Tracing Nutrients and Contaminants in Nearshore Food Web

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A thesis submitted in fulfilment for the degree of

Doctor of Philosophy

University of Otago

December 2018

Abstract

Sewage derived organic matter from domestic and municipal wastewater, promotes enhanced secondary productivity, eutrophication and trace metal contamination, reduction in oxygen levels, and biodiversity. It poses a potential impact on human health, contamination of seafood and water and ecological disturbances in the natural aquatic ecosystem. Thus, this research examined the impact of improvement in treatment and disposal schemes employed by a municipal sewage treatment facility on a previously sewage contaminated coastal marine system. It took a critical look into the influence of the discharged of inadequately treated sewage effluent and the modern improved mitigating efforts put in place to ensure proper treatment and disposal by the municipal wastewater treatment plant on the coastal marine waters. It also investigated the influence of other potential terrestrial organic nutrients and contaminants on the coastal marine waters, the flow of energy and their fate through the natural coastal marine food web. Stable isotope analyses incorporated with mixing models, other independent chemical tracers such as (i.e. faecal sterol, fatty acid and elemental analyses) were utilised to infer change in the sewage derived organic matter dynamics of the coastal marine waters. The detected change was attributed to the modification in wastewater treatment and disposal techniques adopted by the municipal wastewater treatment plant. The differences in the carbon and nitrogen isotope ratios of *Mytilus galloprovincialis* (sentinel organism) were assessed in a preliminary survey to determine the tissues of choice as indicative tools for long-term ecological-based study aimed at tracing the sources and fate of sewage organic materials in the coastal marine waters. The preliminary survey aided the experimental design of the study. Isotope mass balance mixing models were fitted to quantify the contributions of land-based organic materials as part of the diet constituents to examine the influence of terrigenous materials on observed diet switching changes from sewage-derived nutrient source to marine nutrient source in *Mytilus galloprovincialis*, a prevalent resident marine bivalve collected at the nearshore marine waters. Biochemical compositions and elemental concentrations (independent tracers) in *Mytilus galloprovincialis* were analysed and used as independent indicative tools for testing the assumptions obtained from the stable isotope analysis mixing models. The tracers indicated the source of nourishment to the resident organism and provided additional insight into the organic nutrient supply and contaminant dynamics of the coastal marine waters. The independent tracers revealed that the marine particulate organic matter was main source of nourishment to the marine bivalve. The

sea lion colony provided a minor contribution of faecal matter to the nearshore marine waters. The chemical tracers affirmed that the improvement in the treatment and disposal methods had a positive impact on the nearshore marine waters. Trace metal levels and human health risk assessment on the nearshore marine bivalve affirmed the safe human consumption of the nearshore marine fisheries.

Acknowledgement

I would like to express my special thanks to my primary supervisor Professor Russell D. Frew (University of Otago) for his valuable advice and sincere guidance over the course of my thesis. He made himself available and was a great sustenance throughout the entire thesis. Thanks for your encouragements and immense wealth of experience you have put in towards the successful completion of my thesis. My gratitude goes to Dr. Rob Middag, my previous supervisor, who had to move to the Royal Netherlands Institute for Sea Research. He was a great mentor and indeed, a true friend.

My special thanks to the staff at the Isotrace® Ltd., Dr Robert Van Hale and Mrs Dianne Clark for their immense contributions and help with the stable isotope laboratory equipment and making them easily accessible for use. I would like to mention the contributions Mr David Barr (Department of Chemistry) for giving good guidance on the appropriate technical support and assisting with the setting up of the ICP-MS used for trace elemental analysis and Mr Ashely Duncan (Department of Food and Nutrition) giving useful, needed tips and facilitating access to the GC-MS. These people gave quality support with instrumentation and assisted in many ways to make sure everything runs well.

I would also like to thank all the people in my research group for their love and encouragement shown towards me while studying here at the University of Otago. Special thanks to Dr Kiri A. McComb and Tim Jowett (Department of Mathematics and Statistics) for their useful suggestions, guidance and assistance during the data processing and analysis stage of the thesis. My appreciation also goes to Dr Kim Currie research group and her amiable research technical support person, Mrs Judith Murdoch who were both helpful in providing sampling equipment and containers for storing samples. Dr Evelyn Armstrong, Lisa Bucke and Karen Rowley deserve acknowledgment for their support in providing space for analysis and giving their time to help when the need arises. I wish also to thank Professor Claudine Stirling and Drs.Christina McGraw and Amandine Sabadel all part of the Environmental Research Group, University of Otago for their good research ethics and kindly outlook. A big thank you to Don, a good friend and motivator during hard times.

I wish to appreciate the University of Otago for granting me the Doctoral Scholarship for three years without which it would not have been possible to study in New Zealand.

To my family, particularly my lovely two daughters Deborah and Elizabeth Babaranti, aged mother Felicia Temilade Babaranti, brother Evang. Ope-Oluwa Timothy Babaranti, sisters

Boladale, Kemi and Gbemisola Babaranti. I say a big thank you to them all for their love, support, patience and encouragement throughout my PhD study. Finally, great gratitude goes to my late father Snr. Evang. Williams Olayiwola Babaranti who had cultivated in me from infancy the spirit of discipline, honesty and entrepreneurship.

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List of Equations

δ (‰)$_{sample\text{-}standard}$ = (R_{sample}-$R_{standard}$)/ ($R_{standard}$) …Equation 1 9

$E = 12\ hv$ …...Equation 2 11

$AX_L + BX_H \rightleftharpoons AX_H + BX_L$.....Equation 3 13

$\alpha_{X-Y} = RXRY$…...Equation 4.....13

$m_\Sigma\ \delta_\Sigma = m_x\ \delta_x + m_b\ \delta_b$……Equation 5 33

$\delta_\Sigma = \delta_x - m_b\ (\delta_x - \delta_b)/m_\Sigma$…….Equation 6 33

Chlorophyll a concentration (ug/l) = 2.0830 * 1.0769 * (F_O-F_A) * v / V…....Equation 7.....35

RSD (%) = (SD/X) * 100…Equation 8 40

Dilution factor = [25 ml / dry weight (g)]* 1000 (ml/g)…Equation 9 41

$y = mx + c$…….Equation 10 43

r^2 = (n∑xy - ∑x. ∑y) ^{2} / (n∑x^2 – (∑x) ^{2} (n∑y^2 – (∑y) ^{2}….Equation 11 43

t = $r*$ (√n-2)/ (√ 1-r^2)…. Equation 12.....43

RMSE = √ [1/n (∑ (F_i — O_i) ^{2}]…...Equation 13.....44

D = Max [abs (S_1(Y)-S_2(Y))]…...Equation 14 45

$Z^1 = \Phi^{11}X^1 + \Phi^{21}X^2 + \Phi^{31}X^3 + ... + \Phi^{p1}X^p$……Equation 15 47

$\delta X_{tissue} = p\ \delta X_A + (1- p)\ \delta X_B$……Equation 16. 49

$yi = \beta 0 + \beta 1x1i + \beta 2x2i + \beta 3x3i + \beta 4x1ix2i + \beta 5x1ix3i + \beta 6x2ix3i + \alpha j(i) + \varepsilon i$.....

Equation 17 50

$\delta^{15}N_{mussel} = (\delta^{15}N_{POM})\ X + Y(\delta^{15}N_{control})$ ……Equation 18.....91

$\delta^{13}C_{mussel} = (\delta^{13}C_{POM})\ X + Y(\delta^{13}C_{control})$ ……Equation 19 91

[(coprostanol / (coprostanol + 24-ethylcoprostanol)]…...Equation 20.....158

EDC = PTWI × C….Equation 21 173

CCRE = EDC × S…Equation 22.....173

THQ = [(MCM× MCR × EXD× EXF) / (BW × EXT × RFD)] × 10^{-3} …Equation 23.....174

List of Publications & Conferences

Babaranti, O., Horn, S., Jowett, T., & Frew, R. (2019). Isotopic signatures in *Mytilus galloprovincialis* and *Ulva lactuca* as bioindicators for assessing discharged sewage effluent in coastal waters along Otago Peninsula, New Zealand. *Geology, Ecology and Landscapes.* (Published).

Babaranti, O., Frew, R. & McComb, K., (2019). Spatial richness and variance of the isotopes of carbon and nitrogen isotopes in *Mytilus galloprovincialis* from the Otago Coastal Waters, New Zealand. *New Zealand Journal of Ecology* (Submitted and under review).

Babaranti, O., Frew, R., McComb, K. & Van Hale, R., (2018). Stable isotopic inquest: Insight into the flow, fate of nutrients and organic materials at the intertidal mixing zones of two contrasting coastal waters along Otago Peninsula, New Zealand. *Ecological Indicators* (Article in preparation).

