Regional differences driving organic matter and trace metal signatures reflected in temperate reef bivalve communities on the South Island, New Zealand.

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Abstract

Increases in anthropogenic activity along coastal zones has led to an influx of terrestrial particulate matter containing high levels of nutrients and contaminants to coastal ecosystems. In order to manage these detrimental impacts, it is imperative that we understand the factors that drive the spatial variation in pollutants at both regional and local scales. Bivalves are key marine players in the processing of organic matter providing a link between benthic, pelagic, and terrestrial habitats. To this effect, bivalves are recognized as sentinel organisms in evaluating marine ecosystem function as well as detecting pollutants associated with land-based inputs. Therefore, changes to the basal organic matter source pools supporting these species will result in inherent changes in organic matter sources supporting species further up the food chain. Comparisons between Marlborough Sounds, Tasman Bay and Fiordland on New Zealand's South Island, provide a unique study system containing gradients in anthropogenic impacts influencing the uptake of organic matter and accumulation of pollutants in bivalves.

The present study aimed to identify the influence of anthropogenic stressors in driving organic matter flux and trace element concentrations within bivalve communities in Marlborough Sounds, Tasman Bay and Fiordland. We evaluated direct interactions between catchment modifications and salmon farming on feeding strategies, organic matter source pools and trace element signatures of five individual bivalve species.

We established that feeding strategies reveal interspecific differences among sites, while intraspecific differences are reflective of small-scale differences in organic matter resource pools. Further, organic matter source pools shaped by catchment modification and salmon farms revealed the observed differences in proportions of macroalgae supporting primary consumers. Lower proportions of organic matter were correlated with a high degree of catchment modification. Salmon farms influenced nutrient delivery to the surrounding benthos to all subtidal species. However, the intertidal (*Mytilus galloprovincialis*) was uninfluenced.

Spatial difference in trace element concentrations tracked through basal organic matter source pools revealed the influence of catchment modification on the transmission of trace elements into bivalves. Further, aquaculture systems influenced the use of alternate basal organic matter sources (macroalgae versus phytoplankton) which were coincident with trace metal concentrations reflected in sediment dwelling species.

Lastly, as mercury concentrations have not been well resolved for commercially important bivalves in South Island fisheries, using current mercury analysis methodology, we reported baseline mercury concentrations. Further, we illustrated the influence of catchment modification from forestry on spatial variability in mercury concentrations.

The contrast of the relatively developed catchments surrounding Marlborough Sounds and Tasman Bay with Fiordland's relatively pristine forested catchment provides an important test of the links between anthropogenic activity and the contaminant loads reflected in coastal bivalves. Monitoring these coastal bivalves provided essential insight into the movement of particulate organic matter throughout food webs and their implications for ecosystem health.

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Table of Contents

Abstract 2

Acknowledgements 3

Table of Contents..4

List of Tables..8

List of Figure 10

Chapter one: Introduction 15

1.1 Temperate reef communities under threat 15

1.2 The role of suspension feeders in temperate rocky reef communities 16

1.3 Trace metals in bivalves 18

1.4 Bivalves as aquatic ecosystem monitors 19

1.5 Study Sites 20

  Marlborough Sounds 21

  Tasman Bay 22

  Fiordland..22

1.6 Objectives 23

Chapter 2: Influences of land modification and salmon farm on resource use and trophic position of suspension feeding bivalves...26

2.0 Introduction 26

  Feeding modes of different species 27

  Anthropogenic impacts...28

  Regions of study 29

2.1 Methods 30

  2.1.1 Study organisms 30

  2.1.2 Sample collection 34

  2.1.3 Stable isotope analysis...35

  2.1.4 Stable isotopic mixing model 38

  2.1.5 Landcover classification...38

  2.1.6 Statistical analysis...40

    Spatial variation in nitrogen and carbon stable isotopic signatures 40

Salmon farm 40
Landcover classification 41

2.2 Results 41
2.2.1 Organic matter source pools are variable at site and region level

2.2.2 Spatial variation of individual species 44
Overlap of δ^{15}N and δ^{13}C in isotopic space for species 46
Mixing model 50

2.2.2 Community trends with mass balance analysis 51
Spatial variation 51

2.2.3 Anthropogenic influences 56
Salmon farms in Marlborough Sounds 56
Landcover classification 57

2.3 Discussion 63

2.3.1 Macroalgae subsidies 63

2.3.2 Resource overlap and partitioning 66

2.3.3 Influence of anthropogenic stressors to resource subsidies for coastal benthic bivalves 68
Salmon farm 68
Land-cover 69

Chapter 3: Influence of catchments and organic matter source on the variation in concentration of trace metals in five different bivalve species on the South Island of New Zealand 74

3.0 Introduction 74

3.1 Methods 76

3.1.1 Study organisms 77

3.1.2 Sample sites 78

3.1.3 Sample collection 81

3.1.4 Trace metal analysis 81
Sample dissection and preparation 81
Digestion and analysis 81

3.1.5 Landcover classifications 83

3.1.6 Statistical analysis 84
Univariate 84
Multivariate 84

3.2 Results 84

3.2.1 Regional separation in trace metal concentrations 84

3.2.2 Species level differences in trace metal concentrations 89

3.2.3 Organic matter associations with trace metal concentrations 94
Salmon farms 96

Catchment 99

3.3 Discussion 103

3.3.1 General trends in regional trace metal concentrations 104

3.3.2 Associations between organic matter flow and trace metal concentrations among species 105

3.3.3 Land modification impacts 106

Salmon farming 106

Catchment 108

Chapter 4: Spatial variation in mercury concentrations in commercially important bivalves 112

4.0 Introduction 112

4.1 Methods 114

4.1.1 Sample collection 114

4.1.2 Mercury analysis 117

4.1.3 Stable isotopic signatures 119

4.1.4 Landcover classification 119

4.1.5 Statistical analysis 121

4.2 Results 122

4.2.1 Spatial differences for individual species 122

Regional level 122

Catchment level 124

4.2.2 Species spatial overlap 125

4.2.3 Changes associated with organic matter sources using stable isotopic signatures 126

4.2.4 Differences within catchments 127

Salmon farming in Marlborough Sounds 127

Proportion landcover classification 128

4.3 Discussion 129

4.3.1 Spatial variation in mercury concentrations 129

4.3.2 Variations in mercury in relation to stable isotopic signatures 133

4.3.3 Variation with anthropogenic pressures 133

Salmon Farming 133

Catchment Modification 133

Chapter 5: Synthesis 138

Spatial variation in resource use 139

Spatial variation in trace metal fingerprints 139

Spatial variation in mercury concentrations 140

References......144
Appendices 160

List of Tables

Table 2.1 Isotopic signatures (δ^{15}N and δ^{13}C) of the bivalves collected from Marlborough Sounds, New Zealand. 39

Table 2.2 Isotopic signatures (δ^{15}N and δ^{13}C) of the bivalves collected from Tasman Bay, New Zealand. 39

Table 2.3 Isotopic signatures (δ^{15}N and δ^{13}C) of the bivalves collected from Fiordland, New Zealand. 39

Table 2.4 Potential organic matter source supporting suspension feeding bivalves. Site specific averages are provided where available. (-) indicates no data present. 45

Table 2.5 Results from a general linear model reveal significant differences in stable isotopic values (δ^{15}N and δ^{13}C) between species and regions. Significant p-values ($p < 0.05$) are bolded. 46

Table 2.6 Results from a general linear model revealed significant differences in stable isotopic values (δ^{15}N and δ^{13}C) for individual species among regions. Significant p-values ($p < 0.05$) are bolded. 48

Table 2.7 Sensitivity analysis based on the averages among species for (a) proportion of macroalgae in bivalve diets. Values were determined by mixing models first for individuals, for assumed basal carbon isotopic values (δ^{13}C) of (a) macroalgae and (b) SPOM. Values were pooled for all five species present at Motungarara Island.......... 54

Table 2.8 Proportions of landcover classifications and total area for discrete catchments. 60

Table 2.9 Results of discrete general linear models for proportions of macroalgae supporting individual species among catchments. Significant p-values ($p < 0.05$) are bolded. 60

Table 3.1 Limits of detection and percent recovery for 13 trace metals. Recovery is given in a range due to multiple runs with the ICP-MS. 85

Table 3.2 Results of a PERMANOVA on the multivariate similarity among concentrations of conservative metals Mg, Al, Cr, and Sr for species among regions. Significant p-values are bolded. 91

Table 3.3 Results of a PERMANOVA on the multivariate similarity among concentrations of bioactive metals Mn, Co, Fe, Ni, Cu, and Zn for species among regions. Significant p-values are bolded. 91

Table 3.4 Results of a PERMANOVA on the multivariate similarity among concentrations of highly scavenged metals As, Cd, and Pb for species among regions. Significant p-values are bolded. 91

Table 3.5 Individual general linear models revealed species specific differences in trace metal concentrations $\delta^{15}N$ and $\delta^{13}C$ among regions. Significant p-vales are bolded ($p < 0.05$). 97

Table 3.6 Results of a general linear model revealed significant differences in trace metal concentrations with $\delta^{15}N$ and $\delta^{13}C$ for *A. zelandica and T. laticostata* among region. Significant p-values are bolded ($p < 0.05$). 97

Table 3.7 Results of general linear models revealed specific differences in trace metal concentrations with $\delta^{15}N$ and $\delta^{13}C$ among all target species at salmon farm sites and reference sites. 99

Table 3.8 Results of discrete general linear models revealed significant trace metal concentrations among catchments for individual species. Significant p-values ($p < 0.05$) are in bold. 102

Table 3.9 Results of discrete general linear models results revealed significant differences in trace metals with $\delta^{15}N$ and $\delta^{13}C$ among catchments for individual species. Significant p-values ($p < 0.05$) are bold. 103

Table 4.1 Average total mercury concentrations (THg) for individual species at sites within Marlborough Sounds, Tasman Bay and Fiordland. 120

Table 4.2 General linear model results of THg concentrations with stable isotopic values ($\delta^{15}N$ and $\delta^{13}C$) among sites for *P. novaezelandiae*. Bolded p-values ($p < 0.05$) indicate statistical differences. 129

Table 4.3 General linear model results of THg concentrations with stable isotopic values ($\delta^{15}N$ and $\delta^{13}C$) among site for *P. canaliculus*. Bolded p-values ($p < 0.05$) indicate statistical differences. 129

Table 4.4 General linear model results for THg concentrations between salmon farm and reference sites among species. Bolded p-values indicate statistical differences. 130

List of Figure

Figure 2.1 Sample sites within each region: Tasman Bay: 1) Totaranui, 2) Te Karetu Point, 3) Mapua, 4) Delaware Bay, 5) Crousielles Harbour, 6) Ragged Point. Marlborough Sounds: 7) Old Man's Head, 8) Kauaroa, 9) Waitata, 10) Bird Island, 11) Motungarara Island, 12) Ruakaka Bay, 13) Perano Shoal, 14) Dieffenbach Point, 15) Te Pangu Bay 16) Port Underwood. Doubtful Sound: 16) Crayfish Creek, 17) Bradshaw-Thompson, and 18) MacDonald Island. White points indicate salmon farms within Marlborough Sounds.......... 34

Figure 2.2 Landcover classification for each of the five catchments: 1) Tory Channel, 2) Queen Charlotte Sound, 3) Pelorus Sound, 4) Mapua, and 5) Doubtful Sound. 41

Figure 2.3 Average stable isotopic values (δ^{15}N and δ^{13}C) for bivalves from (1) Marlborough Sounds, (2) Tasman Bay and (3) Doubtful Sound, for species: *A. zelandica* (x), *M. galloprovincialis*(diamond), *P. novaezelandiae* (circle), *P. canaliculus* (triangle), and *T. laticostata* (square). Means ± SE. 47

Figure 2.4 Stable isotopic values (δ^{15}N and δ^{13}C) for consumers and organic matter sources from Marlborough Sounds for species: *A. zelandica* (closed triangle), *M. galloprovincialis*(open triangle), *P. novaezelandiae* (open circle), *P. canaliculus* (closed square), and *T. laticostata* (closed rectangle). Shapes display resource use overlap of consumers in isotopic space for: *A. zelandica* (grey), *M. galloprovincialis*(blue), *P. novaezelandiae* (yellow), *P. canaliculus* (green), and *T. laticostata* (orange). 50

Figure 2.5 Stable isotopic signatures (δ^{15}N and δ^{13}C) for consumers and organic matter sources from Tasman Bay for species: *A. zelandica* (closed triangle), *M. galloprovincialis*(open triangle), *P. canaliculus* (closed square), and *T. laticostata* (closed rectangle). Shapes display resource use overlap of consumers in isotopic space for: *A. zelandica* (grey), *M. galloprovincialis*(blue), *P. canaliculus* (green), and *T. laticostata* (orange). 51

Figure 2.6 Stable isotopic signatures (δ^{15}N and δ^{13}C) for consumers and organic matter sources from Fiordland for species: *A. zelandica* (closed triangle), *P. novaezelandiae* (open circle), and *T. laticostata* (closed rectangle). Shapes display resource use overlap of consumers in isotopic space for: *A. zelandica* (grey), *P. novaezelandiae* (yellow), and *T. laticostata* (orange).......... 52

Figure 2.7 Sensitivity of (a) the proportion of macroalgae in diet, and (b) the proportion of SPOM in diet, determined by mixing models, for the assumed base values (δ^{13}C) of (a) macroalgae, and (b) SPOM source pools in the diet of bivalves from Motungarara Island. 53

Figure 2.8 Proportion of macroalgae supporting bivalves for assumed trophic level 1 presented for each region: Marlborough Sounds (grey), Tasman Bay (white), and Doubtful Sound (black).

Average proportions of organic matter from macroalgae for each region are shown here, site specific averages in the mixing model were used to determine organic matter sources for consumers where available. Mean ± 1 SE. 55

Figure 2.9 Proportion of macroalgae supporting bivalves for assumed trophic level 1 from Motungarara Island, Marlborough Sounds. Mean ± 1 SE. Levels not connected by same letters are significantly different.. 56

Figure 2.10 Proportion of macroalgae supporting bivalves for assumed trophic level 1 at sites: Bird Island (black), Perano Shoal (white), and Motungarara Island (grey) within Marlborough Sounds. Mean ± 1 SE. Levels not connected by same letters are significantly different. 57

Figure 2.11 a) Proportion macroalgae supporting *A. zelandica* for assumed trophic level 1 collected from study sites. Mean ± 1 SE. Levels not connected by same letters are significantly different. 58

Figure 2.12 Proportion of macroalgae supporting bivalves for assumed trophic level 1 from reference (grey) and salmon farm (black) sites. Mean ± 1 SE. Levels not connected by same letters are significantly different. 59

Figure 2.13 Significant regression analysis results for proportion of macroalgae and percent landcover for *M. galloprovincialis*among sites: 1. Tory Channel, 2. Queen Charlotte Sound, 3. Pelorus Sound, and 4. Mapua. 61

Figure 2.14 Significant regression analysis results for proportion of macroalgae and percent farming for *P. novaezelandiae* among sites: 1. Tory Channel, 2. Queen Charlotte Sound, 3. Pelorus Sound, and 5. Doubtful Sound.. 62

Figure 2.15 Significant regression analysis results for proportion of macroalgae and percent landcover for *P. canaliculus* among sites: 1. Tory Channel, 2. Queen Charlotte Sound, 3. Pelorus Sound, and 4. Mapua. 63

Figure 2.16 Significant regression analysis results for proportion of macroalgae and percent landcover for *T. laticostata* among sites: 2. Queen Charlotte Sound, 3. Pelorus Sound, 4. Mapua, and 5. Doubtful Sound.. 64

Figure 2.17 Average proportion of macroalgae supporting species for assumed trophic level 1 among study regions: Marlborough Sounds, Tasman Bay and Fiordland. 66

Figure 3.1 Sites and landcover classifications within catchments for each of the study regions. Catchments: a) Port Underwood, b) Queen Charlotte Sound, c) Pelorus Sounds, d) Mapua, and e) Doubtful Sounds. Sites: 1) Perano Shoal, 2) Dieffenbach Point, 3) Ruakaka Bay, 4) Motungarara

Island, 5) Bird Island, 6) Waitata, 7) Clay Point, 8) Old Man's Head, 9) Ragged Point, 10) Crousielles Harbour, 11) Delaware Bay, 12) Mapua, 13) Te Karetu, 14) Totaranui, 15) Crayfish Creek, 16) Bradshaw-Thompson, and 17) MacDonald Island. 82

Figure 3.2 Canonical plot scores and 95% confidence ellipses from discriminant funtion analysis of four conservative metal concentrations among regions: Marlborough Sounds = green, Tasman Bay = blue, and Fiordland = orange. Significanly differ groups display no overlap in the 95% confidence ellipses........ 88

Figure 3.3 Canonical plot scores and 95% confidence ellipses from discriminant funtion analysis of five bioactive metal concentrations among regions: Marlborough Sounds = green, Tasman Bay = blue, and Fiordland = orange. Significanly differ groups display no overlap in the 95% confidence ellipses........ 89

Figure 3.4 Canonical plot scores and 95% confidence ellipses from discriminant funtion analysis of three highly scavenged metal concentrations among regions: Marlborough Sounds = green, Tasman Bay = blue, and Fiordland = orange. Significanly differ groups display no overlap in the 95% confidence ellipses. 90

Figure 3.5 Significantly different concentrations of conservative metals at each region for individual species as determined by general linear models. *A. zelandica, P. canaliculus, M. galloprovincialis, P. novaezelandiae, T. laticostata*. Mean ± 1 SE. Different letters indicate significant differences between regions. 93

Figure 3.6 Concentrations of bioactive metals at each region for individual species. *A. zelandica, P. canaliculus, M. galloprovincialis, P. novaezelandiae, T. laticostata*. Mean ± 1 SE. Different letters indicate significant differences between regions........ 94

Figure 3.7 Concentrations of highly scavenged metals at each region for individual species. *A. zelandica, P. canaliculus, M. galloprovincialis, P. novaezelandiae, T. laticostata.* Mean ± 1 SE. Different letters indicate significant differences between regions........ 95

Figure 3.8 a) Pair-wise analysis of Cu concentrations for target species between salmon farm and references sites and b) average Cu concentrations plotted against δ^{13}C of all species at salmon farm and reference sites. Mean ± 1 SE. Levels connected by different letters indicate statistical differences. 100

Figure 3.9 Significantly different trace metal concentrations with δ^{13}C among catchment for conservative metals in a,b) *P. novaezelandiae*, c) *T. laticostata*, bioactive metals in d) *A. zelandica*, e) *P. novaezelandiae*, f) *P. canaliculus*, and highly scavenged metals in g) *P. canaliculus*. Mean ± 1 SE. Levels connected by different letters indicate statistical differences. 104

Figure 3.10 Concentrations of conservative metal (Sr) in each of the catchments of individual species....105

Figure 4.1 Sample sites within each region: 1) Totaranui, 2) Te Karetu Point, 3) Mapua, 4) Delaware Bay, 5) Crousielles Harbour, 6) Ragged Point, 7) Old Man's Head, 8) Clay Point, 9) Waitata, 10) Bird Island, 11) Motungarara Island, 12) Ruakaka Bay, 13) Perano Shoal, 14) Dieffenbach Point, 15) Te Pangu Bay, 16) Port Underwood, and 17) Bradshaw-Thompson.....118

Figure 4.2 Landcover classification for each of the five catchments: 1) Tory Channel, 2) Queen Charlotte Sound, 3) Pelorus Sound, 4) Mapua, and 5) Doubtful Sound. 122

Figure 4.3 Concentrations of THg for a) *P. novaezelandiae* and b) *P. canaliculus* among sites within regions. Means ± 1SE. Levels connected by different letter indicate significant differences ($p < 0.05$). 125

Figure 4.4 Concentrations of THg for a) *P. novaezelandiae* and b) *P. canaliculus* among sites within catchments. Means ± 1SE. Levels connected by different letter indicate significant differences ($p < 0.05$). 126

Figure 4.5 Concentrations of THg for *P. novaezelandiae* (grey) and *P. canaliculus* (black) among sites within the Marlborough Sounds. Means ± 1SE. Asterix represents significantly different levels ($p < 0.05$). 128

Figure 4.6 Concentrations of THg with a) percent forest and b) percent forestry for *P. novaezelandiae* at sites: 1) Port Underwood, 2) Queen Charlotte Sound, 3) Pelorus Sound, and 4) Doubtful Sound. Means ± 1SE. Levels connected by different letter indicate significant differences ($p < 0.05$). 131

Figure 4.7 Spatial depiction of average THg concentrations (mg kg^{-1}) for *P. novaezelandiae* at sites within Marlborough Sounds and Fiordland. Sites: 1) Old Man's Head, 2) Bird Island, 3) Waitata, 4) Motungarara Island, 5) Ruakaka Bay, 6) Perano Shoal, 7) Dieffenbach Point, 8) Port Underwood, 9) Doubtful Sound. 133

Figure 4.8 Spatial depiction of average THg concentrations (mg kg^{-1}) for *P. canaliculus* at sites within Marlborough Sounds and Tasman Bay. Sites: 1) Clay Point, 2) Bird Island, 3) Waitata, 4) Motungarara Island, 5) Ruakaka Bay, 6) Perano Shoal, 7) Te Pangu Bay, 8) Ragged Point, 9) Crousielles Harbour, 10) Delaware Bay, 11) Mapua, 12) Te Karetu, 13) Totaranui. 134

Chapter one: Introduction

1.1 Temperate reef communities under threat

Rocky reef ecosystems play a key role in the marine environment, providing habitat for ecologically, commercially, recreationally, and traditionally important flora and fauna (Dayton 1985, Shepherd & Edgar 2013). Temperate rocky reef communities, constrained to cool waters, are under a range of increasing stressors that have exacerbated the impacts on this vital marine habitat (Shepherd & Edgar 2013, Smale et al. 2013). Anthropogenic stressors, particularly warming of coastal waters, have led to global changes such as poleward shifts in temperate reef communities (Fowler et al. 2018). In addition, local stressors such as land conversion have increased sedimentation rates resulting in degradation of benthic communities and associated cascading effects on surrounding rocky reef communities (Salomon et al. 2008, Fowles et al. 2018)

Anthropogenic activity from farming, forestry, aquaculture, urbanization, and mining are all contributing to the growing threat humans pose on the world's ecosystems (Haya et al. 2001, Thrush et al. 2004, Medina et al. 2005, Morrison et al. 2009, Tallis 2009, Atkinson et al. 2014). In order to manage these detrimental impacts, it is imperative that we understand the factors that drive the spatial distribution of pollutants on a regional and local scale (Tallis 2009). Ecosystem level analysis has acquired growing attention in recent decades, therefore, resolving stressors linked with anthropogenic modifications is imperative in the context of ecosystem-based management (Minns et al. 2011, Halpern et al. 2010). The principles of ecosystem-based management are centered around considering resource interactions and connectivity within an ecosphere (Long et al. 2015). The recent focus on ecosystem-based management differs from current management practices by utilizing a multispecies approach, whereby focus is expanded on the wider perspective of ecosystem functioning, inclusive of human activity (Garcia & Cochrane 2005, Halpern et al. 2010, Cowan et al. 2012, Kraak et al. 2014, Long et al. 2015).

1.2 The role of suspension feeders in temperate rocky reef communities

Temperate rocky reefs provide an ideal habitat for suspension feeders which represent a large biomass of the organisms present from the intertidal to the subtidal (Stephenson & Stephenson 1949, Newell et al. 1982, Ricciardi & Bourget 1999, Gardner et al. 2006). Bivalves have been identified as key players in the processing of organic matter, and ecosystem engineers in their capacity to modify the environmental conditions in coastal marine systems providing a vital link between pelagic, benthic, and terrestrial habitats (Duggins et al. 1989, Gili & Coma 1997, Post 2002, McLeod et al. 2010). In this capacity they are recognized as sentinel organisms in evaluating marine ecosystem function as well as indicator species for detecting pollutants and land-based inputs (Becker et al. 1992, Kim et al. 2008). Their role as suspension feeders enables them to transport vast amounts of suspended particulate organic matter (SPOM) from the water column to the benthos providing an important channel for energy and organic matter in food webs to higher trophic levels (Tallis 2009, Wing et al. 2012, Atkinson et al. 2014).

Benthic communities on temperate coastal reefs are supported by organic matter from phytoplankton, macroalgal detritus, and terrestrial sources (Jack et al. 2009, McLeod et al. 2010a, McLeod et al. 2010b). Macroalgae, largely in the form of eroded materials from growing algal blades (e.g, Miller et al. 2006, Richards et al. 2011, Wing et al. 2007), become suspended in the water column and settle on the benthos where they are filtered by suspension feeding bivalves (Duggins et al. 1989, Krumhansl & Scheibling 2012, Wing & Jack 2012). Benthic temperate reef communities are also largely supported by phytoplankton in some regions, however, the distribution and abundance of this basal organic matter source and its incorporation into the benthic communities varies seasonally (Murphy et al. 2001, Helson et al. 2006, Wing et al. 2012).

The sedentary or sessile nature of most suspension feeding bivalves requires reliance on particulate matter available in their immediate vicinity. These resources are derived from both autochthonous and allochthonous organic matter inputs. Autochthonous organic matter entering the suspended particulate pool is derived from phytoplankton and macroalgae (Jack & Wing

2011, Miller et al. 2011) while allochthonous inputs are primarily associated with mobile species waste deposits and inputs of terrestrial particulate matter (Hyndes et al. 2014, Atkinson et al. 2014). As a result, organic matter supporting benthic consumers encompasses a range of habitats and is influenced by stressors acting across land, sea, and atmosphere (McLeod & Wing 2007, McLeod & Wing 2009, McLeod et al. 2010, Wing & Leichter 2011, Hyndes et al. 2014, Atkinson et al. 2014)

Five benthic suspension feeding bivalves were chosen for this study based on placement in water column, distance from the benthos and aggregation. All five species share the generally accepted feeding mechanism utilised by suspension feeding bivalves, cirral trapping (Møhlenberg & Riisgård 1978). Slight variations in biological and physical aspects of feeding are present among species, such as the New Zealand scallop, *Pecten novaezelandiae* (Beninger & Le Pennec 2006, MacDonald et al. 2006, Hewitt & Pildich 2004). This feeding mechanism supports filtration of large quantities of water and delivery of both pseudofaeces and waste to the benthos. Mussels, such as the horse mussel, *Atrina zelandica*, green-lipped mussel, *Perna canaliculus*, and blue mussel, *Mytilus galloprovincialis* [also knowns as *Mytilus planulatus* (Zbawicka et al. 2019)], influence hydrodynamics due to density and placement in the water column (Green et al. 1998, Nikora et al. 2002), affecting the enrichment of sediments and microbial activity on reefs (Warwick et al. 1997, Cummings et al. 2001). This results in changes to physical processes such as oxygen availability, sulphate reduction, and denitrification rates (Smaal 1991). In contrast, the scattered distributions of *P. novaezelandiae* and dense distributions of the dog cockle, *Tucetona laticostata* in soft sediment habitats up to 70-80 m^2 (Powell 1979, Kerr & Grace 2005, Marsden & Bull 2006) would have disparate hydrodynamic impacts relative to mussels. As a result, the distribution and abundance of bivalves on temperate reefs make them a key ecological species influencing ecosystem structure and function (e.g. Wing et al. 2018). The varying habitats of these five different species within temperate rocky reefs make them key species in identifying inter- and intraspecific differences among bivalve communities.

1.3 Trace metals in bivalves

Analysis of the composition and concentrations of trace metals, offers a framework in which anthropogenic impacts can be evaluated at an ecosystem-wide level. Trace metals, which are naturally present in minute quantities both in the atmosphere and Earth's crust, can be used to indicate impacts associated with a range of anthropogenic activities. For example, higher concentrations of specific trace metals are associated with anthropogenic activities such as mining, agriculture, and salmon farming (Sneddon & Tremblay 2011, Gall et al. 2015, Langston 2017). Trace metals can be classified into three categories: conservative, bioactive, and highly scavenged. The different classes reflect physical characteristics (ie. salinity, temperature), in the case of conservative metals, and biological (ie. primary productivity, toxicity), in the case of bioactive and highly scavenged metals. The differences reflected in the biochemical signatures within organisms therefore can inform on the variability of physical and biological characteristics across spatial scales (Beer et al. 2011, Jack & Wing 2011, Ruiz-Fernández et al. 2018, Wing et al. 2019).

Conservative trace metals, such as strontium (Sr), magnesium (Mg), and chromium (Cr), reflect differences in salinity (Sadiq 1992, Lannuzel et al. 2011, Wing et al. 2019). The concentrations of these metals can therefore be influenced by freshwater inputs in the environment. Bioactive trace metals, including micronutrients such as iron (Fe), and co-limiting cobalt (Co), nickel (Ni), and zinc (Zn), may be influenced by patterns in primary production as these micronutrients are taken up by primary producers (Wing et al. 2019). Concentrations of toxic, highly scavenged metals such as lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg) decline with distance from point sources. Together, patterns in the concentrations of these three classes of trace metals can provide information on biological and physical differences in the environments influencing marine species.

The toxic, highly scavenged metal, mercury is widely known for its pollutive and bioaccumulative properties (Bargagli et al. 1998, Santos & Monteiro 2007, Chouvelon et al. 2018, Clarkson & Magos 2006, Liu et al. 2011, Scheuhammer et al. 2015). Though mercury is naturally available in the environment, anthropogenic sources largely outweigh the natural production (Serrano et al. 2013). Mass poisoning associated with mercury in seafood in Minimata Bay, Japan (1959) revealed the toxic nature of mercury and the need to monitor this metal in the marine food web (Harada 1995). The neurotoxin, methylmercury (MeHg) biomagnifies up the food web, which is particularly prudent in aquatic ecosystems with the increased number of trophic interactions (Gray 2002). Mercury is widely measured in fish, however fewer studies have been conducted on commercially important bivalves (Sunderland 2007, Díez 2009, Sadhu et al. 2015).

Trace elements make their way into bivalves primarily through organic matter sources (Genç et al. 2018, Ruiz-Fernández et al. 2018). Global macroalgal testing has illustrated hotspots of anthropogenic activity through elevated trace metal signatures (Sanchez-Quiles et al. 2017). Therefore, it is likely that organic matter source pools serve as a source of contamination for consumers in their ability to act as a repository for trace metals (Tsakovski et al. 2012). Studies have been conducted on tracking trace metals through food webs from primary consumers to higher trophic levels (Fey et al. 2019), in addition to organic matter sources to higher trophic levels on coral reefs (Deudero et al. 2009, Briand et al. 2018). We take it one step further by correlating organic matter and trace metal signatures with catchment land cover classifications. Though it is not novel to identify differences in organic matter flow associated with specific land use practices (Tallis 2009), our approach enables influences associated with catchment modification to be evaluated on a large spatial scale.

Though there is some overlap in the categorization of trace metals in the literature, the above classes for individual metals remain consistent throughout the present study. The increased prevalence of trace metals impacts ecological systems due to their inability to degrade (Gall et al. 2015). It is therefore imperative to gain a greater understanding of the transmission of trace metals associated with anthropogenic pressures, particularly to sedentary benthic coastal ecosystems, as these communities fuel higher trophic levels.

1.4 Bivalves as aquatic ecosystem monitors

Bivalves have been used as indicator species in identifying the impacts of human activity since the beginning of the 20th century (Sladecek 1966, Green et al. 1989, Kim et al. 2008, Lanksbury et al. 2014). The ability of bivalves to concentrate contaminants from the environment can be used to reveal changes in water column chemistry to coastal habitats associated largely with anthropogenic stressors (Genç et al. 2018, Ruiz-Fernández et al. 2018). Continuous monitoring is imperative in order to understand changes in contamination patterns (Briant et al. 2017). Therefore, monitoring programs such as NOAA Mussel Watch and French Mussel Watch Program (RNO-ROCCH) have been set up on both a large spatial and temporal scale, tracking the spatial distribution and temporal trends of contaminants of environmental concern (O'Connor & Beliaeff 1995, O'Connor 2002, Kim et al. 2008, Briant et al. 2017).

1.5 Study Sites

Identifying anthropogenic impacts in modern times is difficult due to the extent to which the terrestrial environment has been altered. The present study was based in three regions in South Island, New Zealand, with a range of anthropogenic activities: Marlborough Sounds, Tasman Bay and Fiordland. All regions were characterized by rocky reef habitats with wave exposure at the mouth of the channel or bay, and similar benthic invertebrate and subtidal kelp forest communities (Shears & Babcock 2007, Wing & Jack 2014, Newcombe 2016). Differences in topography and freshwater inputs influenced light availability, salinity, and wave exposure, therefore, impacting patterns in primary production among each of these regions (Miller et al. 2006, Wing et al. 2007, Davidson 2014, Newcombe 2016, Udy et al. 2019a, 2019b, 2019c). In Marlborough Sounds, mixing and resuspension of sediments influences the gradient in light availability, large freshwater inputs and accumulation of sediments in the inner sounds leads to the build-up of particulates from adjoining catchments (Urlich 2015). The shallow depth of Tasman Bay, in addition to large freshwater inputs, led to high levels of sedimentation that influenced the light availability in the shallow subtidal resulting in low levels of primary production (Newcombe et al. 2015). Lastly, primary production in Fiordland is constrained by large freshwater inputs reducing light availability in the inner fiords relative to the outer fiords (Goebel et al. 2005, Wing et al. 2007). The marine environment, specifically subtidal biological communities, at the top of the South Island has been heavily impacted since the arrival of humans in the sixteenth century (Handley 2006), while regions such as Fiordland on the southern part of South Island have remained relatively pristine except for local influences from catchment modification in Doubtful Sound and access from land to Milford Sound (Tallis et al. 2004, Rutger & Wing 2006, Wing & Jack 2014). The gradient in environmental modification with overlapping marine flora and fauna provide the opportunity to investigate the effect of anthropogenic stressors on the influence of organic matter flow and trace metal accumulation reflected in suspension feeding bivalves among these three regions. Further, these results are transferable between temperate ecosystems worldwide, as New Zealand temperate rocky reef communities are not dissimilar in structure and function to those found elsewhere in temperate subtidal habitats (Stephenson & Stephenson 1949, Lipkin & Safriel 1971, Benson 2002, Harley & Helmuth 2003).

Marlborough Sounds

Marlborough Sounds are made up of a vast network of drowned river valleys covering an area of about 4000 km^2 and accounting for approximately 10 percent of New Zealand's coastline (Nicol 2011). Situated on the northeast shore of New Zealand's South Island, this region has been prone to extensive modification since the arrival of humans during the sixteenth century (Handley 2006). First, with the arrival of Mäori people, the burning of native bush (Poole & Adams 1984), and then with the settlement of Europeans and their conversion of native forest to pastures (Wilmshurst et al. 1997). The landcover changes that have been occurring for more than a century in this region have resulted in large increases in fine sediment inputs in Marlborough Sounds (Handley 2006). For example, direct run-off of fine sediment into the coastal environment of Marlborough Sounds are influenced by the extensive areas converted to pine forestry which is exacerbated during tree harvest (Urlich 2015). Therefore, it is theorized that the current composition of marine flora and fauna in Marlborough Sounds are dramatically different to what existed before landcover modification (Handley 2006).

The composition of benthic species in Marlborough Sounds has declined in the last several decades associated with sedimentation and habitat modification from dredging (Thrush et al. 2004, Morrison et al. 2009, Page & Olsen 2016, 'Temporary Closure' 2019). Marlborough Sounds have been subject to extensive scallop dredge fisheries primarily for *P. novaezelandiae* (Marsden & Bull, 2006). The scallop dredge fishery expanded up until recent decades when scallop fisheries closed for the last three seasons following a steady decline in biomass between 2009 and 2015 ('Temporary Closure' 2019).

Tasman Bay

Tasman Bay is characterized by a gently sloping sandy substratum across a shallow depth gradient (max. 50 m) (Mitchell 1986, Handley 2006). The patches of rocky reef kelp forest habitat primarily follows the 1-10 m depth contour, up to 4 km from shore (Handley 2006). A rich history of human settlement and modification has been present in Tasman Bay since the arrival of Mäori peoples in the sixteenth century and European settlers two centuries later (Handley 2006). Human arrival exposed this region to heavy deforestation, exploitation of marine fauna, and extensive landcover alterations in the catchments that dramatically alter the Tasman Bay's subtidal habitats (Handley 2006). In a similar pattern to that observed in Marlborough Sounds, subtidal communities in Tasman Bay are highly modified from documented in the 1960's due to the frequent flooding and heavy sediment loading (McKnight 1969, Handley 2006). The large freshwater inputs from Waimea, Wakapuaka, and Motueka rivers have resulted in extensive sediment plumes that extend across the bay (Newcombe et al. 2015). Sediment both as a direct effect and indirect influence on primary production, in combination with overfishing of the dredge fishery has decimated the scallop fishery in Tasman Bay. The fishery has been in decline for nearly a decade despite reseeding efforts ('Temporary Closure' 2019). It is theorized that the elevated sedimentation has yielded the habitat in Tasman Bay unfavorable to scallop settlement, resulting in recruitment failure ('Temporary Closure' 2019).

Fiordland

Doubtful Sound is located in the Fiordland region on the southwestern coast of New Zealand's South Island. The fiord is characterized by steep valley walls and deep basins formed through glacial carving (Suggate 1990). Fiordland has been a reserve since 1904 and is part of the Te Wahipounami UNESCO World Heritage Area. As a result, fiords such as Doubtful Sound are characterized as relatively pristine with little impact from land-based stressors (Tallis et al. 2004) and intact catchments of native forest (Wing & Jack 2014). However, the development of the Manapouri Hydroelectric Power Plant has caused dramatic changes in freshwater inputs and the water column chemistry in Doubtful Sound, influencing benthic community composition, particularly bivalves (Tallis et al. 2004, Rutger & Wing 2006, Wing & McLeod 2007, McLeod & Wing 2008). Though extensive modification of freshwater inputs exists within Doubtful Sound, the forested catchments and low sediment inputs support a diverse kelp forest community (Miller et al. 2006, Wing et al. 2007, Jack & Wing 2013, Wing & Jack 2013, Wing & Jack 2014, Udy et al. 2019).

