGACG00000011555	groupI	13479008
TIAM1	ENSGACG00000011727	groupI	13903005
	ENSGACG00000011739	groupI	13926650
bach1a	ENSGACG00000011798	groupI	13962476
usp16	ENSGACG00000011804	groupI	13966627
rwdd2b	ENSGACG00000011812	groupI	13971864
FOXO1	ENSGACG00000011918	groupI	14219642
cog6	ENSGACG00000011922	groupI	14261590
lhfp	ENSGACG00000011929	groupI	14281680
slc8a2b	ENSGACG00000012302	groupI	15646519
srsf7a	ENSGACG00000012311	groupI	15672536

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
ccdc9	ENSGACG00000012335	groupI	15743759
gdpd4a	ENSGACG00000013263	groupI	17220794
myo7a	ENSGACG00000013269	groupI	17244095
capn5a	ENSGACG00000013281	groupI	17297365
ompa	ENSGACG00000013293	groupI	17304763
ulk2	ENSGACG00000013524	groupI	18392058
bcas3	ENSGACG00000013586	groupI	18620444
myo18a	ENSGACG00000013682	groupI	19641380
NCAM2	ENSGACG00000014231	groupI	21388343
	ENSGACG00000014241	groupI	21473205
gart	ENSGACG00000014244	groupI	21479070
satb2	ENSGACG00000014437	groupI	22087211
BNC1	ENSGACG00000014360	groupII	3225917
KCTD12	ENSGACG00000015376	groupII	8124609
epha6	ENSGACG00000015384	groupII	8148242
LGR4	ENSGACG00000015514	groupII	8790724
ZNF821	ENSGACG00000015798	groupII	11172021
RFX7	ENSGACG00000015838	groupII	11510431
NEDD4	ENSGACG00000015840	groupII	11521136
PRTG	ENSGACG00000015845	groupII	11545338
PBXIP1	ENSGACG00000015850	groupII	11572026
pigb	ENSGACG00000015853	groupII	11579796
rab27a	ENSGACG00000015855	groupII	11586268
vrk3	ENSGACG00000015858	groupII	11595660
FAM65A	ENSGACG00000015863	groupII	11605084
ctcf	ENSGACG00000015865	groupII	11613372
ush2a	ENSGACG00000016262	groupII	14069329
esrp2	ENSGACG00000016359	groupII	14972131
NFATC3	ENSGACG00000016365	groupII	14997944
	ENSGACG00000021256	groupII	15394094
	ENSGACG00000016462	groupII	15396267
tmed3	ENSGACG00000016467	groupII	15396450
	ENSGACG00000017354	groupII	21973529
	ENSGACG00000017356	groupII	21977401
atp6v0d1	ENSGACG00000017358	groupII	21978560
ppip5k1a	ENSGACG00000017465	groupII	22253581
astn1	ENSGACG00000014303	groupIII	3571387
atp1b3a	ENSGACG00000015750	groupIII	9036495
gk5	ENSGACG00000015760	groupIII	9042783
	ENSGACG00000017965	groupIII	16675147
	ENSGACG00000017967	groupIII	16679542
tmem255a	ENSGACG00000017424	groupIV	6653772
zbtb33	ENSGACG00000017428	groupIV	6662937
nkap	ENSGACG00000017430	groupIV	6667356

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
enox2	ENSGACG00000017504	groupIV	7553451
BEND4	ENSGACG00000017530	groupIV	7960082
ATP8A1	ENSGACG00000017532	groupIV	7972945
scyl1	ENSGACG00000017569	groupIV	8174384
ehd1b	ENSGACG00000017605	groupIV	8233238
MAP1LC3C	ENSGACG00000017618	groupIV	8247807
slc43a1a	ENSGACG00000017810	groupIV	9284842
PPP2R2C	ENSGACG00000017871	groupIV	9490428
wfs1b	ENSGACG00000017874	groupIV	9499826
	ENSGACG00000022555	groupIV	9503554
HRH2	ENSGACG00000017883	groupIV	9586702
DOCK2	ENSGACG00000017885	groupIV	9595096
TRPC7	ENSGACG00000017891	groupIV	9701995
DIAPH1	ENSGACG00000017896	groupIV	9760072
tmco6	ENSGACG00000017990	groupIV	10696737
	ENSGACG00000017993	groupIV	10703030