Babaranti, O., Horn, S., Frew, R., &Van Hale, R., (2018) Isotopic signatures in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* as bio-indicators for assessing the impact of discharged sewage effluent on the nearshore coastal marine ecosystem along Otago Peninsula, New Zealand. *11th Otago Energy Research & Colloquium, November 2017*, New Zealand. (Talk)

Babaranti, O., Frew, R., Van Hale, R., & McComb, K. (2018). Stable isotopic inquest: Insight into the flow, fate of nutrients and organic materials at the intertidal mixing zone of coastal waters along Otago Peninsula, New Zealand. *ASLO, June 2018 Summer Meeting*, Canada. (Poster)

Abbreviations

ANOVA - Analysis of Variance

BSTFA - N, O-Bis (trimethylsilyl) trifluoroacetamide

BW- Body Weight

CCRE - Carcinogenic Risk Effect

CF-IRMS - Continuous Flow-Isotope Ratio Mass Spectrometry

EDC- Estimated Daily Consumption

EA-IRMS - Elemental Analyser-Isotope Ratio Mass Spectrometry

EDTA - Ethylenediaminetetra-acetic acid

EPA- Eicosapentaenoic Acid

EXF – Exposure Frequency

EXD - Exposure Duration

EXT- Exposure Time

FC - Faecal Coliform

FPC - Floodplain Concept

GILF - Green Island Landfill

GC-MS - Gas Chromatography-Mass Spectrometry

GIWWTP - Green Island Wastewater Treatment Plant

GF/F - Glass Fibre Filters

HI - Hazard Index

HPLC - High-Performance Liquid Chromatography

IAEA - International Atomic Energy Agency

ICP-MS - Inductively coupled plasma spectroscopy

KS Test - Kolmogorov-Smirnov Test

LOD - Limits of Detection

LMM - Linear Mixed Effects Model

MCM - Metal Concentration in Mussel

MCR - mussel consumption rate

MSD - Mass Selective Detector

NED - N-(1-naphthyl)-ethylenediamine dihydrochloride

NEP - Net Ecosystem Production

NMDS - Non-Metric Multidimensional Scaling

NIST - National Institute of Standards and Technology

NIWA - National Institute of Water and Atmospheric Research

RFD - Oral Reference Dose

ORS - Octopole Reaction System

OLS - Ordinary Least Squares

OWH - Outwelling Hypothesis

PAST - Paleontological Statistics

PPMCC - Pearson Product-Moment Correlation Coefficient

PCA - Principal Component Analysis

POM - Particulate Organic Matter

POC - Particulate Organic Carbon

PON – Particulate Organic Nitrogen

PTFE - Polytetrafluoroethylene

PSP - Paralytic Shellfish Poisoning

PFAs - Polyunsaturated Fatty Acids

PLFAs – Phospholipid-derived Fatty Acids

RCC- River Continuum Concept

RSD - Relative Standard Deviation

RMSE - Root Mean Square Error

SIM - Selected Ion Monitoring

SIA - Stable Isotope Analysis

SRM - Standard Reference Material

SDOM - Sewage-Derived Organic Matter

SPOM – Suspended Particulate Organic Matter

THQ - Target Hazard Quotient

TMCS - Trimethylchlorosilane

TWWTP - Tahuna Wastewater Treatment Plant

UHMI - Ultra-High Matrix Introduction

USGS - United States Geological Survey

V-SMOW - Vienna Pee Dee Belemnite

V-SMOW - Vienna Standard Mean Ocean Water

WETT - Whole Effluent Toxicity Testing

CHAPTER 1

Introduction

1.0 Overview

This introductory chapter provides the background information on the issue of coastal marine water contamination by human activities and the application of stable isotope tracer tools as indicators for assessing the impacts. The background provides a link to the specific focus of this thesis, i.e. the impact of long-term sewage contamination from the municipal wastewater treatment plant in Tahuna, Dunedin City on the Otago's Coastline of New Zealand and the modification of the municipal treatment plant facility to ameliorate these effects. This chapter took an insight look into previous studies carried out before the upgrade of the municipal wastewater treatment plant and stated the research gap in the previous studies as the basis for this current research. This provides the justification for research with a central focal point on the status of the nearshore marine waters (Otago Coastal System) after the upgrade of the municipal wastewater treatment plant and effect of the influx of terrestrial-based nutrients and contaminants on the coastal marine food web (Figure 1).

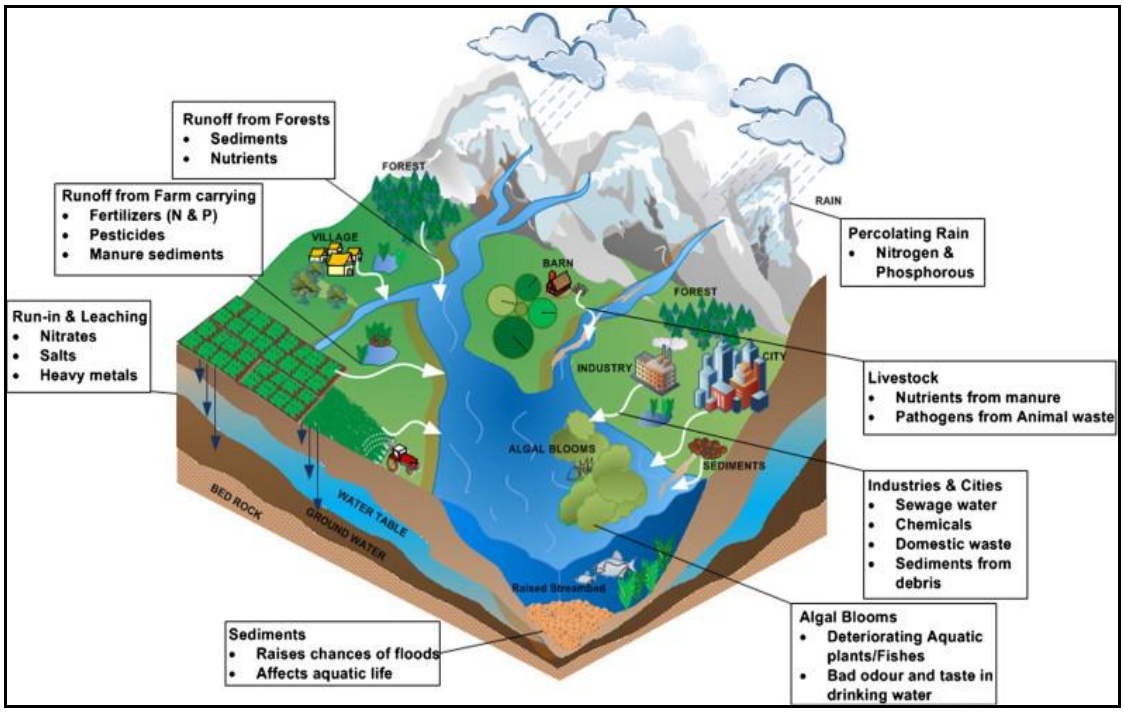


Figure 1: Schematic representation of the nutrient supply and contaminant dynamics of Otago Coastal System. Adapted from Zia et al. (2013).

1.1 Background

The upsurge in human habitation and socio-economic activities around the world's coastal regions has led to an increase in the level of human-derived chemical substances from urban, industrial and agricultural runoff into the coastal marine waters (Cohen et al. 1997; Syvitski et al. 2005). It is estimated that about three-quarters of the large cities of the world are situated along the coastal regions while more than half of the world's population are living within 200 kilometres of the coast (Kennish 2016; Oceans 2008) making the coastal regions the most disrupted portions of the environment (Small and Cohen 2004). The coastal marine zone is generally more productive than other zones of the ocean (Longhurst et al. 1995) but, as the first receiver, it is highly susceptible to the effect of escalated human activities. Such activities have led to an increase in the level of nutrients and contaminants in the coastal marine waters (Scavia and Bricker 2006) (Figure 2) and imbalance in the community structure and modification of the natural coastal marine food web structure.

Many of the exogenous substances from anthropogenic influence pose a threat to the marine biota directly and humans indirectly by producing genotoxic, neurotoxic and endocrine disrupting effects in biological systems (Purdom et al. 1994). For instance, marine organisms can accumulate toxic elements to higher concentrations (bioaccumulation) in their tissues than present in the aquatic environment and be transported higher up the food chain (biomagnification) (Bergman et al. 2013; Rainbow 1993; Voigt et al. 2003) thus posing a threat to the consumers. The influence of anthropogenic substances on the coastal marine environment is the cumulative display of all kinds of human activities.

They cause noticeable and/or unseen disturbances in the natural structure and functions of the nearshore marine biotic communities, anomalies in habitats, alterations in the hydrology and geomorphology of nearshore waters, reduction in fisheries and recreational value. Such impacts will always have negative effects on the ecological, economic, or socioeconomic aspects of the environment.

Human activities such as urbanisation and industrialisation, construction of seaports and harbours, extraction of natural resources (such as mining, oil exploration and over-fishing), marine aquaculture, farming, shipping and recreation activities are the major factors responsible for the transport of point and non-point sources of anthropogenic substances into the coastal marine and shelf zone. The three main channels of entry for these substances are:

- Direct discharge of wastewater effluents and solid wastes into the seas and oceans, e.g. municipal and industrial wastes discharge
- Land runoff mainly from rivers
- Atmospheric deposition, e.g. by-products of fossil fuel combustion (via air masses onto the sea and ocean surfaces)

These substances influence the natural processes and biogeochemical cycles which occur at the sea-land and sea-atmosphere interfaces (Stanislav and Cascio 1999; Vitousek et al. 1997). They can be classified based on their impact on the habitat, water quality and marine biotic communities (Kennish and Paerl 2010) as follows:

- Substances causing mechanical impacts (solid wastes, microplastics) that damage the respiratory organs, digestive system and receptive ability
- Substances provoking eutrophic effects (e.g. mineral compounds of nitrogen and phosphorus and organic substances) that cause the mass growth of phytoplankton and disturbances of the balance, structure and functions of the coastal ecosystem.
- Substances with saprogenic and pathogenic properties (e.g. sewage with a high content of easily decomposing organic matter and disease causing organisms) that cause oxygen deficiency followed by mass mortality of water organisms, and appearance of specific microflora;
- Substances causing toxic effects (e.g., heavy metals, chlorinated hydrocarbons, dioxins, and furans) that damage the physiological processes and functions of reproduction, feeding, and respiration;
- Substances with mutagenic properties (e.g., benzo(a)pyrene and other polycyclic aromatic compounds, biphenyls, radionuclides) that cause carcinogenic, mutagenic, and teratogenic effects.