1.6 Objectives

Anthropogenic impacts such as forestry, aquaculture and mining influence coastal ecosystem connectivity through land-based influences on biogeochemical fluxes in the food web. Accordingly, resolving these influences in bivalve communities will help inform the effective application of ecosystem-based management of coastal communities. The present study aimed to identify the influence of anthropogenic stressors in driving organic matter flow and trace metal concentrations within bivalve communities in Marlborough Sounds, Tasman Bay, and Fiordland.

The objectives were to establish if differences in feeding strategies among bivalve species, particular land cover management practices, and/or aquaculture systems explained the differences in use of alternative organic matter sources and trace metal concentrations reflected in the soft tissues of bivalve species. Initially, we investigated the influences of different feeding mechanisms among species on the uptake of alternative basal organic matter (phytoplankton versus macroalgae). Further, we explored the variations in composition of basal organic matter supporting bivalve communities associated with catchment modification and presence of aquaculture systems. In order to resolve spatial difference in trace metal concentrations, we tracked trace metals through basal organic matter source pools in addition to influences associated with a range of catchment modification and presence of aquaculture systems. Lastly, as mercury concentrations have seldom been recorded for commercially important bivalves in the South Island fisheries, we reported baseline mercury concentrations in addition to spatial variations associated with catchment modification. The contrast of the relatively developed catchments surrounding Marlborough Sounds and Tasman Bay with Fiordland, which has a relatively pristine forested catchment, provided an excellent system for understanding the link between anthropogenic activity and the contaminant loads reflected in coastal bivalves. Monitoring these coastal bivalves provides essential insight into the fluxes of particulate organic matter throughout food webs and their implications for ecosystem health.

The data chapters outlined below have been written as stand-alone papers. Therefore, overlap in content among chapters is present primarily in the Introductions and Methods sections. Chapter 2 will be submitted to Science of the Total Environment, as it explores the interactions between organic matter inputs between the land and coastal habitats. Chapter 3 will be submitted to Estuarine Coastal and Shelf Science as a follow up paper to Chapter 2, as it investigates the flow of trace metals relative to land-based inputs to coastal habitats. Chapter 4, as an initial recording of mercury concentrations of commercial bivalve species, will be submitted to New Zealand Journal of Marine and Freshwater Research.

Chapter 2: Influences of land modification and salmon farms on resource use of suspension feeding bivalves

2.0 Introduction

Suspension feeders represent a large biomass of the organisms present from the intertidal to subtidal habitats of coastal temperate reefs (Newell et al. 1982, Ricciardi & Bourget 1999). As important modifiers of the water column particulate pool, the group is a key ecosystem engineer in coastal environments. They are an integral part of the benthic food web, providing a direct coupling between pelagic pools of suspended particulate organic matter (SPOM) and benthic habitats (Duggins et al. 1989, Gili & Coma, 1998).

As a group, suspension feeders have been considered as opportunistic feeders (Coma et al. 2001). Nevertheless, competition for resources by sessile suspension feeders has led to diverse life forms, feeding behaviors and diet composition within a community (Riisgard & Larsen 2001, Tallis 2009, Wing & Jack 2012). Resource exploitation can vary significantly in adjacent species, illustrating the partitioning of resources within a localized area (Tallis 2009, Wing & Jack 2012). As a result, both habitat partitioning and differences in feeding mode can separate suspension feeders into distinct niches utilizing different components of the suspended particulate pool (e.g. Wing & Jack 2012).

Phytoplankton and macroalgae provide important components of suspension feeder diets as they both contribute to the suspended particulate organic matter pool (Bustamante & Branch 1996, Duggins & Eckman 1997, Salomon et al. 2008). Interspecific variation in resource use by primary consumers among alternate basal organic matter sources has been commonly observed (e.g. Fry et al. 1999, Bolnick et al. 2003, Wing & Jack 2012) illustrating plasticity in diets of consumers across the spectrum of primary producers, providing multiple channels for energy and organic matter to higher trophic levels (Fredriksen 2003, Hill et al. 2006, Nilsen et al. 2008, Tallis 2009, Wing & Jack 2012). Estimates of the extensive contribution of organic matter derived from macroalgae supporting suspension feeders has been challenged by Miller and Page (2012), who proposed that kelp based organic matter sources, upon which diets are calculated, were over-represented in mixing models. Miller and Page (2012) note that the large body of work hypothesising the importance of kelp detritus in coastal suspension feeders, known as the kelp detritus hypothesis (KDH), overlooks the inputs contributed by the often under resolved phytoplankton stable isotopic signatures. The study notes the inconsistency with which basal organic matter sources are collected relative to the study sites and temporal periods of consumer collection. Further, the study underlines the importance of well resolved basal organic matter signatures and testing which is lacking in many studies (Miller & Page 2012). The present study evaluates site-specific macroalgae and SPOM stable isotopic values within the Marlborough Sounds in order to illustrate the potential differences in basal organic matter signatures within a region. The difficulty with which phytoplankton samples can confidently be determined from water samples was combated through evaluation of each filter under a microscope prior to analysis (Udy et al., 2019).

Suspension feeders filter large quantities of seawater and can thus regulate phytoplankton production and water turbidity (Officer et al. 1982, Newell et al. 2007). Therefore, inter- and intra- specific variation in organic matter supporting suspension feeders can likely be tied to differences in localized mixtures of basal organic matter sources which support both benthic food webs and variation in feeding mechanisms among species. Further understanding of the mechanisms behind feeding modes is imperative in order to gain a greater understanding of the organic matter fueling benthic food webs. Naturally occurring differences in stable isotopic values (δ^{15}N and δ^{13}C) of basal organic matter sources, if left unresolved, further obscure our ability to determine the effects of anthropogenic impacts on coastal habitats (Fry & Allen, 2003, Hoffman et al. 2012, Howarth et al. 2012, Wing et al. 2008, Jack & Wing 2011).

Feeding modes of different species

Cirral trapping is the generally accepted mechanism through which bivalve's suspension feed (Møhlenberg & Riisgård 1978). Water containing suspended particles is driven by bands of cilia through interfilamentary canals within the gills and captured on mucus membranes subsequently delivered to the mouth and sorted with labial palps. The sea scallop, *Pecten novaezelandiae*, feed using a variation of cirral trapping, they feed through non-siphonate, ciliary suspension gill structures which filter particles within mucous sheets along the mantle (Beninger

& Le Pennec 2006, MacDonald et al. 2006). Aside from slight variations in the feeding mechanisms among bivalve species, the composition of particles captured and ingested is also influenced by particle size, organism distance from the benthos, inorganic particulate concentrations, and water current strength. For example, filter feeding by *P. novaezelandiae* is limited to high flow conditions (Wildish et al. 1987, Claereboudt et al. 1994). *Pecten novaezelandiae, M. galloprovincialis*, *P. canaliculus*, and *Atrina zelandica* feeding on suspended particulates is also limited in high sediment conditions where sediment clogs the mucus net feeding mechanism (Eckman et al. 1989, Gardner 2000, Pilditch et al. 2001, Hewitt & Pildich 2004). Further, each of the species develop in different habitats where horse mussel, *A. zelandica*, has an upright habit extending beyond the direct sediment surface and mussels *M. galloprovincialis* and *P. canaliculus* are found in dense clusters can therefore alter local hydrodynamics influencing the available organic matter sources to these mussels (Green et al. 1998, Nikora et al. 2002). In contrast, *P. novaezelandiae* and *T. laticostata* feed at the water boundary layer where organic matter settles on the sea floor. Therefore, the habitat of each bivalve species likely influences the benthic-pelagic coupling.

Anthropogenic impacts

Global ocean primary production is being altered by anthropogenic impacts, including nutrient loading, sedimentation, and climate change (Johnson et al. 2011, Koenigstein et al. 2016, Krumhansl et al. 2016). Accordingly, land-based pollution influences benthic marine communities at a global scale (Airoldi & Beck 2007, Edgar & Barrett 2000, Vitousek et al. 2007, Fowles et al. 2018). Impacts of land conversion, intensive farming, and urban development have strongly modified many coastal landscapes, subsequently also altering delivery of nitrogen (Vitousek et al. 1997, Rabalais & Turner 2001) and carbon (Howarth et al. 1991, Smith & Hollibaugh 1993, Correll et al. 2001) to the adjacent marine environment. These impacts are exacerbated by over exploitation of of bivalve filter feeding communities, which can have cascading effects on surrounding rocky reef communities (Fowles et al. 2018, Salomon et al. 2008). Impacts such as forestry have been associated with enriched nitrogen isotopic values in filter feeders, likely due to extensive fertilizer application (Tallis 2009).

Due to the differences in acquisition of inorganic carbon, primarily uptake of CO_2 vs HCO_3, organic matter derived from phytoplankton and macroalgae can be discriminated by characteristic values of $\delta^{13}C$ (e.g. Jack & Wing 2011, Cornelisen et al. 2007). Stable isotope analysis has been applied to determine differences in trophic ecology of benthic organisms and has been used to resolve differences in resource utilization of suspension feeders in the same community (Dubois et al. 2007, Tallis 2009, Wing & Jack 2012). The technique therefore offers a robust and repeatable method for determining contributions of alternative organic matter and nutrient sources to coastal communities.

Regions of study

The present study was based in three contrasting regions, in terms of land-based stressors, on the South Island, New Zealand: Marlborough Sounds, Tasman Bay and Fiordland. The rocky reef habitats harbor benthic invertebrate and macroalgae communities that are comparable across all three regions (Shears & Babcock 2007, Udy et al. 2019a, 2019b, 2019c). The regions experience different levels of wave exposure, Tasman Bay is subject to moderate exposure, while Marlborough Sounds and Fiordland are comparatively sheltered (Shears & Babcock 2007, Wing & Jack 2014).

These three regions provide an ideal study system in which to contrast the likely influences of land-based stressors on organic matter sources supporting suspension feeding communities across a gradient of anthropogenic impacts. The marine environment, specifically subtidal biological communities, at the top of the South Island has been heavily impacted since the arrival of humans in the sixteenth century (Handley 2006), while regions such as Fiordland on the southern part of South Island have remained relatively pristine (Tallis et al. 2004, Wing & Jack 2014).

The catchments that surround each of the study regions are markedly different (Figure 2.2). While a high proportion of landcover is dominated by forest, Tasman Bay and Marlborough Sounds have experienced landcover alteration since the sixteenth century (Handley 2006) and are impacted by farming, forestry and urban developments. In contrast, the relatively pristine Fiordland region, which was established as a reserve in 1904 and gained National Park status in 1952, is characterized by intact catchments of native forest (Miller et al. 2006, Wing & Jack 2014). The amount of freshwater delivered into the inner parts of Doubtful Sound have been greatly increased by the building of the Manapouri Hydroelectric Power Plant. Consequentially, bivalve populations have been modified in these inner fiord regions (Jack et al. 2009, Tallis et al. 2004, Rutger & Wing 2006), in addition to changes in circulation (Sköld et al. 2003), productivity of habitats, and food web structure (Sköld et al. 2003, Rutger & Wing 2006, Jack et al. 2009, Jack & Wing 2011). In the present study we therefore focused on collections in the adjoining Thompsons-Bradshaw Sounds.

Here we provide a well-resolved systematic illustration of the contribution of organic matter derived from macroalgae and phytoplankton to benthic suspension feeders in Tasman Bay, Marlborough Sounds and Fiordland. We compare patterns observed in the composition of organic matter pools with geographic patterns in freshwater sources and landcover classifications, providing an analysis of correlations between the two spatial patterns. Understanding the sources and movement of land-based particulates and their impacts on basal organic matter sources in marine communities is important for pinpointing potential adverse effects pertinent to developing robust ecosystem-based management and mitigation strategies in coastal communities and aquaculture activities (Fowles et al. 2018).

2.1 Methods

2.1.1 Study organisms

In order to identify differences in nitrogen and carbon stable isotopic ratios (δ^{13}C and δ^{15}N) to benthic suspension feeding bivalves among the study regions, five species of bivalves ranging from intertidal rocky reef species to free living and benthic infaunal species were selected from areas with a range of landcover classifications (Figure 2.1).

Horse mussels, *Atrina zelandica* (Gray 1835) reside in the subtidal to depths of ~50m in habitats characterized by muddy to sandy benthos (Powell 1979). This is a large bivalve species, growing to ~30cm. *A. zelandica* has an upright habitat with approximately two thirds of its length buried in the sediment anchored to hard substratum with byssus threads (Powell 1979). The distribution of *A. zelandica* generally form dense patches (eg. >10 m^{-2}) particularly in high flow areas with fine sandy sediment (Cummings et al. 2001). Due to the habitat, this species feeds at roughly 15-20 cm above the seafloor, with feeding currents that extend across the benthic boundary layer.

The New Zealand scallop, *Pecten novaezelandiae* (Reeve 1853, pectinid), is the largest and most abundant species of scallop found in New Zealand (Marsden & Bull 2006). The endemic bivalve is generally found in beds scattered from estuaries to coastal bays and reefs at densities of >1 m^{-2} to depths from subtidal to 80 m (Marsden & Bull 2006). The species has a wide distribution in New Zealand, found from the top of the North Island to the bottom of the South Island (Marsden & Bull 2006, Carbines & Michael 2007). Scallops feed through non-siphonate, ciliary suspension gill structures where they filter particles within mucous sheets along the mantle margin (Beninger & Le Pennec 2006, MacDonald et al. 2006). Feeding ability for pectinids appears to be limited in high flow conditions (Wildish et al. 1987, Claereboudt et al. 1994) in addition to low flow conditions and high presence of seston (Eckman et al. 1989, Pilditch et al. 2001).

The dog cockle, *Tucetona laticostata* (Quoy and Gaimard, 1835) is generally found in higher energy environments, mostly buried in coarse sand or gravel, in a depth range from 2-3 m to about 70 m of water (Powell 1979). The thick-shelled bivalve has a wide distribution extending from the top of the North Island to the bottom of the South Island, forming dense patches of up to more than 80 individuals m^{-2} (Powell 1979, Kerr & Grace 2005). Aside from local abundance and distribution data and paleontological research, few studies have been conducted on the biology and ecology of *T. laticostata* in comparison of many of its neighboring bivalve constituents.

The green-lipped mussel, *Perna canaliculus* are commonly found in dense patches of 160 m^{-1} in farms (Forest & Atalah, 2017) filtering particulate matter from the subtidal and open coast down to 50 m depths, but may extend to the intertidal region, where they play a large role in the temperate coastal community (Powell 1979, Morton & Miller 1968, Gardner 2000). The species often provides habitat for other sessile invertebrates that adhere to its shell. *P. canaliculus* are an endemic species with a wide distribution around New Zealand (Morton & Miller 1968). Studies have indicated that the diet of *P. canaliculus* varies seasonally depending on the presence of phytoplankton and macroalgal abundance, and their relative contribution to the suspended particulate pool (Gardner 2000). Further, feeding is negatively impacted by low quality suspended particulate matter where the rate of particulate consumption decreases with low quality suspended particulates (Gardner 2000).

The blue mussel, *Mytilus galloprovincialis*, has a wide distribution around New Zealand from the top of the North Island to the tip of the South Island (Powell 1955, Shinen & Morgan 2009). This intertidal mussel forms dense patches on rocky substrate at ~1000 m^{-2} (Porri et al. 2008) and will select organic over inorganic particulates (Hawkins et al. 1996). Further, the absorption of ingested particulates is influenced by periodic fluctuation in the available suspended particulate matter and negatively impacted by low quality suspended particulate (Bayne et al. 1987, Navarro et al. 1991, Iglesias et al. 1996, Gardner 2000). Blue mussels have specialized digestive enzymes that allow them to digest bacteria trapped by mucus filter feeding.

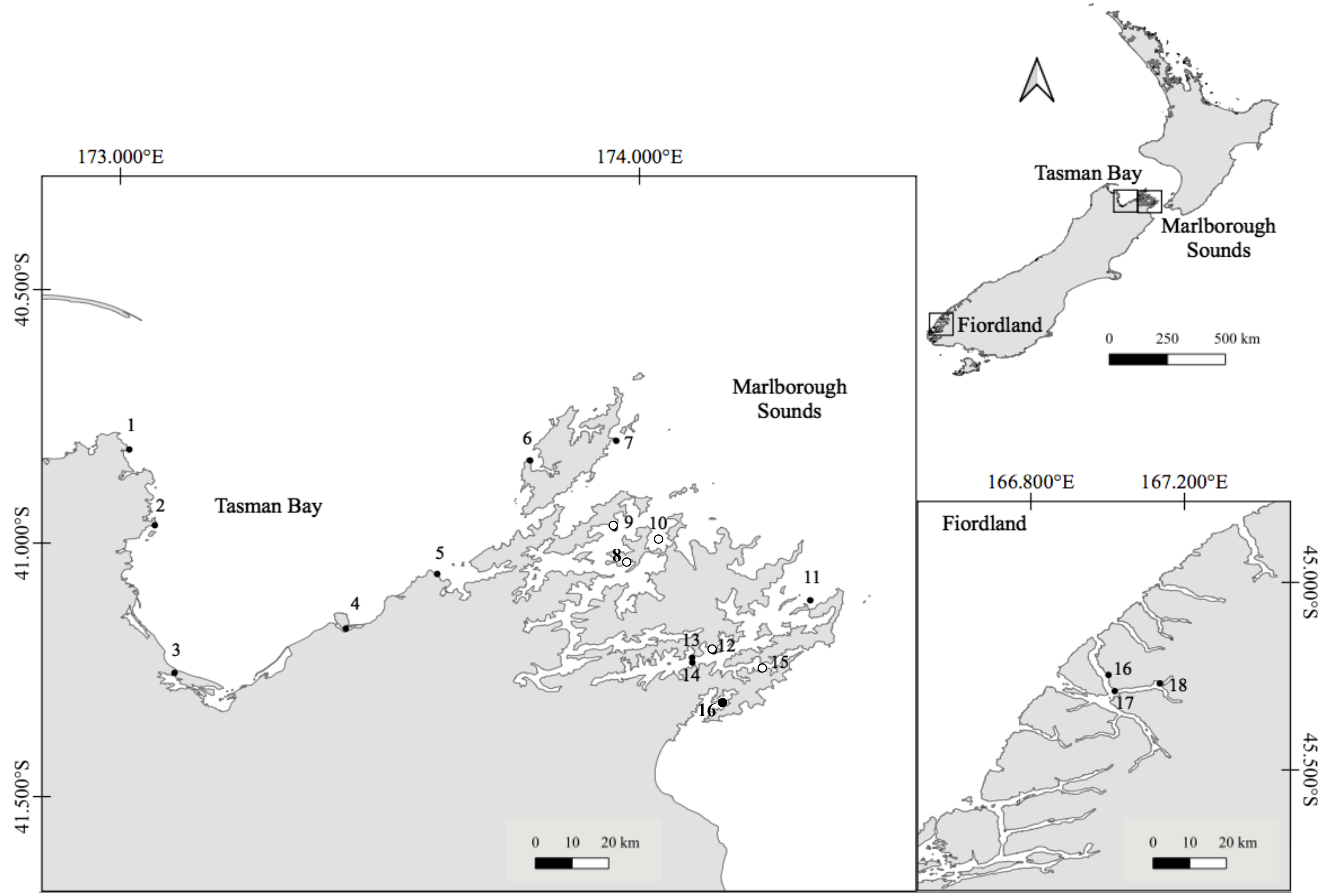

Figure 2.1 Sample sites within each region: Tasman Bay: 1) Totaranui, 2) Te Karetu Point, 3) Mapua, 4) Delaware Bay, 5) Crousielles Harbour, 6) Ragged Point. Marlborough Sounds: 7) Old Man's Head, 8) Kauaroa, 9) Waitata, 10) Bird Island, 11) Motungarara Island, 12) Ruakaka Bay, 13) Perano Shoal, 14) Dieffenbach Point, 15) Te Pangu Bay 16) Port Underwood. Doubtful Sound: 16) Crayfish Creek, 17) Bradshaw-Thompson, and 18) MacDonald Island. White points indicate salmon farms within Marlborough Sounds.

2.1.2 Sample collection

Collections of bivalves were made by scuba divers in Marlborough Sounds during February 2018 and November 2018 from the RV *Polaris* II with sampling sites spanning four regions within Marlborough Sounds: Tory Channel, Queen Charlotte Sound, and Pelorus Sound (Figure 2.1).

Bivalve collections were made by scuba divers from Tasman Bay during February 2018 from the RV *Polaris* II with sites encompassing the perimeter of the bay (Figure 2.1).

The sites from the top of the South Island, Marlborough Sounds and Tasman Bay, are associated with catchments with a range of landcover categories: forest, urban development, forestry, farming, and aquaculture systems (Figure 2.1, Appendix 1). Sites were chosen along the gradient with increasing distance from the mouth of the Sounds in order to compare sites with increased influence of land-based run off from major surrounding catchments.

Bivalve collections were made by scuba divers in Fiordland during February 2019 from the RV Typhoon. Site selection was focused on sampling sites in Thompson's Bradshaw Sound adjacent to Doubtful Sound and were constrained to the 20 m depth contour.

Target species were collected by scuba divers from eighteen sites within Marlborough Sounds (9), Tasman Bay (6) and Fiordland (3) (Figure 2.1). Samples were collected by hand at each site and placed into mesh bags under the New Zealand Ministry of Primary Industries special permit (644-2). Samples were then placed in individual tanks on-board the RV *Polaris* II with a constant surface water flow for 48 hours to allow sediment to be expelled. Samples were then placed in Zip-Lock plastic bags, labeled and frozen until dissection took place at Portobello Marine Laboratory, University of Otago. The number of samples collected was constrained by the abundance of each species at each collection site, therefore the largest sample sizes were from the most abundant species (Table 2.1 – 2.3).

SPOM and macroalgae were collected from the same sites within the Marlborough Sounds. SPOM was collected using a 20µm plankton net towed vertically from 15m to the surface. Samples were subsequently passed through a 250µm filter to eliminate zooplankton and microscopically examined to verify samples were composed primarily of phytoplankton (Udy et al. 2019c). Macroalgal blade tissue samples were taken for dominant species at each site for multiple individuals (n=5) stratified by depth.

2.1.3 Stable isotope analysis

Samples were dissected at Portobello Marine Laboratory, University of Otago, in a trace metal clean laminar flow cabinet (Gelman, HLF series). For each sample a 0.5 g sample of adductor muscle tissue was taken from each sample and placed into a 1.5 ml centrifuge vial. In order to access tissue samples, the left and right valves were separated at the ventral margin using an acid washed ceramic knife and teflon forceps. A piece of adductor muscle tissue was then detached from the posterior end of the bivalve. Tissue samples were frozen for 48 hr at -18 ˚C and subsequently freeze-dried at -60 ˚C for 48 hr (mrc, FDL-10N-50 series). Samples were ground to a fine powder in a mortar and pestle that was cleaned with ethanol (EtOH) between samples to limit cross contamination. Samples of SPOM and macroalgae were processed in the same manner as outline above. Adductor mussel subsamples of 1.0 mg ± 0.1 mg were weighed into 3.5 x 3.0 mm tin capsules and 3 mg subsamples of SPOM and macroalgal were sealed for stable isotopic analysis of $\delta^{15}N$ and $\delta^{13}C$ in the Department of Chemistry, University of Otago.

Samples of adductor mussel, SPOM and macroalgae were analyzed on the Europa 20-20 updated mass spectrometer (Europa Scientific) interfaced with Carlo Erba NA1500 Elemental analyzer (NA1500, Carlo Erba, Milan, Italy) in continuous flow mode (precision: 2‰). The raw sample isotopic ratios were standardized by calibrating against international standards (USGS-40 and USGS-41). In-house laboratory reference material (EDTA-OAS, $\delta^{13}C = -38.93‰ \pm 0.2$, $\delta^{15}N = -0.73 \pm 0.12$) of known carbon and nitrogen isotopic values were measured after every twelfth sample to correct for instrument drift. To ensure obtained isotopic values were not affected by instrumental drift, accuracy and precision for sequential duplicates of every 10th sample was determined by calculating root mean squared error (RMS) (Babaranti et al. 2018).

Sequential duplicate estimates of precision ranged from 0.02 – 0.40. The standard errors of δ^{13}C and δ^{15}N were calculated for each species by region (Table 2.1 – 2.3). Stable isotope ratios are reported in standard delta notation (Peterson & Fry 1987) relative to isotopic ratios of the international reference standards, δ^{15}N (atmospheric air) and δ^{13}C (Vinnea Pee Dee Belemnite).

C:N mol ratio ranged from 3.5 to 5.0 among bivalve species, these values are over the threshold where lipid extraction should be performed (Post et al. 2007). Due to the interference of lipids, decreasing the δ^{13}C, they were mathematically transformed according to the equation from Post et al. 2007:

$$\text{Corrected } \partial^{13}\text{C} = \partial^{13}\text{C} - 3.32 + 0.99 \text{ x } (\text{C:N})$$

Table 2.1 Isotopic values (δ^{15}N and δ^{13}C) of the bivalves collected from Marlborough Sounds, New Zealand.

Species	Common name	Mäori name	**δ^{15}N ± SE**	**δ^{13}C ± SE**	Proportion macroalgae **± SE**	N
Atrina zelandica	Horse mussel	Kua kua	9.85 ± 0.96	-16.29 ± 1.12	0.911 ± 0.117	43
Mytilus galloprovincialis	Blue mussel	Kua kua	9.43 ± 0.81	-17.30 ± 0.38	0.832 ± 0.126	41
Pecten novazelandiae	Scallop	Hururoa	9.15 ± 0.83	-16.14 ± 0.93	0.948 ± 0.092	88
Perna canaliculus	Green-lipped mussel	Kuakua	10.08 ± 0.73	-16.95 ± 0.62	0.888 ± 0.118	43
Tucetona laticostata	Dog cockle	Hururoa	9.48 ± 0.96	-16.93 ± 0.75	0.942 ± 0.076	51

Table 2.2 Isotopic values (δ^{15}N and δ^{13}C) of the bivalves collected from Tasman Bay, New Zealand.

Species	Common name	Mäori name	**δ^{15}N± SE**	**δ^{13}C ± SE**	Proportion macroalgae **± SE**	N
Atrina zelandica	Horse mussel	Kua kua	10.29 ± 0.75	-18.62 ± 0.32	0.521 ± 0.033	5
Mytilus galloprovincialis	Blue mussel	Kua kua	8.7 5± 1.03	-19.12 ± 0.99	0.487 ± 0.147	21
Perna canaliculus	Green-lipped mussel	Kuakua	9.42 ± 0.70	-18.96 ± 0.63	0.496 ± 0.100	39
Tucetona laticostata	Dog cockle	Hururoa	9.87 ± 1.22	-17.98 ± 0.49	0.638 ± 0.058	39

Table 2.3 Isotopic values (δ^{15}N and δ^{13}C) of the bivalves collected from Fiordland, New Zealand.

Species	Common name	Mäori name	**δ^{15}N± SE**	**δ^{13}C ± SE**	Proportion macroalgae **± SE**	N
Tucetona laticostata	Dog cockle	Kuakua	8.45±0.26	-17.33± 0.20	0.833 ± 0.056	6
Atrina zelandica	Horse mussel	Kua kua	9.13±0.59	-18.31± 0.43	0.807 ± 0.080	5
Pecten novazelandiae	Scallop	Hururoa	7.69±0.53	-18.28± 0.22	0.992 ± 0.014	8

2.1.4 Stable isotopic mixing model

Average values of δ^{15}N and δ^{13}C for SPOM and macroalgae derived organic matter were calculated at the site level where possible, when site level averages where not achieved, regional averages were calculated (Table 2.6). For all bivalves, a conservative one-step procedure was used to determine the composition of basal organic matter based on δ^{15}N and δ^{13}C for individuals by site (adjusted from Jack & Wing 2011). Relative contribution of dominant brown macroalgae and SPOM for individual samples was calculated from δ^{13}C from an individual-based 2-source mass balance model for the assumed primary consumer at trophic level 1 (after Phillips and Gregg 2001).

Sensitivity analyses were carried out to test the model's sensitivity to the assumed base δ^{13}C values for macroalgae and SPOM. δ^{13}C data was corrected with plus and minus one and two standard errors from the mean base δ^{13}C values for macroalgae and SPOM.

2.1.5 Landcover classification

The watersheds surrounding Marlborough Sounds, Tasman Bay and Fiordland were divided into the following discrete landcover categories based on proportion landcover: forest, urban, forestry, and farming (Figure 2.2). Presence and absence of salmon farms was also noted for Marlborough Sounds. Landcover information was provided by Land Information New Zealand (LINZ, 2018) and Marlborough District Council (Appendix 2). QGIS maps were constructed by overlaying survey data using the spatial referencing system NZGD 2000.

Catchment areas, defined as the terrestrial region contributing terrigenous particulate to adjacent marine systems, were defined for each sample site by drawing polygons in QGIS and calculating the 2D area (km^2). Boundaries of the catchment were determined using both topographic contours (1 cm : 2.5 km), 8 m digital elevation models and sea-draining catchment layers (LINZ, 2018).

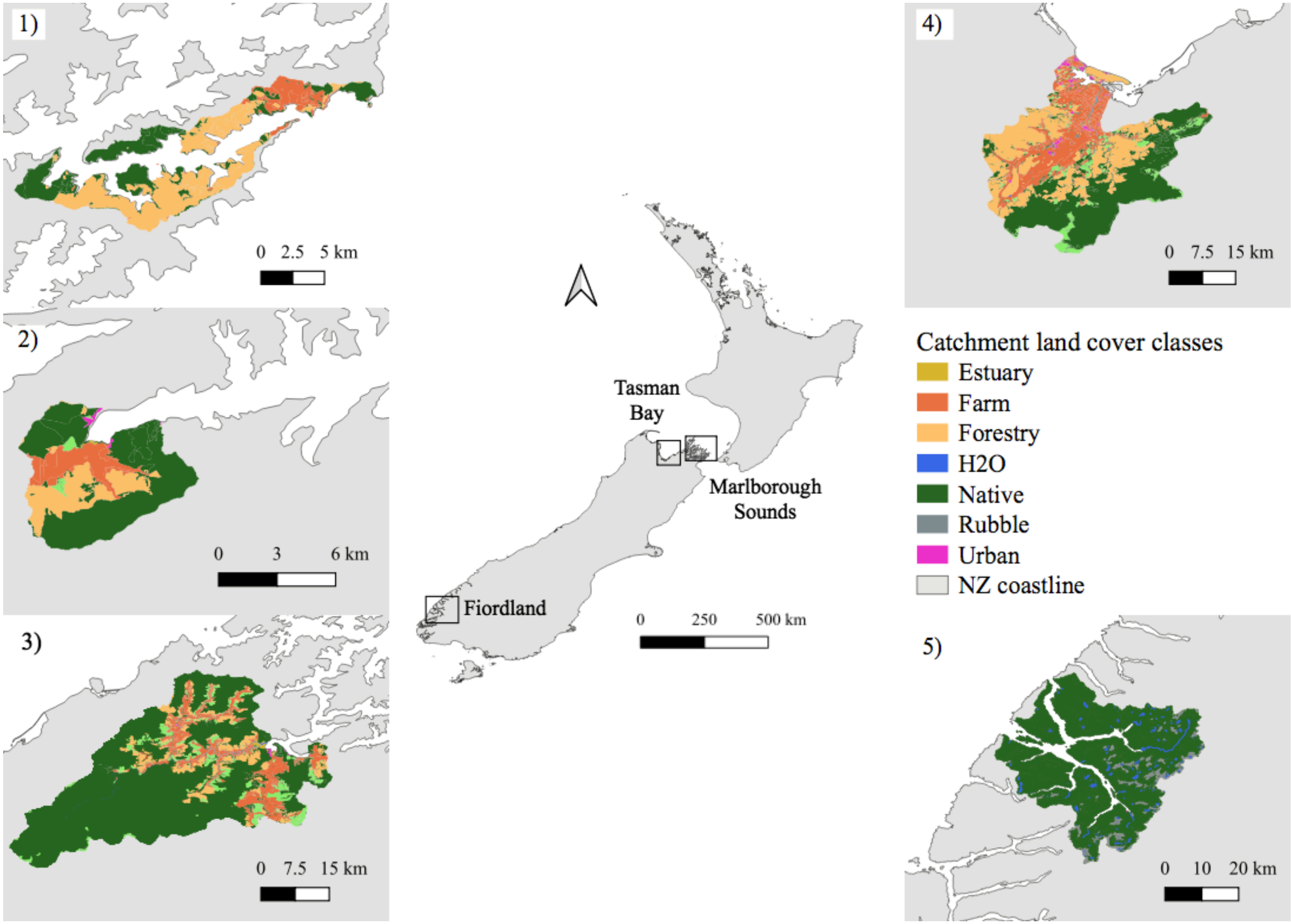

Figure 2.2 Landcover classification for each of the five catchments: 1) Tory Channel, 2) Queen Charlotte Sound, 3) Pelorus Sound, 4) Mapua, and 5) Doubtful Sound.

2.1.6 Statistical analysis

Spatial variation in nitrogen and carbon stable isotopic signatures

Generalized linear models (GLM) with the Standard Least Squares personality in the Fit Model platform in JMP (JMP 14.0 SAS) were used to test the effect of REGION [3 levels, fixed] for individual species on values of δ^{13}C and δ^{15}N. A further GLM was used to test the effect of REGION [3 levels, fixed], SPECIES [2 levels, fixed], SIZE [continuous], and the interaction REGION* SPECIES for *A. zelandica* and *T. laticostata,* present across all regions. Post-hoc tests using Student's t determined where differences among region and species occurred. As size was not taken into account on collection and showed significant correlation with δ^{13}C, size was incorporated as a continuous variable into all subsequent models to account for variability associated with bivalve size (Appendix 3).

Further GLMs were used to test the effect of REGION [3 levels, fixed] and SPECIES [5 levels, fixed] on the proportion of organic matter derived from macroalgae. Separate analyses were run to test the effect of SITE and SPECIES for overlapping species within the Marlborough Sounds. First, at the site where all species were present, we tested the effect of SPECIES [5 levels, fixed]. Second, at sites where the majority of species were present, we tested the effect of SITE [3 levels, fixed], SPECIES [4 levels, fixed], and the interaction between the factors on the proportion of organic matter derived from macroalgae. Lastly, for the species, *A. zelandica*, present at the majority of sites we tested factors SITE [10 levels, fixed] on the proportion of organic matter derived from macroalgae. Post-hoc tests using Student's t were used to determine where differences for site and species occurred.

Salmon farm

To test if the composition of basal organic matter used by bivalves varied significantly with the presence and absence of salmon farms within Marlborough Sounds, factors FARM [2 levels, fixed], SPECIES [5 levels, fixed], and the interaction between the two factors were analyzed using a GML. Post-hoc tests using Student's t were used to determine intraspecific differences in the proportions of organic matter derived from macroalgae between farm and similar reference sites.

Landcover classification

To test if the composition of basal organic matter used by bivalves varied significantly with the different landcover classifications among catchments the factors CATCHMENT (5 levels, categorical) were analyzed for individual species using a GLM. To test if proportion of organic matter derived from macroalgae for different species varied significantly with landcover classes, factors [LANDCOVER, 4 levels, categorical] were analyzed for individual species using a GLM. Size was incorporated as a continuous variable into the models to account for variability in feeding associated with bivalve size within species. Post-hoc tests were run using Student's t to determine where significant differences in proportion of organic matter derived from macroalgae occurred among catchments and landcover classes.

2.2 Results

Stable isotopic analysis of δ^{13}C and δ^{15}N for five species of bivalves was applied to identify differences in the organic matter sources supporting species at sites in Marlborough Sounds, Tasman Bay and Fiordland. Associations between landcover practices and proportion of alternative organic matter sources are indicative of a likely response of changes in bivalve feeding ecology and supply of particulate organic matter due to different landcover practices, presence of salmon farms and regional differences of freshwater inputs.

2.2.1 Organic matter source pools are variable at site and region level

The potential organic matter sources, macroalgae and SPOM, were well discriminated by δ^{13}C and δ^{15}N among both site and region (Table 2.6). Overall highest δ^{15}N and δ^{13}C for macroalgae was from Tasman Bay and lowest was from Doubtful Sound. Highest δ^{15}N for SPOM was in Marlborough Sounds, while lowest recorded was in Doubtful Sound. Highest δ^{13}C for SPOM was recorded in Doubtful Sound and lowest was in Tasman Bay. Moreover, organic matter source pool signatures varied at the site level within Marlborough Sounds. Values for macroalgae ranged between (δ^{15}N: 4.71 to 8.16) and (δ^{13}C: -19.67 to -15.22). Similarly, values for SPOM ranged from (δ^{15}N: 5.60 to 7.17) and (δ^{13}C: -22.52 to -21.74). The range of macroalgal and SPOM stable isotopic values of δ^{15}N and δ^{13}C illustrated the importance of resolving site specific stable isotopic values of organic matter source pools (Wing et al. 2008). Though macroalgae and SPOM are the main organic matter sources investigated in the present study, it should also be noted that alternate potential sources of organic matter include terrestrial inputs and bacterial activity which has been shown to support benthic food webs in some regions (McLeod & Wing 2007, McLeod et al. 2010a). The values of terrestrial organic matter sources including bacterial recycling typically range between (δ^{13}C ~ -29) and (δ^{15}N: ~ -2) (McLeod et al. 2010a). For examples, studies conducted by Wing et al. (2008) and McLeod et al. (2010a) illustrate the importance of terrestrially derived forest litter in supporting benthic food webs in Fiordland. No studies at present reveal terrestrially derived forest litter supporting benthic food webs in Marlborough Sounds or Tasman Bay.

Table 2.4 Potential organic matter source supporting suspension feeding bivalves. Site specific averages are provided where available. (-) indicates no data present.