	ENSGACG00000017995	groupIV	10714052
	ENSGACG00000018041	groupIV	11075050
SIMC1	ENSGACG00000018042	groupIV	11085492
cnot6	ENSGACG00000018046	groupIV	11092615
kctd12	ENSGACG00000018268	groupIV	12353365
	ENSGACG00000018286	groupIV	12451969
znf185	ENSGACG00000018287	groupIV	12459458
fut11	ENSGACG00000018289	groupIV	12463399
nlgn3a	ENSGACG00000018296	groupIV	12500111
unc5a	ENSGACG00000018337	groupIV	13161092
PDLIM7	ENSGACG00000018344	groupIV	13220801
cltb	ENSGACG00000018348	groupIV	13247849
higd2a	ENSGACG00000018350	groupIV	13252044
EBF1	ENSGACG00000018450	groupIV	14093708
	ENSGACG00000018493	groupIV	14882018
nxt2	ENSGACG00000018508	groupIV	15058946
psmd10	ENSGACG00000018509	groupIV	15062301
xiap	ENSGACG00000018511	groupIV	15065812
stag2b	ENSGACG00000018514	groupIV	15071091
tenm1	ENSGACG00000018534	groupIV	15373076
POU3F4	ENSGACG00000018547	groupIV	15610119
slc7a2	ENSGACG00000018601	groupIV	15845227
mtmr7a	ENSGACG00000018602	groupIV	15857017
cnot7	ENSGACG00000018605	groupIV	15866651
zdhhc2	ENSGACG00000018608	groupIV	15871647
her1	ENSGACG00000018614	groupIV	15916641
her5	ENSGACG00000018615	groupIV	15920212
pfdn6	ENSGACG00000018616	groupIV	15923902

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
mmgt1	ENSGACG00000018620	groupIV	15928732
ddx26b	ENSGACG00000018623	groupIV	15932627
	ENSGACG00000021515	groupIV	15981048
	ENSGACG00000022231	groupIV	15981371
gpc3	ENSGACG00000018655	groupIV	15982395
gpc4	ENSGACG00000018656	groupIV	16071855
brd8	ENSGACG00000018659	groupIV	16098569
psd2	ENSGACG00000018679	groupIV	16223245
nrg2	ENSGACG00000018682	groupIV	16245967
	ENSGACG00000018696	groupIV	16331382
GABRA2	ENSGACG00000018709	groupIV	16385527
dcps	ENSGACG00000018734	groupIV	16563045
ids	ENSGACG00000018736	groupIV	16565926
aff2	ENSGACG00000018740	groupIV	16586165
	ENSGACG00000022553	groupIV	16690488
fmr1	ENSGACG00000018742	groupIV	16711643
rab33a	ENSGACG00000018745	groupIV	16726999
casp3b	ENSGACG00000018765	groupIV	17650266
grm8a	ENSGACG00000018829	groupIV	18215045
nampt	ENSGACG00000018838	groupIV	18426809
gsap	ENSGACG00000018841	groupIV	18438511
ccdc146	ENSGACG00000018843	groupIV	18461196
fgl2a	ENSGACG00000018844	groupIV	18488149
strip2	ENSGACG00000018938	groupIV	19685754
ahcyl2	ENSGACG00000018942	groupIV	19707744
tbc1d22a	ENSGACG00000019014	groupIV	20565588
lmo3	ENSGACG00000019264	groupIV	23585527
mgst1.1	ENSGACG00000019267	groupIV	23687033
rint1	ENSGACG00000019309	groupIV	23850745
dennd5b	ENSGACG00000019311	groupIV	23864187
MPPED1	ENSGACG00000019323	groupIV	24031409
SCUBE1	ENSGACG00000019325	groupIV	24111028
samm50l	ENSGACG00000019330	groupIV	24192525
aldh1l2	ENSGACG00000019333	groupIV	24202091
	ENSGACG00000019334	groupIV	24219970
slc41a2b	ENSGACG00000019336	groupIV	24223728
kcnc2	ENSGACG00000019441	groupIV	24763664
	ENSGACG00000019455	groupIV	24852375
nav3	ENSGACG00000019472	groupIV	25318371
arid2	ENSGACG00000019522	groupIV	25849279
igbp1	ENSGACG00000019563	groupIV	26204734
magt1	ENSGACG00000019568	groupIV	26207852
fbxo38	ENSGACG00000019572	groupIV	26215973