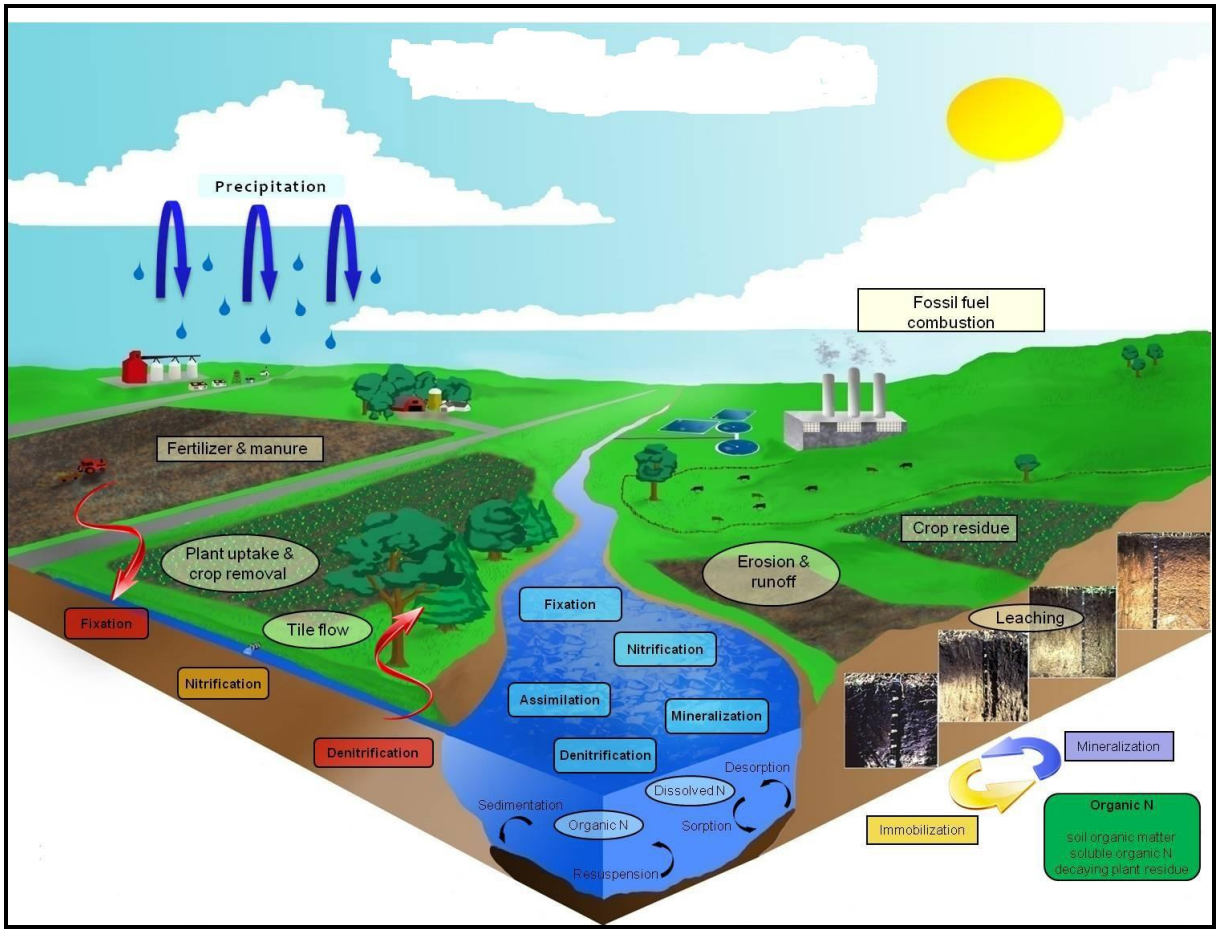


Figure 2: Human and natural processes responsible for the influx of organic nutrients into the nearshore marine ecosytem and various nutrient transformation processes.

(Source: https://swroc.cfans.umn.edu/agricultural-programs/soil-science/nitrogen-cycle)

Quantifying some of these substances in nearshore marine waters can be challenging due to their complex nature, low-level concentration over time and sporadic nature, which make them hard to detect via spot sampling. The uncertainty in the variation of their concentrations at each sampling period may also arise due to poor laboratory protocol during analysis. Thus, several independent methodologies that take into cognisance the complex multifactorial challenges are required to assess the impact of these anthropogenic substances on the nearshore marine waters and biota.

The natural biogeochemical cycling and variability of elements such as carbon, nitrogen, phosphorus and sulpur in the coastal marine environment are altered by fluxes of anthropogenic substances from human-driven chemical perturbations. For example; higher loads of nitrogen and phosphorus documented in marine coastal waters are attributed to coastal improvement and aggresive farming activities (Anderson et al. 2010; Galloway et al. 2008; Gruber and Galloway 2008; Howarth 2008).

Though, the marine coastal zone can also receive nutrients through rivers, groundwater, the atmosphere and opean ocean-via upwelling nutrient-rich deep waters (Doney 2010). Nutrient inflows through these channels can be influenced by human perturbations and are usually altered at the areas (estuaries) where there is the mixing of saltwater and freshwater inputs. These areas are characterised by high turbidity due to the trapping and resuspension of both marine and freshwater sediments (Pilskaln et al. 1998) which declines light penetration within the water column and limits primary productivity (phtyoplankton growth). Fluvial sediments around these areas are sources and sink for contaminants (Cave et al. 2005; Chapman and Wang 2001; Ip et al. 2007; Larrose et al. 2010).

1.2 Nutrient Cycling and Contaminant Dynamics of Nearshore Marine Waters

The dynamics of the biogeochemical cycling of elements in the coastal marine waters are somewhat related to the biogeochemistry of the seas and oceans. Both are controlled by physical and biological processes, sources and sinks across boundaries with the land, atmosphere and ocean floor. In the marine coastal environment, the nitrogen and phosphorus cycles are linked to the carbon cycle in contrast to the open ocean biogeochemical cycles (Bolin and Cook 1983a) owing to the fact that the levels of nitrate and phosphate are low in the open ocean as under natural conditions these are generally the bio-limiting nutrients.

The excessive influx of nutrients (nitrates) from land-based sources (resulting from increased human activities) into the coastal waters has been linked to coastal eutrophication (Anderson et al. 2002). This encourages massive biomass growth ensuing in the excessive utilisation of available oxygen in the water column and decomposition of organic matter (planktonic organisms and macroscopic plants) (Hallegraeff 2003).

In partially stratified coastal marine water, the decaying organic matter accumulates in the bottom waters leading to hypoxia (oxygen depletion) or anoxia (no dissolved oxygen). This causes mass mortality of benthic (bottom dwelling) organisms and if prolonged, can lead to a biological desert, creating a seabed with no animal life. Such conditions alter water quality, reduce water clarity and light penetration, promote algal blooms and over growth of epiphytic algae, loss of seagrass meadows (in shallow coastal waters), modify food chains, diminished survival rate of fish larvae and promote increased production of jellyfish (Shumway 1990).

Atmospheric deposition rates of nutrients and contaminants resulting from anthropogenic emissions into coastal marine waters are higher than oceanic waters because of their proximity to sources. The magnitude of deposition depends on the level of habitation and

industrial activity around the coastal region. For instance; the magnitude of atmospheric fluxes varied over at least two orders of magnitude between relatively pristine environments such as the Alaskan Shelf (0.9 mmol N m^{-2} yr^{-1}) to areas close to major centres of habitation and industrial activity as the North Sea (70 mmol N m^{-2} yr^{-1}) (Jickells et al. 2005).

In the global ocean, the level of nutrients and contaminants increases during the transition from the southern parts of all oceans to the north, where the main industrial centres and main pollution sources are concentrated. This general pattern of distribution of nutrients and contaminants may be influenced by two underlying factors; the relative confinement of large-scale water circulation within the limits of each hemisphere and the predominance of the zonal transport moving along the geographic parallels of the trace substances in the atmosphere.

Another distinctive and repeatedly recorded feature of the general pattern of nutrient and contaminant distribution in the marine environment is their localization at the water-atmosphere and water-bottom sediment boundaries. These boundaries provide the biotopes for the communities of hyponeuston / nekton (near surface dwellers) and benthos (bottom dwellers) respectively. The occurrence of elevated levels of nutrients and contaminants in these zones of high biological productivity is of ecological importance.

1.2.1 Nutrients and Contaminants in Nearshore Marine Waters

The coastal marine zone is an area influenced by the nearshore currents extending seaward from the low water mark well beyond the littoral and breaker zones. It is part of the coastal water; “the surface water on the landward side of a line, every point of which is at a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured, extending where appropriate up to the outer limit of transitional waters.” (as defined in the European Union Water Frame Directive Article 2 (7) (2000/60/EC) (Directive 2003). The ‘transitional waters’ so referred to in the definition are partly saline water bodies.