Region	Site	Macroalgae **δ^{15}N± SE**	Macroalgae **δ^{13}C ± SE**	SPOM **δ^{15}N± SE**	SPOM **δ^{13}C ± SE**
Marlborough Sounds	Bird Island	5.10 ± 0.64	-18.14 ± 1.88	-	-
	Waitata	4.71 ± 0.33	-19.67 ± 0.27	7.17 ± 0.66	-21.74 ± 0.44
	Kauaroa	5.43 ± 0.92	-19.23 ± 0.70	7.09 ± 0.59	-21.43 ± 0.32
	Motungarara Is.	6.88 ± 0.61	-15.56 ± 4.65	5.60 ± 1.34	-22.20 ± 0.34
	Ruakaka Bay	7.70 ± 0.64	-15.99 ± 0.67	7.06 ± 0.62	21.96 ± 0.87
	Dieffenbach Point/ Perano Shoal	6.55 ± 1.06	-16.94 ± 3.90	6.61 ± 1.06	-22.34 ± 0.81
	Te Pangu Bay	6.37 ± 0.37	-17.98 ± 1.54	6.59 ± 1.28	-22.40 ± 0.66
	Te Ahi Ihi	6.77 ± 0.85	-15.22 ± 1.18	6.39 ± 1.15	-22.52 ± 0.72
Tasman Bay	Tasman Bay	7.57 ± 0.39	-16.21 ± 6.90	6.57 ± 1.77	-22.63 ± 1.02
Fiordland	Doubtful Sound	5.40 ± 0.35	-17.48 ± 0.24	3.86 ± 0.95	-21.60 ± 0.19

2.2.2 Spatial variation of individual species

The values of $\delta^{13}C$ and $\delta^{15}N$ were significantly different among species and regions, as tested by a general linear model (GML) in the program JMP Pro 14.0v (SAS) (Table 2.4). Highest $\delta^{15}N$ were found in *A. zelandica* from Tasman Bay while lowest $\delta^{15}N$ were found for *P. novaezelandiae* from Fiordland. Highest $\delta^{13}C$ were found for *P. novaezelandiae* from Marlborough Sounds and lowest $\delta^{13}C$ were found for *M. galloprovincialis* from Tasman Bay. Overall lowest $\delta^{15}N$ values are revealed for species collected in Fiordland while lowest $\delta^{13}C$ values are from species collected in Tasman Bay. The difference in isotopic values in bivalves collected among regions is evident by the distinct groupings of species, with slight overlap in isotopic values of species between Marlborough Sounds and Fiordland (Figure 2.3).

Table 2.5 Results from a general linear model reveal significant differences in stable isotopic values ($\delta^{15}N$ and $\delta^{13}C$) between species and regions. Significant p-values ($p < 0.05$) are bolded.

Stable Isotope	Factor	df	F-ratio	p-value
$\delta^{15}N$	Species	4	14.9551	**<0.0001**
	Region	3	9.5454	**<0.0001**
$\delta^{13}C$	Species	4	22.2165	**<0.0001**
	Region	3	94.5373	**<0.0001**

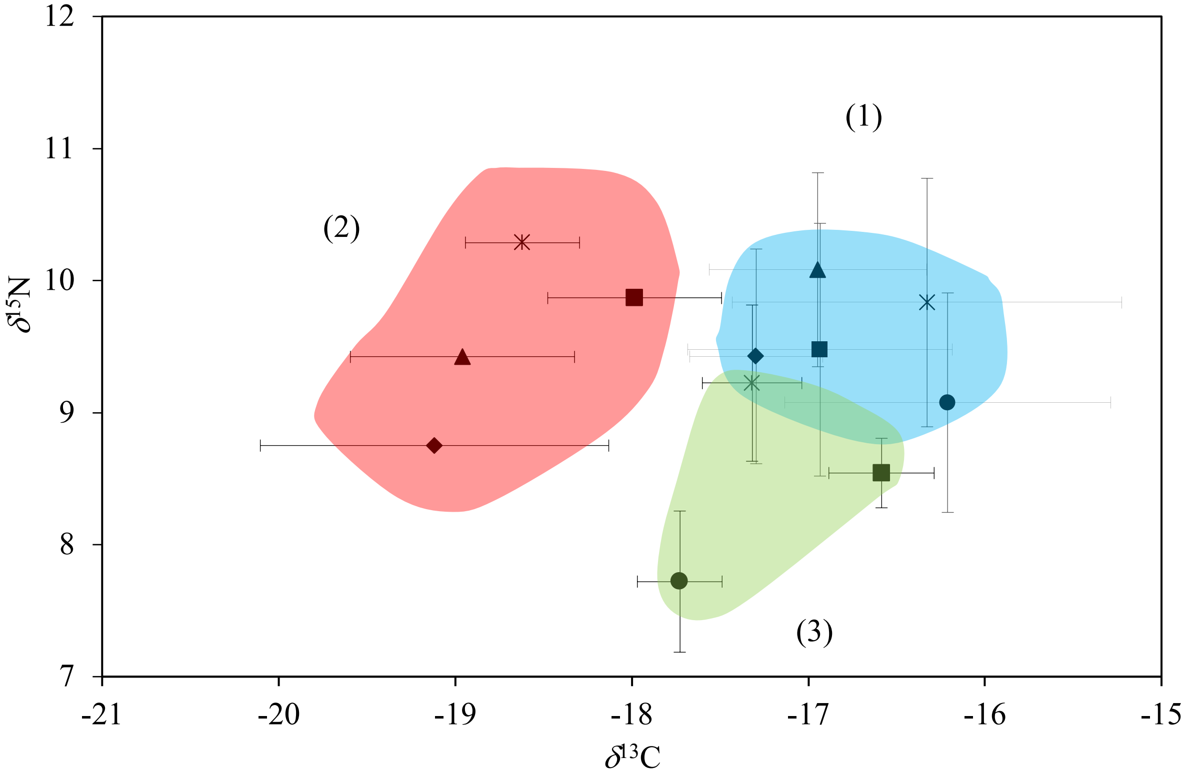

Figure 2.3 Average stable isotopic values (δ^{15}N and δ^{13}C) for bivalves from (1) Marlborough Sounds, (2) Tasman Bay and (3) Doubtful Sound, for species: *A. zelandica* (x), *M. galloprovincialis*(diamond), *P. novaezelandiae* (circle), *P. canaliculus* (triangle), and *T. laticostata* (square). Shapes outline the stable isotopic values of species collected from each region. Means ± SE.

The interaction between species and region could not be tested due to uneven sample distributions. Instead differences for δ^{13}C and δ^{15}N were tested for individual species across regions. Values of δ^{15}N were significantly different between respective regions for all species except *A. zelandica* (Table 2.5). Values of δ^{13}C were significantly different between respective regions for all species (Table 2.5).

Table 2.6 Results from a general linear model revealed significant differences in stable isotopic values (δ^{15}N, δ^{13}C) for individual species among regions. Significant p-values ($p < 0.05$) are bolded.

Factor	Stable Isotope	Species	df	F-ratio	p-value
Region	δ^{15}N	*A. zelandica*	2	1.72	0.1858
		M. galloprovincialis	1	7.99	**0.0064**
		P. novaezelandiae	1	20.48	**<0.0001**
		P. canaliculus	2	8.84	**0.0003**
		T. laticostata	2	4.68	**0.0115**
	δ^{13}C	*A. zelandica*	2	12.62	**<0.0001**
		M. galloprovincialis	1	109.85	**<0.0001**
		P. novaezelandiae	1	21.35	**<0.0001**
		P. canaliculus	2	114.84	**<0.0001**
		T. laticostata	2	34.63	**<0.0001**

To identify differences between regions, same species collected from Marlborough Sounds, Tasman Bay and Fiordland were analysed for the interaction between factors REGION and SPECIES. There was no significant interaction for δ^{15}N among *A. zelandica* and *T. laticostata* (Main test: $F_{5,167} = 3.18$, $p = 0.009$, $r^2 = 0.09$. δ^{15}N: REGION, $p = 0.0092$. SPECIES, $p = 0.07$. REGION * SPECIES. $p = 0.9$). There was a significant interaction for δ^{13}C for *A. zelandica* and *T. laticostata* (Main test: $F_{5,167} = 23.70$, $p < 0.0001$, $r^2 = 0.42$. δ^{13}C: REGION, $p < 0.0001$. SPECIES, $p = 0.28$. REGION * SPECIES, $p = 0.0011$).

Overlap of δ^{15}N and δ^{13}C in isotopic space for species

Overlap among species within regions is evident in the values of δ^{13}C and δ^{15}N mainly in samples collected from the Marlborough Sounds and Tasman Bay (Figure 2.4 – 2.6). Clear species overlap of δ^{13}C and δ^{15}N are evident among all species in the Marlborough Sounds (Figure 2.4). The same trend is evident for species collected from Tasman Bay, with the exception of two outliers (Figure 2.5). Analysis of samples collected in Fiordland illustrate overlap in the values of δ^{13}C and δ^{15}N between *A. zelandica* and *P. novaezelandiae* while *T.*

laticostata remains distinct (Figure 2.6). The high incidence of overlap in isotopic values among species within regions provides an indication of overlap in use of basal organic matter sources, phytoplankton versus particulate matter derived from macroalgae.

The basal organic matter sources supporting different bivalve species within each of the regions illustrates higher proportions of organic matter derived from macroalgae among species from Marlborough Sounds and Fiordland relative to those resolved in the Tasman Bay (Figure 2.4 – 2.6).

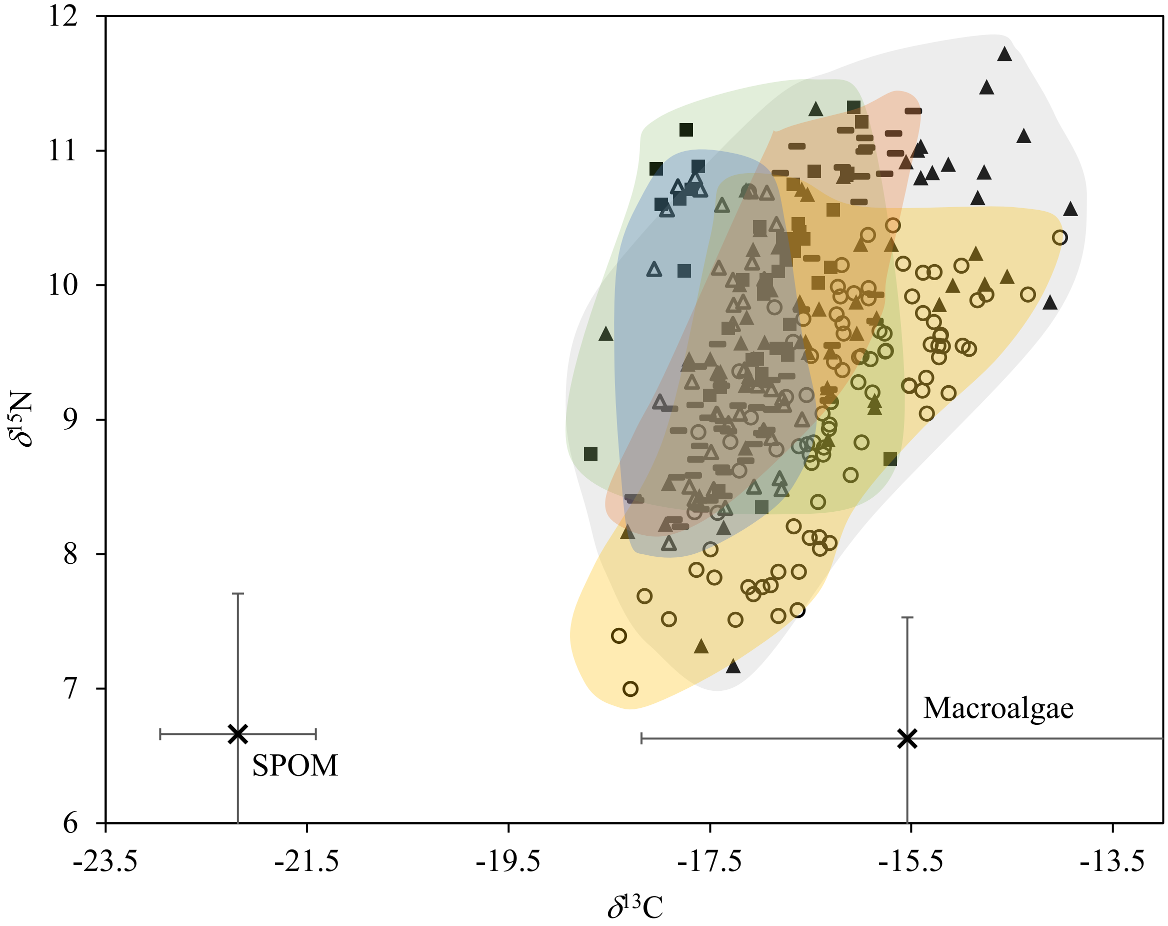

Figure 2.4 Stable isotopic values (δ^{15}N and δ^{13}C) for consumers and organic matter sources from Marlborough Sounds for species: *A. zelandica* (closed triangle), *M. galloprovincialis*(open triangle), *P. novaezelandiae* (open circle), *P. canaliculus* (closed square), and *T. laticostata* (closed rectangle). Shapes display resource use overlap of consumers in isotopic space for: *A. zelandica* (grey), *M. galloprovincialis*(blue), *P. novaezelandiae* (yellow), *P. canaliculus* (green), and *T. laticostata* (orange). Shapes outline the stable isotopic values of species collected within Marlborough Sounds.

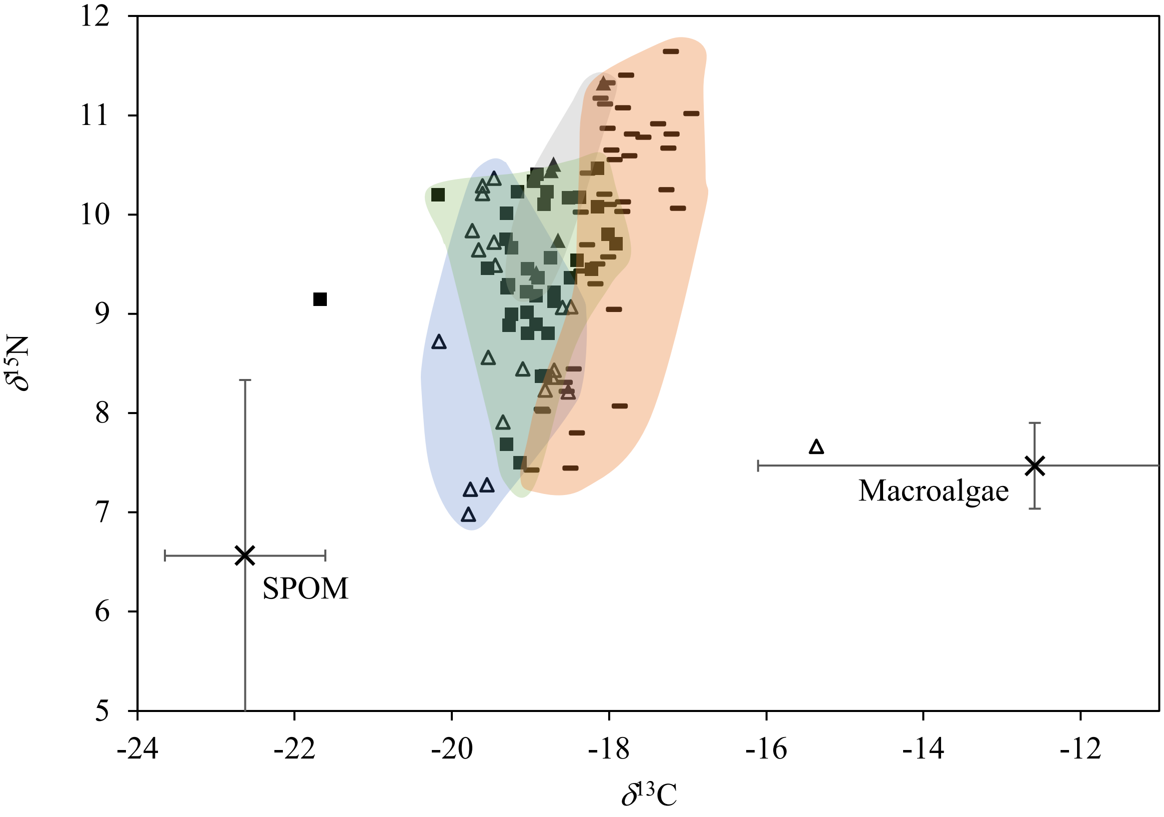

Figure 2.5 Stable isotopic signatures (δ^{15}N and δ^{13}C) for consumers and organic matter sources from Tasman Bay for species: *A. zelandica* (closed triangle), *M. galloprovincialis* (open triangle), *P. canaliculus* (closed square), and *T. laticostata* (closed rectangle). Shapes display resource use overlap of consumers in isotopic space for: *A. zelandica* (grey), *M. galloprovincialis* (blue), *P. canaliculus* (green), and *T. laticostata* (orange). Shapes outline the stable isotopic values of species collected within Tasman Bay.

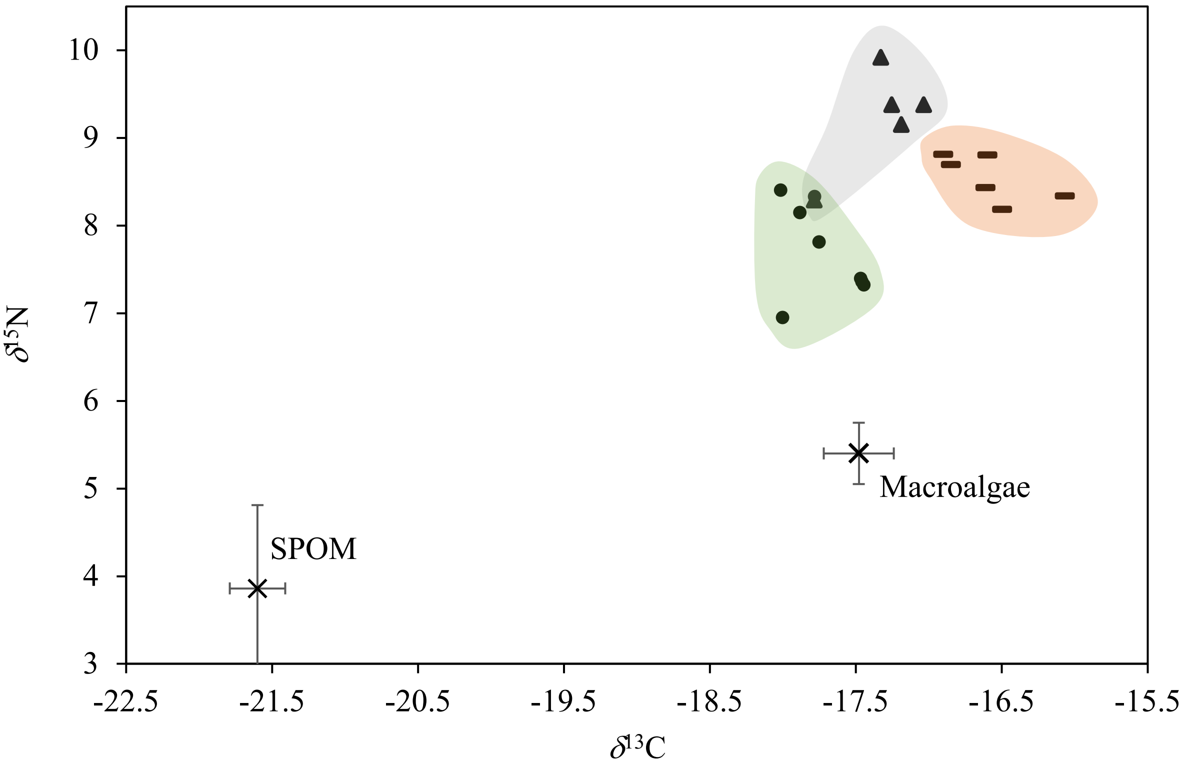

Figure 2.6 Stable isotopic signatures (δ^{15}N and δ^{13}C) for consumers and organic matter sources from Fiordland for species: *A. zelandica* (closed triangle), *P. novaezelandiae* (open circle), and *T. laticostata* (closed rectangle). Shapes display resource use overlap of consumers in isotopic space for: *A. zelandica* (grey), *P. novaezelandiae* (yellow), and *T. laticostata* (orange). Shapes outline the stable isotopic values of species collected within Fiordland.

Mixing model

Individual-based estimates of proportion of organic matter supporting bivalves suggests diets are largely supported by organic matter derived from macroalgae. The observed pattern is consistent at both the site and regional level. The results of the mass balance mixing model revealed the model's sensitivity to the assumed base δ^{13}C values for macroalgae and SPOM.

The sensitivity analysis revealed that the mixing model is sensitive to assumed basal carbon isotopic value (δ^{13}C) for macroalgae and SPOM source pools. The average proportion of organic matter derived from macroalgae in the diet of bivalves varied between 8% and 26% when assumed base values of macroalgae and SPOM were increased or decreased up to two standard errors from the average (Figure 2.7). The result indicated that the contributions of alternative organic matter sources as determined by the mixing model in the present study are highly sensitive to the assumed δ^{13}C value for both macroalgae and SPOM source pools.

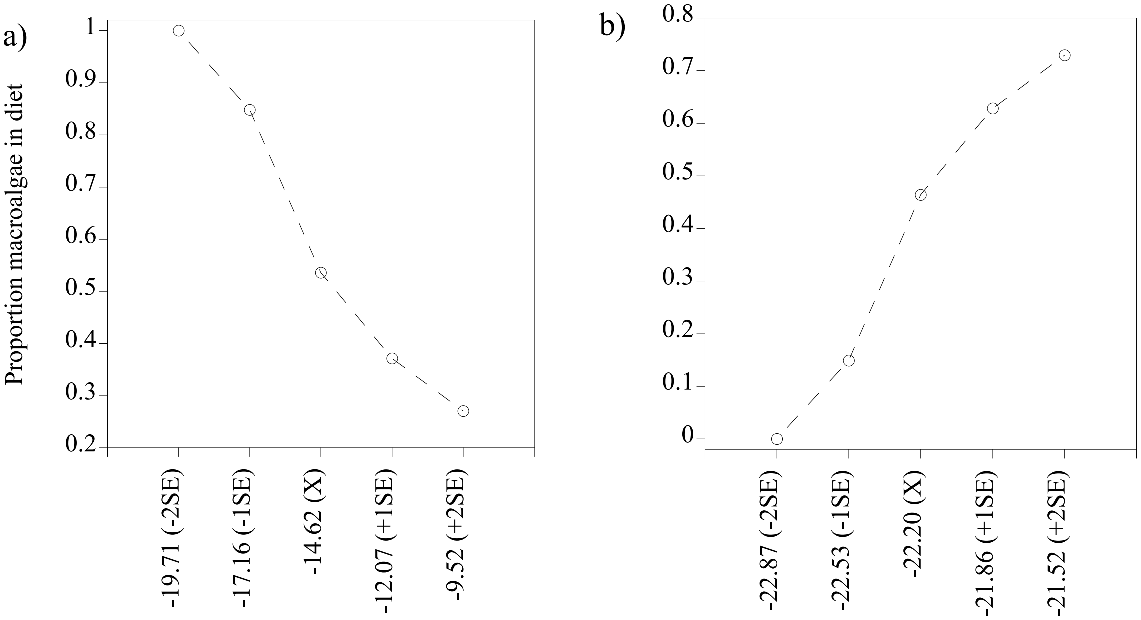

Figure 2.7 Sensitivity analysis based on the averages among species for proportion of macroalgae in bivalve diets. Values were determined by mixing models first for individuals, for assumed basal carbon isotopic values (δ^{13}C) of (a) macroalgae and (b) SPOM. Values were pooled for all five species present at Motungarara Island.

2.2.3 Community trends with mass balance analysis

Spatial variation

The proportion of organic matter derived from macroalgae supporting bivalves varies significantly between the three regions (Table 2.7). Highest contributions macroalgae to bivalve diets were identified for samples collected from Marlborough Sounds and Doubtful Sound where diets are composed of between 57% and 86% organic matter derived from macroalgae or another isotopically similar source (Figure 2.8). The lowest contributions of macroalgae to bivalve diet were consistently identified from samples collected in the Tasman Bay where the bivalves were supported by nearly equal proportions of organic matter derived from macroalgae and phytoplankton (between 39% and 53%) (Figure 2.8). Differences in organic matter use among species indicated that contributions of organic matter derived from macroalgae were highest for *T. laticostata* and lowest for *A. zelandica* in both Doubtful Sound and Tasman Bay (Figure 2.8). However, in Marlborough Sounds, *P. novaezelandiae* were supported by the highest contribution of organic matter derived from macroalgae while *M. galloprovincialis* was supported by the lowest (Figure 2.8). These patterns suggested that the contributions of organic matter vary strongly among regions for individual species.

Table 2.7 Results of general linear models for proportion of macroalgae supporting individual species among regions. Significant p-values ($p < 0.05$) are bolded.

Species	F ratio	r^2	p
A. zelandica	$F_{2,67} = 12.91$	0.28	**<0.0001**
M. galloprovincialis	$F_{1,56} = 19.67$	0.26	**<0.0001**
P. novaezelandiae	$F_{1,87} = 8.01$	0.09	**0.0058**
P. canaliculu	$F_{1,75} = 70.66$	0.49	**<0.0001**
T. laticostata	$F_{2,74} = 24.78$	0.41	**<0.0001**

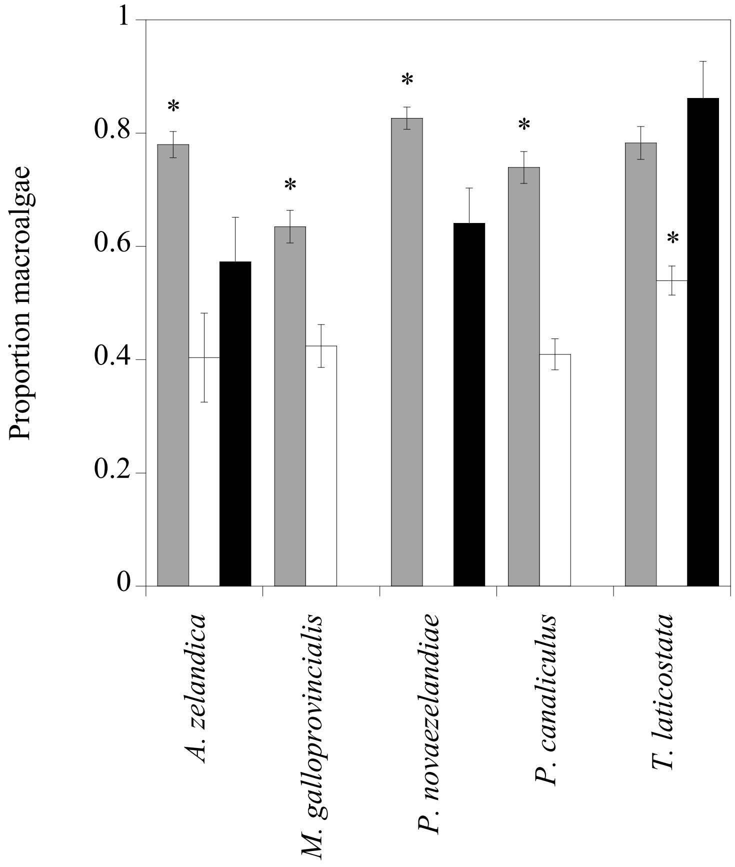

Figure 2.8 Proportion of macroalgae supporting bivalves for assumed trophic level 1 presented for each region: Marlborough Sounds (grey), Tasman Bay (white), and Doubtful Sound (black). Average proportions of organic matter from macroalgae for each region are shown here, site specific averages in the mixing model were used to determine organic matter sources for consumers where available. Mean ± 1 SE.

From the single site where all species could be found, proportions of macroalgae supporting species are significantly different for assumed trophic level 1 (Main test: $F_{5,36} = 3.40$, $p = 0.0145$, $r^2 = 0.35$. Species, $F = 4.13$, $p = 0.0085$. Shell length, $F = 0.71$, $p = 0.4066$). *P. novaezelandiae* derived significantly higher proportions of organic matter from macroalgae relative to all other species (Figure 2.9). The lowest proportions of organic matter from macroalgae are consistent among *A. zelandica*, *M. galloprovincialis*, *P. canaliculus*, and *T. laticostata* (Figure 2.9).

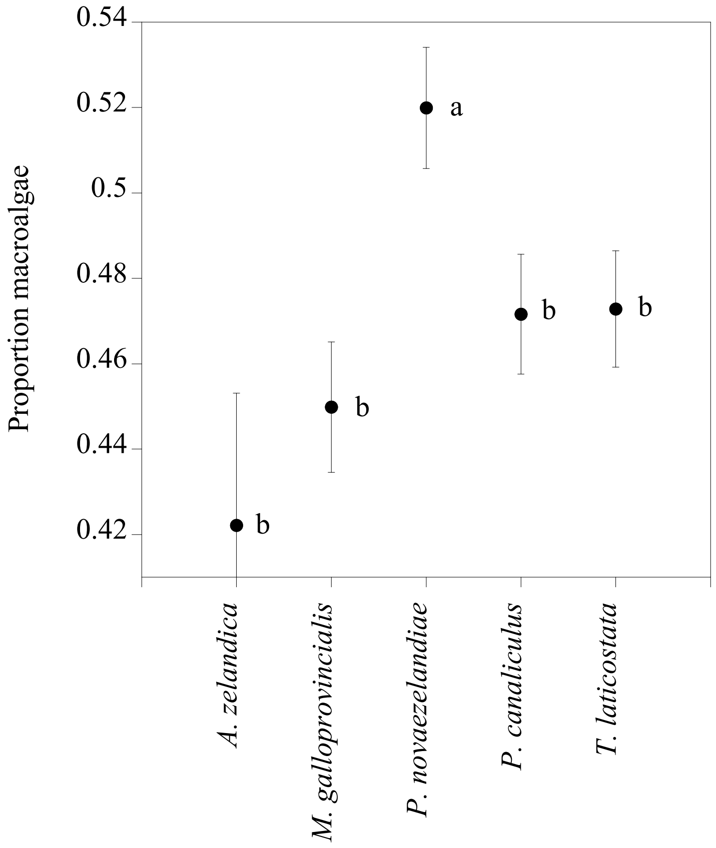

Figure 2.9 Proportion of macroalgae supporting bivalves for assumed trophic level 1 from Motungarara Island, Marlborough Sounds. Mean ± 1 SE. Levels not connected by same letters are significantly different.

A similar trend was identified when considering bivalves collected from several sites within Marlborough Sounds where a majority of the species were found. Significant differences were identified for factors SITE and SPECIES, but not for the interaction between SITE * SPECIES on the proportion of organic matter derived from macroalgae (Main test: $F_{12,103} = 107.74$, $p < 0.0001$, $r^2 = 0.93$. Site, $F = 431.65$, $p <0.0001$. Species, $F = 4.07$, $p = 0.0092$. Site * Species, $F = 1.93$, $p = 0.0839$. Shell length, $F = 6.12$, $p = 0.0152$). The highest proportion of organic matter derived from macroalgae was present for species collected from Bird Island while lowest was recorded for Motungarara Island (Figure 2.10). Highest proportions of organic matter derived from macroalgae were consistent for *P. novaezelandiae* and lowest for *A. zelandica* among sites. Overall similar patterns in the proportion of macroalgae supporting *P. novaezelandiae*, *P. canaliculus*, and *T. laticostata* were consistent for samples collected from Bird Island and Motungarara Island. Further, similar proportions of organic matter derived from macroalgae were present for *A. zealandica*, *P. canaliculus* and *T. laticostata* at Perano Shoal. Differences in proportion of organic matter derived from macroalgae supporting bivalves were more pronounced between sites than within sites (Figure 2.10).

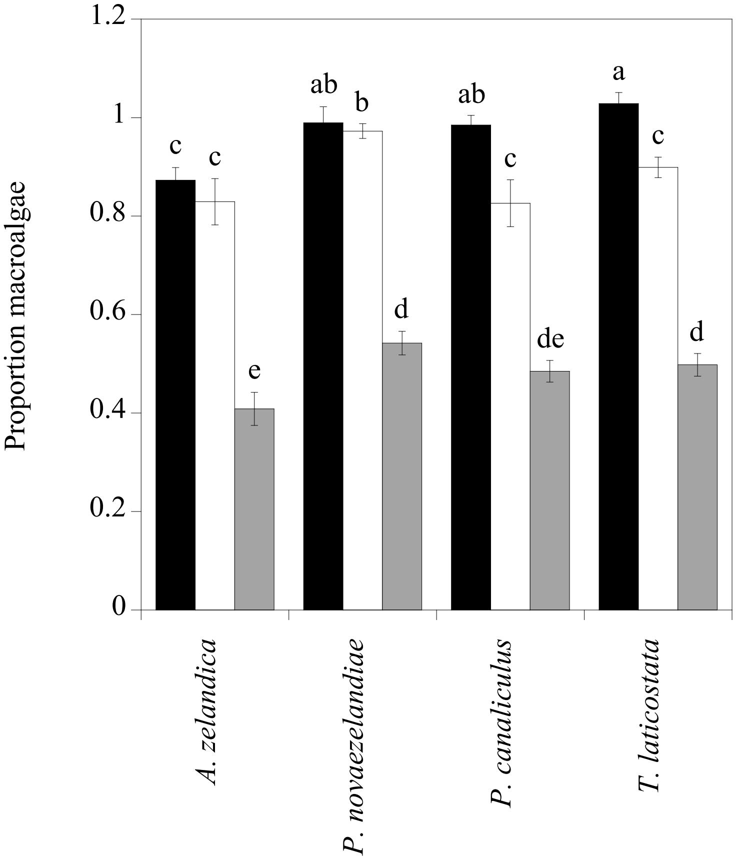

Figure 2.10 Proportion of macroalgae supporting bivalves for assumed trophic level 1 at sites: Bird Island (black), Perano Shoal (white), and Motungarara Island (grey) within Marlborough Sounds. Mean ± 1 SE. Levels not connected by same letters are significantly different.

Atrina zelandica, collected from each of the study sites displayed significant differences in the proportion of organic matter derived from macroalgae ($F_{9,67} = 12.41$, $p < 0.0001$, $r^2 = 0.66$). Individuals collected at sites within Marlborough Sounds derive the highest proportion of organic matter from macroalgae relative to those collected from Tasman Bay and Doubtful Sound (Figure 2.11). However, proportions of organic matter derived from macroalgae significantly differed among sites within Marlborough Sounds (Figure 2.11).

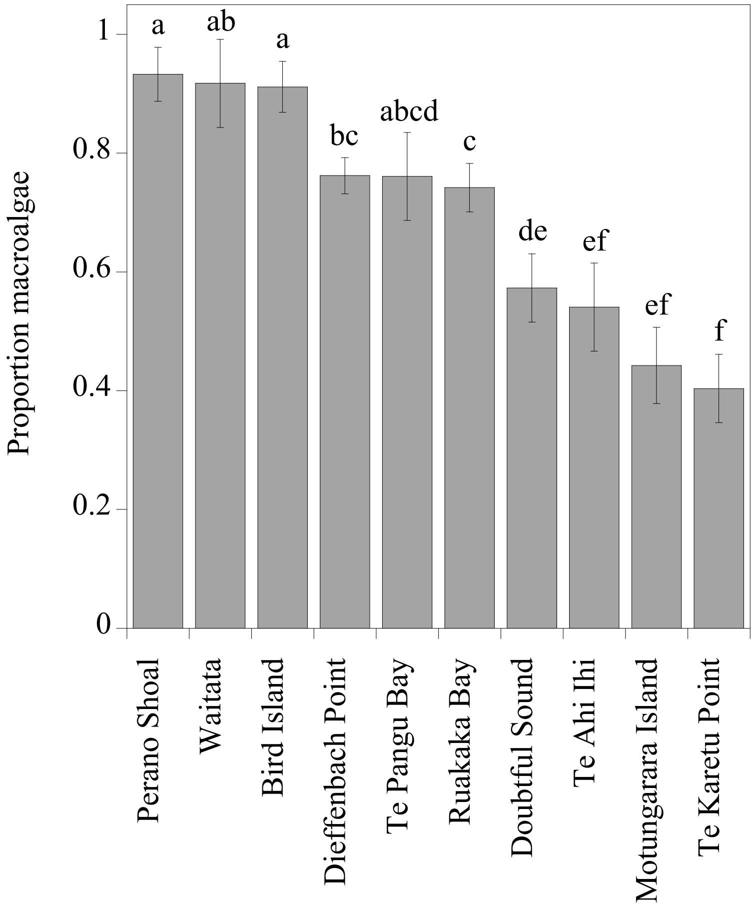

Figure 2.11 a) Proportion macroalgae supporting *A. zelandica* for assumed trophic level 1 collected from study sites. Mean ± 1 SE. Levels not connected by same letters are significantly different.

2.2.4 Anthropogenic influences

Salmon farms in Marlborough Sounds

Significant differences were revealed for the proportion of organic matter derived from macroalgae supporting bivalves at salmon farms and reference sites for factors SALMON FARM, SPECIES and the interaction between SALMON FARM * SPECIES (Main test: $F_{10,240} = 10.22$, $p < 0.0001$, $r^2 = 0.31$. Salmon farm, $F = 44.17$, $p < 0.0001$. Species, $F = 15.15$, $p < 0.0001$. Salmon farm * Species, $F = 3.72$, $p = 00.0059$. Shell length, $F = 20.78$, $p < 0.0001$). Highest proportion of organic matter derived from macroalgae was revealed at salmon farm sites for *T. laticostata* and lowest for *A. zelandica* at reference sites relative to other species (Figure 2.12). Proportions of organic matter from macroalgae are significantly different for *A. zelandica*, *P. novaezelandiae*, *P. canaliculus*, and *T. laticostata* collected between salmon farm sites and similar reference sites (Figure 2.12).