	ENSGACG00000021313	groupIV	26242542

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
	ENSGACG00000021446	groupIV	26243268
csnk1a1	ENSGACG00000019578	groupIV	26250725
stox2b	ENSGACG00000019586	groupIV	26297130
enpp6	ENSGACG00000019588	groupIV	26317520
fgf4	ENSGACG00000019608	groupIV	26392351
SLC7A11	ENSGACG00000019609	groupIV	26452010
il1rapl2	ENSGACG00000019644	groupIV	27845019
	ENSGACG00000019647	groupIV	28304039
cspp1b	ENSGACG00000019648	groupIV	28308294
snx25	ENSGACG00000019651	groupIV	28316948
	ENSGACG00000019652	groupIV	28330947
fgl1	ENSGACG00000019653	groupIV	28334582
TMEM60	ENSGACG00000019659	groupIV	28429103
phtf2	ENSGACG00000019660	groupIV	28438400
adipor2	ENSGACG00000019662	groupIV	28457178
syt1a	ENSGACG00000020017	groupIV	31925264
cyb5r3	ENSGACG00000020019	groupIV	31945908
	ENSGACG00000020041	groupIV	32090728
igf1	ENSGACG00000020042	groupIV	32098461
KCNMA1	ENSGACG00000002248	groupV	434056
xpo1b	ENSGACG00000002743	groupVI	1376813
alox5a	ENSGACG00000007762	groupVI	9151832
slc25a16	ENSGACG00000007784	groupVI	9159664
spock2	ENSGACG00000008600	groupVI	9823329
	ENSGACG00000018640	groupVII	406357
CLEC19A	ENSGACG00000018643	groupVII	411335
col4a5	ENSGACG00000018775	groupVII	976428
	ENSGACG00000019337	groupVII	4136442
lphn3	ENSGACG00000019498	groupVII	5325617
PTPRD	ENSGACG00000019528	groupVII	5878944
USP5	ENSGACG00000019533	groupVII	6050601
myoz2	ENSGACG00000019535	groupVII	6072903
SYNPO2	ENSGACG00000019536	groupVII	6086475
sec24d	ENSGACG00000019537	groupVII	6100518
dhx15	ENSGACG00000019549	groupVII	6551731
	ENSGACG00000019590	groupVII	6623513
rpl34	ENSGACG00000019593	groupVII	6663776
ostc	ENSGACG00000019601	groupVII	6666220
etnppl	ENSGACG00000019605	groupVII	6668657
NFIB	ENSGACG00000019677	groupVII	7005184
SNORD22	ENSGACG00000022786	groupVII	7725103
SNORD29	ENSGACG00000022602	groupVII	7727427
SNORD31	ENSGACG00000022446	groupVII	7727697
SNORD22	ENSGACG00000022711	groupVII	7728434

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
	ENSGACG00000019819	groupVII	7739783
	ENSGACG00000019888	groupVII	8020685
	ENSGACG00000019893	groupVII	8044555
	ENSGACG00000019895	groupVII	8092741
rpgrip1	ENSGACG00000019896	groupVII	8099373
fam113	ENSGACG00000019900	groupVII	8107491
TDRD7	ENSGACG00000019913	groupVII	8130248
wrap53	ENSGACG00000019924	groupVII	8570726
gigyf1	ENSGACG00000019925	groupVII	8583416
ENDOD1	ENSGACG00000019927	groupVII	8599185
tmem256	ENSGACG00000019937	groupVII	8805329
TMEM102	ENSGACG00000019941	groupVII	8813564
fgf11	ENSGACG00000019942	groupVII	8870275
chrnb1	ENSGACG00000019943	groupVII	8932781
chrnb1l	ENSGACG00000019946	groupVII	8950083
cldn7a	ENSGACG00000019947	groupVII	8968580
	ENSGACG00000019950	groupVII	8975875
ponzr5	ENSGACG00000019951	groupVII	8986296
	ENSGACG00000019952	groupVII	8987681
	ENSGACG00000019953	groupVII	8995661
	ENSGACG00000019954	groupVII	9000505
	ENSGACG00000019958	groupVII	9005340
PTGDR2	ENSGACG00000019959	groupVII	9007774
kirrela	ENSGACG00000020005	groupVII	9351489
	ENSGACG00000020029	groupVII	9728033
NRXN2	ENSGACG00000020030	groupVII	9805143