The coastal marine zones comprise of the waters present in the surface layer (photic zone) ranging from 20 to 200 metres and boundaries of natural environment such as the water-atmosphere and water-bottom sediment within the seas, estuaries, coastal and shelf waters. The shelf and coastal zones make up 10 % of the World Ocean surfaces and less than 3 % of its volume, where the most intense processes of biological production, including the self-reproduction of the main living resources of the sea, take place.

The main pressures of anthropogenic impacts are mostly concentrated in these zones due to their vulnerability to land-based chemical substances from urban and rural runoff, storm water discharges, sewer discharges from municipal outfalls, and seepage from malfunctioning or poorly maintained septic tank systems (Figure 3). These substances usually contain nutrients, contaminants, sediments, bacteria, viruses and pathogens, pesticides and trace metals that can cause chronic and acute effects to the benthic and pelagic species, alter nutrient dynamics thereby degrading the coastal marine ecosystem. Therefore, it is significant that at the regional and local levels, the intensity of anthropogenic pressures on the nearshore marine environment generally decreases and is properly monitored.

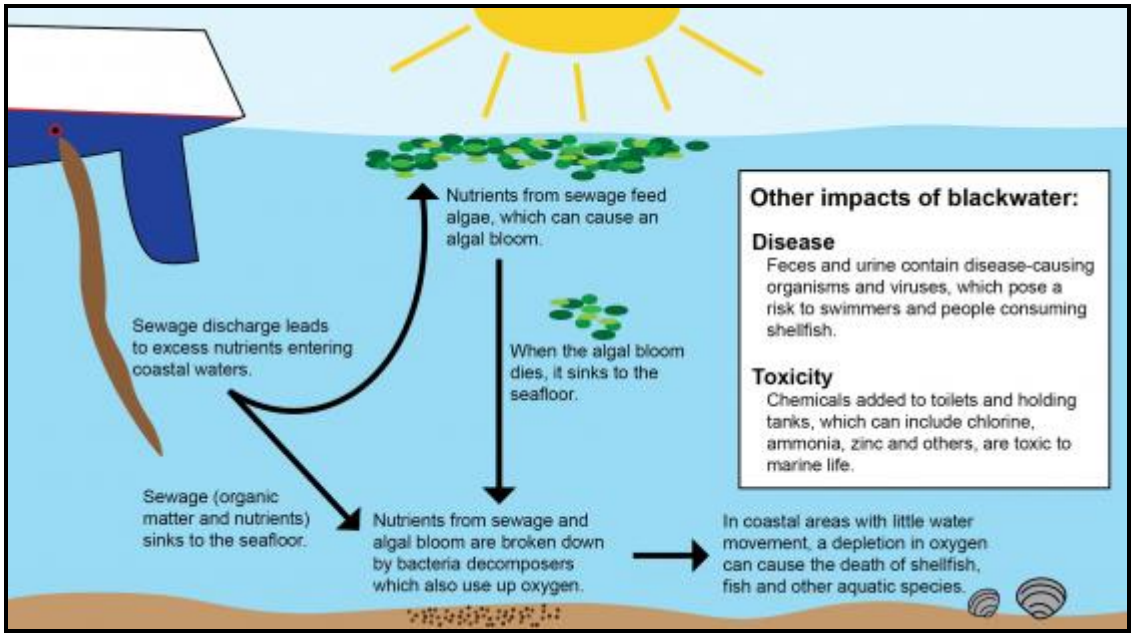


Figure 3 : Impact of discharged sewage on nearshore marine ecosystem.

(Source: https://www.sailorsforthesea.org/programs/green-boating-guide/blackwater)

Major nutrient loading and contamination observed in the nearshore marine waters are associated with terrestrial-based activities and are therefore apposite indicators for evaluating the impact of land-use practices and management on the nutrient supply and contamination dynamics in nearshore waters (Ameztegui et al. 2016; Crawford 2006; Scheltinga et al. 2004).

1.2.2 Sewage in Nearshore Marine Ecosystem

Sewage is one of the major anthropogenic contaminants in municipal wastewater discharged into the coastal marine ecosystem. Wastewater treatment plants play a key role in the

modification of nutrient and other organic contaminant concentrations in wastewater effluent before being released into receiving marine and estuarine waters. Elevated nutrients (nitrogen and phosphorous) from wastewater induce eutrophication, a condition that alters the coastal ocean balance by changing food web structure, increasing primary production and the prevalence and severity of pathogenic diseases (Lapointe et al. 2000; Wear and Thurber 2015).

The susceptibility of marine ecosystems to nutrients from various chemical perturbations from storm water, untreated septic tanks and ships, improper or damaged sewerage connections, and other non-point sources such as surface run-off, faecal pellets from marine animals, agricultural inputs, and industrial pollutants, makes the identification of sources of nutrients that contribute to persistent eutrophic conditions in marine systems unknown. Therefore, stable isotope analysis of carbon and nitrogen fills this gap, serving as tracers for sources of sewage in the receiving environment (Archana et al. 2016)

Even though nitrogen is rapidly diluted, diffused and transported by ocean currents, the source of δ^{15}N values are usually preserved in primary producers and can be used to indicate the isotope baseline in the marine food web (Archana, Li *et al*. 2016). Furthermore, δ^{15}N measurements are especially valuable in contaminant monitoring studies because each source of nitrogen has a distinctive δ^{15}N signature associated with it. For instance, sewage has different δ^{15}N signatures relative to natural sources; a typical δ^{15}N for sewage derived organic material from a chronic sewage sludge contaminated marine environment range from -1.1 to 7.2 ‰ (Van Dover *et al.* 1992), while treated sewage effluent is estimated to be between 10 and 20 ‰ (McClelland and Valiela 1998). An enrichment in δ^{15}N values from microbial mediated nitrification and denitrification reactions vary from enrichment factors (ε) of 10 ‰ to 40 ‰ depending on the type of wastewater treatment employed (Risk et al. 2009). Other examples of nitrogen sources that have unique δ^{15}N values are synthetic fertilizer (~ 3 ‰), combustion products (~ 1 ‰), and wet deposition (~ − 7 ‰) (Fry 2006).

1.3 Stable Isotope Concepts and Principles

An isotope is an atom whose nuclei contain the same number of protons but a different number of neutrons. Isotopes are broken into two specific types: stable (non-radioactive) and unstable (radioactive). There are approximately 300 known naturally occurring stable isotopes (Hoefs 1997). The stable isotopes of light elements such as hydrogen, carbon, oxygen and nitrogen are tools used in ecological research. Physical, chemical and biological

processes in the environment influence the isotope abundance and variability of these elements in naturally occurring materials. These light elements contain different proportions of at least two isotopes.

Usually, one isotope is predominantly an abundant isotope. For example, the average natural abundance of ^{12}C is 98.89 %, while the average abundance for ^{13}C is 1.11 %. Table 1 illustrates the average isotopic abundances of these elements. Stable isotopic variations/ratios of these elements are usually minuscule and measured using an elemental analyser. An isotope ratio mass spectrometer transforms a sample of material (e.g., soil, waste or drinking water, bodily fluids, minerals, chemical compounds) via thermal combustion quantitatively to a suitable purified gas (typically CO_2, N_2, or H_2) easily detected and determined isotope ratio mass spectrometer.

Table 1: Natural abundances of the stable isotopes of hydrogen, carbon, nitrogen and oxygen

Hydrogen	Carbon	Nitrogen	Oxygen
^{1}H - 99.984 %	^{12}C - 98.89 %	^{14}N - 99.64 %	^{16}O - 99.763 %
^{2}H - 0.0156 %	^{13}C - 1.11 %	^{15}N - 0.36 %	^{17}O - 0.0375 %
-	-	-	^{18}O - 0.1995 %

Isotope compositions of sample materials are reported relative to an internationally accepted standard and are expressed in parts per thousand deviations from the standard.

For instance, the average difference in the isotope composition between a sample material and the reference gas (standard material) is determined using Equation 1 described in Coplen (2011).

$$\delta\,(‰)_{sample\text{-}standard} = (R_{sample}\text{-}R_{standard})/\,(R_{standard}) \quad \ldots\text{Equation 1}$$

Where;

R_{sample} is the ratio of the heavy isotope to light isotope in the sample gas prepared from chemical conversion of the sample, if required,

$R_{standard}$ is the ratio of the heavy isotope to light isotope in the working reference gas, calibrated against internationally accepted standards to tie to the relevant international scale. The IAEA (International Atomic Energy Agency) maintains the international scales. The

IAEA and other agencies such as the United States Geological Survey (USGS) supply the certified standards.

δ (‰)$_{\text{sample-standard}}$ is the difference in the isotope composition of the sample material relative to that of the reference, expressed and reported in per mil (‰) (after being multiplied by 1000). The primary reference scales and the absolute ratios (illustrated in Table 2) of these standards are:

- V-SMOW (Vienna Standard Mean Ocean Water) - used for δ^{2}H and δ^{18}O isotope measurement. The standard is an average of different ocean samples from around the world.
- V-PDB (Vienna Pee Dee Belemnite) - used for δ^{13}C measurement. The standard is a $CaCO_3$ from a belemnite from the Pee Dee formation in South Carolina.
- Atmospheric Nitrogen - used for δ^{15}N measurement. The air has a very homogeneous isotopic composition making this an ideal reference.

Table 2: The $R_{standard}$ absolute ratio values for the international standards for measuring stable isotopes of hydrogen, carbon, oxygen and nitrogen.