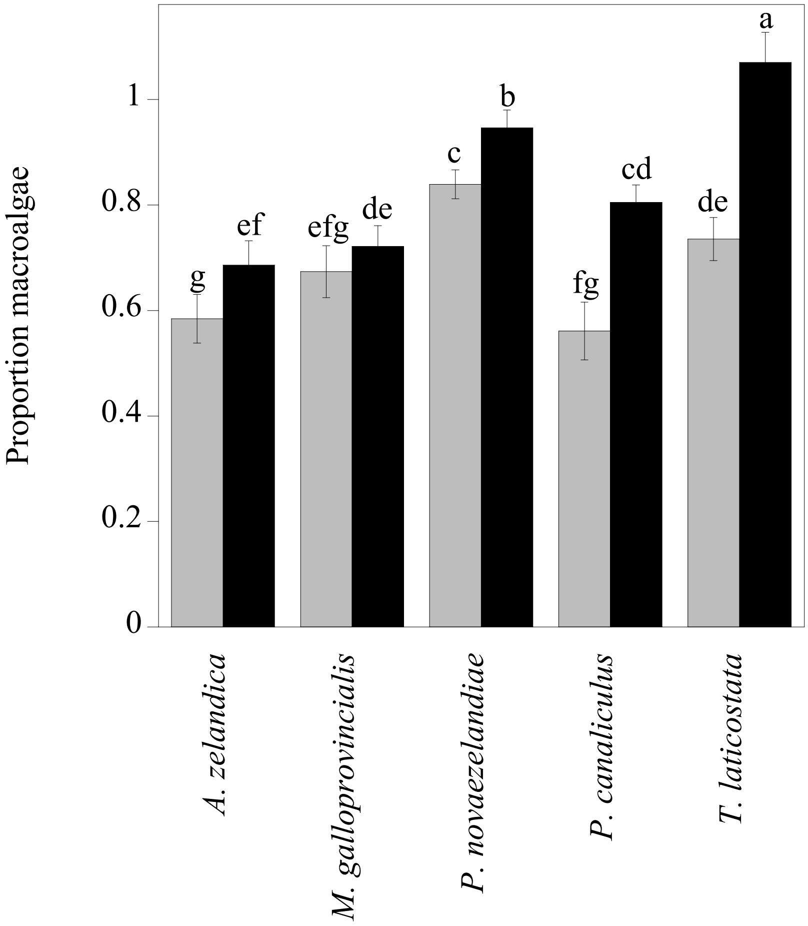

Figure 2.12 Proportion of macroalgae supporting bivalves for assumed trophic level 1 from reference (grey) and salmon farm (black) sites. Mean ± 1 SE. Levels not connected by same letters are significantly different.

Landcover classification

Significant differences were revealed for proportions of organic matter derived from macroalgae supporting individual species among catchments characterized be a range of land modification (Table 2.8,2.9).

Table 2.8 Proportions of landcover classifications and total area for discrete catchments.

Catchment	%Forest	%Urban	%Forestry	%Farming	Catchments size (km^2)
Doubtful Sound	0.949446	1.08E-05	0	0	947.492417
Mapua	0.463407	0.010584	0.301777	0.219149	871.6436599
Pelorus Sound	0.790336	0.000833	0.083039	0.120129	1096.489307
Queen Charlotte Sound	0.631024	0.006929	0.208192	0.152431	32.25880051
Tory Channel	0.363658798	0	0.521617823	0.114723379	62.32033168

Table 2.9 Results of discrete general linear models for proportions of macroalgae supporting individual species among catchments. Significant p-values ($p < 0.05$) are bolded.

Species	F ratio	r^2	p
A. zelandica	$F_{5,67} = 16.35$	0.57	**<0.0001**
M. galloprovincialis	$F_{4,56} = 15.93$	0.55	**<0.0001**
P. novaezelandiae	$F_{4,87} = 32.62$	0.61	**<0.0001**
P. canaliculu	$F_{4,75} = 73.99$	0.81	**<0.0001**
T. laticostata	$F_{4,74} = 68.16$	0.80	**<0.0001**

Significant regression analyses between proportion of macroalgae and landcover classifications revealed positive linear associations for percent forest for *M. galloprovincialis*, *P. canaliculus* and *T. laticostata*. Further, negative associations were observed in the previous species for percent landcover from: urban, forestry and farming (Figure 2.13, 2.15 – 2.16). The only significant regression analysis for *P. novaezelandiae* revealed a negative association between proportion of organic matter derived from macroalgae and percent farming in the associated catchment (Figure 2.14). No significant regressions were revealed for *A. zelandica.*

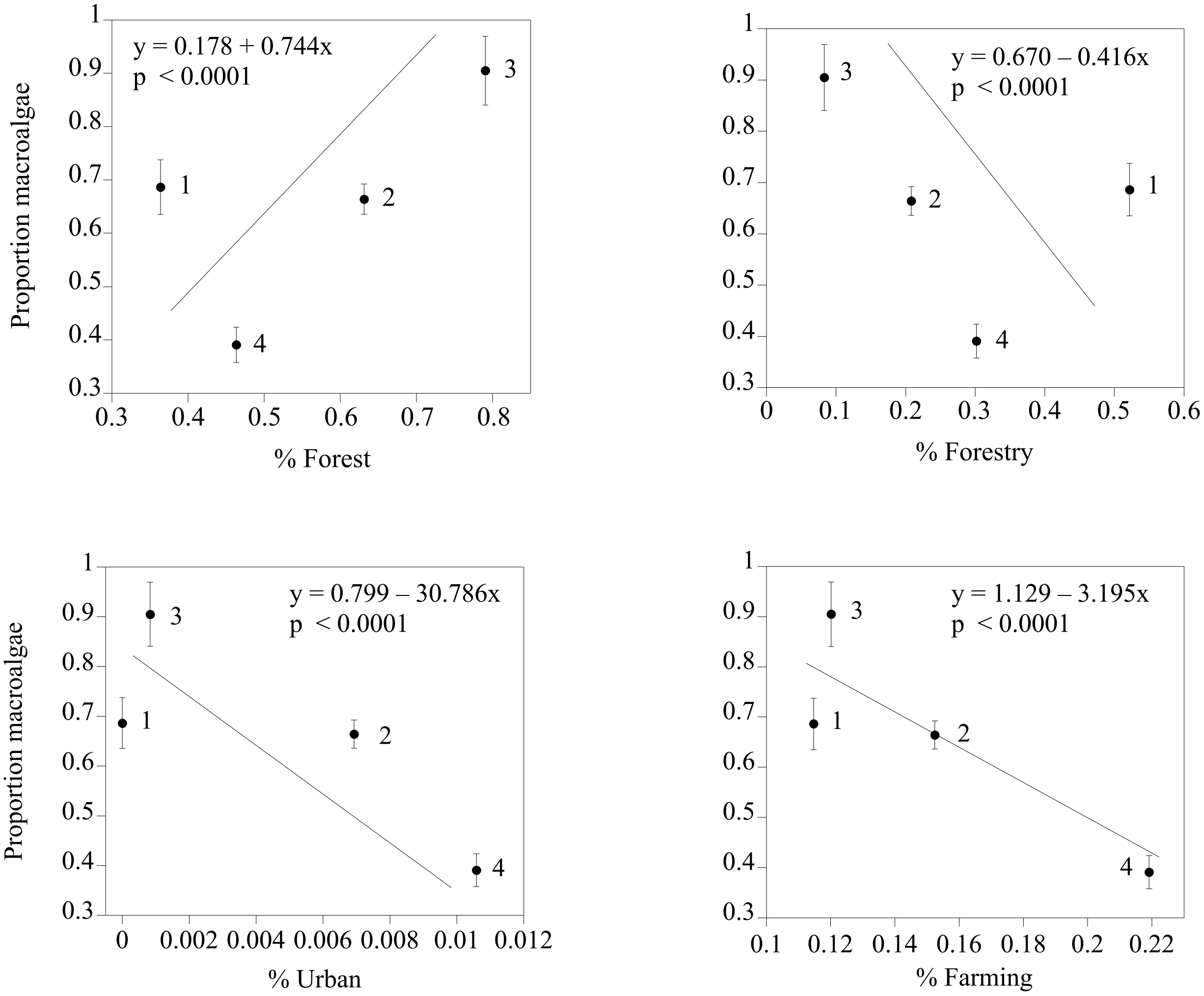

Figure 2.13 Significant regression analysis results for proportion of macroalgae and percent landcover for *M. galloprovincialis* among sites: 1. Tory Channel, 2. Queen Charlotte Sound, 3. Pelorus Sound, and 4. Mapua.

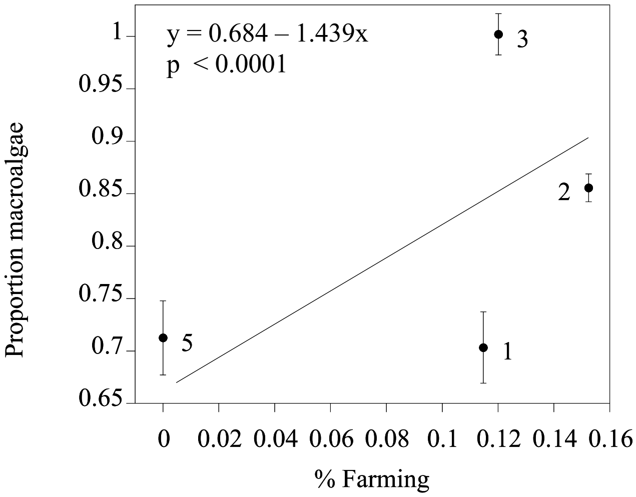

Figure 2.14 Significant regression analysis results for proportion of macroalgae and percent farming for *P. novaezelandiae* among sites: 1. Tory Channel, 2. Queen Charlotte Sound, 3. Pelorus Sound, and 5. Doubtful Sound.

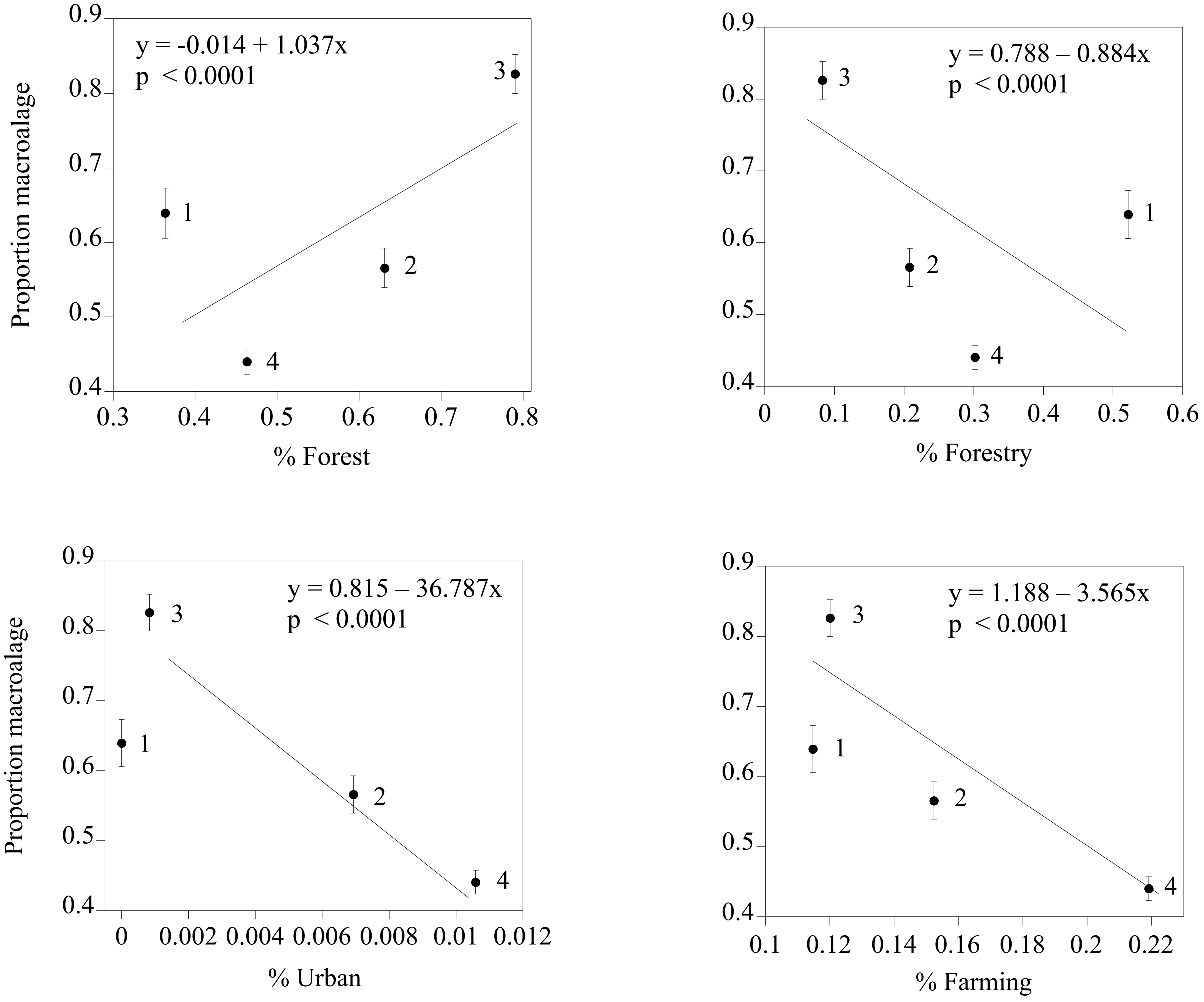

Figure 2.15 Significant regression analysis results for proportion of macroalgae and percent landcover for *P. canaliculus* among sites: 1. Tory Channel, 2. Queen Charlotte Sound, 3. Pelorus Sound, and 4. Mapua.

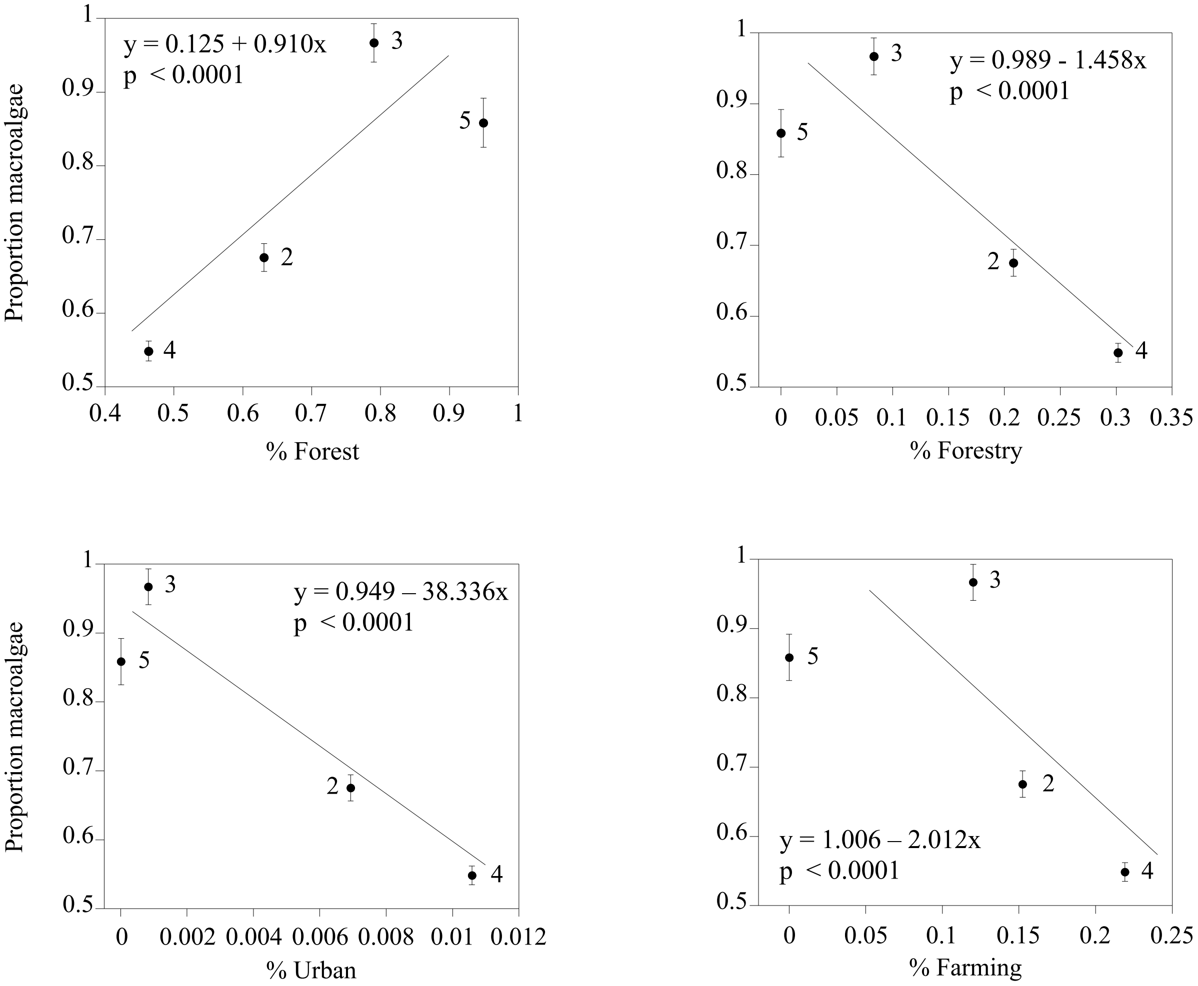

Figure 2.16 Significant regression analysis results for proportion of macroalgae and percent landcover for *T. laticostata* among sites: 2. Queen Charlotte Sound, 3. Pelorus Sound, 4. Mapua, and 5. Doubtful Sound.

2.3 Discussion

The data and results presented here demonstrate that the composition of alternative basal organic matter sources supporting benthic bivalves was likely shaped by anthropogenic activity in adjoining catchment systems. While the intraspecific differences in the diet of temperate benthic bivalve communities were largely related to land modification and salmon farming, interspecific differences resulted from contrasting feeding strategies. Marlborough Sounds, Tasman Bay, and Fiordland with different intensities of anthropogenic influence, provided a range of contrasting conditions for assessing the effects of land modification on benthic food webs. Accordingly, among these regions, the degree of catchment modification and salmon farming revealed the strongest relationships with variations in alternative basal organic matter use by bivalves among regions.

2.3.1 Macroalgae subsidies

We focused on two primary organic matter sources supporting temperate reef communities, phytoplankton and macroalgae (Fredriksen 2003, Koenigs et al. 2015, von Biela et al. 2016, Udy et al. 2019c). Macroalgae has specifically been identified in fueling food webs by supporting primary consumers, such as benthic suspension feeding bivalves, that then provide prey for higher trophic levels (Tallis 2009, Wing & Jack 2012). The widespread idea that macroalgae are a pervasive supporter of marine food webs through suspension feeding has been challenged by Miller and Page (2012), who contended that stable isotope analysis resulted in overestimating the macroalgal contribution to bivalve species. Here we reveal that suspension feeding bivalves rely heavily on subsidies of macroalgae. Bivalves in the present study were supported by as much as ~90% organic matter derived from macroalgae, depending on the species and site sampled. Stable isotopic values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) illustrated that all species from the northern and southern study regions of South Island, New Zealand relied on organic matter from macroalgae to a significant degree (Figure 2.17).

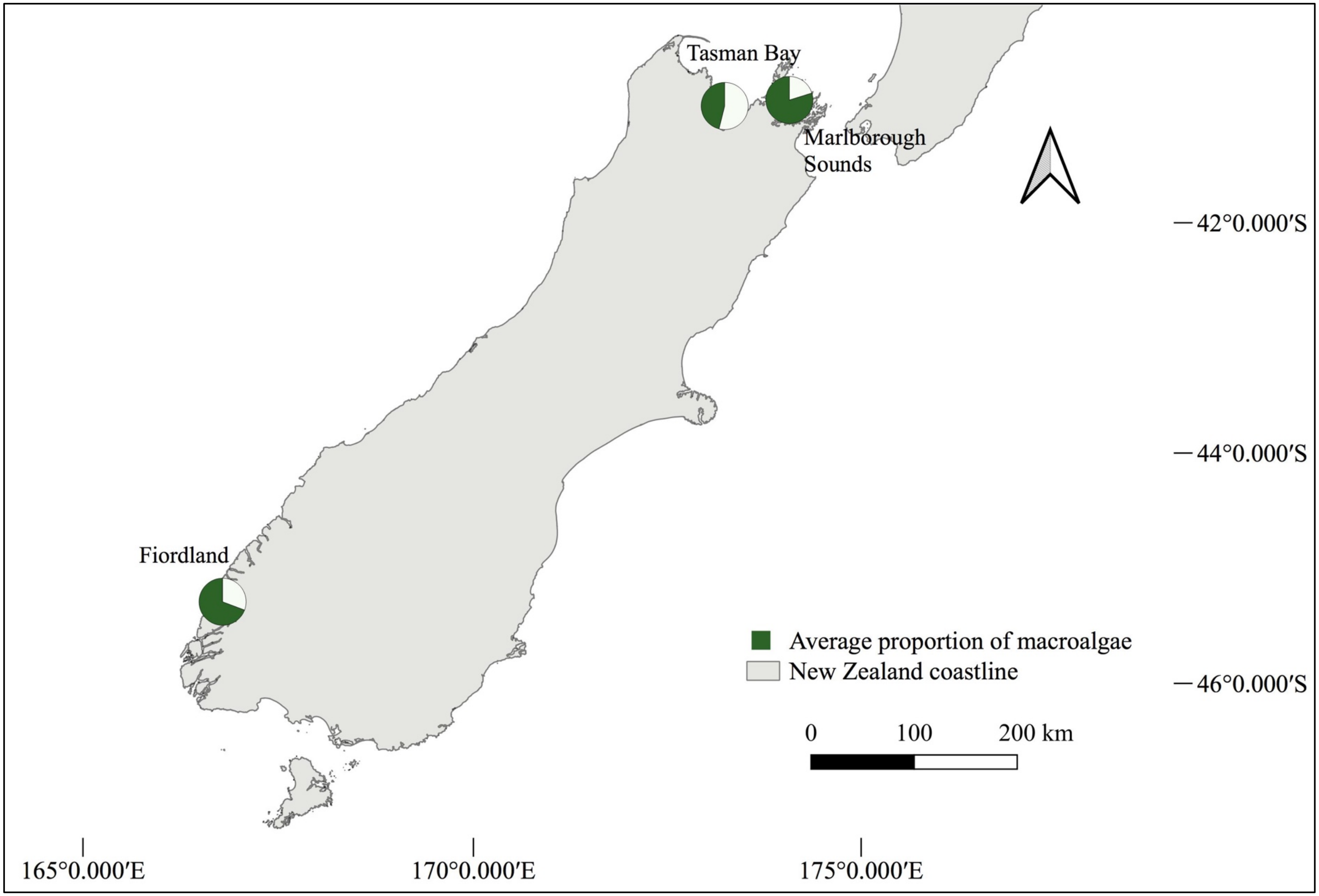

Figure 2.17 Average proportion of macroalgae supporting species for assumed trophic level 1 among study regions: Marlborough Sounds, Tasman Bay and Fiordland.

The factor, REGION, best explained the variation in organic matter derived from macroalgae supporting individual species (Table 2.7, Figure 2.8). A likely explanation for the observed differences in source pools has previously been linked to the abundance of macroalgae among regions (Udy et al. 2019a, 2019c). Highest contributions of organic matter derived from macroalgae in Marlborough Sounds and Doubtful Sound reflect the higher abundance of macroalgae and / or a more active detrital pathway of macroalgae derived organic matter to suspension feeders in these regions, relative to that observed in the Tasman Bay. Previous studies illustrated the contribution of macroalgae to higher trophic level fish in food webs in Marlborough Sounds and Doubtful Sound (Wing & Jack 2012, Udy et al. 2019c). We found that though Fiordland has a larger biomass of macroalgae relative to Marlborough Sounds, contributions of organic matter to the detrital pathways supporting bivalves in these regions appears equal or significantly greater in Marlborough Sounds (Figure 2.8). Though the carbon and nitrogen values fell within the macroalgae and SPOM signatures, it is likely that some organisms draw organic matter from alternate sources (Figure 2.4 – 2.6). For example, detrital feeding invertebrates in Fiordland have been identified as accessing organic matter from terrestrial sources and microbially influenced organic material derived from forest litter (Wing & Jack 2012, McLeod et al. 2010).

In contrast to the patterns in organic matter use by bivalves in the Marlborough Sounds and Fiordland, the lowest contribution of organic matter derived from macroalgae are consistent for all species in Tasman Bay (Figure 2.8). This pattern may be reflective of the lower abundance of kelp forest habitats in Tasman Bay relative to the other regions, and high concentrations of phytoplankton in the Bay (Newcombe et al. 2015). Due to the lack of kelp forests in Tasman Bay and the high phytoplankton abundance, the primary sources of organic matter are derived from phytoplankton in the water column and benthic microalgae (Newcombe et al. 2015). The phytoplankton productivity between Fiordland and Marlborough Sounds is similar, however during bloom events Fiordland possesses slightly higher phytoplankton biomass (Ogilvie et al. 2000, Goebel et al. 2005). Therefore, regional differences observed in the present study are likely driven by differences in the detrital pathways from macroalgal production into the suspended particulate organic matter pool.

2.3.2 Resource overlap and partitioning

Site-specific samples of macroalgae and SPOM within Marlborough Sounds provided a localized perspective on trophodynamics among individual bivalve species. The observed variability in organic matter derived from macroalgae among primary consumers were explained by factors, SITE and SPECIES, but not the interaction between SITE * SPECIES. Interspecific differences in organic matter derived from macroalgae at individual sites reflected differences in feeding modes while intraspecific differences among sites were likely tied to variability in the abundance of alternative resource pools.

Interspecific differences revealed contributions of organic matter from macroalgae varied significantly among species (Figure 2.9, 2.10). Highest proportions of organic matter from macroalgae are consistent for *P. novaezelandiae* among discrete sites within Marlborough Sounds relative to other species (Figure 2.10). These findings were likely due to the selective feeding strategy identified for *P. novaezelandiae*, where more nutritious particulate matter is often selected over particulates with lower nutritional values captured in the mucus net along the mantle margin (Macdonald & Ward 1994). The mechanisms that support selectivity in this species are rejection channels that allow particles to be discarded (Liang & Morton 1988, Macdonald & Ward 1994). For example, *P. novaezelandiae* increase filtration rates and biodeposit build-up in turbid conditions, illustrating the pathway through which nutrients are selected (Macdonald & Ward 1994). Further, the position of *P. novaezelandiae* on the seafloor reveals the utilization of organic matter build-up reflected in higher abundance of macroalgae and / or a more active detrital pathway of macroalgae derived organic matter.

Though all other species, aside from the understudied *T. laticostata*, have also been identified as selective feeders (Hawkins et al. 1996, James et al. 2001, Ellis et al. 2002, Hewitt & Pilditch 2004), it is likely that the resource pools at discrete sites vary relative to the composition of the benthic community. The similar proportions of organic matter supporting *A. zelandica, P. canaliculus*, and *M. galloprovincialis* reflect overlap in resource pools by these species that have a feeding position away from the benthos in the water column. Further, it is likely that mussel beds, which have previously been identified as influential to the hydrodynamics within the benthic boundary layer along rocky reefs (Green et al. 1998, Nikora et al. 2002), enrichment of sediments, and microbial activity (Warwick et al. 1997, Cummings et al. 2001) alter the available particulates in the water column. In addition, suspension feeding epibionts primarily present on *A. zelandica* may influence the resource pool available to their host (Cummings et al. 2001). Studies on other species of large sediment dwelling cockles, *Glycymeris*, indicate periodic feeding activity with active filtration only about 60% of the time and longer digestion periods (Savina & Pouvreau 2004). As an alternative to active filtration, passive feeding facilitated by strong water currents provides increased delivery of suspended particulates, particularly those of the detrital pathway to *T. laticostata* (Powell 1979).

Intraspecific differences reflect the composition of resource pools among sites where influences acting on organic matter source pools may explain the observed variation. Differences in bottom stress and wave activity which has been associated with abundance of primary production among sites may account for the observed differences in the resource pools (Handley 2015, Urlich 2015). Of the sites within Marlborough Sounds, lowest proportions of macroalgae were present at Motungarara Island. Though this site was characterized by high macroalgal cover, the low contribution of macroalgae to bivalves may have been a result of the influence of high wave activity on the retention of suspended particulate organic matter (Neil et al. 2018). In contrast, Bird Island and Perano Shoal were sheltered from direct oceanic wave exposure and may therefore have retained suspended particulate matter leading to higher concentrations of detrital leaf particulates in the SPOM pool.

While bivalve species sampled in the present study revealed both site and species level differences in use of organic matter source pools, the observed variability reflects resource partitioning likely leading to the reduction in competition for food (Tallis 2009). The differences among species identified here were supported by studies identifying interspecific differences in resource utilization among benthic suspension feeding communities (Dubois et al. 2007, Tallis 2009, Wing & Jack 2012). Further, these observations are supported by large-scale patterns in origins of organic matter, to regional changes in nutrient availability (Hill et al. 2006). Smaller scale differences in hydrology, coastal currents, and wave exposure can all contribute to the differences in organic matter production and suspended particulate transport to rocky shores

(Schaal et al. 2009). Overall, the differences in resource use from the suspended particulate pool are reflective of the divergent feeding mechanisms allowing species to access different particulates among the available resource pools.

2.3.3 Influence of anthropogenic stressors to resource subsidies for coastal benthic bivalves

Salmon farm

Results of the current study reveal factors, SALMON FARM, SPECIES, and the interaction between the factors, significantly alters the utilization of alternate organic matter sources by all subtidal species *A. zelandica*, *P. novaezelandiae*, *P. canaliculus*, and *T. laticostata*, but not by the intertidal, *M. galloprovincialis* (Figure 2.12). The significantly higher proportion of organic matter derived from macroalgae likely reflected nutrient enrichment from salmon farm associated inputs, such as salmon feed, waste, and resulting stimulation of macroalgal production in the vicinity of salmon farms (Gao et al. 2006). The elevated proportions of macroalgae associated with salmon farm sites reflected distinctive alteration of organic matter and nutrients within benthic food webs.

The present study clearly indicates that salmon farms directly influence the basal organic matter sources likely altering the pathways for organic matter entering benthic communities. The stressors on benthic communities in the vicinity of salmon farms have been documented in Marlborough Sounds, with community collapses associated with heavy sedimentation (Davidson & Richards 2014). With a total cage area of 0.1 km^2 and delivery of approximately 19,700 tons of fish feed per year (Knight 2012, Taylor et al. 2015), the aquaculture systems along the coastal Marlborough Sounds are likely to alter pathways for organic matter entering benthic communities. For benthic dwelling species, *P. novaezelandiae* and *T. laticostata*, this likely enhanced contributions of organic matter to the detrital pathways at salmon farm sites relative to similar references sites. While the alteration of organic matter supporting benthic communities may result in changes to trophodynamics, the influence of sedimentations could reduce benthic habitat near salmon farms. Therefore, the alteration of organic matter sources at the benthic level will have indirect effects on the flow of organic matter and nutrients within benthic food webs (Salomon et al. 2008, Udy et al. 2019b).

Land-cover

The data and results presented here demonstrate that proportions of organic matter supporting bivalve communities were significantly different among catchments within Marlborough Sounds, Tasman Bay and Fiordland (Table 2.9). We demonstrated that organic matter derived from macroalgae supporting bivalve communities in Southern New Zealand correlated with the degree of catchment modification (Figure 2.13 – 2.16). We found that bivalves were supported by a lower proportion of organic matter derived from macroalgae in heavily modified catchments: Mapua, Queen Charlotte Sound, and Tory Channel relative to the more intact: Pelorus Sound and Doubtful Sound. As bivalve feeding modes can be heavily influenced by composition of suspended particulate matter (Eckman et al. 1989, Hawkins et al. 1999, Gardner 2000, Pilditch et al. 2001, Ellis et al. 2002, Safi et al. 2007), these observations are likely the result of sedimentation from catchments imposed on coastal ecosystems influenced by catchment size, modification and vegetation cover (Waters 1995, Wemple et al. 2001, Wemple et al. 2003, Suttle et al. 2004, Coe 2006, MacDiarmid et al. 2012).

The observed variation in uptake of organic matter derived from macroalgae by bivalves within catchments is further revealed by the influences associated with the range of catchment modification. Influences of landcover classifications have been associated with the increased delivery of particulate and nutrients. Forestry in Marlborough Sounds alters the nutrients and organic matter sources available to benthic communities (Thrush et al. 2004, Morrison et al. 2009) through the deliverance of high sedimentation along the coastlines recently evaluated by Urlich (2015). These effects can be identified as small-scale variability in sources of organic matter within regions (Hamilton et al. 2011), which likely impacts sedentary species (Wing et al. 2012). Farming in Marlborough Sounds and Tasman Bay introduce nitrogen inputs, which have likely escalated in the last 40 years through increased development of the Pelorus and Mapua catchment resulting in increases of nitrogenous fertilizer application in intensified farming practices (Parfitt et al. 2012).

The influences associated with the different landcover classifications appear to have the most pronounced impacts in Tasman Bay where collectively urban development, farming and forestry make up over 50% of the catchment (Table 2.8). The major rivers, Waimea and Wakapuaka, empty into estuaries where nutrients, contaminants and sediments are absorbed or retained. The exception is the Motueka river which dumps directly onto coastal habitats (Tuckey et al. 2006, Newcombe et al. 2015). The rapid tidal flushing of the shallow Tasman Bay estuaries (Newcombe et al. 2015) frequently brings the land-based pollutants into the bay where they can be incorporated into the coastal ecosystem (Gillespie et al. 2011, Handley & Mclean 2016).

Though Pelorus Sound was also influenced by a large freshwater input, the opposite trend to Tasman Bay was present for the contribution of macroalgae supporting bivalves. Though Pelorus Sound was influenced by a high degree of forestry and receives large freshwater inputs (Table 2.8) ('Freshwater' 2017) the bivalve community here were supported by a high proportion of organic matter from macroalgae. The high riverine inputs of nitrogen have been reported at the head of Pelorus Sound since the 1970s (Carter 1976, Boynton et al. 1982). At the time, Boynton et al. (1982) demonstrated that around 60% of the nitrogen inputs going into Pelorus Sound were derived from riverine inputs, predominantly from the Pelorus River. It is likely that the strong tidal currents and depth of Pelorus Sound mediate the impacts of the resuspension of benthic sediment contributed to the bivalve community in this catchment (Carter 1976, Handley 2015).

In contrast to Pelorus Sound and Tasman Bay, there are no large riverine inputs to Queen Charlotte Sound (QCS) and the Tory Channel (Lucas 1997). The land-based inputs of particulates to QCS and Tory Channel were likely due to extensive forestry modification resulting in direct land runoff and small riverine inputs throughout the sound. Hydrodynamic models that have been generated for QCS illustrate widespread dispersal of particulates (Knight & Beamsley 2012, Hadfield et al. 2014). Therefore, differences in bottom stress and wave activity drive the build-up of particulate from catchments throughout QCS and Tory Channel influencing organic matter resource pools and detrital pathways in the food web supporting to coastal bivalves (Handley 2015, Urlich 2015).

Forested catchments generally have lower rates of land-based input of fine sediments to the marine environment, except for the case where forestry harvest leads to extensive erosion (Davidson et al. 2011). Increased sediment loading to marine habitats may have pervasive impacts on the marine community (Schiel & Foster 1986, Estes et al. 1989, Cole & Babcock, Airoldi & Virgilio 1998). The relatively pristine Fiordland region was characterized by intact catchments of native forest (Miller et al. 2006, Wing & Jack 2014). Therefore, it is likely that the bivalve communities within Doubtful Sound, with observed proportion of organic matter derived from macroalgae of ~85%, have not been influenced by the same magnitude of inputs from runoff as seen in Marlborough Sounds and Tasman Bay.

High rates of land-based suspended particulate matter found within modified catchments, corroborate the results of the present study on the negative impacts on bivalve feeding (Hawkins et al. 1999, Gardner 2000, Ellis et al. 2002, Safi et al. 2007). The differences in observed proportions of organic matter among bivalves associated with catchments with different landcover illustrated the potential for land-based influence on ecosystem function. Changes in landcover ultimately influence the available organic matter particulate pools to bivalves through the increase of sedimentation. Sedimentation increases turbidity and reduces light transmission, subsequently influencing primary production and in turn impacting benthic consumers, both in resource availability but also through negative effects of suspended sediment on bivalves feeding rates (Hewitt & Pilditch, 2004, Thrush et al. 2004, Gibbs et al. 2005, Morrison et al. 2009). The effects of land modification and alterations from urban development, farming and forestry can strongly affect trophodynamics of benthic bivalves which play a key role in benthic pelagic coupling between shallow water ecosystems and pelagic productivity (Griffiths & Hokey 1987).

In the present study, we show clear differences in the use of alternative organic matter sources supporting bivalve species with different feeding strategies inhabiting three regions characterized by large differences in land modifications and aquaculture development. The influence of land modification and salmon farms on bivalve trophodynamics are consistent with shifts in resource utilization among regions, while differences in feeding strategies among species underlie the observed intraspecific differences in resource partitioning. Changes in landcover and modification of catchments in Marlborough Sounds were closely linked to changes in food web structure in the region (Udy et al. 2019a). In the present study we provide more evidence that Marlborough Sounds may have a strongly modified detrital pathway in the food web leading to coastal bivalves. These results highlight the key role of bivalves in the routing of basal carbon sources between land and coastal habitats, and that physical modification to terrestrial habitats have ecosystem level consequences.

Chapter 3: Influence of catchments and organic matter source on the variation in concentration of trace metals in five different bivalve species on the South Island of New Zealand

3.0 Introduction

Environmental changes influence the structure and patterns of organic matter fluxes through food webs, as demonstrated in Chapter 2 by the influence of anthropogenic activity on organic matter resource use in bivalves. Environmental changes can be observed in both physical and biological processes and are reflected within the skeletal structure and tissues of organisms. For example, shell material in bivalves can resolve interannual changes in sea ice cover in Antarctica (Dick et al. 2007, Wing et al. 2019) while soft tissues of the same species provide a snapshot of pollution levels with potential health implication to humans (Deudero et al. 2009, Melwani et al. 2014, Ruiz-Fernández et al. 2018). Trace metal concentrations reflected in the soft tissues of suspension feeding bivalves are influenced by environmental conditions through direct exposure to the water column as well as through ingested food particulates (Rainbow 2002, Shulkin et al. 2003, Wang 2003, Deudero et al. 2009b, Melwani et al. 2014, Genç et al. 2018, Ruiz-Fernández et al. 2018). In this context, the environmental influences on coastal habitats can be explored through the information reflected in the biochemical signatures from bivalve tissue to resolve important ongoing changes in coastal ecosystems.