nrip1b	ENSGACG00000020154	groupVII	12304843
wscd1b	ENSGACG00000020156	groupVII	12410732
SIM2	ENSGACG00000020158	groupVII	12494791
hlcs	ENSGACG00000020159	groupVII	12512681
B3GAT1	ENSGACG00000020191	groupVII	13248581
	ENSGACG00000020192	groupVII	13266860
STT3A	ENSGACG00000020193	groupVII	13275786
prkrir	ENSGACG00000020194	groupVII	13295494
	ENSGACG00000020195	groupVII	13300100
wnt11r	ENSGACG00000020196	groupVII	13318639
uvrag	ENSGACG00000020197	groupVII	13374257
dgat2	ENSGACG00000020198	groupVII	13452421
mogat2	ENSGACG00000020199	groupVII	13463877
map6	ENSGACG00000020200	groupVII	13472213
umodl1	ENSGACG00000020201	groupVII	13489019
zbtb21	ENSGACG00000020202	groupVII	13505638
cxadr	ENSGACG00000020209	groupVII	13593320
auts2a	ENSGACG00000020210	groupVII	13627454

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
cltca	ENSGACG00000020216	groupVII	14183650
dhx33	ENSGACG00000020229	groupVII	14498720
c1qbp	ENSGACG00000020230	groupVII	14507117
MLXIPL	ENSGACG00000020231	groupVII	14513150
SRRM3	ENSGACG00000020232	groupVII	14555092
YWHAG	ENSGACG00000020234	groupVII	14603675
SSC4D	ENSGACG00000020235	groupVII	14614771
BZRAP1	ENSGACG00000020236	groupVII	14658991
	ENSGACG00000022230	groupVII	14692593
	ENSGACG00000022453	groupVII	14692704
supt4h1	ENSGACG00000020237	groupVII	14706030
hpd	ENSGACG00000020238	groupVII	14778775
MTMR4	ENSGACG00000020239	groupVII	14793405
ca4	ENSGACG00000020240	groupVII	14824723
gusb	ENSGACG00000020241	groupVII	14852692
vkorc1	ENSGACG00000020242	groupVII	14865123
NUPR1	ENSGACG00000020243	groupVII	14871252
znhit3	ENSGACG00000020244	groupVII	14873239
MYO19	ENSGACG00000020245	groupVII	14876168
pigw	ENSGACG00000020246	groupVII	14888367
ggnbp2	ENSGACG00000020247	groupVII	14891145
dhrs11	ENSGACG00000020248	groupVII	14896973
	ENSGACG00000020249	groupVII	14910165
flot2	ENSGACG00000020250	groupVII	14919931
	ENSGACG00000021516	groupVII	14937030
	ENSGACG00000021526	groupVII	14937156
eral1	ENSGACG00000020251	groupVII	14938208
fam222b	ENSGACG00000020252	groupVII	14964767
trpv1	ENSGACG00000020253	groupVII	14974131
shpk	ENSGACG00000020254	groupVII	14983177
emc6	ENSGACG00000020255	groupVII	14987555
p2rx5	ENSGACG00000020256	groupVII	14990159
ubc	ENSGACG00000020257	groupVII	14996045
	ENSGACG00000020258	groupVII	14996446
cenpv	ENSGACG00000020259	groupVII	15002338
	ENSGACG00000020271	groupVII	15260522
stip1	ENSGACG00000020272	groupVII	15340712
rps6ka4	ENSGACG00000020273	groupVII	15349635
FLRT1	ENSGACG00000020274	groupVII	15405552
vps51	ENSGACG00000020279	groupVII	15448825
tm7sf2	ENSGACG00000020280	groupVII	15454238
adssl	ENSGACG00000020288	groupVII	15567186
	ENSGACG00000022183	groupVII	15576697
	ENSGACG00000021274	groupVII	15576872

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
ammecr1	ENSGACG00000020290	groupVII	15598480
gabra3	ENSGACG00000020292	groupVII	15640600
thoc2	ENSGACG00000020367	groupVII	17555779
gria3	ENSGACG00000020368	groupVII	17580031
atp6v1e1a	ENSGACG00000020388	groupVII	17841023
NYAP1	ENSGACG00000020389	groupVII	17857676