Standard	$R_{standard}$	Atom
V-SMOW	0.0001558	^{2}H
V-PDB	0.0112372	^{13}C
V-SMOW	0.0020052	^{18}O
N-AIR	0.0036765	^{15}N

1.3.1 Isotope Fractionation and Effects

The isotopes of an element display similar chemical properties but vary in their physical properties. The differences in the physical properties due to masses differences (i.e. bond strength and velocity) give rise to fractionation (separation of the lighter isotope from the heavier isotope) among isotopes of an element during chemical reactions (Figure 4). Natural processes, such as evaporation, condensation, diffusion (kinetic isotope effect) or ordinary mixing processes, can also cause isotopic fractionation. The major types of isotope fractionation effects are equilibrium fractionation, kinetic fractionation, mass-independent fractionation (or Non-mass-dependent fractionation) and transient kinetic isotope fractionation. Although all of the fractionation effects are related, the principles of the first two are essential for this thesis.

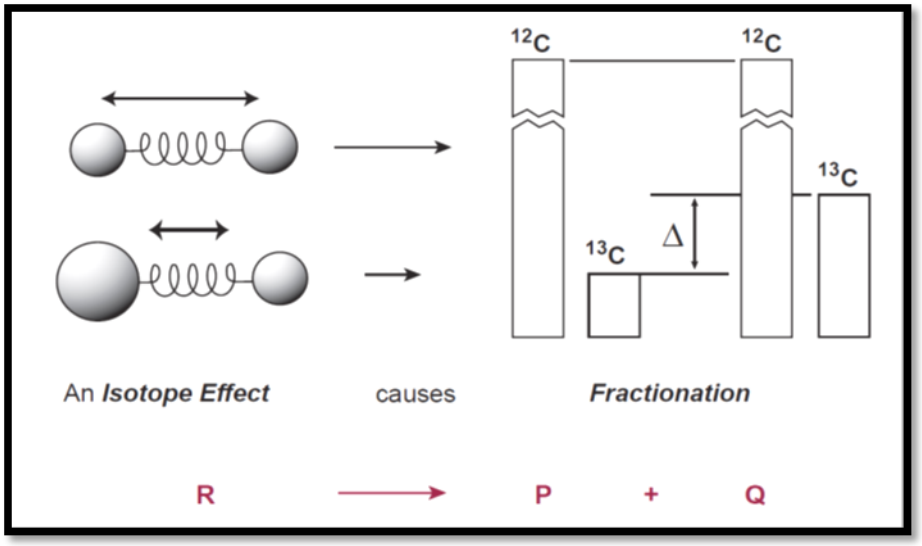


Figure 4 : Schematic representation of the relationship between isotope effect and isotopic fractionation [Adapted from Hayes (2004)].

Quantum theory states that the energy of an atom or molecule is restricted to certain discrete energy levels. Atoms and molecules constantly vibrate at a given temperature; the frequency with which they vibrate (v) depends on their mass. The differences in bonding energy result in varying molecular physicochemical properties. The lowest level of energy that an atom of a molecule has is proportional to its vibrating frequency and defined as expressed in Equation 2. Figure 5 illustrates where the upper horizontal line (EL) represents the dissociation energy of the light molecule and the lower line (EH), that of the heavy one. EL is actually not a line, but an energy interval between the zero-point energy level and the "continuous" level. This means that the bonds formed by the light isotope are weaker than bonds involving the heavy isotope (Hoefs 2018). Therefore, during a chemical reaction, molecules bearing light isotope will react slightly more readily than those containing the heavy isotope. The difference in zero-point is, therefore, the basis for isotopic fractionation (Criss 1999)

$$E = {}^{1}/_{2}\ hv \quad \text{.....Equation 2}$$

Where

E is the potential energy of the atom or molecule

h is the Planck's constant

ν is the frequency with which the atoms in the molecule vibrate with respect to one another

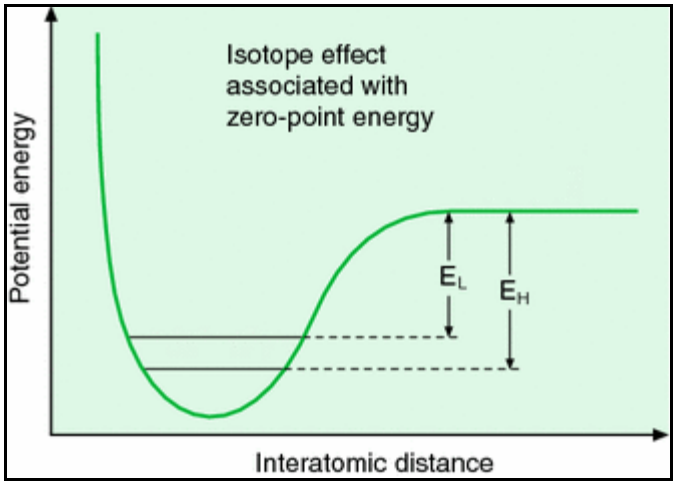


Figure 5: Schematic potential energy *curve* for the interaction of two atoms in a stable molecule or between two molecules in a substance or material. Adapted from Hoefs (2018)

1.3.2 Kinetic Fractionation

Kinetic isotope effects are associated with incomplete and unidirectional processes like evaporation, dissociation reactions, biologically mediated reactions and diffusion. A kinetic isotope effect also occurs when the rate of a chemical reaction is sensitive to atomic mass at a particular position in one of the reacting species. Biological processes are generally unidirectional and are very good examples of "kinetic" isotope reactions. All organisms preferentially use lighter isotopic species, because they require lesser energy to split up, resulting in a significant fractionation between the substrate (heavier) and the biologically mediated product (lighter). For instance, photosynthesis preferentially takes up the light isotope of carbon ^{12}C during the assimilation of an atmospheric CO_2 molecule (Criss 1999). This kinetic isotope fractionation explains why plant material (and thus fossil fuels, which are derived from plants) is typically depleted in ^{13}C by 25 ‰ comparative to most inorganic carbon on Earth (Kendall and Caldwell 1998). Kinetic fractionation had been reported to occur in biological systems involving discrimination of stable isotopes by mass in a biomechanical reaction such as hydrogenation of fatty acids in macro algae (Chikaraishi et al. 2004). Kinetic isotope fractionation effects are vital; they can provide unique information on the biochemical reaction pathways in biological systems (Kendall and Caldwell 1998; Schoeller 1999; Wunderlin et al. 2013).

1.3.3 Equilibrium Isotope Effect

The equilibrium isotope fractionation is a type of mass-dependent isotope fractionation effect. This effect occurs between two different phases containing common compounds and

elements. Most equilibrium fractionations are thought to ensue from the decrease in vibrational energy (especially zero-point energy) when a heavier isotope is substituted for a lighter one. This leads to higher concentrations of the massive isotopes in substances where the vibrational energy is most sensitive to isotope substitution, i.e., those with the highest bond force constants. However, there is no net reaction; the isotopic distribution changes between different phases or molecules. It can be expressed as in Equation 3:

$$AX_L + BX_H \rightleftharpoons AX_H + BX_L \quad \text{.....Equation 3}$$

The reaction involves the exchange of two isotope X_L and X_H of the element in molecules AX and BX each reactant molecule is identical to a product except for the distribution of isotopes (i.e., they are isotopologues)

The subscripts denote species A and B that contains either the heavy (H) or light (L) isotopes. The magnitude of the equilibration fractionation is dependent upon the different isotopes of element X.

1.3.4 Isotope Fractionation factor (α)

The fractionation factor is defined as the ratio of the numbers of any two isotopes in one chemical compound X divided by the corresponding ratio for another chemical compound Y (see Equation 4). The factor represents the partitioning of isotope ratio between two different parts of a system ideally between reactants and products during a given isotopic exchange reaction (Hoefs 2009).

$$\alpha_{X-Y} = \frac{R_X}{R_Y} \quad \text{.....Equation 4}$$

The R denotes the ratio of heavy to light isotope, X and Y represent the two different parts of the system (i.e. reactant and product, respectively).

A fractionation factor of 1 indicates the isotopes are distributed evenly in both systems, greater than 1 indicates the isotopes are concentrated in R_X and lesser than 1 indicates the isotopes are concentrated in R_Y.

1.3.5 Stable Isotopes as Tracers of Nutrients and Contaminants in Aquatic Ecosystems

Isotope fractionation due to isotope effects ensuing in isotope variations that ensure the temporal stability of stable isotopes in materials make it possible for them useful chemical tracers for the studying diverse environmental processes (Fry 2006; Sánchez-Carrillo and Álvarez-Cobelas 2017). They are useful in studying several ecological and biogeochemical processes such as characterisation and tracing of nutrient and contaminant sources within the mixing zones of estuaries, coastal water, and shelf water (Drake et al. 2009; Povinec et al. 2008a; Skei et al. 2000). ^{15}N biochemical tracer can indicate N-cycle processes. It was used as an indicative tool to assess N uptake, turnover, and retention processes at a whole-ecosystem scale in wetlands, lakes, and streams, estuaries, underflow zones and floodplains (Gribsholt et al. 2005; Holmes et al. 2000; Hubbard Jr et al. 2010; Mulholland et al. 2009; Zarnetske et al. 2011).