Bivalves can bioaccumulate trace metals from both the water column and from food particles, with concentrations up to 1000 times greater than metal concentrations observed in the surrounding seawater (Naimo 1995). Bioaccumulation of metals is species specific and may vary significantly with both physical (e.g. water quality, habitat, salinity and temperature) and biotic (e.g. composition of food particulates, growth, reproduction, size) factors (Rainbow & Phillips 1993, Wu et al. 2005). Turn-over times of soft tissues, in addition to tissue type, influences the concentrations of different trace metals (Deudero et al. 2009a, Peake et al. 2010). However, due to the small size of some species, whole sample digestions are required in order to make comparisons among species (Marsden et al. 2014, Ruiz-Fernández et al. 2018).

Some bivalves show similar patterns in the uptake of metals, indicating similar responses to bioavailability and accumulation among species (Ruiz-Fernández et al. 2018). Further differences in concentrations among species are related to storage of trace metals. Metals in bivalves can be stored in two different compartments, the metabolically available compartment, and the stored detoxified compartment. Variability in processing of metals among these compartments may contribute to the observed differences in trace metal concentrations among species (Rainbow 2002, Voets et al. 2009).

Due to the observed interspecific variability in bioaccumulation of trace metals it is important to consider multiple species across a region in order to resolve how trace metal concentrations vary as a result of both natural and anthropogenic influences (Nriagu 1994, Callender & Rice 2000, Dimitrakakis et al. 2014, Briant et al. 2017, Sánchez-Quiles et al. 2017). For example, trace metal concentrations in macroalgae have been associated with hotspots of anthropogenic activity (Sánchez-Quiles et al. 2017), but these may be reflected differently by bivalves with different feeding mechanisms.

Conservative trace metals, such as Sr, Mg, and Cr, are found in sea water at concentrations proportional to salinity (Sadiq 1992, Lannuzel et al. 2011, Wing et al. 2019). The concentrations in the environment can therefore be influence by factors such as riverine inputs delivering freshwater to the coastal marine ecosystem. Bioactive trace metals including micronutrients such as Fe, and co-limiting Co, Ni, and Zn may be influenced by patterns in primary production (Wing et al. 2019). Concentrations of toxic, highly scavenged metals such as Pb, As, and Cd, decline with distance from point sources. Together, patterns in the concentrations of these three classes of trace metals can provide information on food sources, environmental conditions, and trace metal pollutants that each influence bivalve species.

As a result of anthropogenic activity, landcover has been dramatically altered globally with few habitats left in a pristine condition. Therefore, conducting studies of land-based impacts on coastal communities can be difficult due to the lack of baseline knowledge. Marlborough Sounds and Tasman Bay are influenced by a high degree of land conversion dating back to Mäori settlement (Handley 2006). In contrast, the relatively pristine, native forest covered catchments of Fiordland have been protected from land conversion since 1904 as a reserve, and since 1952 as New Zealand's largest National Park (Miller et al. 2006, Wing & Jack 2014). Previous studies have illustrated the higher degree of land-based impacts on reef fish communities and the food webs that are supported by in Marlborough Sounds, relative to those observed in Fiordland (Udy et al. 2019a, 2019b, 2019c). In these studies on reef fish communities, extensive conversion of land in the catchments of Marlborough Sounds were associated with a decline and simplification of kelp forest based food webs and lower fluxes of kelp derived organic matter into high trophic level fishes. Consequentially, these regions provide a useful contrast for identifying differences associated with landcover in how trace metals may be delivered and taken up in bivalve communities.

Previous studies indicate that trace metal signatures vary among bivalve species as a result of differences in physiology and compartmentalization of toxic elements. Therefore, a single species cannot be used to understand patterns of metal concentrations in the aquatic environment, therefore, a multispecies approach should be employed if possible (Ruiz-Fernández et al. 2018). Limits on the natural distribution of bioindicator organisms makes it difficult to compare metal concentrations over wide geographic areas (Wu & Lau 1996). To address both of these apparent problems we sample five different species ranging from intertidal to sediment dwelling, each with wide geographic distributions around New Zealand. We aim to resolve how trace metal concentrations are likely shaped by anthropogenic activity around the South Island of New Zealand. We predict that bivalves from the heavily impacted Tasman Bay and Marlborough Sounds will reflect higher concentrations of trace elements relative to Fiordland. Further, we hypothesize that sediment dwelling species will reveal higher trace element concentrations relative to those residing nearer to the surface.

3.1 Methods

In order to identify differences in trace metal concentrations in benthic suspension feeding bivalves among the three study regions, five species of bivalves ranging from intertidal to sediment dwellers were selected from areas with a range of landcover classifications (Figure 3.1).

3.1.1 Study organisms

Horse mussels, *Atrina zelandica* (Gray 1835) reside in the subtidal to depths of ~50 m in habitats characterized by muddy to sandy benthos (Powell 1979). *Atrina zelandica* are a large bivalve species, growing to ~30 cm. *A. zelandica* has an upright habit with at least two thirds of its shell length are buried in the sediment. The shell is attached to rocks or other anchor points in the sediment with byssus threads (Powell 1979). The distribution of *A. zelandica* can generally be found as a series of dense patches (eg. >10 m^2) (Cummings et al. 2001). Due to the upright posture of its large shell, which can modify the benthic boundary layer, *A. zelandica* feeds at roughly 15-30 cm above the seafloor.

The New Zealand scallop, *Pecten novazelandiae* (Reeve 1853, pectinid), is the largest and most abundant species found in New Zealand (Marsden & Bull 2006). The endemic bivalve is generally found in beds scattered from estuaries to coastal bays and reefs at densities of >1 m^{-2} to depths from subtidal to 80 m (Marsden & Bull 2006). The species has a wide distribution around New Zealand, found from the top of the North Island to the bottom of the South Island (Marsden & Bull 2006, Carbines & Michael 2007). Scallops feed through non-siphonate, ciliary suspension gill structures where they filter particles within mucous sheets along the mantle (Beninger & Le Pennec 2006, MacDonald et al. 2006). Feeding ability for pectinids appears to be limited in very high flow conditions (Wildish et al. 1987, Claereboudt et al. 1994) as well as very low flow conditions along with high concentrations of seston (Eckman et al. 1989, Pilditch et al. 2001).

The dog cockle, *Tucetona laticostata* (Quoy and Gaimard, 1835) are generally found in higher energy environments, mostly buried in coarse sand or gravel, across a depth range from ~2-3 m to about 70 m of water (Powell 1979). The thick-shelled bivalve has a wide distribution extending from the top of the North Island to the bottom of the South Island forming dense patches of up to more than 80 individuals m^{-2} (Powell 1979, Kerr & Grace 2005). Aside from local abundance and distribution data and paleontological research, few studies have been conducted on the biology and ecology of *T. laticostata* in comparison of many of its neighboring bivalve species.

The green-shelled mussel, *Perna canaliculus* are commonly found in dense patches of 160 m^{-1} on farms (Forest & Atalah, 2017) filtering particulate matter from the subtidal and open coast, but may extend to the intertidal region, where they play a large role in the temperate coastal community (Morton & Miller 1968, Gardner 2000). The species often provide habitat for other sessile invertebrates that adhere to mussel shells. *Perna canaliculus* are an endemic species with a wide distribution around New Zealand (Morton & Miller 1968). Studies have indicated that the diet of *P. canaliculus* is seasonal depending on the presence of phytoplankton and macroalgal abundance (Gardner 2000). Further, feeding is negatively impacted by low quality suspended particulate where the rate of particulate consumption decreases with low quality suspended particulates (Gardner 2000).

The blue mussel, *Mytilus galloprovincialis*, have a wide distribution around New Zealand from the top of the North Island to the tip of the South Island (Powell 1955, Shinen & Morgan 2009). This intertidal mussel forms dense patches on rocky substrate at ~1000 m^{-2} (Porri et al. 2008) and will select organic over inorganic particulates (Hawkins et al. 1996). Further, the absorption of ingested particulates is influenced by periodic fluctuation in the available suspended particulate matter and negatively impacted by low quality suspended particulate (Bayne et al. 1987, Navarro et al. 1991, Iglesias et al. 1996, Gardner 2000). Densities range average 100 m^{-2} (Porri, Jordaan and McQuaid, 2008)

3.1.2 Sample sites

Bivalve collections were carried out by scuba divers in Marlborough Sounds during February 2018 and November 2018 from the RV *Polaris* II with sampling sites spanning four regions within the Sounds: Port Underwood, Queen Charlotte Sound, and Pelorus Sound (Figure 3.1).

Collections from Tasman Bay were conducted during February 2018 from the RV *Polaris* II with sites encompassing the perimeter of the bay (Figure 3.1).

The sites from the top of the South Island, Marlborough Sounds and Tasman Bay, fall within catchments with varied landcover categories: forest, urban development, forestry, farming, and aquaculture systems (Figure 3.1, Appendix 1). Sites were chosen along the gradient with increasing distance from the mouth of the Sounds in order to compare sites with increased influence from major surrounding catchments.

Collections of bivalves in Fiordland were carried out by scuba divers during February 2019 from the RV Typhoon. Site selection was focused on sampling sites in Thompson's Bradshaw Sound adjacent to Doubtful Sound and were constrained to the 20 m depth contour (Figure 3.1).

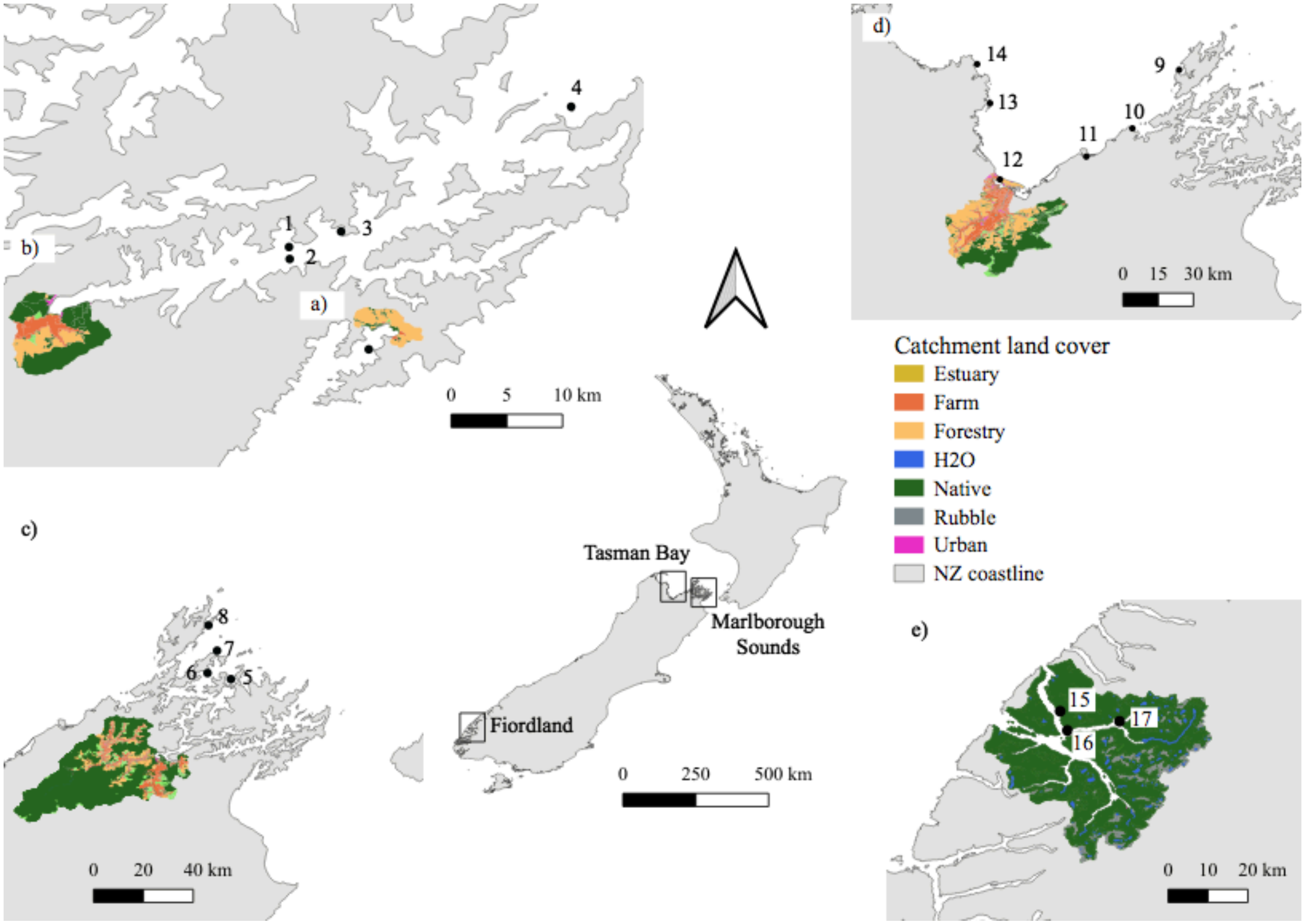

Figure 3.1 Sites and landcover classifications within catchments for each of the study regions. Catchments: a) Port Underwood, b) Queen Charlotte Sound, c) Pelorus Sounds, d) Mapua, and e) Doubtful Sounds. Sites: Marlborough Sounds 1) Perano Shoal, 2) Dieffenbach Point, 3) Ruakaka Bay, 4) Motungarara Island, 5) Bird Island, 6) Waitata, 7) Clay Point, 8) Old Man's Head. Tasman Bay 9) Ragged Point, 10) Crousielles Harbour, 11) Delaware Bay, 12) Mapua, 13) Te Karetu, 14) Totaranui. Doubtful Sound 15) Crayfish Creek, 16) Bradshaw-Thompson, and 17) MacDonald Island.

3.1.3 Sample collection

Target bivalve species were collected from seventeen sites within Marlborough Sounds (10), Tasman Bay (6) and Fiordland (1) (Figure 3.1). Samples were collected by hand at each site and placed into mesh bags under the New Zealand Ministry of Primary Industries special permit (644-2). Samples were then placed in individual tanks on-board the RV *Polaris* II with a constant surface water flow for 48 hrs to allow sediment to be dispelled. Samples were then placed in Zip-Lock plastic bags, labeled and frozen until dissection took place at the Portobello Marine Laboratory, University of Otago. The number of samples collected was constrained by the abundance of each species at each collection site, therefore, the largest sample size was from the most abundant species.

3.1.4 Trace metal analysis

Sample dissection and preparation

Samples were dissected at Portobello Marine Laboratory, University of Otago, in a laminar flow cabinet (Gelman, HLF series). All tissue, aside from the ≤ 0.5 g sample of adductor muscle tissue used for stable isotopic analysis, was placed in labeled 50 ml falcon tube. In order to access tissue, the left and right valves were separated at the ventral margin using teflon forceps or a ceramic knife if forceps were insufficient. Tissue samples were frozen for 48 hr at -18 ˚C and subsequently freeze dried at -60 ˚C for 48 hr (mrc, FDL-10N-50 series). Based on methods tested in a pilot study for homogenizing sample tissues, samples were ground to as fine a powder as could be achieved using a mortar and pestle in compliance with standard method (Jack et al. 2011). To limit cross contamination of samples, the agate mortar and pestle was cleaned between each sample by rinsing with ethanol (EtOH) and drying thoroughly with lint-free paper.

Digestion and analysis

Trace metal samples were acid digested in trace metal clean Teflon tubes. Teflon tubes were cleaned prior to each digestion by adding 400 ml of quartz-distilled nitric acid (QD HNO_3) and microwaving Teflon tubes in CEM MARS6 microwave under “clean,” heating to 180 ˚C over 20 min and holding for 5 min. Tubes were allowed to cool in the microwave for an additional 30 min. Once cooled, Teflon tubes were rinsed 3x with Milli-Q and set to dry overnight (10-12 hrs) under the laminar flow cabinet (Gelman, HSL series).

Samples weighing 250 ± 20 mg were transferred from Falcon tubes into clean microwave vessels with plastic spoons soaked in 5% hydrogen chloride (HCL), which were changed between samples. Three replicates of each sample were analyzed to identify instrument precision. Samples were digested by adding 1 ml Milli-Q, 10 ml QD HNO_3 and 1 ml hydrogen peroxide (H_2O_2) and microwaved (CEM MARS6) under the "animal tissue" setting which programmed the oven to reach 200 ˚C in 10 min and held at temperature for 15 min. Samples cooled for 30 min and were subsequently poured into pre-weighed and labeled DigiTubes. Teflon tubes were rinsed 3 x with ~5 ml Milli-Q and poured into DigiTube to collect any remaining sample. Final sample volumes of ~25 ml were placed on a hot block at 90 ˚C overnight (10-12 hrs). One ml of QD HNO_3 was added to dried samples, swirled and replaced on the hot block for an additional 15 min. DigiTubes were diluted to 10 ml with Milli-Q and weighed. Sample densities were calculated for a random subset and used to calculate final sample volumes (method adjusted from Sander et al. 2009, Ng et al. 2007, Wing et al. 2014).

An Agilent 7900 ICP-MS (Agilent Technologies, U.S.A) in the Department of Chemistry, University of Otago, was used to analyze trace metal concentrations in digested tissue samples. The trace metals of particular interest were Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Zn, and Sr, however a range of metals were analyzed for identification of possible instrument interferences. Blanks (Milli-Q) and reference material (DOLT-2 dogfish liver, National Research Council Canada) were incorporated into the autosampler pot in order to identify drift or possible matrix effects in sample trace metal concentrations. In addition, a combination of six reference metals (NIST traceable) were added online and used to construct calibration standards. The ICP-MS was tuned according to manufacture instructions to enhance instrumental precision and limit interference and instrumental drift. All trace metals of interest had recoveries within 5% of the expected values, therefore the results are considered reliable.

Trace metal concentrations were recorded at 100% of the study sites. All organisms recorded trace metal concentrations above the limit of detection with the exception of three *A. zelandica* samples for Pb concentrations (Table 3.1).

Table 3.1 Limits of detection and percent recovery for 13 trace metals. Recovery is given in a range due to multiple runs with the ICP-MS.

Trace metal group	Trace metal	Concentrations (mg/kg)	Recovery range (%)
Conservative	Mg	5	92-105
	Al	5	97-106
	Cr	0.15	91-93
	Sr	0.02	94-103
Bioactive	Mn	0.05	93-102
	Fe	1	95-99
	Co	0.02	93-102
	Ni	0.2	84-95
	Cu	0.1	92-106
	Zn	1	91-99
Highly scavenged	As	0.15	90-97
	Cd	0.05	93-102
	Pb	0.2	72-89

3.1.5 Landcover classifications

The watersheds surrounding Marlborough Sounds, Tasman Bay and Fiordland were divided into the following discrete landcover categories based on proportion: forest, urban, forestry, and farming (Figure 3.1). Presence and absence of salmon farms was also noted for Marlborough Sounds. Landcover information was provided by Land Information New Zealand (LINZ, 2018) and Marlborough District Council (Appendix 2). GIS maps were constructed by overlaying survey data using the spatial referencing system NZGD 2000.

Catchment areas, defined as the terrestrial region contributing terrigenous particulate matter to adjacent marine systems, were defined for each sample site by drawing polygons in QGIS and calculating the 2D area (km^2). Boundaries of the catchment were determined using both topographic contours (1 cm : 2.5 km), 8 m digital elevation models and sea-draining catchment layers (LINZ, 2018).

3.1.6 Statistical analysis

Univariate

To test for differences in concentrations of conservative (Mg, Cr, Al and Sr), bioactive (Mn, Fe, Cu, Co, Zn and Ni), and highly scavenged (As, Cd and Pb) metals within individual species among regions with varying influence of freshwater input, landcover classification and salmon farm presence, we ran general linear mixed models for the factors: REGION (3 levels fixed), SPECIES (5 levels, fixed) with presence of SALMON FARMS (2 levels, fixed) and varying LANDCOVER (4 levels, continuous) using JMP Pro (Version 14). Shell length was included in each of the models because trace metal concentrations have been demonstrated to increase with size in some species. Further, a post hoc test using Student's t identified pairwise differences among SPECIES and REGION with presence of SALMON FARMS and varying LANDCOVER. Homogeneity of variance was tested for each model within the general linear model platform in JMP Pro (Version 14).

Multivariate

A discriminant analysis in JMP compared trace metal concentrations within the three classes between Marlborough Sounds, Tasman Bay and Fiordland. To test significant differences in trace metal concentrations among regions, a two-way MANOVA design with SPECIES nested in REGION using a permutational analysis of variance in the program PRIMER v6 with PERMANOVA+ version 1.02. The PERMANOVA design was applied to the Euclidian adjusted matrix using a maximum of 9999 permutations with unrestricted permutations on raw data.

3.2 Results

3.2.1 Regional separation in trace metal concentrations

Discriminant function analysis indicated significant separation in trace metal groups among regions (Figure 3.2 – 3.4). The 95% confidence intervals around each region indicated significant differences for all trace metal groups. Conservative metals, Sr, Mg and

Al contributed most to the separation between regions with Cr also contributing to the discrimination (Figure 3.2). The bioactive metals, Ni, Co and Cu contributed most to the separation of bioactive metals among regions with concentrations of Mn and Zn also contributing to discrimination (Figure 3.3). All three highly scavenged metals, As, Cd and Pb contributed to the separation among regions (Figure 3.4). It was clear that concentrations of metals from each class contributed to the differences in metal concentrations between the regions. PERMANOVA analysis revealed that differences in trace metal concentrations were primarily the result of differences in bivalve species composition among regions (Table 3.2 – 3.4).

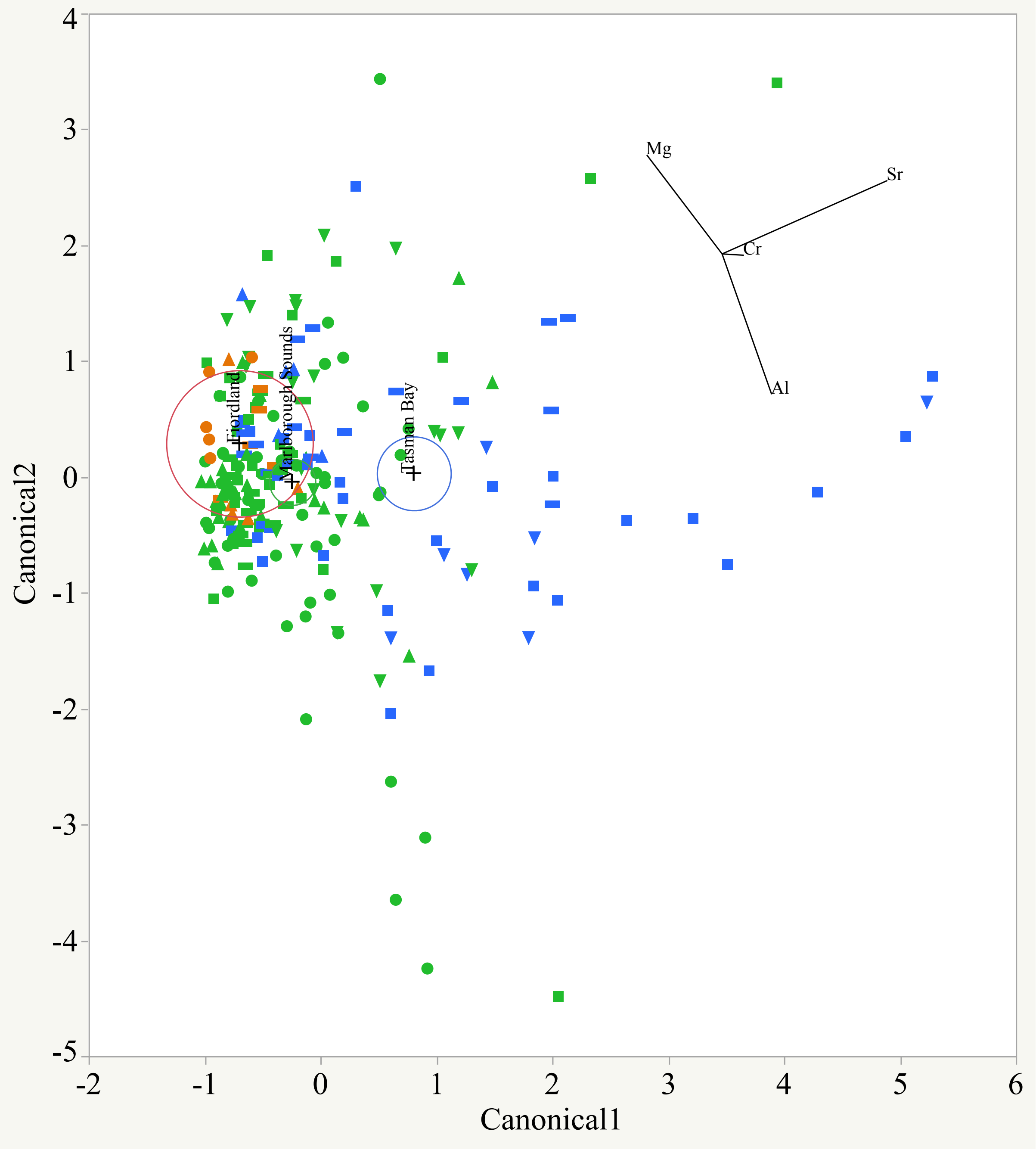

Figure 3.2 Canonical plot scores and 95% confidence ellipses from discriminant funtion analysis of four conservative metal concentrations among regions; Marlborough Sounds = green, Tasman Bay = blue, and Fiordland = orange. 95% confidence ellipses do not overlap for significantly different groups.

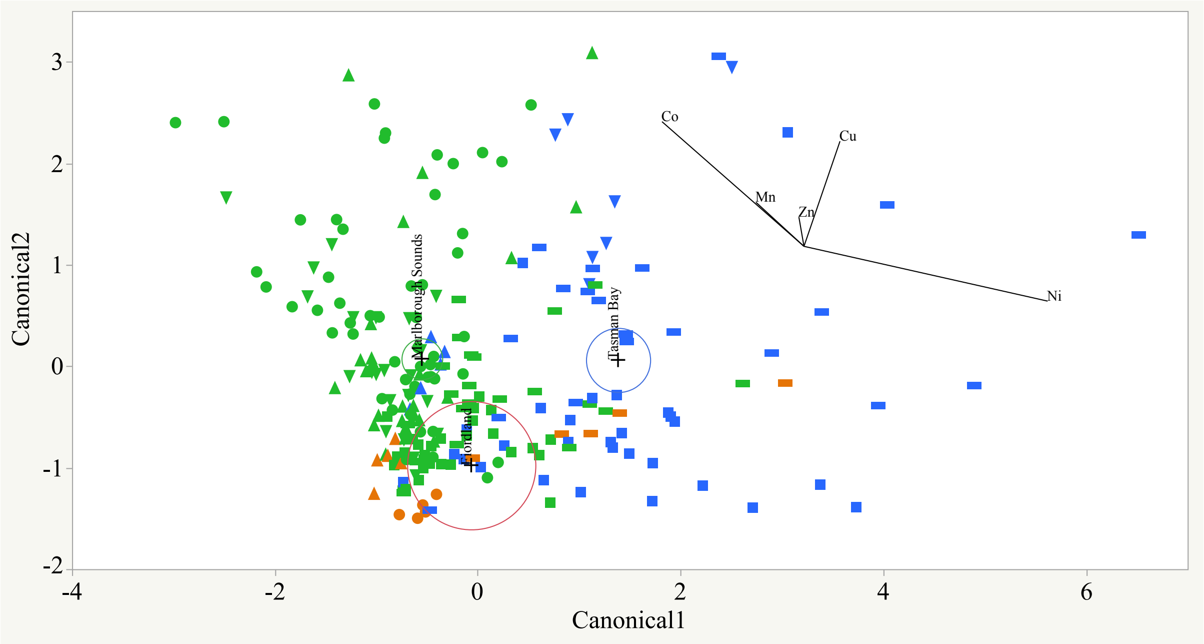

Figure 3.3 Canonical plot scores and 95% confidence ellipses from discriminant funtion analysis of five bioactive metal concentrations among regions; Marlborough Sounds = green, Tasman Bay = blue, and Fiordland = orange. 95% confidence ellipses do not overlap for significantly different groups.

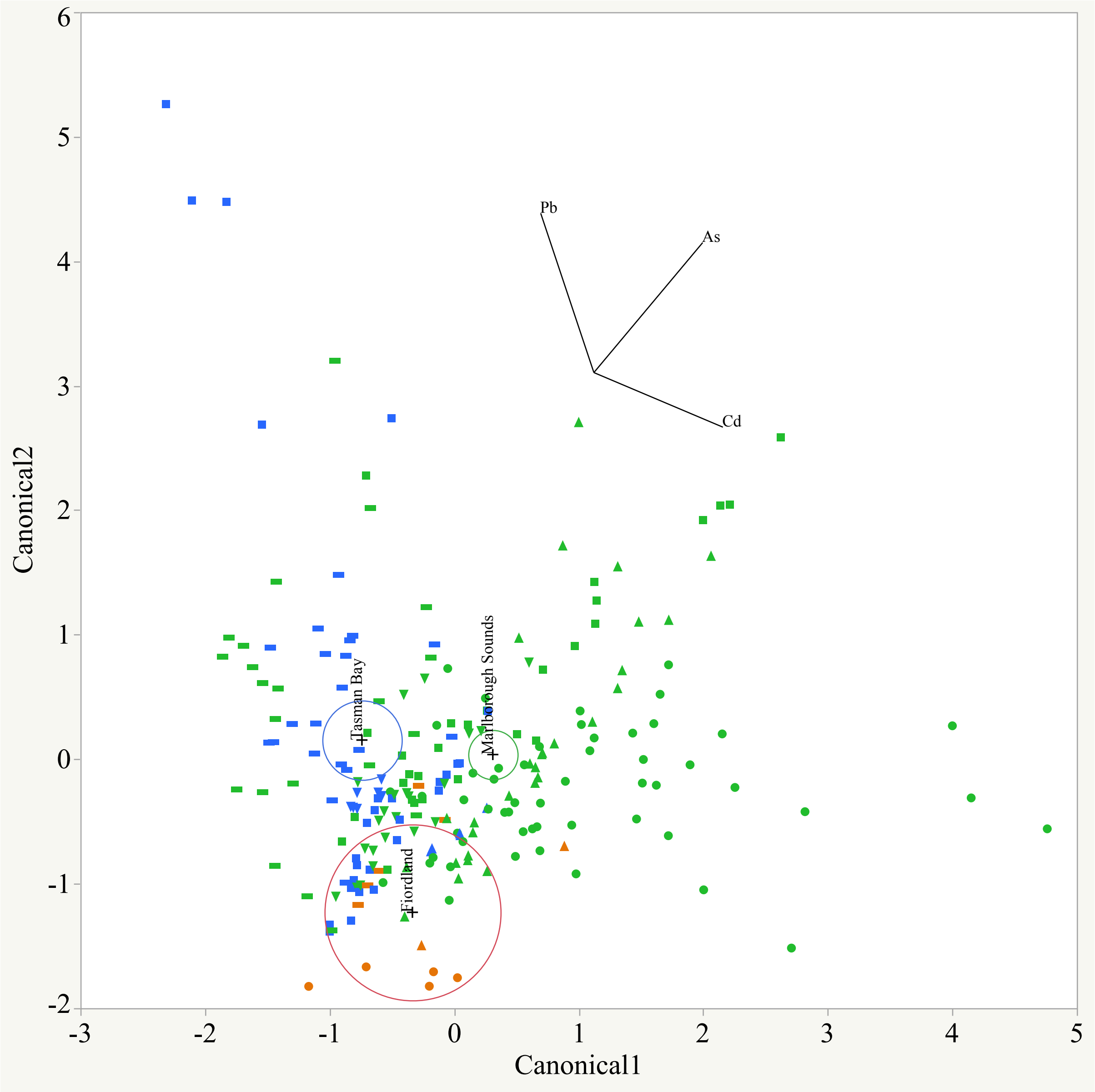

Figure 3.4 Canonical plot scores and 95% confidence ellipses from discriminant funtion analysis of three highly scavenged metal concentrations among regions; Marlborough Sounds = green, Tasman Bay = blue, and Fiordland = orange. 95% confidence ellipses do not overlap for significantly different groups.

Table 3.2 Results of a PERMANOVA on the multivariate similarity among concentrations of conservative metals Mg, Al, Cr, and Sr for species among regions. Significant p-values are bolded.

Source	df	SS	MS	Pseudo-F	P(perm)
Region	2	2.8964E7	1.4482E7	1.103	0.3689
Species(Region)	9	2.0588E8	2.2876E7	10.352	**0.0001**
Residual	217	4.7951E8	2.2097E6		
Total	228	6.8938E8			

Table 3.3 Results of a PERMANOVA on the multivariate similarity among concentrations of bioactive metals Mn, Co, Fe, Ni, Cu, and Zn for species among regions. Significant p-values are bolded.

Source	df	SS	MS	Pseudo-F	P(perm)
Region	2	9.6337E5	4.8169E5	0.42807	0.8472
Species(Region)	9	1.8213E7	2.0237E6	17.057	**0.0001**
Residual	217	2.5745E7	1.1864E5		
Total	228	4.5274E7			

Table 3.4 Results of a PERMANOVA on the multivariate similarity among concentrations of highly scavenged metals As, Cd, and Pb for species among regions. Significant p-values are bolded.

Source	df	SS	MS	Pseudo-F	P(perm)
Region	2	2160.5	1080.3	0.6204	0.6756
Species(Region)	9	28654	3183.8	25.477	**0.0001**
Residual	217	27118	124.97		
Total	228	62361			

3.2.2 Species level differences in trace metal concentrations

Further differences within species among regions explored with general linear models revealed significantly different concentrations within individual species among regions for all groups of metals. The highest concentrations of conservative metals were identified overall for bivalves collected from Tasman Bay while the lowest concentrations were found in bivalves collected in Marlborough Sounds (Figure 3.5). The highest concentrations of bioactive metals were identified for bivalves collected from the Tasman Bay while lowest concentrations were identified in bivalves collected in both Marlborough Sounds and Fiordland (Figure 3.6). Highest concentrations of highly scavenged metals were present in bivalves collected from Marlborough Sounds while lowest concentrations were found in bivalves collected from Fiordland (Figure 3.7).

Conservative metal concentrations (Mg, Al, Cr and Sr) were different among regions for *T. laticostata* (Figure 3.5). Bioactive metal concentrations (Mn, Fe, Co, Ni, and Cu) in *M. galloprovincialis* were each different among regions except Zn (Figure 3.6). Highly scavenged metal concentrations (As, Cd and Pb) in *A. zelandica* were different among regions (Figure 3.7).

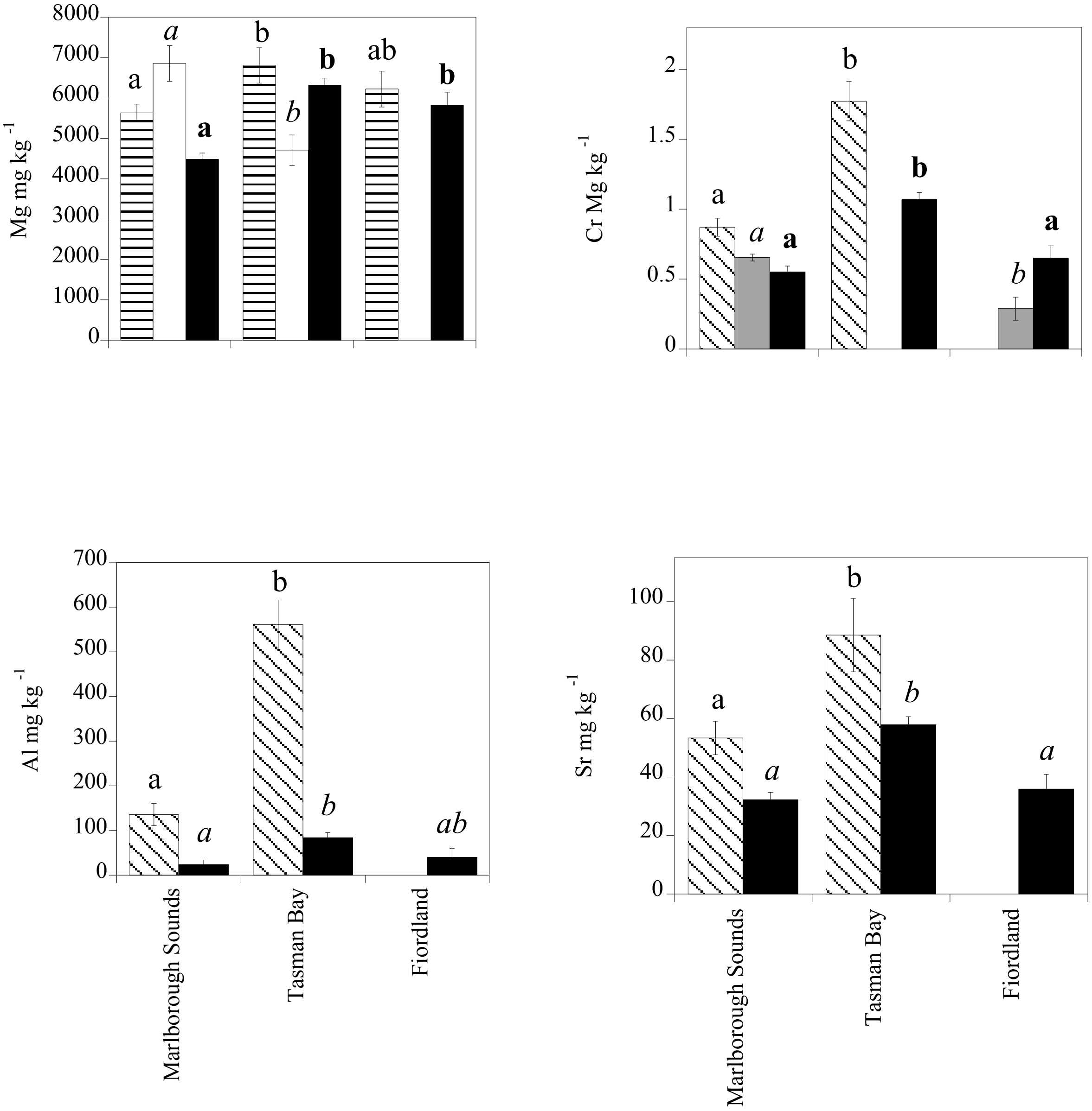

Figure 3.5 Significantly different concentrations of conservative metals at each region for individual species as determined by general linear models. (▤) *A. zelandica*, (☐) *P. canaliculus*, (▧) *M. galloprovincialis*, (▩) *P. novaezelandiae*, (■)*T. laticostata*. Mean ± 1 SE. Different letters indicate significant differences between regions.