atp1b2b	ENSGACG00000020390	groupVII	17868985
gltpd2	ENSGACG00000020391	groupVII	17880340
chrne	ENSGACG00000020392	groupVII	17887230
adamts15	ENSGACG00000020418	groupVII	18219272
bco2a	ENSGACG00000020420	groupVII	18286645
fhl1b	ENSGACG00000020432	groupVII	18527504
slc9a6b	ENSGACG00000020433	groupVII	18539190
il2rgb	ENSGACG00000020434	groupVII	18548109
snx12	ENSGACG00000020435	groupVII	18553876
abhd11	ENSGACG00000020497	groupVII	19269612
cldnh	ENSGACG00000020498	groupVII	19279730
rxfp2	ENSGACG00000020550	groupVII	19898579
fry	ENSGACG00000020551	groupVII	19937626
trpc4a	ENSGACG00000020568	groupVII	20270318
synj1	ENSGACG00000020575	groupVII	20539803
tiam1	ENSGACG00000020582	groupVII	20634688
map3k7cl	ENSGACG00000020584	groupVII	20695666
GRM5	ENSGACG00000020586	groupVII	20721798
TYR	ENSGACG00000020587	groupVII	20737985
chordc1a	ENSGACG00000020588	groupVII	20741461
	ENSGACG00000020589	groupVII	20749176
cdh2	ENSGACG00000002928	groupVIII	95049
irf4b	ENSGACG00000004966	groupVIII	3510444
exoc2	ENSGACG00000004977	groupVIII	3518916
lrp8	ENSGACG00000006827	groupVIII	7401543
kank4	ENSGACG00000006846	groupVIII	7475544
FCHO1	ENSGACG00000007515	groupVIII	8519939
rfx2	ENSGACG00000007541	groupVIII	8542911
acsbg2	ENSGACG00000007559	groupVIII	8571604
mllt1	ENSGACG00000007614	groupVIII	8587594
acer1	ENSGACG00000007629	groupVIII	8601701
myo1f	ENSGACG00000007641	groupVIII	8608236
NR2F6	ENSGACG00000007766	groupVIII	8767315
ptprsa	ENSGACG00000008773	groupVIII	10168569
nr5a2	ENSGACG00000008896	groupVIII	10503534
HYKK	ENSGACG00000009062	groupVIII	11269682
nmur1a	ENSGACG00000009069	groupVIII	11278291

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
	ENSGACG00000009076	groupVIII	11289636
HTR3E	ENSGACG00000009077	groupVIII	11296839
	ENSGACG00000009078	groupVIII	11301927
KIAA0226	ENSGACG00000009086	groupVIII	11312464
tsc22d2	ENSGACG00000009134	groupVIII	11336696
lrrc15	ENSGACG00000010035	groupVIII	12743498
CCDC50	ENSGACG00000010040	groupVIII	12761712
p3h2	ENSGACG00000010066	groupVIII	12787013
	ENSGACG00000010465	groupVIII	13126242
arhgef18b	ENSGACG00000010468	groupVIII	13135192
slc1a6	ENSGACG00000011239	groupVIII	14314442
LTBP	ENSGACG00000011786	groupVIII	15151890
CYP2J2	ENSGACG00000011790	groupVIII	15158424
uqcrh	ENSGACG00000011853	groupVIII	15173417
	ENSGACG00000011857	groupVIII	15177443
clockb	ENSGACG00000015939	groupIX	489361
polq	ENSGACG00000016100	groupIX	1429086
ptpn9b	ENSGACG00000016107	groupIX	1443105
usp53b	ENSGACG00000017796	groupIX	8669923
myoz2b	ENSGACG00000017804	groupIX	8678717
ctnna2	ENSGACG00000017985	groupIX	9146194
DLC1	ENSGACG00000018034	groupIX	9720025
kcnq5b	ENSGACG00000018097	groupIX	9900166
scrn2	ENSGACG00000018155	groupIX	10153416
	ENSGACG00000018157	groupIX	10162047
CBX4	ENSGACG00000018219	groupIX	10496079
inab	ENSGACG00000018244	groupIX	10749285
nt5c2	ENSGACG00000018247	groupIX	10750399
jup	ENSGACG00000018328	groupIX	10988528
kcnip2	ENSGACG00000018330	groupIX	10996164
rgs12b	ENSGACG00000018356	groupIX	11144114
dok7	ENSGACG00000018358	groupIX	11180226