The isotopes of light elements (hydrogen, carbon, nitrogen, oxygen, sulphur, and chlorine) in combination with other conventional techniques are some of the most frequently used. The stable isotopes of carbon and nitrogen can identify N sources based on the notion that these elements are interconnected in the biochemical N cycle (Bolin and Cook 1983b; Werner 1981) and that measurable differences in the isotopic composition of N-source materials will persist as N-containing compounds are transported from the source. However, the isotopic compositions and forms of C and N in soil and water may resemble those of a nearby N source. The composition of soils and waters not only reflects the composition of the original source, or of mixed sources having different compositions (for example, biologically fixed N in soil, synthetic fertilizer, and animal waste), but can be influenced by isotopic fractionation during the transport and chemical transformation of C and N compounds. Thus, the $\delta^{13}C$ and $\delta^{15}N$ values of the material from which a compound formed establish an isotopic "baseline" that can be subsequently shifted by isotopic fractionation.

For isotopes to be most useful as tracers of N sources, fractionation should occur prior to transport, causing sources to have unique isotopic ratios, and fractionation should be minimal during transport from the source to nearby surface waters so that the transported products will inherit the source isotopic ratios. The partial loss of volatile species formed under reducing conditions such as denitrification and hydrogenation due to microbial actions (with the formation of methane of (CH_4), NH_3, N_2,) could cause major fractionations in C and N in most anthropogenic N sources (Toran 1982).

The measurements of $\delta^{13}C$ values in aquatic consumers can be used to characterise algal and terrestrial energy sources in unproductive streams with supersaturated dissolved CO_2 concentrations, and some productive rivers where CO_2 concentrations are low relative to photosynthetic rates. Since the relative contribution of terrestrial and algal carbon sources often varied within and between functional feeding communities and terrestrial and aquatic plants often have different $\delta^{13}C$ values (Finlay 2001; Rounick and Hicks 1985).

Particulates are non-reactive in comparison to most dissolved species. Ideally, suspended particulates in stream water consist of small fragments of the original N-source material(s) and that have an isotope composition similar to the source (Allan and Castillo 2007; Finlay 2004; Finlay et al. 1999). However, the clarification of isotope compositions of particulates may be unpredictable due to biological processes such as denitrification which could add particles of other materials to the bulk of suspended load (Liu et al. 2013).

Phytoplankton can constitute a significant part of the particulate material (Berg and Staaf 1981; Fairchild et al. 1983; James et al. 1988), especially during base flow conditions in the summer and fall. Hence, measurement of the isotope composition of particulate matter can be an indicative tool to mark out the sources of nutrients that constitute primary and secondary productivity as sustenance for the consumers within the aquatic ecosystem. However, the bulk $\delta^{15}N$ and $\delta^{13}C$ signatures of particulate materials are the combination of the $\delta^{15}N$ and $\delta^{13}C$ signatures of land plant detritus, primary production by aquatic organisms, and microbial biomass which can appropriately indicate sources of organic matter composition derived from primary and secondary inputs.

1.4.0 Wastewater Management in Dunedin

New Zealand has many wastewater outfalls, the majority of which discharge to the marine environment either at the shoreline, into harbours and estuaries or as offshore ocean outfalls. An estimated 70 % of New Zealand's treated wastewater is discharged directly into the marine environment. The majority of the remainder is discharged into the freshwater environment (24 %) and about 6 % to land (NZWERF, 2002). Municipal wastewater contains a variety of inorganic substances from domestic and industrial sources including a number of potentially toxic elements such as arsenic, cadmium, chromium, copper, lead, mercury, zinc, etc. A typical component of sewage effluent and metal concentrations of various degree municipal sewage sludge based on wastewater treatment schemes are depicted in Table 3 and 4. The strong, medium and weak went through primary, secondary and tertiary treatment

scheme respectively. Typical New Zealand untreated wastewater total N concentrations are in the range 7 - 60 mg/l, with an average concentration of 35 mg/l while total phosphorus (TP) concentrations in between 3.3 - 13 mg/l, with an average concentration (across the WWTPs surveyed) of 7 mg/l. The total suspended solids level is in the range 50-800 mg/l with an average of 300 mg/l, pH is 7.5 and BOD range between 150 - 450 mg/l, with an average value of 250 mg/l (Hauber 1995). Industrial and domestic waste sources contribute to a large proportion of metals and metalloids in New Zealand wastewater. Industry-specific metals and metalloids include chromium (tanning), arsenic, copper, zinc/ nickel (metal plating), aluminium (metal smelting) and boron. Metals contributed from domestic sources come from water treatment, detergents, soap, cosmetics, household dust, medicines, toilet paper and old pipe network (copper).

In Hastings and Dunedin (Tahuna), treated wastewater contacts rocks for Maori cultural purposes before discharge into the marine environment. New Zealand also has numerous outfalls that discharge into freshwater. The two largest municipal outfalls are Hamilton with a full width multiport diffuser into the Waikato River and Palmerston North with a bankside rock wall discharge into the Manawatu River (Bradley 2016).

Table 3: Major constituents of typical domestic wastewater

Constituent	Strong mg/l	Medium mg/l	Weak mg/l
Total solids	1200	700	350
Dissolved solids (TDS)	850	500	250
Suspended solids	350	200	100
Nitrogen (as N)	85	40	20
Phosphorus (as P)	20	10	6
Chloride	100	50	30
Alkalinity (as $CaCO_3$)	200	100	50
Grease	150	100	50
BOD^5	300	200	100

Modified from Henze and Comeau (2008)

Table 4: Elemental contaminants and concentration thresholds in dry weight of sewage sludge.

Metals (mg/kg)	Strong	Medium	Weak
Arsenic	30	27	20
Cadmium	32	5	3
Chromium	600	500	250
Lead	500	420	150
Mercury	19	15	1
Nickel	300	125	60
Zin	3500	700	200

Modified from Ogilvie (1998)

The Dunedin City Council owns and manages seven separate wastewater treatment plants of varying waste treatment methods. The Middlemarch, Waikouaiti/Karitane and Warrington wastewater treatment facilities utilise the oxidation pond (stabilization pond technology), the effluent obtained is used for land irrigation. The Seacliff wastewater treatment facility uses a rapid sand filtration method, and the resultant effluent derived is used for land irrigation. The volume of wastewater effluent discharged by four of the wastewater treatment facilities is nearly 187,300 m^3. The Mosgiel and Port Chalmers wastewater treatment facilities send their wastewater effluent to the Green Island Wastewater Treatment Plant (GIWWTP) and the Tahuna Wastewater Treatment Plant (TWWTP) respectively for secondary and tertiary treatment (Bouman and Archer 2014). The Tahuna Wastewater Treatment Plant (TWWTP) and the Green Island Wastewater Treatment Plant (GIWWTP) are the two major wastewater treatment plants in Dunedin that release treated wastewater effluents through an ocean outfall. The discharged wastewater is in treated solids and liquid form (Figure 6).

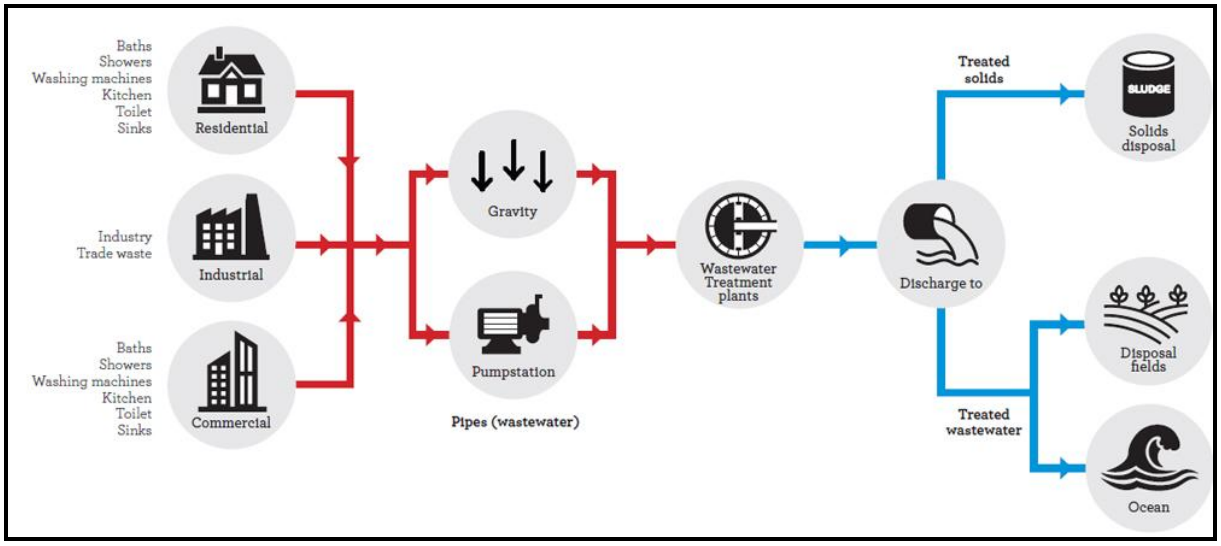


Figure 6: The wastewater treatment scheme employed by Dunedin City Council involving the collection of wastewater from residential, industrial and commercial facilities via pipes to the wastewater treatment plants for processing into treated less toxic solids and liquids that are discharged into disposal fields and ocean outfalls. (http://www.dunedin.govt.nz)

Since the 1950s, Dunedin Water Pollution Control Plant (now called Tahuna Wastewater Treatment Plant) discharged raw sewage directly into the Pacific Ocean at Lawyers Head. Presently, the Green Island Wastewater Plant and Tahanu Wastewater Treatment Plant (TWWTP) serving over 120,000 people in Dunedin, New Zealand, discharge adequately treated wastewater effluent from Waldronville and Lawyers Head, respectively into the Pacific Ocean from two ocean outfalls pipes extended from 550 to 1100 m in 2009. Between 2010 and 2013, the wastewater treatment plant was upgraded to handle both primary and secondary wastewater treatment processes. The upgrade, aimed at improving the flow capacity of the plant and the introduction of Biological Trickling Filters and UV disinfection as oppose to the previously used chlorination method of disinfection to ensure secondary wastewater treatment. The advancement was desirable to protect public health and improve nearshore water quality. The Tahanu Wastewater Treatment Plant (TWWTP), the second largest wastewater treatment plant in the South Island serves over 120,000 people and has a peak wet weather flow of 4000 litres per second turning out 18,993,000 m^3 of wastewater effluent discharges annually at Lawyers Head. The Green Island Wastewater Treatment Plant (GIWWTP) facility provides secondary treatment and UV disinfection for wastewater effluent from Mosgiel and Green Island. The GIWWTP discharges 2,447,000 m^3 wastewater effluent discharges annually at Waldronville (Figure 7)

Figure 7: Otago Coastline (Dunedin, New Zealand) highlighting the position of the two ocean outfalls (drawn with Google Earth, 2018).