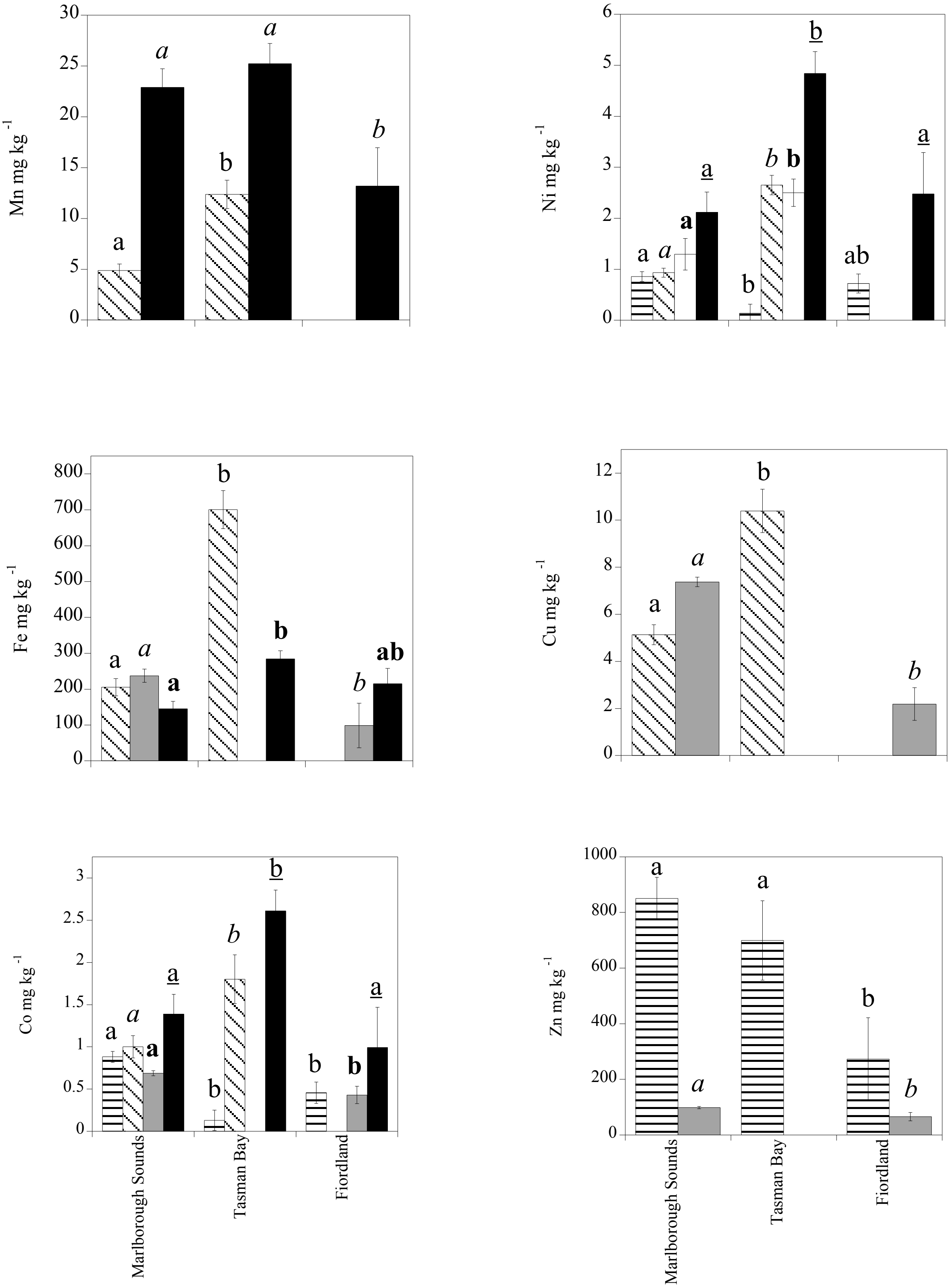

Figure 3.6 Concentrations of bioactive metals at each region for individual species. (▤) *A. zelandica*, (☐) *P. canaliculus*, (▧) *M. galloprovincialis*, (▩) *P. novaezelandiae*, (■)*T. laticostata*. Mean ± 1 SE. Different letters indicate significant differences between regions.

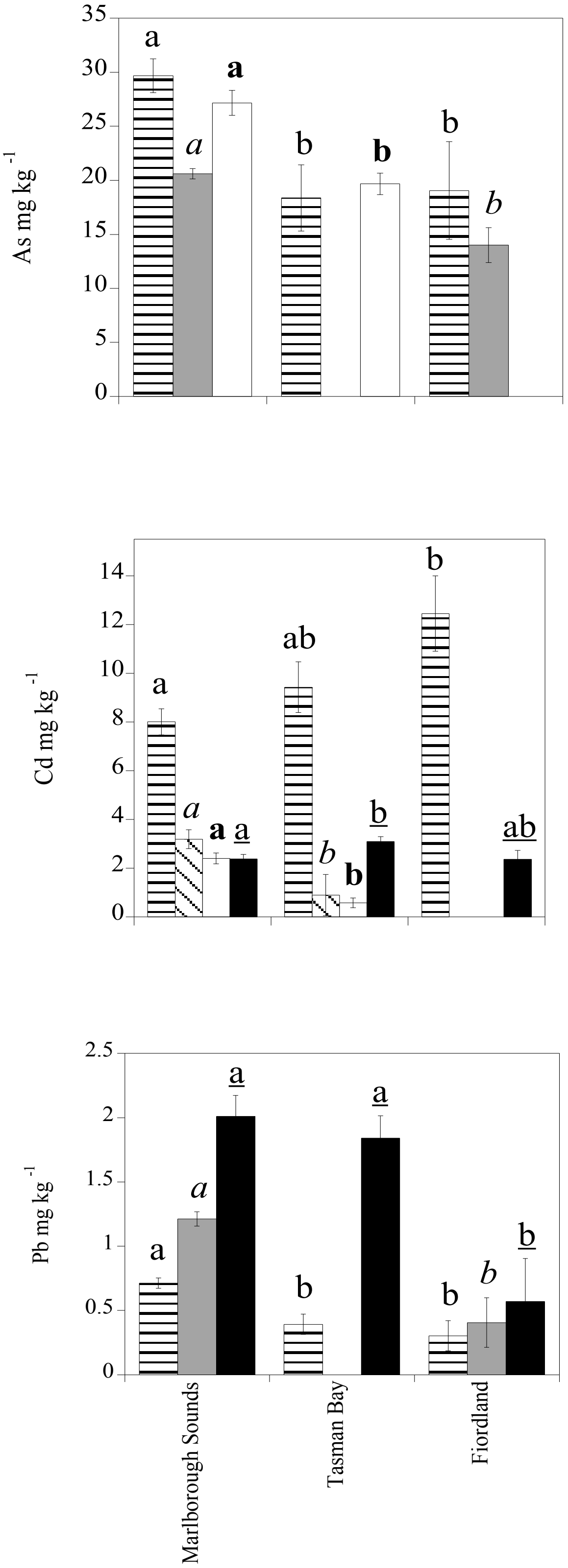

Figure 3.7 Concentrations of highly scavenged metals at each region for individual species. (▤) *A. zelandica*, (□) *P. canaliculus*, (▧) *M. galloprovincialis*, (▒) *P. novaezelandiae*, (■)*T. laticostata*. Mean ± 1 SE. Different letters indicate significant differences between regions.

3.2.3 Organic matter associations with trace metal concentrations

General linear models indicated that concentrations of trace metals varied with stable isotopic values (δ^{15}N, δ^{13}C) differently among species. The micronutrient, Co varied significantly in *A. zelandica* with δ^{13}C, while *P. canaliculus* varied significantly with δ^{15}N (Table 3.5). The highly scavenged metals, As and Pb, varied significantly with δ^{15}N in *P. canaliculus* while concentrations of As varied significantly with δ^{13}C in *T. laticostata* (Table 3.5). For the species, *A. zelandica* and *T. laticostata* which were abundant and collected from all study regions, the concentrations of the conservative metal, Mg, bioactive metals, Ni and Co, and highly scavenged metals, Cd and Pb were significantly different with stable isotope values (δ^{15}N, δ^{13}C) (Table 3.6).

Table 3.5 Individual general linear models revealed species specific differences in trace metal concentrations with δ^{13}C and δ^{15}N among regions. Significant p-vales are bolded ($p < 0.05$).

Species	Metal	Main test			δ^{13}C		δ^{15}N		Site[Region]		Region		Shell length	
A. zelandica		$F_{12,26}$	P	r^2	F	P	F	P	F	P	F	P	F	p
	Co	24.57	**<0.0001**	0.94	7.53	**0.0144**	2.50	0.1337	31.6997	**<0.0001**	21.9889	**<0.0001**	2.49	0.1345
P. canaliculus		$F_{13,47}$												
	Co	1.2164	0.3103	0.32	0.07	0.7891	4.82	**0.0350**	0.69	0.7130	0.13	0.7213	0.09	0.7685
	As	15.19	**<0.0001**	0.85	3.94	0.0554	11.28	**0.0019**	5.45	**0.0001**	0.13	0.7236	1.46	0.2357
	Pb	13.9029	**<0.0001**	0.84	1.15	0.2918	5.75	**0.0221**	17.71	**<0.0001**	0.16	0.6893	0.43	0.5146
T. laticostata		$F_{10,38}$												
	As	2.95	**0.0117**	0.51	6.72	**0.0150**	1.60	0.2170	4.93	**0.0023**	3.26	0.0535	0.11	0.7452

Table 3.6 Results of a general linear model revealed significant differences in trace metal concentrations with δ^{13}C and δ^{15}N for *A. zelandica* and *T. laticostata* among region. Significant p-values are bolded ($p < 0.05$).

Metal	Main test			δ^{13}C		δ^{15}N		Species		Region		Species*Region		Shell length	
	$F_{8,72}$	P	r^2	F	P	F	P	F	P	F	P	F	p	F	p
Cr	3.22	**0.0039**	0.29	3.93	0.0518	4.72	**0.0335**	1.24	0.2693	1.56	0.2180	3.63	0.0321	0.33	0.5687
Fe	2.68	**0.013**	0.25	5.19	**0.0261**	1.51	0.2230	1.38	0.2441	2.06	0.1358	1.69	0.1932	1.16	0.2845
Cu	10.28	**<0.0001**	0.56	10.51	**0.0019**	0.23	0.6343	5.54	**0.0216**	3.97	**0.0236**	4.99	**0.0097**	5.88	**0.0181**
As	6.78	**<0.0001**	0.45	6.10	**0.0161**	0.21	0.6511	0.03	0.8720	6.59	**0.0025**	4.33	**0.0172**	2.84	0.0968

Salmon farms

Concentrations of Mn, Co, Cu and Zn among all target bivalves significantly differed between sites with active salmon farms and similar reference sites (Table 3.7). Pari-wise analysis revealed significant intraspecific differences for *P. novaezelandiae* where significantly higher concentrations of Cu were present at salmon farm sites relative to reference sites (Figure 3.8a). Further, a positive linear relationship was found for concentrations of Cu relative to δ^{13}C in Marlborough Sounds among all species (Figure 3.8b).

Table 3.7 Results of general linear models revealed specific differences in trace metal concentrations with δ^{13}C and δ^{15}N among all target species at salmon farm sites and reference sites.

Metal	Main test			δ^{13}C		δ^{15}N		Salmon farm		Species		Salmon farm * Species		Shell length	
	$F_{12,101}$	P	R2	F	P	F	P	F	P	F	P	F	p	F	p
Mn	10.26	**<0.0001**	0.58	2.19	0.1421	6.80	**0.0107**	2.26	0.1367	12.86	**<0.0001**	0.48	0.7519	4.48	**0.0371**
Co	4.61	**<0.0001**	0.38	1.12	0.2922	3.96	**0.0497**	0.05	0.8273	7.05	**<0.0001**	0.28	0.8915	2.45	0.1208
Cu	11.62	**<0.0001**	0.61	4.86	**0.0300**	0.01	0.9091	3.49	0.0649	16.63	**<0.0001**	4.35	**0.0029**	6.62	**0.0117**
Zn	19.15	**<0.0001**	0.72	4.28	**0.0415**	1.66	0.2005	3.91	0.0510	11.84	**<0.0001**	2.32	0.0634	50.72	**<0.0001**

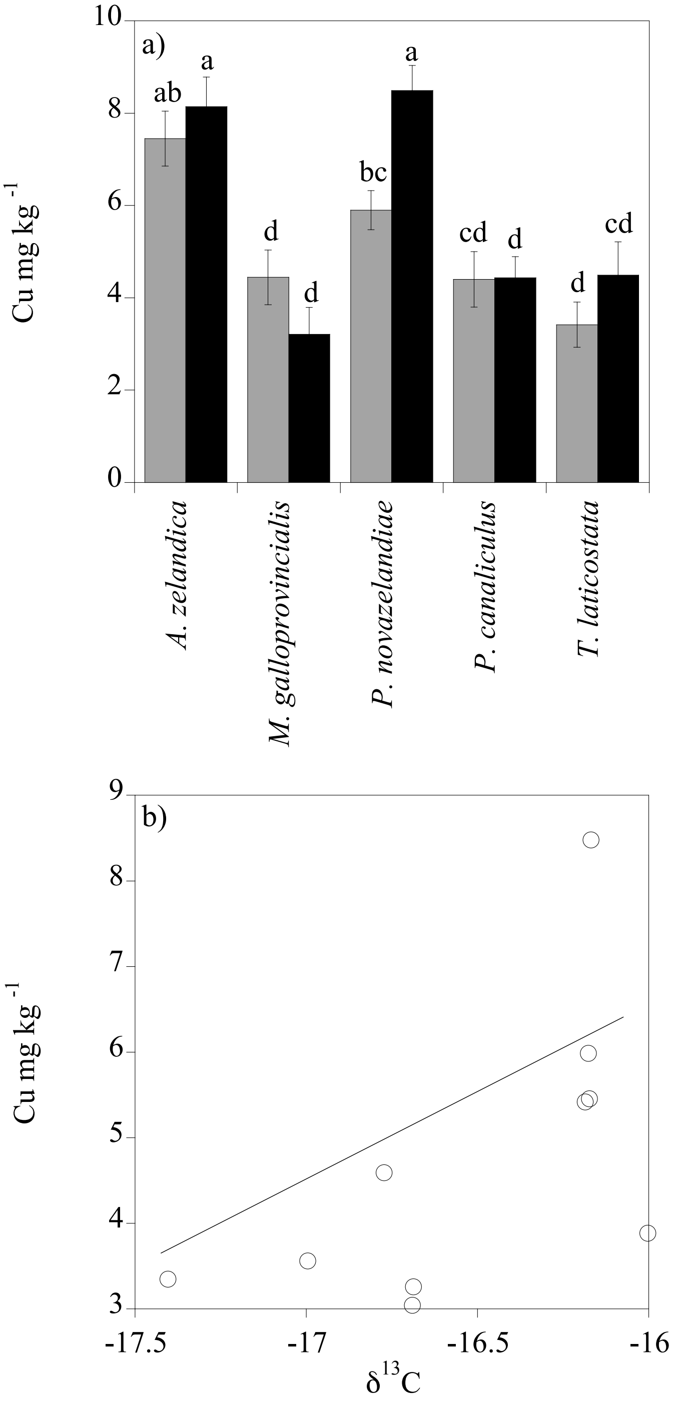

Figure 3.8 a) Pair-wise analysis of Cu concentrations for target species between salmon farm (black) and references (grey) sites and b) average Cu concentrations plotted against δ^{13}C of all species at salmon farm and reference sites. Mean ± 1 SE. Levels connected by different letters indicate statistical differences.

Catchment

Linear models illustrated significant differences in trace metal concentrations in bivalves collected adjacent to specific catchments (Table 3.8). Additional linear models were used to further explore these differences in relation to the varying proportion of landcover classification among catchments (Table 3.9). Significant variability in the accumulation of trace metal concentrations was observed for individual species within specific catchment systems (Figure 3.9).

The variation in landcover among catchments resolved differences in concentrations of trace metals with clear difference in conservative, bioactive and highly scavenged metals. Of the conservative metals, Sr, significantly differed among catchments for nearly all species. The concentration of Sr displayed a slight negative trend with catchments receiving decreasing levels of riverine inputs (Figure 3.10).

Table 3.8 Results of discrete general linear models revealed significant trace metal concentrations among catchments for individual species. Significant p-values ($p < 0.05$) are in bold.

Metal class	Metal	*A. zelandica*	*M. galloprovincialis*	*P. novaezelandiae*	*P. canaliculus*	*T. laticostata*
Conservative	Mg	**0.0263**	**0.001**	0.0881	0.1295	**0.0009**
	Al	0.1234	**0.001**	**0.0003**	0.3913	**0.0339**
	Cr	0.2837	**< 0.0001**	**<0.0001**	0.0551	**<0.0001**
	Sr	0.5368	**0.016**	**0.0051**	**0.0274**	**0.0002**
Micronutrients	Mn	0.5513	**0.001**	0.4246	0.6754	**0.0009**
	Fe	0.1806	**< 0.0001**	**<0.0001**	0.2320	**0.0086**
	Co	0.1795	0.872	**0.0020**	0.2214	**0.0009**
	Ni	0.0557	**< 0.0001**	**0.0045**	**0.0122**	**0.0002**
	Cu	0.3239	**< 0.0001**	**<0.0001**	0.2563	0.1160
	Zn	**<0.0001**	**0.0001**	**<0.0001**	**0.0283**	**0.0008**
Highly scavenged	**As**	**0.0049**	**0.024**	**<0.0001**	**<0.0001**	**<0.0001**
	Cd	0.1771	**< 0.001**	**0.0439**	**<0.0001**	**0.0280**
	Pb	0.0644	**0.0004**	**0.0032**	0.05060	**0.0013**

Table 3.9 Results of discrete general linear models results revealed significant differences in trace metals with δ^{13}C and δ^{15}N among catchments for individual species. Significant p-values ($p < 0.05$) are bold.

Species	Metal	Main test			δ^{13}C		δ^{15}N		Channel		Shell length	
A. zelandica		$F_{7,26}$	p	r^2	F	p	F	p	F	p	F	p
	Cu	4.27	**0.0055**	0.61	7.52	**0.0130**	1.17	0.2935	3.03	0.0545	1.63	0.2167
M. galloprovincialis		$F_{5,23}$										
	Mg	3.50	**0.0219**	0.49	0.07	0.7925	5.05	**0.0374**	0.15	0.7006	1.26	0.2755
P. novaezelandiae		$F_{6,34}$										
	Al	7.47	**<0.0001**	0.62	5.34	**0.0284**	0.58	0.4534	12.75	**0.0001**	3.83	0.0605
	Cr	9.86	**<0.0001**	0.68	6.05	**0.0204**	6.56	**0.0161**	2.21	0.1286	0.17	0.6873
	Fe	8.70	**<0.0001**	0.65	5.91	**0.0217**	0.73	0.4016	14.90	**<0.0001**	0.94	0.3406
P. canaliculus		$F_{6,47}$										
	Fe	1.80	0.1222	0.21	4.16	**0.0478**	0.13	0.7191	2.15	0.1292	3.71	0.0609
	As	31.05	**<0.0001**	0.82	12.65	**0.001**	6.78	**0.0128**	5.31	**0.0089**	1.77	0.1910
T. laticostata		$F_{7,38}$										
	Mg	6.96	**<0.0001**	0.61	4.27	**0.0472**	0.33	0.5674	11.17	**<0.0001**	0.81	0.3764
	Sr	9.32	**<0.0001**	0.68	0.26	0.6145	9.03	**0.0052**	11.47	**<0.0001**	5.73	**0.0229**

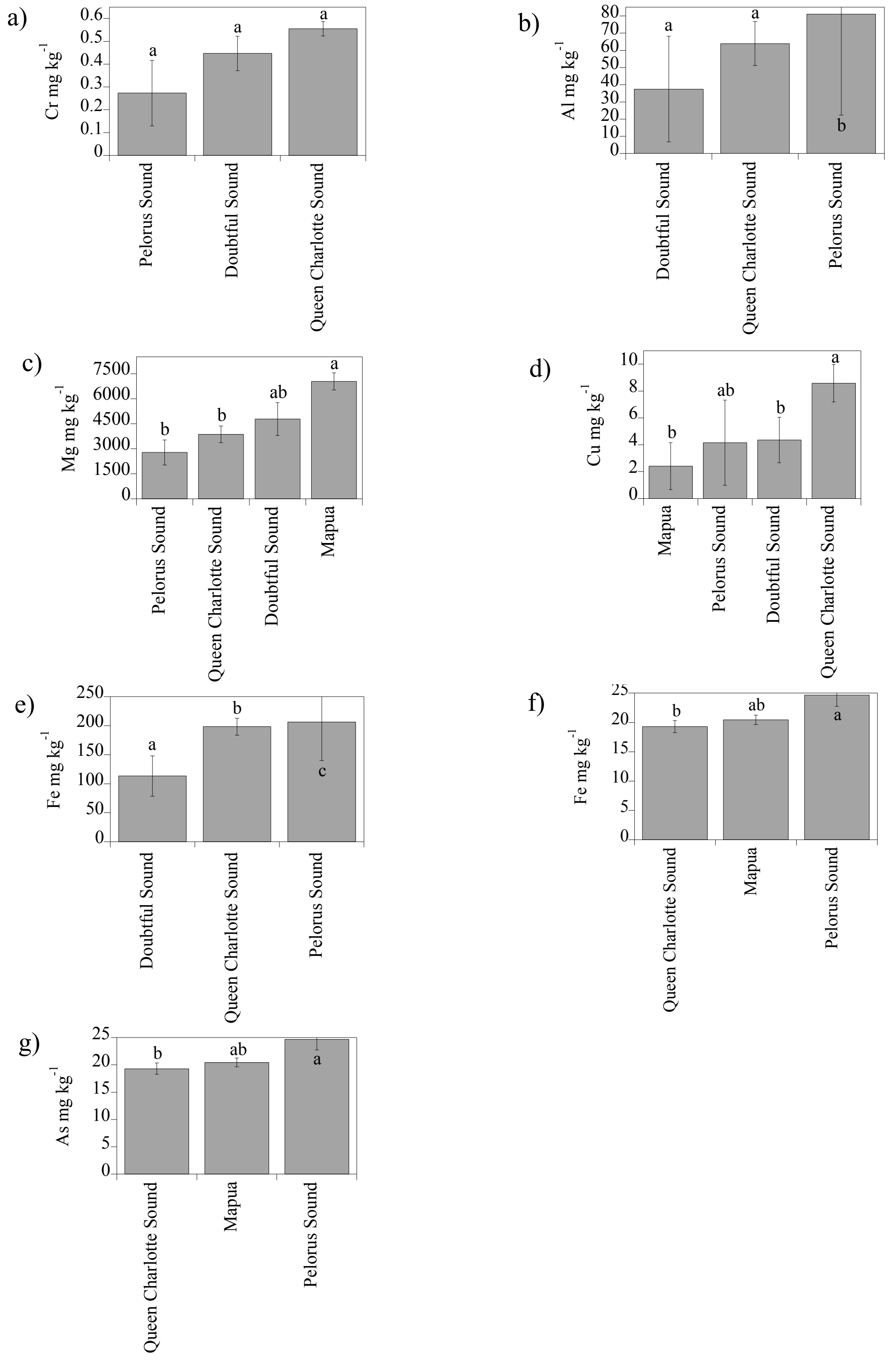

Figure 3.9 Significantly different trace metal concentrations with among catchment for conservative metals in a,b) *P. novaezelandiae*, c) *T. laticostata*, bioactive metals in d) *A. zelandica*, e) *P. novaezelandiae*, f) *P. canaliculus*, and highly scavenged metals in g) *P. canaliculus*. Mean ± 1 SE. Levels connected by different letters indicate statistical differences.

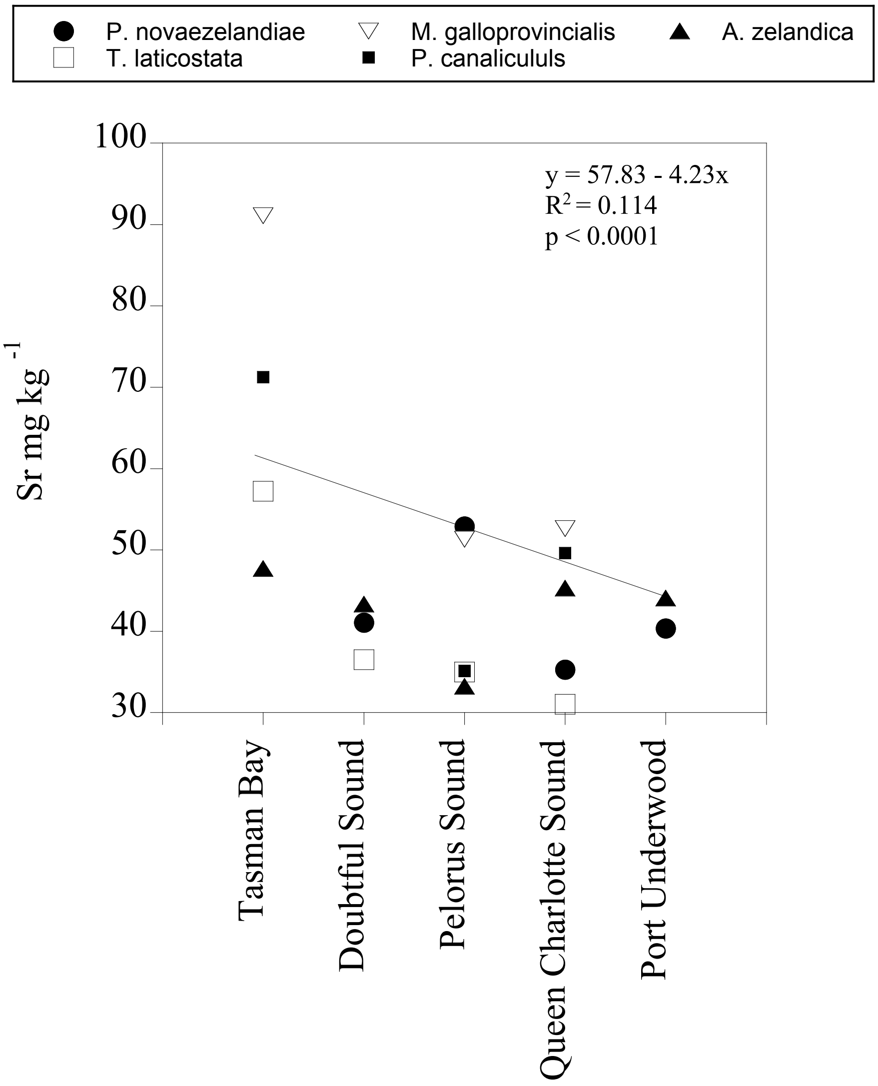

Figure 3.10 Concentrations of conservative metal (Sr) in each of the catchments of individual species.

3.3 Discussion

The data from the present study provide strong evidence that organic matter sources and differences among particulate pools associated with discrete catchments in each of the study regions were reliably reflected by the concentrations of trace metals in the tissues of the five bivalve study species. The variation in organic matter sources supporting bivalves as identified in Chapter 2, freshwater input and landcover, provided an opportunity to test whether the target species tissues, as a community, could be used to identify local and regional differences in trace metal concentrations. The catchment systems within

Marlborough Sounds, Tasman Bay and Fiordland, each with different intensities of anthropogenic influences, provided a testing platform for assessing how concentrations of trace metals in benthic food webs varied in response to land modification. Statistical relationships that were resolved between trace metal concentrations for bivalve species among regions were consistent with the ideas that spatial variability in the concentrations of conservative, bioactive, and highly scavenged trace metals in target bivalve tissue were reflective of water column chemistry linked to landcover modification affecting whole catchment systems and local modification associated with salmon farms.

3.3.1 General trends in regional trace metal concentrations

Significant multivariate differences in trace metal concentrations of bivalves were revealed among regions (Table 3.2 – 3.4). Among the metals analysed, conservative metals, Sr, Mg and Al, bioactive metals Ni, Co and Cu, and highly scavenged metals As, Cd and Pb explained the largest amount of multivariate dissimilarity among regions (Figure 3.2 – 3.4). Spatial differences in trace metal concentrations have previously been attributed to natural variation molded by geographically and chemically disparate water masses (Becker et al. 2005, Carson 2010, Sorte et al. 2013, Cathey et al. 2014) and localized anthropogenic activity (Callender & Rice 2000, Bonnet et al. 2013, Dimitrakakis et al. 2014, Sánchez-Quiles et al. 2017, Ruiz-Fernández et al. 2018).

Significant differences in the three classes of trace metals among species revealed by the factor REGION, uncover patterns associated with contrasts in freshwater inputs, primary productivity and contaminants. Conservative metal concentrations in bivalves were consistent with differences in water column chemistry between Tasman Bay and Marlborough Sounds. The observed pattern may have reflected the high riverine inputs to Tasman Bay relative to the minimal riverine inputs in Marlborough Sounds (Lucas 1997, Tukey et al. 2006, Forrest et al. 2007, Newcombe 2016). Bioactive metal concentrations can be indicative of regional differences in water column productivity. The observed high bioactive metal concentrations in bivalves collected from Tasman Bay may have reflected the influence of local upwelling, that accounted for up to 90% of the nitrogen pool, as well as the influence of land-based runoff on productivity which was high in the region (MacKenzie & Gillespie 1986, Newcombe et al. 2015). However, the seasonality of phytoplankton production would need to be tested over a larger temporal scale in order to confidently report differences associated with upwelling events. The resulting differences may reflect the more variable organic matter source pools in Tasman Bay relative to a the potentially more stable organic matter source pools supporting bivalves in Marlborough Sounds and Fiordland. The stability of the organic matter sources from Marlborough Sounds and Fiordland may be attributed to the lack of upwelling events and the typically lower biomass of phytoplankton among these two regions (Zeldis et al. 2008). Concentrations of highly scavenged trace metals in bivalves can reflect their proximity to a point source. We observed elevated concentrations of the highly scavenged metals in both Tasman Bay and Marlborough Sounds relative to those observed in bivalves collected from Fiordland (Figure 3.7). The observed pattern was consistent with inputs of highly scavenged metals (As and Pb) from land-based catchments. The observed spatial variation in trace metal signatures reveal how land-based influences associated with particular regions help to shape trace metal concentrations in the bivalve communities.

3.3.2 Associations between organic matter flow and trace metal concentrations among species

The variation in trace metal concentrations among species have been well studied. Different species have affinities for particular metals and uptake trace metals both dissolved in seawater and with ingested organic matter sources (Rainbow 2002, Shulkin et al. 2003, Genç et al. 2018, Ruiz-Fernández et al. 2018). The variation among species can be influenced by both biological (diet, metabolism, growth, and reproduction) and environmental factors (temperature and salinity) (Wang & Fisher 1996, Casas & Bacher 2006, Degger et al. 2011, Melwani et al. 2014, Genç et al. 2018). It is therefore imperative to interpret results in the context of how different sources of basal organic matter are taken up by the different species.

The results of the present study reveal clear associations between trace metal concentrations from each of the three classes and uptake of alternative basal organic matter source pools supporting *A. zelandica*, *P. canaliculus* and *T. laticostata* (Table 3.5). For example, *A. zelandica* and *T. laticostata*, which were collected from all three regions in the study, had significant correlations between isotopic values (δ^{15}N, δ^{13}C) and concentrations of Cr, Fe, Cu, and As (Table 3.6). These results are consistent with the idea that concentrations of conservative, bioactive, and highly scavenged metals are associated with the composition of organic matter resource pools supporting individual species. Previous studies by Genç et al. (2018) and Ruiz et al. (2018) concluded that food and suspended particulate matter are major sinks of metals and the primary method of transport into suspension feeders. The overlap in associations between stable isotopic signatures (δ^{15}N, δ^{13}C) and trace metal concentrations further support the associations between composition of basal organic matter sources in the particulate pool and trace metal concentrations. Further, these concentrations likely reflect the bioavailability of Cr, Fe, Cu and As in the marine environment. Our results are consistent with the idea that trace metals are concentrated in bivalve species through differences in diet. Further, the observed differences in diet are coincident with variation among landcover categories. These linkages are consistent with the idea that landcover and runoff into the coastal ocean have a strong influence on the trace metal inventory of local bivalve species, and further that landcover strongly influences the base of the food web in these systems.

3.3.3 Land modification impacts

Concurrent measurements of stable isotopic values (δ^{15}N, δ^{13}C) and trace metal concentrations indicated significant associations between trace metal concentrations in bivalves and use of alternate basal organic matter sources. These data reveal both regional scale associations and local influences of salmon farming on routing of trace metals and organic matter through benthic food webs.

Salmon farming

The factor SALMON FARM explained variation in δ^{13}C and δ^{15}N values correlated with concentrations of Mn, Co, Cu, and Zn in bivalves within Marlborough Sounds (Table 3.7). A positive trend was identified for concentrations of Cu and δ^{13}C among all species (Figure 3.8b). The significant associations between stable isotopic values (δ^{15}N, δ^{13}C) with bioactive metals were consistent with the effect of increases in available nutrients at salmon farms relative to references sites for benthic dwelling species.

Copper is a primary agent in anti-fouling paint which is used on nets to reduce the build-up of epibionts (Burridge et al. 2010) and Zn is added to enrich fish feed (Sneddon &

Tremblay 2011). The weathering of nets leads to the gradual reduction of anti-fouling paint resulting in the build-up of Cu in the sediment near salmon farms. Similarly, uneaten fish feed and fecal waste settle on the seafloor near fish farms leading to elevated levels of Zn (Sneddon & Tremblay 2011). Brooks and Mahnken (2003) and Dean et al. (2007) identified salmon farms as the main anthropogenic contributor of Zn and Cu to coastal systems, though the extent of impacts would be farm dependent and influenced by local circulation patterns. The patterns associated with the accumulation of Cu and Zn at salmon farms relative to reference sites therefore reveal the increased nutrients availability, particularly to sediment dwelling species, *P. novaezelandiae*, near salmon farms.

The impacts on structure, compositon and organic matter use by benthic communities from salmon farm activity can be identified up to ~150 m from a salmon farm (Sneddon & Tremblay 2011) (McMullin et al. in prep). However, hydrodynamics influence distance affected by increased concentrations of Zn and Cu associated with salmon farms (Dean et al. 2007, Brooks & Mahnken 2003, Chou et al. 2002, Schendel et al. 2004). For metals to be toxic to organisms they need to be biologically available. The bioavailable form of Cu and Zn are the free ions Cu2+ and Zn2+ (Kim et al. 1999, Eriksen et al. 2001, Burridge et al. 2008) the association between carbon source and trace metals suggest the metals are available in a biologically accessible form. Metals are complex, they are also influenced by biological, physical, and chemical processes (Eriksen et al. 2001, Chapman & Wang 2001). Each of these metals tend to be more biologically available to burrowing organisms or filter feeders at the sediment boundary (Chapman et al. 2002). The idea that bioavailable Zn and Cu were sourced from farms were supported by the observed patterns of high concentrations of Cu and Zn observed in infaunal bivalves in the present study. Though other trace metals may be associated with salmon farms, the consistency with which concentrations of Cu and Zn are significantly higher at salmon farm sites versus those at references sites have been documented world-wide (Morrisey et al. 2000, Brooks et al. 2003, Dean et al. 2007).

Though Cu and Zn are micronutrients in trace amounts and are responsible for a range of enzymatic processes (Thurberg et al. 1973, WHO 2001), these bioactive metals may become toxic to benthic communities at higher concentrations influencing both the benthic primary consumers and the food webs they support. Concentrations above an organism's physiological threshold can lead to toxic impacts associated with physiological processes (Thurberg et al. 1973). The significant increase in Cu and Zn concentrations associated with use of different basal organic matter sources for *P. novaezelandiae* reveal how resource pools around salmon farms are different for sediment dwelling bivalves in comparison with those that feed further up in the water column. Further, the significant association between Cu and Zn and the alternate basal organic matter source pools indicates that the composition of the particulate pool likely drives the observed elevated levels of bioactive metals to bivalves. The significant differences in the concentrations to sediment dwelling suspension feeders can have knock-on effects for subsequent trophic levels enhancing bioaccumulation (Fey et al. 2019).

Catchment

The observed differences in use of alternate organic matter source pools for each bivalve species indicated that trace metal concentrations in all three classes were strongly associated with differences in landcover in the local catchments (Table 3.9). The observed differences in the relationship between trace metal concentrations and δ^{13}C of bivalves among catchments are consistent with differences in the accumulation of trace metals in bivalves among catchments associated with differences in uptake of alternate basal organic matter sources. The significant differences in relation to organic matter revealed a handful of trace metals correlated with stable isotopic values of δ^{13}C and δ^{15}N. Of particular interest is δ^{13}C as it is reflective of the resource pool supporting bivalves (Table 3.9). Conservative metals were significantly higher for *T. laticostata* collected from Mapua and Doubtful Sound (Figure 3.9c). The highest observed levels of bioactive metals for *A. zelandica*, *P. novaezelandiea*, and *P. canaliculus* were found adjacent to large catchments within Marlborough Sounds (Figure 3.9d - f). The only highly scavenged metal that varied significantly with δ^{13}C was As for *P. canaliculus*. Highest concentrations of As were found in Pelorus Sound and Mapua (Figure 3.9g).