lrpap1	ENSGACG00000018360	groupIX	11194695
	ENSGACG00000018365	groupIX	11287767
cpz	ENSGACG00000018366	groupIX	11321370
htra3a	ENSGACG00000018376	groupIX	11334493
pi4k2b	ENSGACG00000018377	groupIX	11339606
sclt1	ENSGACG00000018401	groupIX	11484494
prkca	ENSGACG00000018519	groupIX	12793088
cacng5	ENSGACG00000018521	groupIX	12900795
cacng4a	ENSGACG00000018524	groupIX	12915523
cacng1	ENSGACG00000018528	groupIX	12926489
helz	ENSGACG00000018530	groupIX	12938360
gne	ENSGACG00000018604	groupIX	13630506

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
clta	ENSGACG00000018609	groupIX	13638390
evpla	ENSGACG00000019260	groupIX	16159871
ten1	ENSGACG00000019266	groupIX	16171112
acox1	ENSGACG00000019268	groupIX	16175263
pik3r5	ENSGACG00000019372	groupIX	16835067
ntn1a	ENSGACG00000019374	groupIX	16854580
hn1a	ENSGACG00000019394	groupIX	17081308
trim71	ENSGACG00000008309	groupX	12801925
abat	ENSGACG00000004710	groupXI	304385
prrg2	ENSGACG00000004841	groupXI	428273
nags	ENSGACG00000005126	groupXI	764494
b4galnt2.2	ENSGACG00000005131	groupXI	798847
rnd2	ENSGACG00000009070	groupXI	6291989
hs3st3b1a	ENSGACG00000010816	groupXI	8484883
pmp22a	ENSGACG00000010819	groupXI	8512176
	ENSGACG00000010914	groupXI	8839051
GPRC5C	ENSGACG00000010915	groupXI	8840666
btbd17b	ENSGACG00000010921	groupXI	8847136
zgc:171489	ENSGACG00000011523	groupXI	10379462
cdr2a	ENSGACG00000011526	groupXI	10385184
sdr42e2	ENSGACG00000011530	groupXI	10392237
	ENSGACG00000011652	groupXI	10494542
mkl2b	ENSGACG00000013988	groupXI	13726297
slc16a7	ENSGACG00000003483	groupXII	2152217
	ENSGACG00000003497	groupXII	2156242
PNPLA8	ENSGACG00000003501	groupXII	2161697
kcnc4	ENSGACG00000007907	groupXII	10128185
SLC6A17	ENSGACG00000007913	groupXII	10154388
adora1b	ENSGACG00000008072	groupXII	10258103
def6	ENSGACG00000008311	groupXII	10464460
	ENSGACG00000008347	groupXII	10475511
DTX3	ENSGACG00000008546	groupXII	10679024
rnd1l	ENSGACG00000008549	groupXII	10697368
cacnb3	ENSGACG00000008568	groupXII	10710520
ADCY6	ENSGACG00000008575	groupXII	10731068
ARHGEF25	ENSGACG00000008996	groupXII	11150617
ankrd33aa	ENSGACG00000009005	groupXII	11188599
hoxc13b	ENSGACG00000009389	groupXII	11575429
hoxc10	ENSGACG00000009394	groupXII	11606252
csrp1	ENSGACG00000009706	groupXII	12100734
phlda3	ENSGACG00000009727	groupXII	12103970
tnni1a	ENSGACG00000009730	groupXII	12109940
	ENSGACG00000009740	groupXII	12113808
lad1	ENSGACG00000009743	groupXII	12117810

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
tnnt2a	ENSGACG00000009747	groupXII	12120260
pkp1a	ENSGACG00000009752	groupXII	12129960
tead3	ENSGACG00000009758	groupXII	12143626
ube2t	ENSGACG00000009813	groupXII	12215552
ETV7	ENSGACG00000009831	groupXII	12218917
KCNA10	ENSGACG00000009836	groupXII	12234412
KCNA2	ENSGACG00000009837	groupXII	12251007
	ENSGACG00000009842	groupXII	12262938
ngfa	ENSGACG00000009843	groupXII	12310358
tspan2b	ENSGACG00000009844	groupXII	12315252
	ENSGACG00000009866	groupXII	12325491
TSHB	ENSGACG00000009897	groupXII	12329010
CACNA2D3	ENSGACG00000010123	groupXII	12488249