1.4.1 Monitoring of Otago's Coastline

In 2009, before the extension of the ocean outfall pipe from 550 m to 1100 m long at the Lawyers Head, wastewater effluent discharge through ocean outfall at Lawyers Head had negative health and environmental effect on the water quality of the nearshore ecosystem along Otago coastline. In Dunedin, a large portion of the coastline was contaminated by the sewage effluent due to the geographical conditions and coastal position of the ocean outfall for the sewage effluent discharge. The water mass structure near and offshore the Otago coast especially the Southland Current is a northward flow of water along the south-east coast of New Zealand (Heath 1972; Sutton 2003) which intrudes on the sub-Antarctic surface water, and the neritic waters have a profound effect on the movement and mixing of the sewage effluent discharged. Consequently, the deposition of sewage effluent coupled with the Otago shoreline also been greatly influenced by runoff from farmlands and river levels raised a concern on the water quality of some portions of the Otago shoreline (Selvarajah 2008).

In November 1998 and October 2000, Ryder (2000) carried out bacteriological studies on shellfish (blue mussels) and seawater samples. An elevated level of faecal coliforms (FC) at various sites along the Otago coastline was noted and was ascribed to contamination from wastewater effluent from the Tahuna Wastewater Treatment Plant (TWWTP) discharged through Lawyers Head and that of the Green Island Wastewater Treatment Plant (GIWWTP) wastewater discharged through Waldronville. The observed high level of faecal coliform (FC) exceeded the stipulated benchmark set by both the Ministries of Health and Environment. Portions of the coastal beaches along Otago coastlines were considered not suitable for recreational activities and gathering of shellfish.

The Otago Regional Council relies on Resource Consent Monitoring as stipulated by the Resource Management Act (1991) for an insight into the state of Otago's coastal water quality to ascertain whether the coastlines are suitable for recreational activities and collection of seafood (Council 2004). Mussels and seawater are collected from fifteen sites extending from Akatore to Victory Beaches on a weekly basis. They are tested for microbiological (i.e. faecal coliforms (FC) (*Enterococci spp*. for marine and *Escherichia coli* for freshwater) and trace metal contamination (Council 2005). A whole effluent toxicity testing (WETT) is also carried out annually by the National Institute of Water and Atmospheric Research (NIWA) to assess the water quality of the east and west end of Green Island outfall, the Lawyers Head and beaches along the Otago shoreline.

Based on the results obtained, warning signs are displayed (Figure 8) at strategic locations near the beaches to alert the public on the prevailing water quality of the beaches in accordance with the microbiological water quality guidelines for marine and freshwater recreational areas by Ministry for the Environment.

Figure 8: Warning sign for the public on the dangers of swimming and collecting seafood due to sewage contamination at Tomahawk Beach. (Photo was taken during sampling exercise at the site).

The Surveillance/Green Mode indicates the water is safe for recreational activities. The Alert/Amber Mode indicates possible water contamination and source of contamination needs verification. The Action/ Red Mode indicates the water is unsafe for reactional activities and closed to the public. The natural occurrence of algal blooms when wind and ocean currents are favourable at the nearshore marine coastal waters was another human health and environmental concern (MacKenzie 2014). Toxic blooms led to the extensive closures of North Island and South Island west coast shellfish area in the summer of 2000 and 2001. It has been a major environmental issue hence the commencement of a nationwide shellfish biotoxin monitoring programme in 1993 (Trusewich et al. 1996). The attributed possible causes are slow water circulation, unusually high water temperatures and high nutrient runoffs (mainly phosphorus, nitrogen and carbon) from water catchment areas due to human activities. Blooms may occur after extreme weather events such as cyclones, floods, or drought. The effect of such blooms includes; production of toxins (called biotoxins or phycotoxins) that can kill shellfish, marine mammals, birds, and humans via consumption of

contaminated with paralytic shellfish poisoning (PSP). The formation of large blooms (high concentrations of cells) may clog the gills of fish, cover beaches, and deplete oxygen in the water as they die and decompose and disrupt the nearshore marine food web by reducing the ability of herbivores to graze. Shellfish such as oysters, mussels, pipis, clams and cockles (mainly filter feeders) filter any harmful bacteria, viruses, toxins and chemicals from the seawater, accumulated in their flesh and gut.

The use of faecal coliform (FC) as a monitoring tool fails to identify the main and other possible sources but only attempt to give a snap shot for conditions at the time of testing. Apart from the sewage effluent, other sources of faecal coliforms could be attributed to faeces from marine mammals (such as seals and penguins) birds, farm runoff carrying faecal matter of agricultural animals into coastal marine areas. Therefore, there is the need to explore the use of a more reliable monitoring tool such as stable isotope analyses to assess the impact of organic matter, identify its source and contribution to the isotopic signatures of marine organisms, which make up the nearshore ecosystem food web structure.

Stable isotopic signatures of resident nearshore marine organisms will offer a time-integrated measurement of assimilated carbon and nitrogen rather than an instantaneous measurement of ingested materials. In general, human sewage (4 major and about 8 minor outfalls), storm water, and agricultural pollutants such as fertilisers, and animal wastes mostly affect Otago's coastal seas. Industrial wastes may have a considerable impact on a small scale, but there are only a few sources. There is potential for hydrocarbon spills in port areas and on wharves with diesel pumps (Council 2001).

1.4.2 The Rationale for Stable Isotopic Study of Otago's Coastline

The main human health and environmental concerns of the long-term wastewater disposal on nearshore marine waters of Otago's coastline are the:

- Accumulation and transfer of metals and xenobiotic compounds in the coastal marine food webs.
- Toxic effects of contaminants on the survival and reproduction of marine organisms.
- Uptake and accumulation of microbial pathogens in commercially harvested species destined for human consumption

- Discharge of degradable organic matter (mainly carbonaceous organic materials) and nutrients to the nearshore coastal waters resulting in localised eutrophication and organic enrichment.

Therefore, a short- and long-term monitoring scheme of biotic communities is essential for decision makers to devise sound solutions to acute and insidious nearshore coastal waters pollution. Such monitoring scheme can assist in the management of nearshore marine amenity, exploitation of natural nearshore marine resources; identify sites for conservation priority and basic scientific research. Van Dover et al. (1992), using the stable isotope ratios of carbon, nitrogen and sulphur as tracers of sewage-derived organic material showed that sewage organic matter could reach the deep sea floor and enter the benthic food web, specifically via surface-deposit feeding organisms. Once sewage organic matter route into the marine food web is through absorption of dissolved inorganic nutrients by primary producers such as micro- and macro-algae consumed by filter or suspension feeders (Tucker et al. 1999).

Carbon and nitrogen isotopic ratios are of interest as isotopic tracers because they are the major components of dietary material. In order to trace sewage isotopically in the coastal waters, the sewage endmember must have a characteristic isotopic ratio. The greater the differences among the carbon and nitrogen isotopic ratios for the sewage endmember and other sources of organic matter, the more accurate the process of tracing and identifying sewage matter in species diet. The overlap in the isotope values of endmembers from diffuse contamination through other sources and nutrient transformation processes (i.e. nitrification and denitrification) described in Kendall et al. (2015) (see Figure 9) can be resolved by the use of complex isotopic models and independent chemical tracers.