The significantly higher conservative metal concentration, Mg, identified for *T. laticostata* reveal the large fresh water inputs into Tasman Bay and Doubtful Sound (Gibbs 2001, Newcombe et al. 2015). The elevated levels of Mg identified for *T. laticostata* revealed the freshwater influences on the benthic community within Doubtful Sound. In a study conducted on another species of sediment dwelling suspension feeder, *Austrovenus stutchburyi*, populations were severely depleted by the freshwater inputs (Tallis et al. 2004).

The significant relationship of Mg and δ^{13}C indicated source pools supporting benthic suspension feeders are influenced by freshwater inputs, while the high levels of Mg in bivalves collected from Tasman Bay reflected the large riverine inputs from the Waimea catchment (Newcombe et al. 2015). No studies have identified direct connections between freshwater inputs and population decline in this case.

Significantly higher concentrations for bioactive metals were revealed for bivalves collected from catchments within Marlborough Sounds. Elevated concentrations of Cu for *A. zelandica* in Queen Charlotte Sound and Pelorus Sound may have reflected the influence of salmon farms as observed in the section above. In addition, Cu, as an anti-fouling agent is used on ships which are pervasive within Marlborough Sounds (Davidson et al. 2017). In addition, elevated concentrations of Fe observed for *P. canaliculus* in Pelorus Sound may have reflected the input of iron-rich minerals delivered as a result of upland forestry (O'Loughlin 1979, Coker 1994, Handley et al. 2017).

The observed elevated concentrations for highly scavenged metal, As, in *P. canaliculus* with δ^{13}C may have reflected high land-based inputs associated with Pelorus Sound and Mapua. A previous study identified As in benthic habitats are primarily inorganic (Price et al. 2012), however the significant association with δ^{13}C are consistent with the ideas that concentrations of As varied with alternative carbon sources for *P. canaliculus*. The significant differences associated with the single species support previous studies on the species-specificity of As accumulation (Price et al. 2012).

Results of the variability in trace metal concentrations with stable isotopic signatures for individual species revealed the influence of organic matter sources on trace metal concentrations for the three trace metal classes. Here we observed that these associations were associated by the range of land modification among catchments and by presence of salmon farms.

The present chapter demonstrated that patterns in the variability of conservative (Mg, Al, Cr, Sr), bioactive (Mn, Fe, Co, Ni, Cu, Zn) and highly scavenged metal (As, Cd, Pb) concentrations in bivalve tissues could be statistically explained by the observed spatial variability in anthropogenic stressors associated with salmon farming and catchment modifications. We provided evidence that salmon farms are likely associated with the availability of bioactive trace metal concentrations through their influence on differences in alternate basal organic matter sources supporting individual bivalve species. The three classes of trace metals provide a basis for evaluating physical, chemical, and biological changes associated with catchment modification and salmon farming. Future studies may focus on diet experimentation to further untangle the underlying mechanisms responsible for trace metal associations with different organic matter sources that make up the particulate pool. Our results have important implications for understanding changes in anthropogenic stressors driving trace metal concentrations in bivalve tissue through alternate organic matter source pools. This is particularly pertinent with the increasing development and aquaculture activities which, if not monitored, may pose detrimental physiological and biological impacts on benthic dwelling bivalves.

Chapter 4: Spatial variation in mercury concentrations in commercially important bivalves

4.0 Introduction

Mercury is a non-essential metal for biological organisms and is one of the top toxic metals concentrated by marine species as it bioaccumulates with increasing trophic interactions (Gray 2002, Sunda 2012, WHO 2020). The natural and anthropogenic sources responsible for mercury availability vary spatially, however, due to the volatility of mercury it is able to pass easily from the atmosphere into the ocean (Fitzgerald et al. 2007, Driscoll et al. 2013). Natural sources such as geological weathering, volcanic eruptions and oceanic evasion (Kim & Fitzgerald 1986, Pirrone et al. 2010, Mason et al. 2012) contribute to atmospheric emissions of mercury. In addition, anthropogenic sources such as primary coal-burning, waste incineration, and mining have led to 2 - 5 fold increase in mercury inputs to the environment since the industrial era (Serrano et al. 2013). The primary method of mercury pollution to aquatic systems is through atmospheric deposition (Semkin et al. 2005). The complexity within dynamic marine systems influenced by resources from the land and atmosphere make the influence of toxic metals such as mercury difficult to trace. In this sense, determining the inputs to coastal marine environments is integral in ensuring the health of marine resources.

Mercury is present in three different forms in nature: elemental, inorganic and organic. Organic mercury in the form of methylmercury (MeHg) is the most toxic to biological processes and bioaccumulates up the food web (Bargagli et al. 1998, Sarkar et al. 2008, Chouvelon et al. 2018). The neurotoxin MeHg, can lead to nervous system damage from children to adults and a variety of adverse health impacts (Dadar et al. 2016, Mergler et al. 2007, Choi & Grandjean 2008, Hong et al. 2012, Karagas et al. 2012). Further impacts have been observed in marine organisms (Hutcheson et al. 2014, Ruus et al. 2017, Bakar et al. 2017). Methylmercury production is mediated in aquatic sediments by the activity of sulphate- and iron-reducing anaerobic bacteria, where methylation primarily transforms inorganic mercury to mono-methyl mercury (Compeau & Bartha 1985, Gilmour & Henry 1991, Gilmour et al. 1992, Branfireun et al. 1999, King et al. 1999). Sulphate-reducing bacteria appears to be the primary mediator or MeHg in sediments, however, iron-reducing bacteria have been tied to methylation of mercury when sulfate reduction is inhibited, and iron-reducing conditions are prevalent (Fleming et al. 2006). Coastal sediments supporting microbial activity are heavily influenced by anthropogenic activity where catchment modification increases sedimentation to coastal habitats (Jonsson et al. 2017, Taylor et al. 2019). The build-up of sediment provides a favourable environment (abiotic conditions, anoxic habitats and high degree of organic matter) for microbial activity where increased levels of MeHg have been observed (Chen et al. 2018, Fitzgerald et al. 2007, Taylor & Calabrese 2018). This has been revealed particularly in estuaries as they capture and retain catchment particulate (Lambourg et al. 2003, Balcom et al. 2004, Balcom et al. 2008).

Suspension feeding bivalves, such as mussels, are commonly used as bioindicators of chemical contamination in the aquatic environment (Rainbow & Phillips 1993, Romeó et al. 2005, Kim et al. 2008, Lanksbury et al. 2014, Melwani et al. 2014, Taylor et al. 2019). Global monitoring programs such as the Mussel Watch Program have been monitoring coastal contamination using mussels since the 1970s (Goldberg et al. 1978). Long term monitoring is crucial to track changes in pollution levels over time (Briant et al. 2017). The ability of sedentary suspension feeders to filter large quantities of water and absorb contaminants reflect the bioavailability of contaminants in marine environments. Further, the sedentary nature allows for chemical fingerprints at specific locations underpinning the bioavailability of contaminants within specific localities (Elskus et al. 2020).

Mercury is commonly tested on commercial fish species that bioaccumulate mercury at levels that may be toxic to humans (Sunderland 2007, Díez 2009, Sadhu et al. 2015). Fewer studies have been conducted on commercially important bivalves and to our knowledge none have been conducted using current mercury methodology at the top of the South Island where bivalve fisheries have existed since the 1970s (Page 2017). Identifying mercury concentrations in bivalves is key for revealing point sources responsible for elevated mercury levels in marine resources. The position of sessile bivalves at the base of the food web supporting higher trophic levels makes them an ideal species for identifying origins of mercury to the marine environment. Further the evaluation of commercially important bivalves reveals mercury concentrations for public health benefit.

The commercially important bivalves on the South Island of New Zealand are primarily *Pecten novaezelandiae* and *Perna canaliculus*. The *P. novaezelandiae* fisheries at the top of the South Island have been declining in recent years. The commercial fishery in

Tasman Bay has been closed for ten years and reseeding stocks has been undertaken, though to no avail ('Temporary Closure' 2019). The natural commercial fishery in Marlborough Sounds and Port Underwood have been closed over the past three seasons. Though there are some large populations in the outer Marlborough Sounds, the surveyed biomass has not change ('Temporary Closure' 2019). Long line mussel farms of *P. canaliculus* have been present in NZ since the 1970s and is New Zealand's largest aquaculture fishery (Page 2017).

The key questions concerning contaminants such as mercury are 1) what are the sources and are they localized or expansive? and 2) are concentrations changing over time? In the present chapter we investigate site specific variability of mercury concentrations within commercially important bivalves, *P. novaezelandiae* and *P. canaliculus* on the South Island of New Zealand. We investigate site specific variation in mercury concentrations relative to catchments with a range of landcover modification and resources supporting benthic bivalves. As the first measurement of mercury on commercially important species, we provide the baseline measurements for future monitoring of mercury in commercially important bivalves on the South Island of New Zealand.

4.1 Methods

In order to identify the spatial distribution of mercury in commercial bivalves, *P. novaezelandiae* and *P. canaliculus*, total mercury (THg) concentrations were recorded from Marlborough Sounds, Tasman Bay, and Fiordland. Total mercury concentrations were measured for *P. novaezelandiae* at nine sites within Marlborough Sounds and Fiordland, while concentrations of THg were measured in *P. canaliculus* at 13 sites within Marlborough Sounds and Tasman Bay (Figure 4.1). Further, all samples were selected from sites encompassing a range of landcover classifications (Figure 4.2).

4.1.1 Sample collection

Bivalve collections were carried out by scuba divers in Marlborough Sounds during February 2018 and November 2018 from the RV *Polaris* II with sampling sites spanning five regions within the Sounds: Tory Channel, Queen Charlotte Sound, Pelorus Sound, and Port Underwood (Figure 3.1).

Bivalve collections were made by scuba divers from Tasman Bay during February 2018 from the RV *Polaris* II with sites encompassing the perimeter of the bay (Figure 4.1). The sites from the top of the South Island, Marlborough Sounds and Tasman Bay, fall within catchments with a range of landcover categories: forest, urban development, forestry, farming, and aquaculture systems (Figure 4.2, Appendix 1). Sites were chosen along the gradient with increasing distance from the mouth of the Sounds in order to compare sites with increased influence of land-based run off from major surrounding catchments.

Bivalve collections were made by scuba divers in Fiordland during February 2019 from the RV Typhoon. Site selection was focused on sampling sites in Thompson's Bradshaw Sound adjacent to Doubtful Sound and were constrained to the 20 m depth contour (Figure 3.1).

Target species were collected using SCUBA from 18 sites within Marlborough Sounds (10), Tasman Bay (6) and Fiordland (1) (Figure 4.1). Samples were collected by hand at each site and placed into mesh bags under the New Zealand Ministry of Primary Industries special permit (644-2). Samples were then placed in individual tanks on-board the RV *Polaris* II with a constant surface water flow for 48 hours to allow sediment to be expelled. Samples were then placed in Zip-Lock plastic bags, labeled and frozen until dissection took place at Portobello Marine Laboratory, University of Otago.

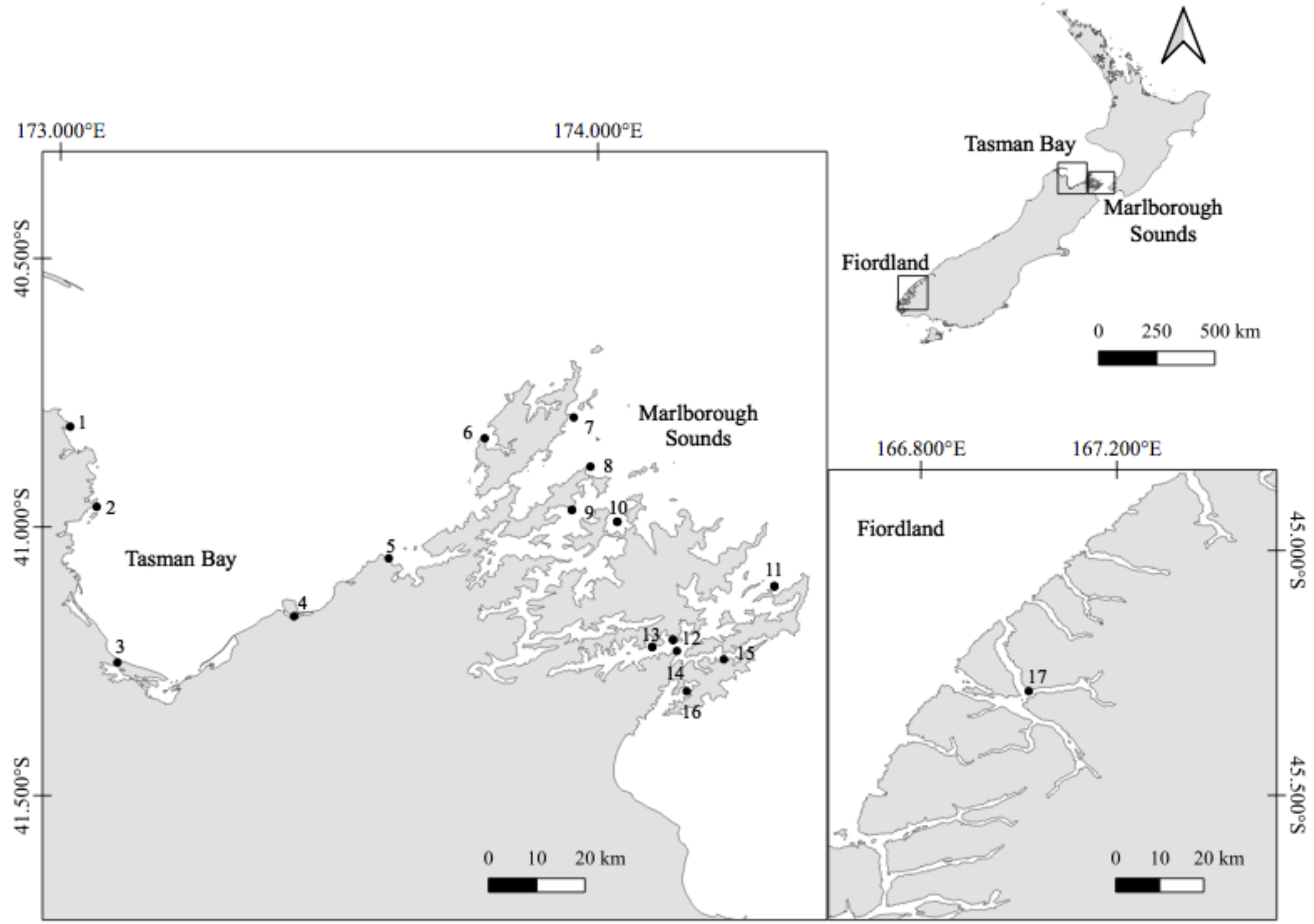

Figure 4.1 Sample sites within each region. **Tasman Bay:** 1) Totaranui, 2) Te Karetu Point, 3) Mapua, 4) Delaware Bay, 5) Crousielles Harbour, 6) Ragged Point. **Marlborough Sounds:** 7) Old Man's Head, 8) Clay Point, 9) Waitata, 10) Bird Island, 11) Motungarara Island, 12) Ruakaka Bay, 13) Perano Shoal, 14) Dieffenbach Point, 15) Te Pangu Bay, 16) Port Underwood. **Doubtful Sound:**17) Bradshaw-Thompson.

4.1.2 Mercury analysis

For each of the target species a random subset of n = 3 were selected for mercury measurements. Samples were dissected at Portobello Marine Laboratory in a laminar flow cabinet (Gelman, HLF series). All tissue, aside from the ≤ 0.5 g sample of adductor muscle tissue used for stable isotopic analysis, was placed in labeled 50 ml falcon tubes. In order to access tissue, the left and right valves were separated at the ventral margin using teflon forceps or a ceramic knife if forceps were insufficient. Tissue samples were frozen for 48 hr at -18 ˚C and subsequently freeze dried at -60 ˚C for 48 hr (mrc, FDL-10N-50 series). Based on methods tested in a pilot study for homogenizing sample tissue, samples were ground to as fine a powder as could be achieved using a mortar and pestle in accordance standard methods (Jack et al. 2011). To limit cross contamination of samples, the agate mortar and pestle was cleaned between each sample by rinsing with ethanol (EtOH) and drying thoroughly with lint-free paper.

Mercury analysis was conducted by colleagues at Vrije Universiteit Brussel in Belgium. Total mercury concentrations of samples were measured on an AMA 254 analyzer (Altec, Ltd. Czech Republic). All samples were measured twice in order to ensure reliability of analysis. Blank values were not included in the data as they were automatically corrected during analysis and subtracted from the THg mass measured. The analysis is founded on *in situ* dry decomposition of, in our case, solid samples (max. 300 mg) that are combusted through reactions in a catalytic column. Subsequently, THg from the reaction is trapped on the gold amalgamator. The amalgamator is then heated which readily evaporates preconcentrated mercury, where its atomic absorption is measured. The analysis is carried out automatically without the need of prefatory dissolution and / or decomposition of the sample. The detection limit of the AMA 254 analyzer is 0.01 ng THg, with a working range between 0.05 to 500 ng THg, and sample reproducibility < 1.5 %. (Száková et al. 2004, Sysalová et al. 2013).

Table 4.1 Average total mercury concentrations (THg) for individual species at sites within Marlborough Sounds, Tasman Bay, and Fiordland.

Species	Region	Site	THg mg kg $^{-1}$	± SE
P. canaliculus	Marlborough Sounds	Bird Island	0.05 ±	0.004
		Clay Point	0.06 ±	0.012
		Moturanga Island	0.05 ±	0.002
		Perano Shoal	0.12 ±	0.037
		Ruakaka Bay	0.07 ±	0.019
		Te Pangu Bay	0.09 ±	0.016
		Waitata	0.07 ±	0.018
	Tasman Bay	Crousielles Harbour	0.08 ±	0.004
		Delaware Bay	0.08 ±	0.014
		Mapua	0.12 ±	0.008
		Pepin Island	0.05 ±	0.004
		Ragged Point	0.08 ±	0.004
		Te Karetu	0.07 ±	0.004
		Totaraniu	0.05 ±	0.004
P. novaezelandiae	Fiordland	Doubtful Sound	0.05 ±	0.002
	Marlborough Sounds	Bird Island	0.07 ±	0.016
		Dieffenbach Point	0.05 ±	0.005
		Moturanga Island	0.07 ±	0.010
		Old Man’s Head	0.04 ±	0.001
		Perano Shoal	0.06 ±	0.003
		Port Underwood	0.09 ±	0.026
		Ruakaka Bay	0.04 ±	0.008
		Waitata	0.06 ±	0.019

4.1.3 Stable isotopic signatures

Stable isotopic signatures were used to reveal potential differences in THg for species relative to basal carbon sources and trophic level for the subset of samples. A ≤ 0.5 g sample of adductor muscle tissue was taken from each sample and placed into a 1.5 ml centrifuge vial and dried at 60˚C for 48 hr (mrc, FDL-10N-50 series). Samples were ground to a fine powder in a mortar and pestle that was cleaned with ethanol (EtOH) between samples to limit cross contamination. Subsamples of 1.0 mg ± 0.1 mg were weighed into 3.5 x 3.0 mm tin capsules and sealed for stable isotopic analysis of δ^{15}N and δ^{13}C in the Department of Chemistry, University of Otago. The full detailed description of this process is provided in Ch. 2.

4.1.4 Landcover classification

The watersheds surrounding Marlborough Sounds, Tasman Bay and Fiordland were divided into the following discrete landcover categories based on proportion landcover: forest, urban, forestry, and farming (Figure 4.2). Presence and absence of salmon farms was also noted for Marlborough Sounds. Landcover information was provided by Land Information New Zealand (LINZ, 2018) and Marlborough District Council (Appendix 2). GIS maps were constructed by overlaying survey data using the spatial referencing system NZGD 2000.

Catchment areas, defined as the terrestrial region contributing terrigenous particulates to adjacent marine systems, were defined for each sample site by drawing polygons in QGIS and calculating the 2D area (km^2). Boundaries of the catchment were determined using both topographic contours (1cm : 2.5km), 8 m digital elevation models and sea-draining catchment layers (LINZ, 2018).

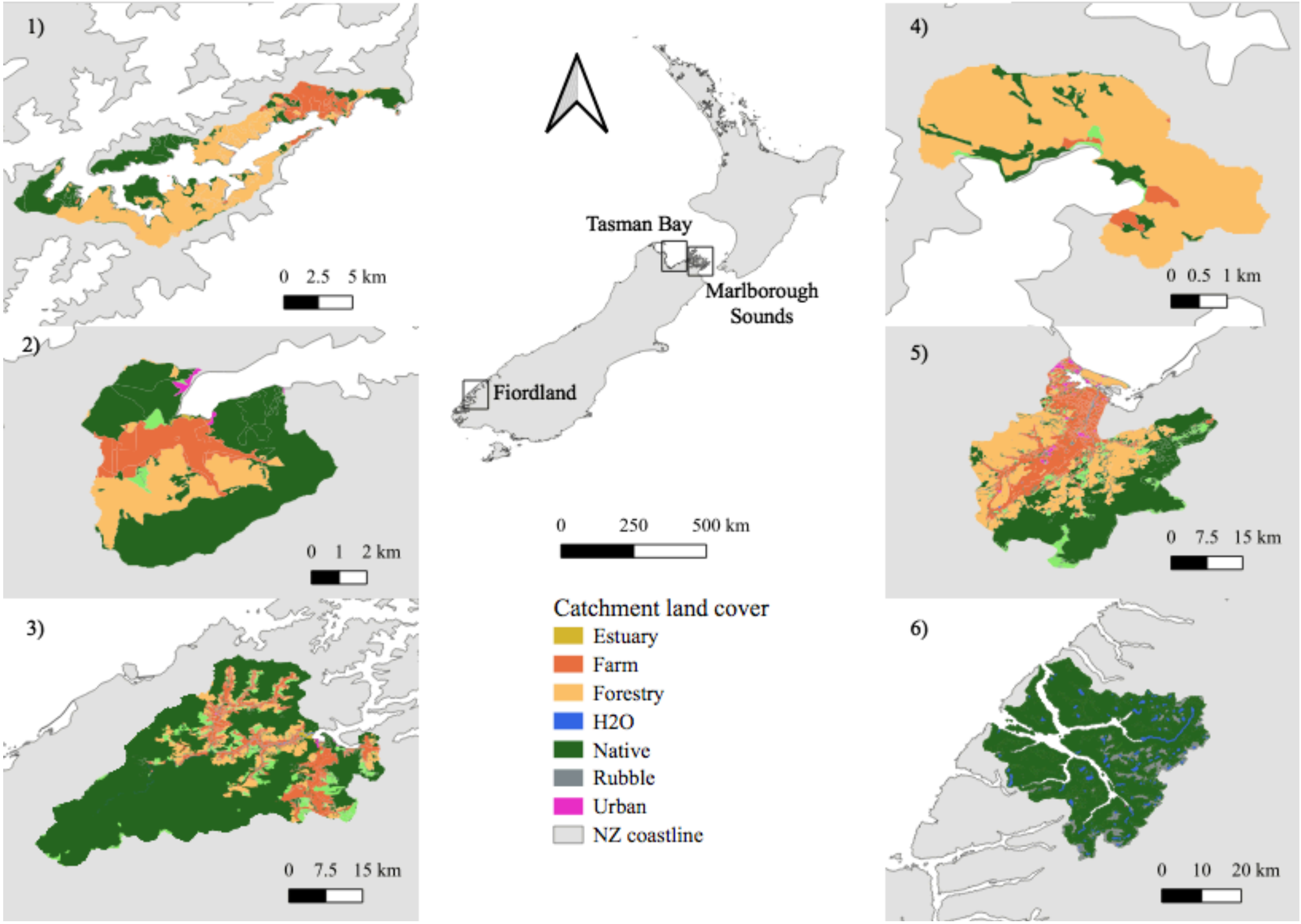

Figure 4.2 Landcover classification for each of the five catchments: 1) Tory Channel, 2) Queen Charlotte Sound, 3) Pelorus Sound, 4) Mapua, and 5) Doubtful Sound.

4.1.5 Statistical analysis

Generalized linear mixed models with the Standard Least Squares personality in the Fit Model platform in JMP (JMP 14.0 SAS) were used to test the effect of SITE [9 levels, random] nested in REGION [2 levels, fixed] for *P. novaezelandiae* to identify spatial variation in THg among sites. The same analysis was carried out for *P. canaliculus* with SITE [13 levels, random] nested in REGION [2 levels, fixed]. Identifying a significant difference for the interaction of site and species was constrained to sites where species overlapped in Marlborough Sounds. A separate analysis was run for the factors SITE [5 levels, random], SPECIES [2 levels, fixed], and the interaction SITE * SPECIES on THg for *P. novaezelandiae* and *P. canaliculus*. Pair-wise differences were determined using a Student's t post-hoc tests. SIZE [continuous] was incorporated as a variable to account for variance associated with bivalve shell length as this was not taken into account on collection and significantly correlated with δ^{13}C (Appendix 3).

Further generalized linear mixed model were used to test the effect of SITE [9 levels, random] nested in CATCHEMNT [4 levels, fixed] for *P. novaezelandiae* to identify spatial variation in THg among catchments. The same analysis was carried out for *P. canaliculus* with SITE [13 levels, random] nested in CATCHEMNT [4 levels, fixed]. Pair-wise differences were revealed using Student's t post-hoc test.

In order to identify if THg concentrations varied relative to stable isotopic signatures, a generalized linear mixed model was used to test the effect of factors SITE [5 level, random], SPECIES [2 levels, fixed], δ^{15}N [continuous], δ^{13}C [continuous], and the interaction SITE * SPECIES in THg for *P. novaezelandiae* and *P. canaliculus*. For individual species, the effect of SITE [9 level, random], δ^{15}N [continuous], and δ^{13}C [continuous] was tested for *P. novaezelandiae*. The same analysis was conducted for *P. canaliculus* for the effect of SITE [13 level, random], δ^{15}N [continuous], and δ^{13}C [continuous].

To test if THg varied significantly in the presence and absence of salmon farms, factors FARM [2 levels, fixed] within Marlborough Sounds for individual SPECIES [2 levels, fixed] and the interaction FARM * SPECIES, were analysed using a linear model.

To test if THg varied significantly for individual species with different landcover classifications, the factors LANDCOVER [4 levels, categorical] and CATCHEMNT [4 levels, fixed] were analysed in a linear model for *P. novaezelandiae*. The same analysis was conducted for *P. canaliculus* with LANDCOVER [4 levels, categorical] and CATCHEMNT [3 levels, fixed]. Pair-wise differences were determined using a Student's t post-hoc tests.

4.2 Results

4.2.1 Spatial differences for individual species

Regional level

Pecten novaezelandiae collected from Marlborough Sounds and Fiordland had significantly different concentrations of THg at the site level, but not between regions (Main test: $F_{9,26} = 4.74$, $p = 0.0029$, $r^2 = 0.72$. SITE [REGION], $F = 4.92$, $p = .0034$. REGION, $F = 0.76$, $p = 0.3954$. SIZE, $F = 1.86$, $p = 0.1903$). Total mercury concentrations were significantly different at Port Underwood, Bird Island and Motungarara Island within Marlborough Sounds (Figure 4.3a).

Perna canaliculis collected from Marlborough Sounds and Tasman Bay had significantly different concentrations of THg at the site level, but not between regions (Main test: $F_{13,37} = 8.73$, $p < 0.0001$, $r^2 = 0.83$. SITE [REGION], $F = 9.20$, $p < 0.0001$. REGION, $F = 0.08$, $p = 0.7722$. SIZE, $F = 1.58$, $p = 0.2210$). Significantly higher concentrations of THg were observed for *P. canaliculus* at Perano Shoal within Marlborough Sounds and Mapua within Tasman Bay relative to other sites (Figure 4.3b).

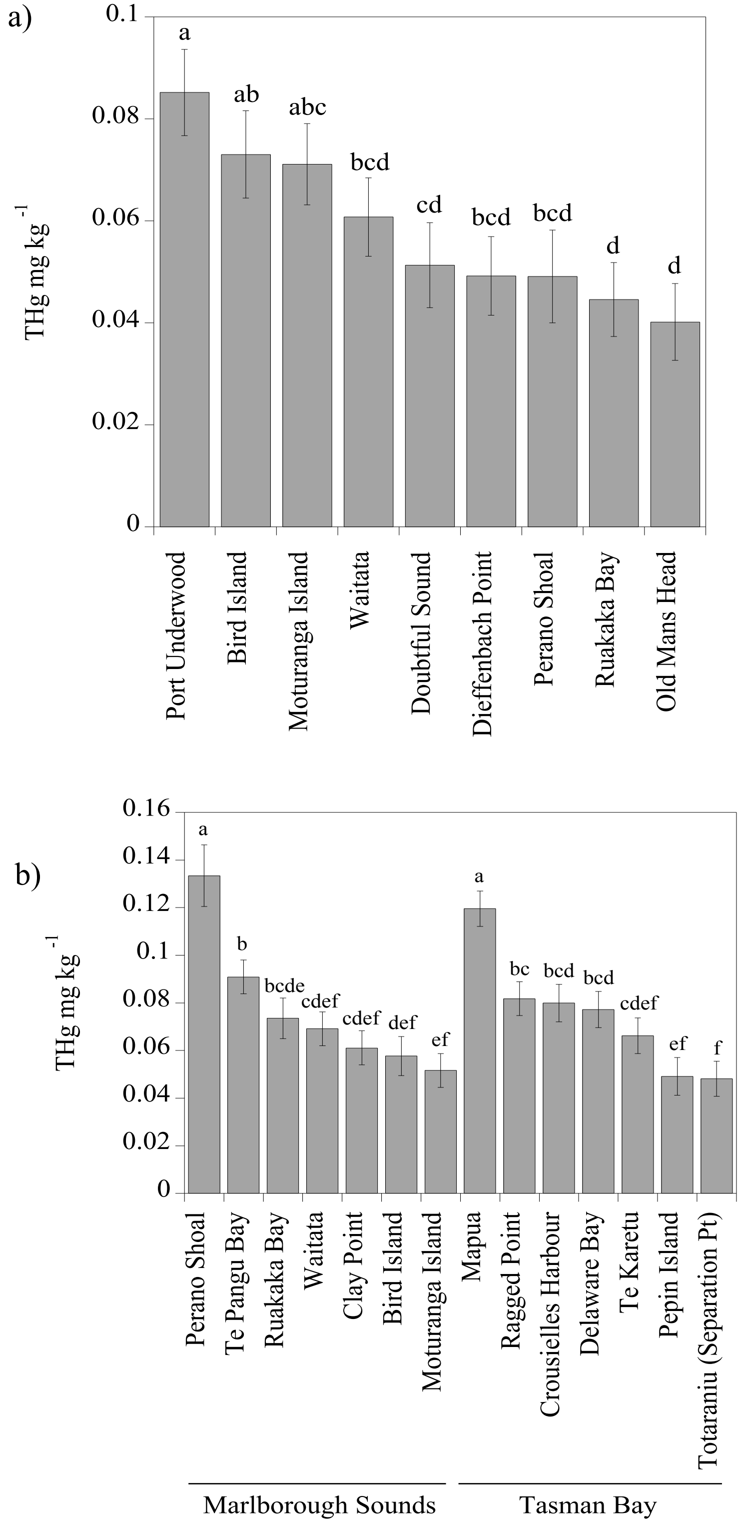

Figure 4.3 Concentrations of THg for a) *P. novaezelandiae* and b) *P. canaliculus* among sites within regions. Means ± 1SE. Levels connected by different letter indicate significant differences ($p < 0.05$).

Catchment level

Concentrations of THg are significantly different for *P. novaezelandiea* among catchments (Main test: $F_{9,26} = 4.74$, $p = 0.0029$, $r^2 = 0.72$. SITE[CATCHMENT], $F = 3.40$, $p = 0.0261$. CATCHMENT, $F = 4.46$, $p = 0.0175$. SIZE, $F = 1.86$, $p = 0.1903$). Concentrations of THg were highest at sites within each of the three Marlborough Sounds catchments (Figure 4.4a).

Concentrations of THg were significantly different for *P. canaliculus* among catchments (Main test: $F_{13,37} = 8.73$, $p < 0.0001$, $r^2 = 0.83$. SITE[CATCHMENT], $F = 9.68$, $p < 0.0001$. CATCHMENT, $F = 6.45$, $p = 0.0023$. SIZE, $F = 1.58$, $p = 0.2210$). Concentrations of THg were highest at sites with Queen Charlotte Sound in Marlborough Sounds and Mapua catchment in Tasman Bay (Figure 4.4b).

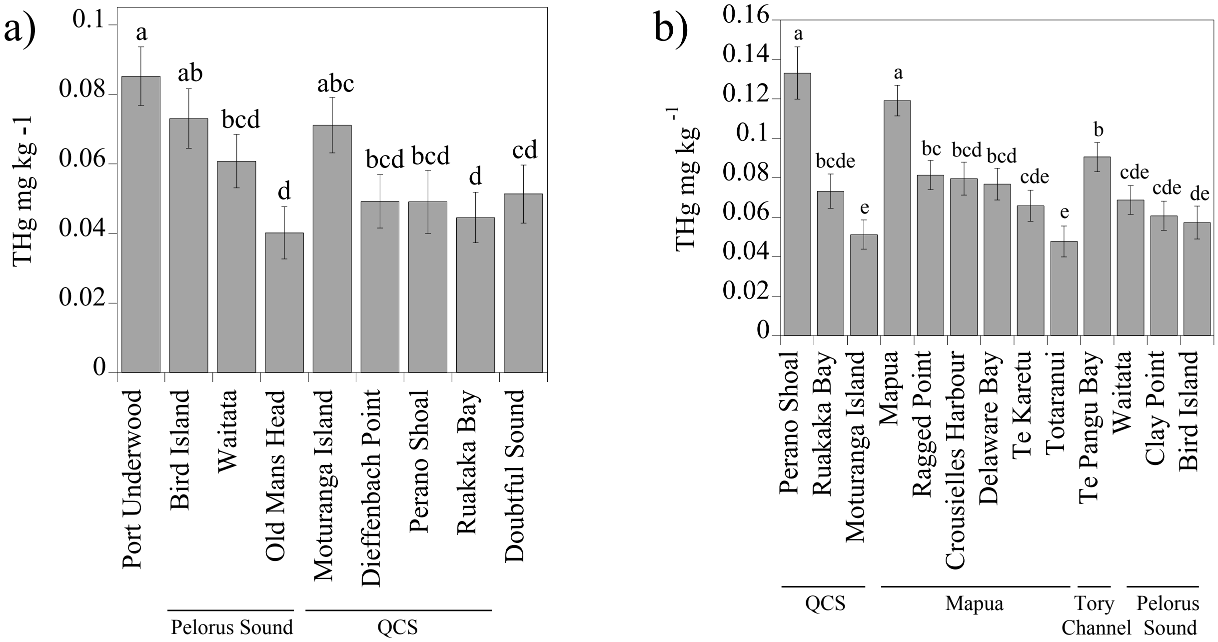

Figure 4.4 Concentrations of THg for a) *P. novaezelandiae* and b) *P. canaliculus* among sites within catchments. Means ± 1SE. Levels connected by different letter indicate significant differences ($p < 0.05$).

4.2.2 Species spatial overlap

Factor SITE reveals significantly different concentrations with significantly higher concentrations of THg for Perano Shoal relative to the rest of the sites where both *P. novaezelandiae* and *P. canaliculus* was present. Factor SPECIES has no significant influence on the observed THg concentrations between *P. novaezelandiae* and *P. canaliculus*. The interaction between site and species, however, was significant. (Main test: $F_{10,28} = 3.92$, $p = 0.0058$, $r^2 = 0.69$. SITE, $F = 3.67$ $p = 0.0235$. SPECIES, $F = 4.08$ $p = 0.0585$. SITE * SPECIES, $F = 5.96$, $p = 0.0031$. SIZE, $F = 0.65$, $p = 0.4319$). These results reveal concentrations of THg are more strongly influenced by the interaction of site and species where the highest concentration reported was for *P. canaliculus* from Perano Shoal (Figure 4.5).

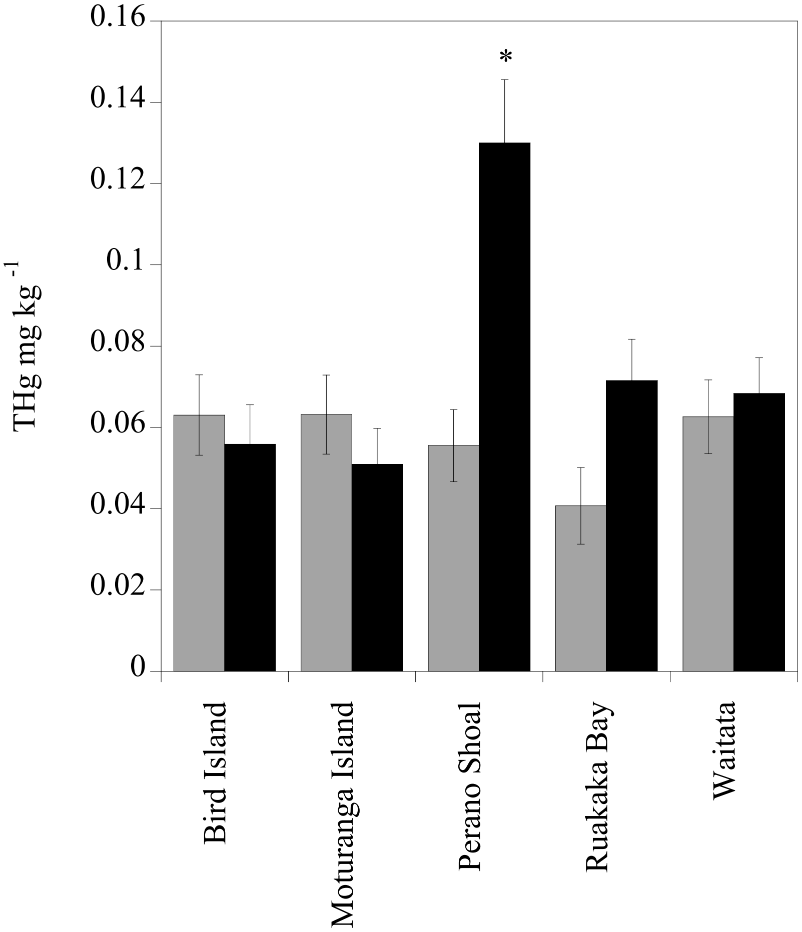

Figure 4.5 Concentrations of THg for *P. novaezelandiae* (grey) and *P. canaliculus* (black) among sites within the Marlborough Sounds. Means ± 1SE. An asterix represents significantly different levels ($p < 0.05$).