LRTM1	ENSGACG00000010150	groupXII	12569996
wnt5a	ENSGACG00000010153	groupXII	12609079
ERC2	ENSGACG00000010158	groupXII	12648568
tlr9	ENSGACG00000010164	groupXII	12738331
apeh	ENSGACG00000010172	groupXII	12743186
capza1b	ENSGACG00000010181	groupXII	12750847
cttnbp2nlb	ENSGACG00000010190	groupXII	12758253
	ENSGACG00000010196	groupXII	12771425
acss2	ENSGACG00000010216	groupXII	12779521
mapre1b	ENSGACG00000010256	groupXII	12798594
dnmt3	ENSGACG00000010262	groupXII	12806093
dnmt4	ENSGACG00000010273	groupXII	12823810
commd7	ENSGACG00000010283	groupXII	12844822
lama5	ENSGACG00000010405	groupXII	13049591
tuba1b	ENSGACG00000010436	groupXII	13123145
RAB29	ENSGACG00000010473	groupXII	13152549
NPBWR2	ENSGACG00000010477	groupXII	13219071
oprl1	ENSGACG00000010479	groupXII	13262291
FAM187B	ENSGACG00000010484	groupXII	13309389
sox18	ENSGACG00000010505	groupXII	13373226
xkr7	ENSGACG00000010506	groupXII	13403771
ccm2l	ENSGACG00000010511	groupXII	13448683
	ENSGACG00000010523	groupXII	13483912
hsd17b10	ENSGACG00000010525	groupXII	13489301
	ENSGACG00000022263	groupXII	13493981
	ENSGACG00000022179	groupXII	13494464
tfcp2	ENSGACG00000010929	groupXII	13828331
csrnp2	ENSGACG00000010943	groupXII	13840252
itga5	ENSGACG00000010945	groupXII	13865837
PLXNA2	ENSGACG00000011007	groupXII	14055012
rgmb	ENSGACG00000009766	groupXIII	9546174

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
mef2cb	ENSGACG00000010270	groupXIII	10417033
	ENSGACG00000021300	groupXIII	19683692
ptrh1	ENSGACG00000014682	groupXIII	19684305
slc27a6	ENSGACG00000018349	groupXIV	14021776
lmnb1	ENSGACG00000018353	groupXIV	14039862
	ENSGACG00000005133	groupXV	1444644
fosaa	ENSGACG00000007617	groupXV	4764418
mlh3	ENSGACG00000007623	groupXV	4768572
crlf1a	ENSGACG00000009968	groupXV	8210188
snap23.1	ENSGACG00000011055	groupXV	10429950
sptb	ENSGACG00000011100	groupXV	10442747
asmt	ENSGACG00000006621	groupXVI	12969734
fer1l6	ENSGACG00000008352	groupXVI	16508075
prkag3b	ENSGACG00000008373	groupXVI	16525600
parp4	ENSGACG00000008521	groupXVI	16908539
	ENSGACG00000008710	groupXVI	17405163
	ENSGACG00000008714	groupXVI	17408026
	ENSGACG00000008715	groupXVI	17411595
ppcs	ENSGACG00000007405	groupXVII	6273370
utp3	ENSGACG00000007417	groupXVII	6274869
	ENSGACG00000007429	groupXVII	6279497
fam83e	ENSGACG00000007430	groupXVII	6282073
	ENSGACG00000007437	groupXVII	6285207
SLC2A9	ENSGACG00000009129	groupXVII	8676250
znf395	ENSGACG00000004727	groupXVIII	1364627
ufl1	ENSGACG00000006287	groupXVIII	4128680
HS3ST5	ENSGACG00000006332	groupXVIII	4276771
mllt4a	ENSGACG00000006700	groupXVIII	4873161
	ENSGACG00000006716	groupXVIII	4892806
	ENSGACG00000006718	groupXVIII	4904826
ANO1	ENSGACG00000002381	groupXIX	959942
	ENSGACG00000011039	groupXIX	13049496
kcnj11	ENSGACG00000011042	groupXIX	13049738
mob2	ENSGACG00000011046	groupXIX	13053541
osbpl5	ENSGACG00000011081	groupXIX	13227769
ldha	ENSGACG00000011270	groupXIX	13757826
tsg101a	ENSGACG00000011311	groupXIX	13764439
hrasa	ENSGACG00000011340	groupXIX	13775246
rag2	ENSGACG00000011461	groupXIX	14489909
rag1	ENSGACG00000011465	groupXIX	14493756
polr3b	ENSGACG00000011794	groupXIX	15049715
rfx4	ENSGACG00000011830	groupXIX	15070736
SPIRE1	ENSGACG00000004577	groupXX	2462673
atp6v1c1a	ENSGACG00000004615	groupXX	2501237

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
TMEM74	ENSGACG00000005301	groupXX	3788628
rbms3	ENSGACG00000006377	groupXX	6102582
grb10	ENSGACG00000006438	groupXX	6202336
nrsn1	ENSGACG00000006587	groupXX	6912058
SLC6A3	ENSGACG00000006614	groupXX	7033258
cdkal1	ENSGACG00000006622	groupXX	7344820
tcea3	ENSGACG00000007286	groupXX	8195753
kcnk5b	ENSGACG00000007871	groupXX	9218218
EPB41	ENSGACG00000007879	groupXX	9233627
	ENSGACG00000007893	groupXX	9249276
CSNK2B	ENSGACG00000007897	groupXX	9251806
cyp21a2	ENSGACG00000007916	groupXX	9263386
	ENSGACG00000007933	groupXX	9276661
	ENSGACG00000021296	groupXX	9290682
	ENSGACG00000007971	groupXX	9292404
rxrbb	ENSGACG00000007982	groupXX	9297024
fhod3b	ENSGACG00000007994	groupXX	9307622
sp8a	ENSGACG00000008062	groupXX	9481109
macc1	ENSGACG00000008067	groupXX	9490944
twist2	ENSGACG00000008070	groupXX	9505813
	ENSGACG00000008075	groupXX	9526242
hdac9b	ENSGACG00000008076	groupXX	9526503
ankrd28b	ENSGACG00000008084	groupXX	9563160
fam188a	ENSGACG00000008767	groupXX	10155793
	ENSGACG00000008776	groupXX	10173184
cdh17	ENSGACG00000009113	groupXX	10687628
rad54b	ENSGACG00000009128	groupXX	10704434
rnf41l	ENSGACG00000009133	groupXX	10715828
	ENSGACG00000009138	groupXX	10719553
esrp1	ENSGACG00000009169	groupXX	10732757
epb41l4b	ENSGACG00000009175	groupXX	10747464
ptpn3	ENSGACG00000009182	groupXX	10758440
fam171a1	ENSGACG00000009230	groupXX	10816365
nmt2	ENSGACG00000009238	groupXX	10834107
styk1	ENSGACG00000010432	groupXX	11900072
phc1	ENSGACG00000010438	groupXX	11905700
m6pr	ENSGACG00000010442	groupXX	11911904
NAT14	ENSGACG00000010519	groupXX	12046215
aicda	ENSGACG00000010521	groupXX	12050972
NECAP1	ENSGACG00000010530	groupXX	12053199
epn1	ENSGACG00000010556	groupXX	12077130
foxj2	ENSGACG00000010572	groupXX	12085828
isoc2	ENSGACG00000010580	groupXX	12090387
atg12	ENSGACG00000010597	groupXX	12092527

Gene Name	ENSEMBL gene id	Chromosome	Gene Start Position
rcv1	ENSGACG00000010601	groupXX	12100423
	ENSGACG00000010614	groupXX	12112453
ccdc106	ENSGACG00000010622	groupXX	12120567
u2af2	ENSGACG00000010632	groupXX	12125987
klf6a	ENSGACG00000001778	groupXXI	1563702
smyhc2	ENSGACG00000002145	groupXXI	2929787
C3orf19	ENSGACG00000000764	Un	
	ENSGACG00000000832	Un	
COL7A1	ENSGACG00000000833	Un	
GNAI2	ENSGACG00000000839	Un	
kbp	ENSGACG00000001219	Un	
	ENSGACG00000001222	Un	
	ENSGACG00000001226	Un	
kbp	ENSGACG00000001229	Un	
	ENSGACG00000001237	Un	
MON1B	ENSGACG00000001246	Un	
TCTA	ENSGACG00000001247	Un	
GLYCTK	ENSGACG00000001248	Un	
BSN	ENSGACG00000001561	Un	
	ENSGACG00000001670	Un	
	ENSGACG00000000873	Un	
KIAA0495	-	Un	
	ENSGACG00000001002	Un	
SLC12A7	ENSGACG00000001024	Un	
	ENSGACG00000001055	Un	
SASS6	-	Un	
TG	ENSGACG00000000896	Un	
CRYGN	ENSGACG00000018096	Un	
NYREN18	ENSGACG00000018101	Un	
	ENSGACG00000018093	Un	