4.2.3 Changes associated with organic matter sources using stable isotopic signatures

No significant differences in concentrations of THg with stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$) were revealed for *P. novaezelandiae* and *P. canaliculus* among sites within Marlborough Sounds (Main test: $F_{12,28} = 2.98$, $p = 0.0219$, $r^2 = 0.69$. $\delta^{15}N$, $F = 0.0112$, $p = 0.9172$. $\delta^{13}C$, $F = 0.18$, $p = 0.6792$. SITE, $F = 3.02$, $p = 0.0494$. SPECIES, $F = 0.55$, $p = 0.4709$. SITE * SPECIES, $F = 3.51$, $p = 0.0307$. SIZE, $F = 0.13$, $p = 0.7233$).

Significant differences in THg concentrations with stable isotopic values of δ^{13}C were revealed for *P. novaezelandiae* (Table 4.2). No significant relationships were observed for THg with stable isotopic values (δ^{13}C and δ^{15}N) for *P. canaliculus* among sites (Table 4.3).

Table 4.2 General linear model results of THg concentrations with stable isotopic values (δ^{15}N and δ^{13}C) among sites for *P. novaezelandiae*. Bolded p-values ($p < 0.05$) indicate statistical differences.

Level	df	F ratio	p
δ^{15}N	1	0.0159	0.9013
δ^{13}C	1	4.5893	**0.0490**
SITE[REGION]	7	5.2814	**0.0034**
REGION	1	1.7743	0.2027
SIZE	1	2.7838	0.1160

Table 4.3 General linear model results of THg concentrations with stable isotopic values (δ^{15}N and δ^{13}C) among site for *P. canaliculus*. Bolded p-values ($p < 0.05$) indicate statistical differences.

Level	df	F ratio	p
δ^{15}N	1	3.0984	0.0923
δ^{13}C	1	0.0057	0.9406
SITE[REGION]	11	7.0209	**<.0001**
REGION	1	0.3474	0.5616
SIZE	1	0.9846	0.3319

4.2.4 Differences within catchments

Salmon farming in Marlborough Sounds

No differences in THg concentrations were detected between salmon farm sites and references sites among species (Main test: $F_{4,43} = 1.89$, $p = 0.1319$, $r^2 = 0.16$) (Table 4.4).

Table 4.4 General linear model results for THg concentrations between salmon farm and reference sites among species. Bolded p-values indicate statistical differences.

Level	**df**	**F ratio**	**p**
FARM	1	2.2344	0.1430
SPECIES	1	0.0238	0.8782
FARM * SPECIES	1	0.0191	0.8907
SIZE	1	2.8923	0.0970

Proportion landcover classification

Concentrations of THg were significantly different with proportion of landcover: forest, urban, forestry and farming among catchments for *P. novaezelandiae* (Main test: $F_{4,26} = 4.15$, $p = 0.0118$, $r^2 = 0.43$). A negative association is observed between THg concentrations and proportion forest while a positive relationship is observed between THg concentrations and proportion forestry (Figure 4.6). Neither positive nor negative relationships were revealed for proportion urban or farming landcover classifications. Pair-wise analysis revealed significantly higher concentrations of THg in Port Underwood relative to all other catchments (Figure 4.6).

Concentrations of THg did not differ with proportion of landcover: forest, urban, forestry and farming cover among catchments for *P. canaliculus* (Main test: $F_{4,37} = 1.96$, $p = 0.1234$, $r^2 = 0.19$).

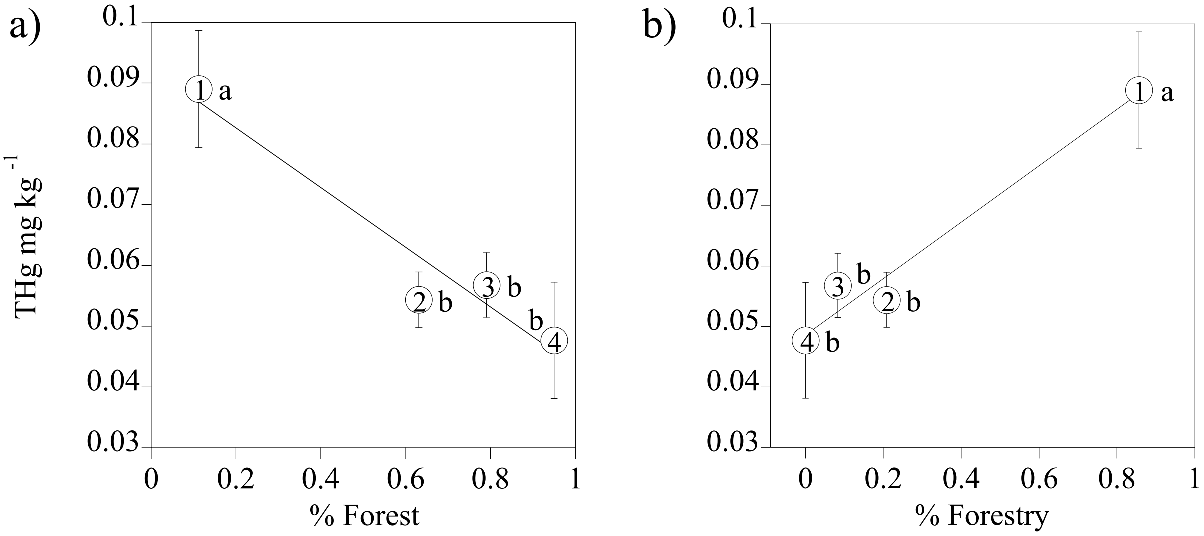

Figure 4.6 Concentrations of THg with a) percent forest and b) percent forestry for *P. novaezelandiae* at sites: 1) Port Underwood, 2) Queen Charlotte Sound, 3) Pelorus Sound, and 4) Doubtful Sound. Means ± 1SE. Levels connected by different letter indicate significant differences ($p < 0.05$).

4.3 Discussion

Results presented here reveal the first measures of total mercury (THg) concentrations for commercially important species *P. novaezelandiae* and *P. canaliculus* in Marlborough Sounds, Tasman Bay and Fiordland. The observed patterns in the variation of THg concentrations associated with site and catchment revealed differences in THg concentrations associated with localised inputs. Since mercury bioaccumulates up the food web, it is imperative that we understand the sources shaping mercury concentrations in benthic organisms that are ultimately consumed by top predators, including humans.

4.3.1 Spatial variation in mercury concentrations

The factor SITE revealed significant differences in observed concentrations of THg for *P. novaezelandiae* and *P. canaliculus*. The spatial variations in THg concentrations for *P. novaezelandiae* and *P. canaliculus* is illustrated in Figures 4.7 and 4.8. No significant differences were explained by REGION for either species supporting differences in THg concentrations related to point source inputs of mercury. Sources responsible for mercury emissions are largely associated with industrial discharge, such as coal burning and mining. Further, mercury emissions are able to travel large distances in the atmosphere where they are deposited across catchments and enter the sea through both atmospheric deposits and catchment hydrology (Pacyna et al. 2006, Lovett et al. 2009, Ward et al. 2011, Serrano et al. 2013). Therefore, although mercury inputs may originate from activities far away, the inputs from riverine catchments may be responsible for localised variability (Elskus et al. 2020). The results from the present study support localised inputs as the main mechanism responsible for the spatial variability in mercury concentrations on the South Island of New Zealand.

Sites where both species were collected was constrained to Marlborough Sounds as Tasman Bay population decline of *P. novaezelandiae* has led to a fishery closure ('Temporary Closure' 2019). Both site and the interaction between site and species revealed significant differences in THg concentrations. However, pair-wise analysis revealed that *P. canaliculus* from Perano Shoal, Marlborough Sounds had the highest mercury concentrations relative to all other species among sites (Figure 4.5). This result indicates *P. canaliculus* may have been more susceptible to mercury concentrations than *P. novaezelandiae*. Do to their global distribution, mussels have long been used as bioindicators of chemical contamination in the aquatic environment, where they have displayed their sensitivity to aquatic contaminants on temporal and spatial scales (Goldberg et al. 1978, Melwani et al. 2014, Briant et al. 2017, Elskus et al. 2020). The significantly higher concentrations of mercury in *P. canaliculus* at Perano Shoal is consistent with their sensitivity to contaminants and ability to reflect acute sources of mercury inputs.

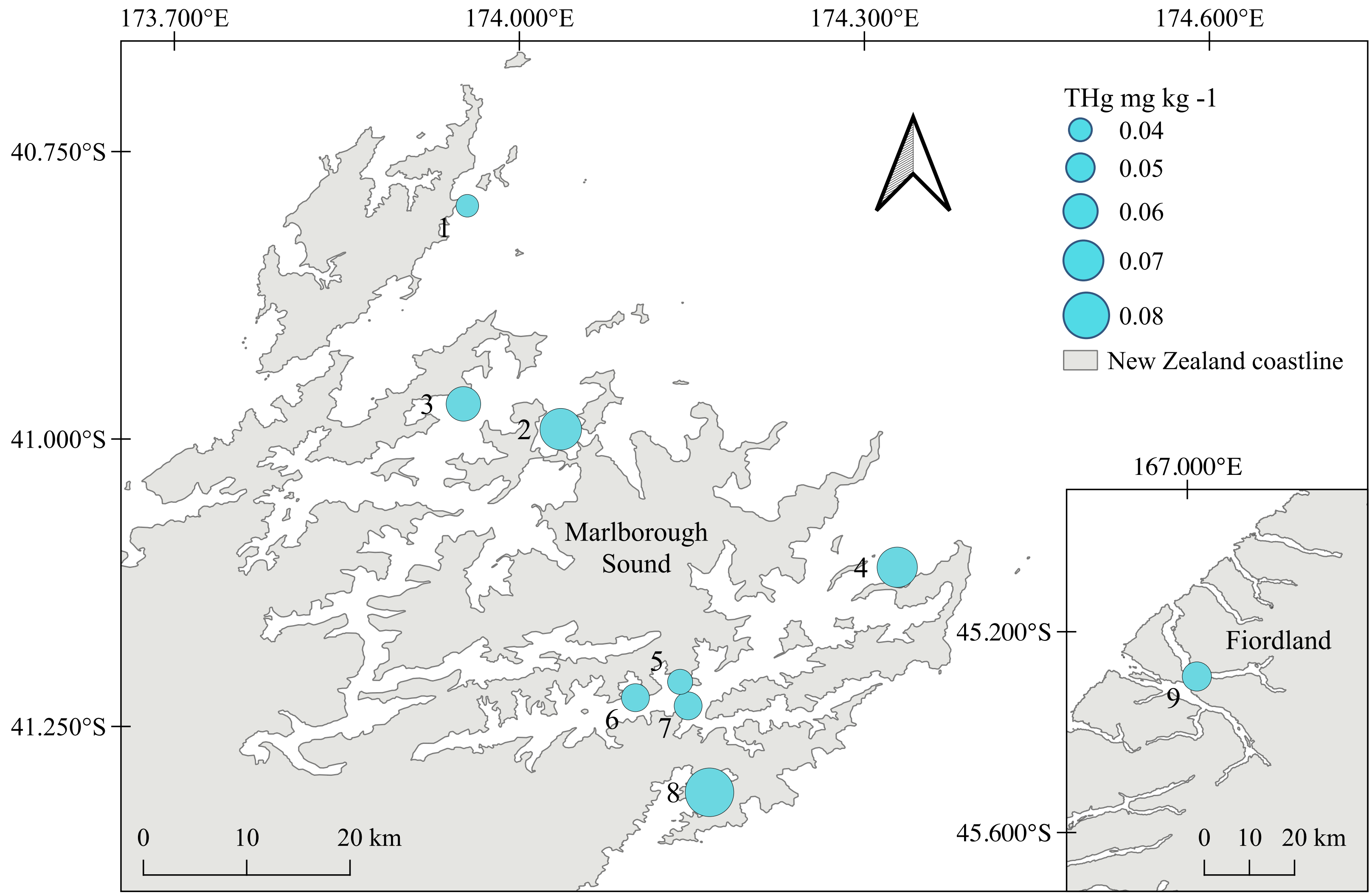

Figure 4.7 Spatial depiction of average THg concentrations (mg kg $^{-1}$) for *P. novaezelandiae* at sites within Marlborough Sounds and Fiordland. Sites: 1) Old Man's Head, 2) Bird Island, 3) Waitata, 4) Motungarara Island, 5) Ruakaka Bay, 6) Perano Shoal, 7) Dieffenbach Point, 8) Port Underwood, 9) Doubtful Sound.

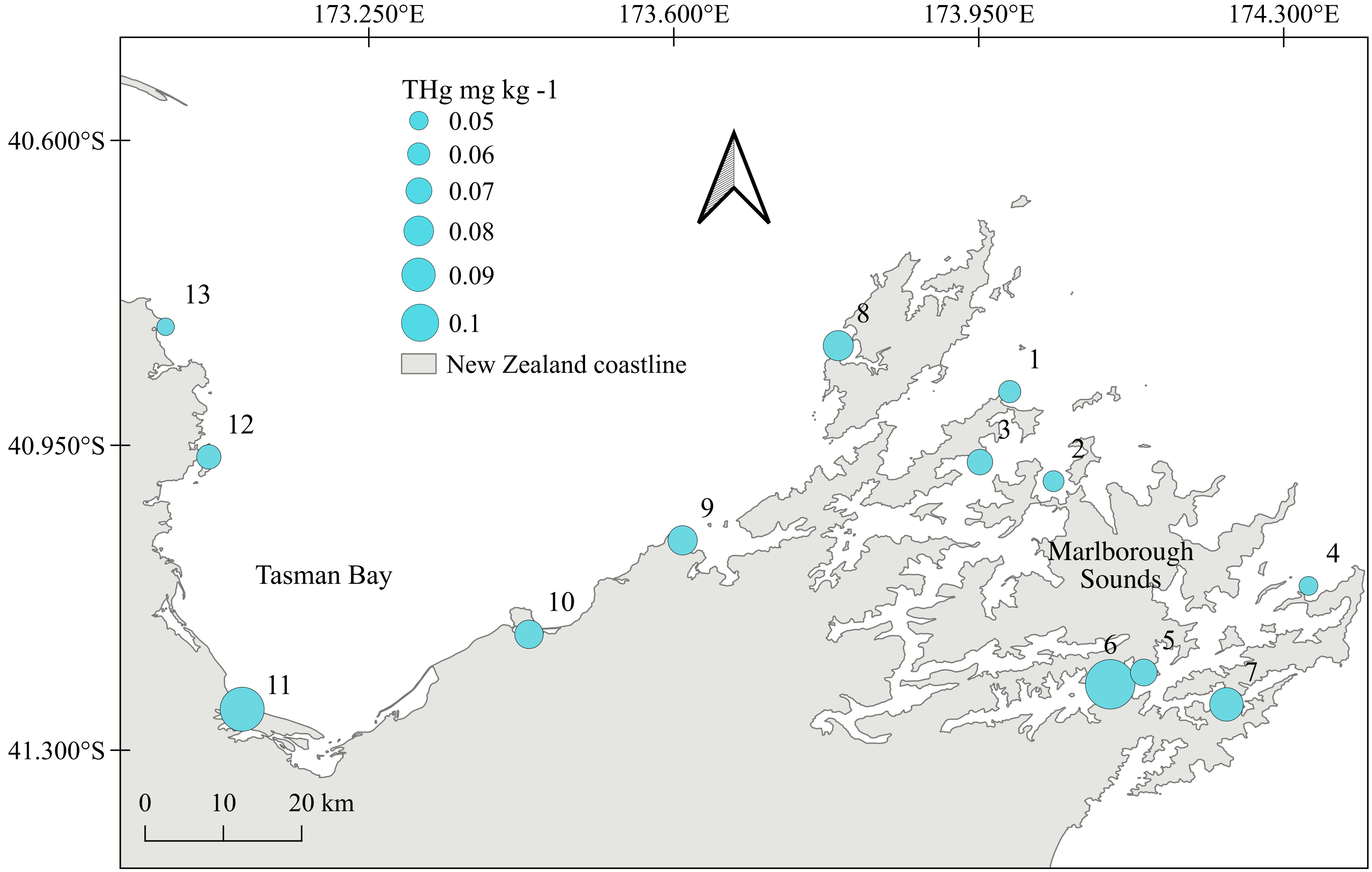

Figure 4.8 Spatial depiction of average THg concentrations (mg kg $^{-1}$) for *P. canaliculus* at sites within Marlborough Sounds and Tasman Bay. Sites: 1) Clay Point, 2) Bird Island, 3) Waitata, 4) Motungarara Island, 5) Ruakaka Bay, 6) Perano Shoal, 7) Te Pangu Bay, 8) Ragged Point, 9) Crousielles Harbour, 10) Delaware Bay, 11) Mapua, 12) Te Karetu, 13) Totaranui.

4.3.2 Variations in mercury in relation to stable isotopic signatures

In order to understand how mercury enters the benthic food web, variation in THg concentrations were investigated relative to differences in stable isotopic values (δ^{15}N and δ^{13}C). No differences in THg concentrations were observed for the interaction between site and species. However, for individual species, *P. novaezelandiae* revealed a significant relationship in THg with δ^{13}C (Table 4.2). The observed trend in THg associated with δ^{13}C indicate mercury concentrations may vary with carbon sources supporting *P. novaezelandiae*. A prior study by Taylor et al. (2019) revealed mercury concentrations in marine organisms were significantly influenced by organic matter from terrestrial sources, however, no prior connection has been drawn to marine derived organic matter. Therefore, the present study reveals marine basal carbon sources, primarily composed of macroalgae as revealed in Chapter 2, influenced mercury concentrations observed for *P. novaezelandiae*.

4.3.3 Variation with anthropogenic pressures

Salmon Farming

Salmon farming was identified as a contributor to the variation in trace metal concentrations reflected in bivalves within Marlborough Sounds in Chapter 3. Salmon farms have no apparent relationship with concentrations of mercury in *P. novaezelandiae* and *P. canaliculus*. The uniform concentrations of mercury among salmon farm and reference sites reveal the benign nature of salmon farming within Marlborough Sounds on THg concentrations. Our results revealed similar findings to previous studies where salmon farming has not resolved variations in mercury concentrations to farm adjacent marine communities (Bustnes et al. 2011, Onsanit et al. 2012).

Catchment Modification

Though no significant differences were revealed at the regional level, catchments with a range of landcover modification explained significant differences in THg concentrations to *P. novaezelandiae* and *P. canaliculus*. The effect of CATCHMENT was significant for individual species, where Port Underwood revealed significantly higher concentrations of THg in *P. novaezelandiae* relative to all other catchments (Figure 4.4a). Further, Mapua and

Queen Charlotte Sound (QCS) revealed significantly higher concentrations of THg in *P. canaliculus* (Figure 4.4b).

The Port Underwood catchment was dominated by the highest proportion of landcover from forestry within Marlborough Sounds (Urlich 2015) (Figure 4.2). The extensive forestry delivers runoff high in sediment, particularly after harvest when the soil is vulnerable to weather events (Urlich 2015). The muddy bottom of Port Underwood is illustrative of the continuous build-up of sediment and low circulation rate with which the bay is flushed. The health of the benthic invertebrate community in Port Underwood has been monitored for almost a decade. The observed decline in health has been attributed to sedimentation and filamentous diatom blooms (Page & Olsen 2016). The positive association recorded between proportion of forestry and mercury concentrations in *P. novaezelandiae* revealed elevated levels of mercury may be explained by forestry sedimentation (Figure 4.6a). Recent studies corroborate this finding as high inputs of terrestrial organic matter have been linked to elevated levels of mercury, both in the sediment and among infaunal organisms (Jonsson et al. 2017, Elskus et al. 2020). Therefore, the large inputs of terrestrial organic matter associated with forestry in Port Underwood provided further evidence of the influences of heavy sedimentation on the accumulation of mercury in benthic suspension feeder *P. novaezelandiae*.

Similarly, Mapua and QCS, characterized by a high degree of catchment modification revealed highest THg concentrations for *P. canaliculus*. Both the Mapua and QCS catchments have been modified by forestry, farming and urban development (Figure 4.2). Further, ports are present at the base of each catchment and the marine environments experience heavy boat traffic (Newcombe et al. 2015, Newcombe & Johnston 2016). In contrast to QCS, Mapua catchment delivers large inputs of runoff from the Waimea river situated within a largely modified catchment (Newcombe 2016). Though QCS does not experience the same riverine inputs as Tasman Bay, it is the major hub of transport between the South and North Island where South Island train networks come together was well as cars and supply trucks. Though these results cannot definitely determine localised sources of mercury inputs to these systems, it is clear that elevated mercury concentrations mirror catchments influenced by a high degree of anthropogenic pressure. Therefore, we provide evidence that elevated mercury concentrations observed for *P. novaezelandiae* and *P.*

canaliculus reflect modified catchments and a high degree of anthropogenic activity on the South Island of New Zealand.

Contrast to the above catchments, Doubtful Sound, characterized by intact catchments of native forest (Miller et al. 2006, Wing & Jack 2014) returns similar THg concentrations to those observed in QCS and Pelorus for *P. novaezelandiae.* Though Doubtful Sound is not influenced by riverine inputs, it received freshwater from Manapouri Hydroelectric Power Plant. Studies have identified the considerable changes in freshwater inputs and water column chemistry in Doubtful Sound, influencing benthic community composition (Tallis et al. 2004, Rutger & Wing 2006, Wing & McLeod 2007, McLeod & Wing 2008). Further, hydroelectric power plants have been tied to elevated THg concentrations in other fiord systems (Hylander et al. 2006, Kasper et al. 2014, Schartup et al. 2015, Azad et al. 2019). It is presumed that the mechanism supporting elevated THg concentrations from hydropower plants is similar to that of coastal sedimentation through riverine inputs. Hydropower stations generally use water from the hypolimnium layer, or dense lower water layer, which has favorable conditions for methylation (Azad et al. 2019). It is therefore likely that the THg concentrations reflected in *P. novaezelandiae* in Doubtful Sound are influenced by the freshwater inputs associated with Manapouri Hydroelectric Power Plant. Previous records of THg analyses in this region would allow a more concrete link between increased anthropogenic activity and THg concentrations in Doubtful Sound to be established.

The results from the present chapter provided evidence that the variation in THg concentrations of benthic bivalves are likely influenced by point source mercury inputs on the South Island, New Zealand. Preliminary conclusions from the present study linking elevated THg concentration to extensive modification of catchments are particularly prudent as MeHg may increase up to 30% in coastal areas, resulting from increased runoff associated with climate change (Jonsson et al. 2017). Currently, levels of mercury measured in *P. novaezelandiae* and *P. canaliculus* are safe for consumers of these species within Marlborough Sounds, Tasman Bay, and Fiordland. Concentrations measured for all organisms fell below the food safety limit as indicated by FAO/WHO and ANZFS at 0.5 mg kg $^{-1}$ (Table 1). Further, the observed concentrations in this study are lower than many THg concentrations recorded globally (Deudero et al. 2009, Apeti et al. 2012, Lanksbury et al. 2014, Sadhu et al. 2015, Briant et al. 2017, Ruiz-Fernández et al. 2018, Elskus et al. 2020).

Though current levels of THg in *P. novaezelandiae* and *P. canaliculus* are not cause for great concern, the projected increases in runoff associated with climate change reveal the importance of future monitoring of these key marine resources.

To our knowledge, these results illustrate the first measurements of mercury for commercially important bivalves encompassing a large spatial scale on the South Island of New Zealand, therefore, providing a baseline for future monitoring. In order to remain confident that THg concentrations will not become detrimental to both ecological and human health, further laboratory and field studies are required to monitor and confidently pinpoint sources contributing to the variability of THg concentrations among sites.

Chapter 5: Synthesis

Bivalves are important sentinel species that can help us make inferences about how anthropogenic modification of the natural environment may affect a wider range of species, and in turn the structure and stability of entire temperate rocky reef food webs. In the present study, we evaluated direct interactions between catchment modifications and salmon farming on both resource composition and availability to benthic bivalve communities. Further, intraspecific differences in feeding strategies were investigated to identify how these interactions influence community dynamics on the whole. Our objective was to gain a broader understanding of how basal organic matter source pools are altered by a range of anthropogenic stressors, in addition to the implications for the composition of organic matter and availability of trace metal concentrations reflected in key primary consumers. Here, spatial patterns revealed catchment modification and salmon farming significantly influenced use of alternative basal organic matter sources and the availability of trace metal concentrations in bivalve species among Marlborough Sounds, Tasman Bay and Fiordland. The presented findings may help inform associations between land use and bivalves as indicator taxa on a global scale to evaluate food web dynamics on temperate rocky reefs worldwide.

The factor REGION could explain large spatial scale variation in organic matter and trace metal signatures, however, mercury concentrations remained consistent among regions. Analysis of the factor CATCHMENT consistently explained observed differences in organic matter, trace metal, and mercury concentrations for benthic suspension feeding bivalves. The range in landcover classifications among catchments revealed the influence of modified catchments in driving the organic matter resource pools available to benthic bivalves. Further, FORESTRY revealed patters associated with organic matter and mercury concentrations, while SALMON FARMS explained species specific differences in organic matter availability and trace metal concentrations. The results further supported site level variation in organic matter use and mercury concentrations influenced by resource availability on the scale of a few kilometers. The results of the present thesis illustrate the pervasive influence of catchment modification and salmon farms on benthic coastal communities from regional to local scale differences in resource composition and availability.

Spatial variation in resource use

In the present study we suggest that environmental changes influence the structure and patterns of organic matter fluxes through food webs. The variability in organic matter source pools was reflected by the composition of macroalgal communities among Marlborough Sounds, Tasman Bay, and Fiordland. In this case, low availability of organic matter derived from macroalgae in Tasman Bay, coupled with an overall higher abundance of macroalgae in Marlborough Sounds and Fiordland, support the idea that contribution of bivalve diet from organic matter sources varies with the composition of organic matter resource pools. This trend was further observed at the site level within Marlborough Sounds. Discrete feeding mechanisms associated with individual species revealed resource partitioning among species may be responsible for the composition of organic matter supporting individual species within a community, further revealing that feeding strategies among individual species likely minimize food competition through niche partitioning as suggested by Tallis (2009).

The clear patterns associated with the degree of catchment modification and resource variability among species revels that the composition of resource pools is relative to the impacts associated with catchment modification across species. Catchment modification associated with a higher degree of urban development, farming, and forestry were associated with lower proportions of organic matter derived from macroalgae than intact catchments. This has been corroborated Further, the elevated proportions of macroalgae in species near salmon farm revealed the increased productivity of macroalgae associated with an influx of nutrients surrounding farms (Haya et al. 2001). The influences associated with modified catchments and salmon farming revealed the role of anthropogenic activity in shaping resource pools available to benthic communities and the alteration of detrital pathways.

Spatial variation in trace metal fingerprints

The unique opportunity to identify patterns in trace metal concentrations for multiple species with different feeding strategies across the South Island, New Zealand, revealed how trace metal concentrations were likely shaped by anthropogenic stressors. Together, patterns in the concentrations of three classes of trace metals revealed information on food sources, environmental conditions, and trace metal pollutants representative of the regions.

Concentrations of conservative, bioactive, and highly scavenged trace metals in target bivalve tissues were reflective of water column chemistry, primary production, and contaminants consistent with local catchment modification and salmon farm practices.

Though limitations of trace metal analysis exist due to the variability in biologically available and species-specific physiological mechanisms, variations in trace metal concentrations associated with carbon sources ($\delta^{13}C$) could be explained by a range of anthropogenic stressors. Significant differences in trace metal concentrations among catchments with a range of modification was contingent on intraspecific differences. Further, elevated levels of bioactive metals among salmon farms in association with stable isotopic values ($\delta^{15}N$ and $\delta^{13}C$) were consistent with the effect of increases in available nutrients at salmon farms relative to references sites for benthic dwelling species. Therefore, supporting the influence of food sources of the reflected trace metal concentrations (Rainbow 2002, Genç et al. 2018, Ruiz-Fernández et al. 2018) in addition to anthropogenic stressors driving the observed differences in resource composition. Each of these metals tend to be more biologically available to burrowing organisms or filter feeders at the sediment boundary (Chapman et al. 2002), reflecting the importance of a multi-species approach in documenting spatial variability in trace metal fingerprints.

These data reveal the influence of both regional scale differences in catchment modification and local scale influences of salmon farming on the alternate organic matter source pools driving variability in trace metal concentration among bivalves. Further, variability in the associations between trace metals and organic matter revealed species specific physiological mechanism driving uptake from the environment.

Spatial variation in mercury concentrations

The effect of CATCHMENT and SITE explained variation in mercury concentrations among study regions. However, SITE explained the largest inter- and intraspecific variation. Mercury concentrations were consistently highest in catchments influenced by a high degree of modification, where forestry could be tied to the elevated levels of mercury for *P. novaezelandiae* in the Marlborough Sounds. This may be explained by the methylation of mercury in coastal benthic habitats influenced by a high degree of sedimentation resulting in anoxic sediments (Jonsson et al. 2017). Further, as the first comprehensive record of mercury concentrations to commercially important bivalve species on the South Island of New Zealand, we provide a baseline for future measurement of *P. novaezelandiae* and *P. canaliculus* for the benefit of public health.

Further directions of study

Though site-specific steps were taken to resolve stable isotopic signatures of two distinct basal organic matter sources within Marlborough Sounds, further temporal sampling should be conducted in future studies to evaluate organic matter signatures over time. Frequent temporal sampling throughout the year may benefit the identification of potential diet shifts. Further, site-specific sampling within Fiordland and Tasman Bay would provide additional resolution in basal organic matter source pools within these discrete regions.

We provide evidence in Chapter 3 supporting the influence of salmon farms and modified catchment on the uptake of trace metals in coastal shellfish. We make connections to the organic matter sources supporting these species, however, in order to more accurately identify pathways, we would like to analyse trace element concentrations on basal organic matter source pools. This would enhance the conclusions we were able to draw regarding transfer of trace elements from basal organic matter source pools to consumers.

In Chapter 4 we provide strong evidence supporting the role of forestry and freshwater inputs on the increased THg concentrations of *P. novaezelandiae.* It would be beneficial to conduct this aspect of the study with an increased number of species encompassing a larger habitat range as we did in Chapters 2 and 3. However, due to the cost of THg analysis, consumer species encompassing both soft sediment and subtidal habitats were an effective starting point for this study. In addition, though THg is an effective measure of Hg in shellfish, MeHg would resolve the total bioavailable Hg concentrations reflected in shellfish (Bargagli et al. 1998, Sarkar et al. 2008, Chouvelon et al. 2018). In future studies, it would be prudent to conduct MeHg analyses on both basal organic matter source pools and consumers.

Bivalves are an integral part of rocky reef communities and a key link in food webs between benthic, pelagic organic matter source pools, in addition to terrestrial organic matter sources. Therefore, changes to the basal organic matter source pools supporting these species will result in inherent changes in organic matter sources supporting species further up the food chain, perhaps resulting in population declines in areas of high anthropogenic pressures (Udy et al. 2019a, 2019b). The pressures associated with anthropogenic activity are not ubiquitous across temperate rocky reef ecosystems, but contingent upon environmental modifications and biological community dynamics within catchments.

In the present study we evaluated direct interactions between catchment modifications and salmon farming on feeding strategies of individual species and further evaluated how these interactions influence the community on the whole. With the increase in development of land and aquaculture endeavors for economic gain, we can expect the impacts to coastal reefs to increase. Therefore, further altering resource pools supporting rocky reef communities. We provide evidence that development of a robust and holistic understanding of catchment modification associated with food web dynamics is imperative for modeling of ecosystem health.

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Appendices

Appendix 1

Bivalve species description table

Scientific Name	Mäori Name	Common Name	Taxonomy (Class: Family)	Description
Pecten novaezelandiae	Kuakua	Common scallop	Bivalvia: Pectinidae	Subtidal, sediment habitat, orange/pink shell, edible
Tucetona laticostata	Kua kua	Dog cockle	Bivalvia: Glycymerididae	Subtidal, sediment habitat, dorsal shell prominently ribbed, edible
Atrina zelandica	Hururoa	Horse mussel	Bivalvia: Pinnidae	Subtidal, sediment habitat, brittle shell with jagged protrusions, edible
Mytilus galloprovincialis	Kuku	Blue mussel	Bivalvia: Mytilidae	Rocky intertidal habitat, Dark blue shell with dense byssus threads, edible
Perna canaliculus	Kuku	Green-lipped mussel	Bivalvia: Mytilidae	Rocky intertidal and subtidal dwellers, vibrant green shell, edible

Appendix 2

Landcover descriptions (LINZ Data Service, Marlborough District Council)

Landcover class	Description
Farming	Short-rotation crop, orchards, vineyards, and other perennial crops
Urban development	Commercial, industrial or residential buildings, including associated infrastructure and amenities, not resolvable as other classes. Low density 'lifestyle' residential areas are included where hard surfaces, landscaping and gardens dominate

	other landcovers. Open, mainly grassed or sparsely-treed, amenity, utility and recreation areas. Class includes parks, playing fields, public gardens, cemeteries, golf course, berms and other vegetated areas usually within or associated with build-up areas.
Forestry	Predominantly bare ground arising from harvested exotic forest or, less commonly the clearing of indigenous forest. Replanting of exotic trees (or conversion to a new landcover) is not evident and nor is the future of land cleared of indigenous forest.
Forest	Deciduous hardwoods, exotic forest, Gorse and/or Broom, herbaceous vegetation, high producing exotic grassland, low producing grassland, mixed exotic shrubland, tall Tussock grassland, flaxland, fernland, Manuka, Kanuka, broadleaved indigenous hardwoods, Matagouri, indigenous forest
Marine farms	Salmon farms

Appendix 3

Size justification

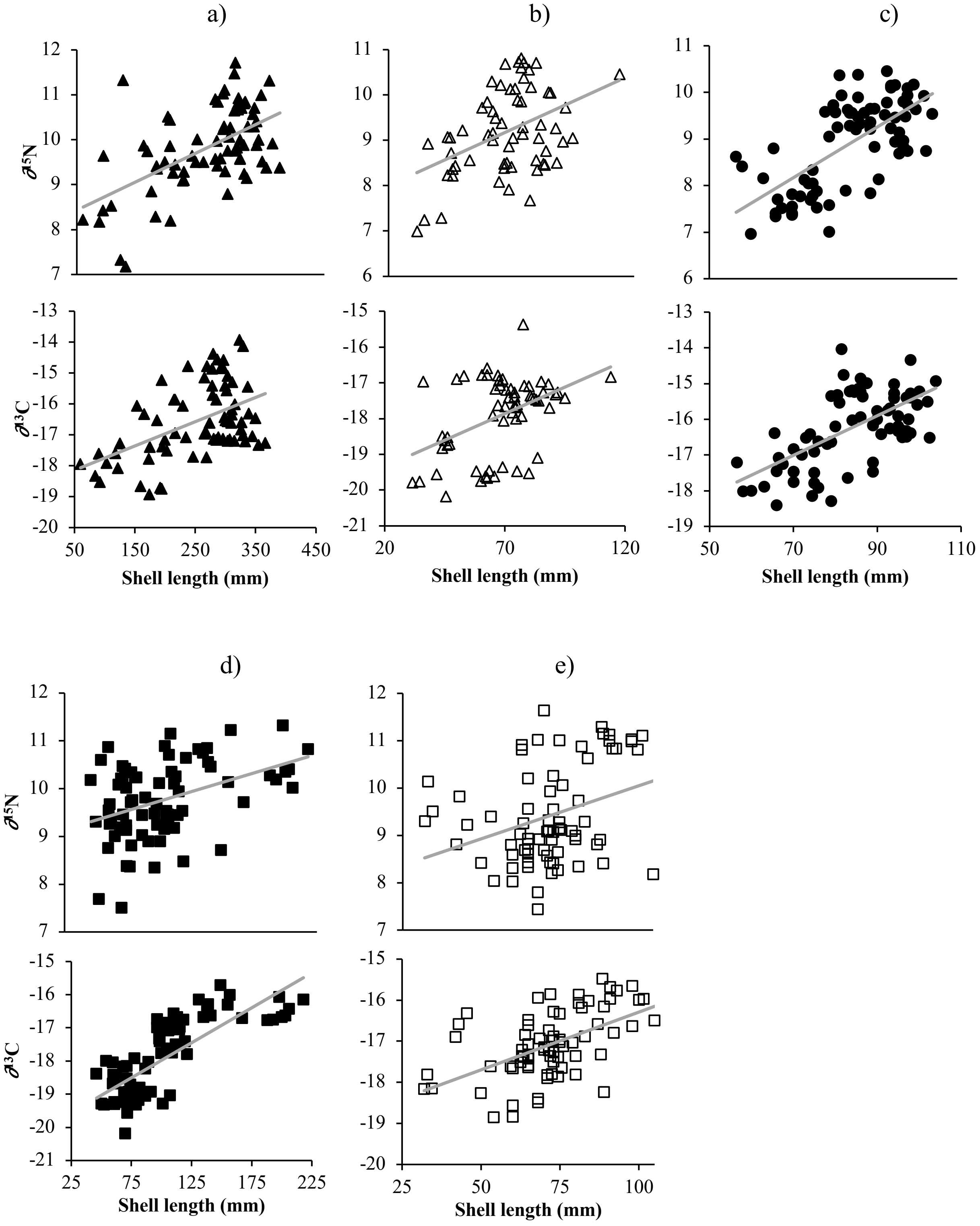

Figure 2. Positive linear relationship between shell length and stable isotopic signatures (δ^{13}C and δ^{15}N) for a) *A. zelandica*, b) *M. galloprovincialis*, c) *P. novazelandiae*, d) *P. canaliculus*, e) *T. laticostata.*