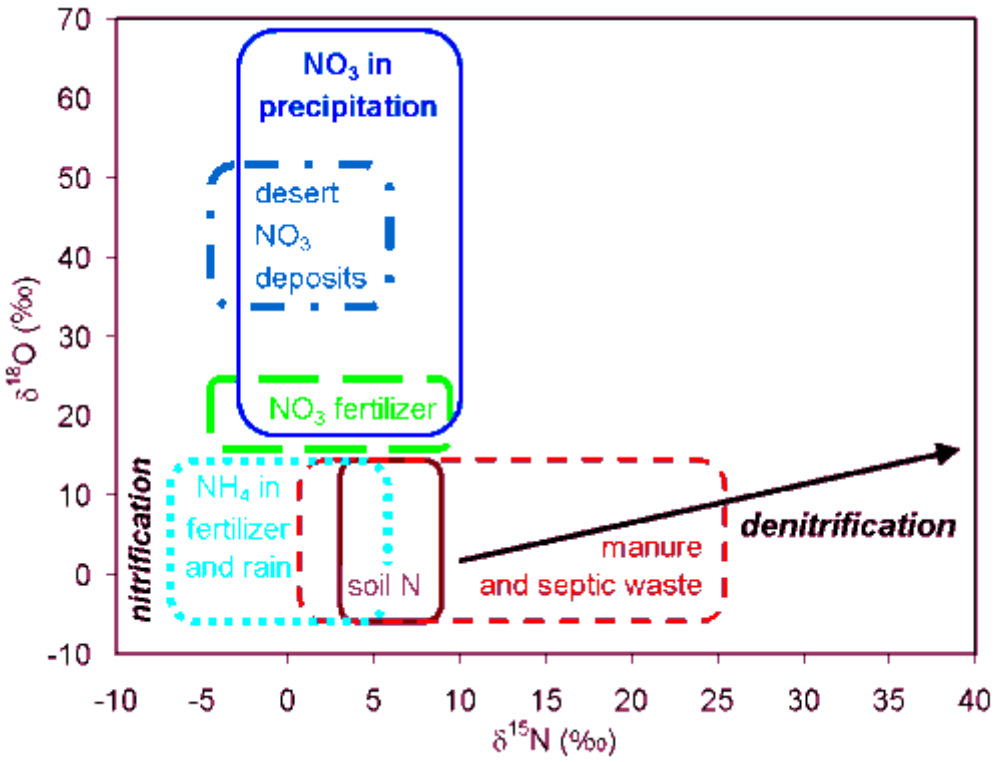


Figure 9: Overlap of the dual isotopic ratio for nitrate in the samples collected for testing hypotheses about sources of nutrients and algae, and biogeochemical processes in a section of the San Francisco Estuary (Kendall et al. 2007).

A pilot study into the feasibility of using carbon and nitrogen stable isotope analysis on indicator organisms (*Mytilus galloprovincialis* and *Ulva lactuca*) was carried out. This was done to identify sewage derived organic matter entry into the coastal marine food web. Ryder Consulting Limited conducted this pilot study in 1999 for the Dunedin City Council (Ryder 2000). The results obtained looked promising with a general enrichment observed in *Mytilus galloprovincialis*, and *Ulva lactuca* collected at the sites near the Lawyers Head outfall site. There was an abnormality in that no uptake of sewage derived organic matter was observed for mussels collected at Lawyers Head. Because the ocean is a charged electrolyte solution, the rapid entry of the dispersed sewage effluent into the sea may cause the dissolved organic matter to form a colloidal fraction of the sewage effluent that does not readily settle out (Buswell et al. 1928; Johnson et al. 2014; Schrader et al. 2005). The carbon and nitrogen species may turn out to be insoluble on entry to the ocean and then be removed from colloidal suspension through aggregation with distance.

The sea lettuce (*Ulva lactuca*) assimilated dissolved nitrogen and carbon and found to indicate the presence of sewage derived organic matter despite the possibility of the

formation of colloids in the coastal waters (Ryder 2000). Horn (2001) also conducted an isotopic monitoring study on seawater samples, the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* across various beaches along the Otago Peninsula to trace the pattern and distribution of sewage effluent dis-charged from Lawyers Head. Horn observed that the nitrogen isotopic ratios of the digestive tissues of the end member mussels vary considerably. He reported that major sewage contamination occurred at Lawyers Head and Tomahawk Beach while there was minor contamination at St. Kilda and Smaills Beaches. He found out that more than 60 % of the mussels and seaweeds sampled at Lawyers Head and Tomahawk Beach had their isotopic ratios were affected by discharged sewage effluent.

Since Ryder (2000) and Horn (2001), there has been no stable isotopic studies to assess the impact of the modifications in the sewage treatment and disposal on the nearshore marine waters and resident biota along the Otago Peninsula had been carried out. The variability of the carbon and nitrogen ratios in the suspended particulate organic matter (phytoplankton biomass), tissues of *Ulva lactuca* (producer) and *Mytilus galloprovincialis* (consumer) are useful indicators for tracing organic matter sources, elucidating nutrient enhancement and contaminant dynamics of the nearshore waters of Otago Peninsula. *Mytilus galloprovincialis*, a filter feeder consumes and metabolises phytoplankton (primary producer) in its tissues while the *Ulva lactuca* (microalgae) as well as the phytoplankton biomass in the particulate load growth limited by nutrients such as nitrogenous compounds (nitrates, nitrites and ammonium).

Therefore, they were selected as indicator organisms for temporal and spatial ecosystem monitoring studies of nutrients and contaminants in the nearshore coastal waters. This research applied isotopic ratios and other independent analytical techniques on sentinel organisms to examine the status of the organic matter in the nearshore marine waters along the Otago Peninsula.

Previous work gave strong indications of sewage derived organic matter impact from the municipal wastewater treatment plant, they fail to account for the possibility of sewage derived organic matter from farmlands and other nutrient and contaminant sources of ecological interest in the nearshore marine waters. Hence, this study tends to examine the influence of the improvement in the municipal wastewater treatment plant on the nearshore marine waters and other possible fluxes of nutrients and contaminants of ecological interest.

1.5.0 Aims and Objectives of the Research

The aims and objectives of this thesis are to:

(1) Evaluate the possibility of using the differences in stable isotope compositions of carbon and nitrogen in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* as indicators of organic matter sources as nutrient and contaminants in the coastal marine waters.

(2) Examine the impact of discharged of sewage-derived organic materials on the nearshore marine waters by way of using the resident marine flora (*Ulva lactuca*) and fauna (*Mytilus galloprovincialis*) as indicator organisms for exploring the nutrient supply and contaminant fluxes in the coastal marine waters.

(3) Appraise the outcome of the advancement in the municipal waste treatment plant and disposal process (extension of sewage outfall) on the sewage derived organic matter deposited in the nearshore waters and preserved in the coastal marine flora (*Ulva lactuca*) and fauna (*Mytilus galloprovincialis*).

(4) Set up isotope mixing mass balance models on measured bulk stable isotope compositions of carbon and nitrogen suspended particulate organic matter in water and land based materials to quantify the contributions of organic materials and infer change in organic matter sources in the resident fauna (*Mytilus galloprovincialis*) and probably in the coastal marine waters.

(5) Quantify the biochemical and elemental compositions (as chemical tracers) in resident organisms as independent assessors to infer and ascertain the changes in the organic matter sources (nutrient supply) and contaminant dynamics in the nearshore marine waters.

(6) Assess the potential human health risks associated with the consumption of the coastal marine fisheries (i.e. *Mytilus galloprovincialis*) collected from the Otago coast.

CHAPTER 2

Materials and Analytical Techniques

Overview

This chapter focuses on the description of the study sites, sampling protocols and methodology used for the study. Emphasis was placed on the perfection of instrumentation, method development and validation of the analytical techniques employed to quantify the various key biochemical tracers as indicators of nutrient enrichment and contamination in the biological samples collected from the study sites. A conceptual background and justification of the various statistical approaches and stable isotope mass balance models used to elucidate the major biogeochemical processes in the subsequent chapters are discussed herein.

2.1 Otago's Coastline

The Otago Coastal Marine Area extends from the line of mean high water to the limits of the territorial sea at 12 nautical miles (22.2 kilometres), from the Waitaki River in the north to Wallace Beach in the south (Figure 10). There are more than 80 protected areas along the landward edge of Otago's coastline, ranging from scenic, recreational, and historical reserves to wildlife and bird sanctuaries.

Most of these fall under the jurisdiction of the Department of Conservation with the rest managed by territorial authorities. The Otago coastline waters are affected by water masses of different salinities, temperatures and different densities, which tends to inhibit mixing. Roughly, 20-30 kilometres offshore of Otago, the warm and salty Southland Current intrudes on the "normal" cool and less saline Sub-Antarctic surface water. Nearshore coastal water is extremely variable and strongly influenced by runoff and river levels. The boundary between warm subtropical water and cool Sub-Antarctic water (the Subtropical Convergence) is a large-scale feature of the Southern Ocean (Heath 1972).

The Otago coastal waters of the South Island is one of the very few places in the world where this global front approaches land. The longshore drift, tidal flow, large-scale currents, overriding wave pattern and winds move water northeast along the Otago coast. Sediment transport in a northeasterly direction is a direct result of these forces. There are a few local southerly eddies, but the net transfer of water and sediment in Otago is to the northeast. Next to the land, there are the intertidal areas such as estuaries, wetlands, beaches and rocky shores. These are important feeding and breeding habitats for seabirds and marine mammals.

Pollution at detectable levels (and therefore subject to study) in New Zealand tends to be concentrated in estuaries or near cities. Human sewage (from 4 major and about 8 minor outfalls), storm water, and agricultural pollutants such as fertilisers, and animal wastes mostly affect Otago's coastline. Industrial wastes might have a considerable impact on a small scale, but there are relatively few sources. There is potential for hydrocarbon spills around the port areas and on wharves with diesel pumps. The coastal environment is a very important place where the people of Otago can pursue various recreational activities. The coast is used for many active recreational pursuits such as yachting and rowing. The coast also has significant amenity value to the people actively using the coast and to those people who choose to live in locations from where they can observe the coast.

Figure 10: Otago's Coastal Marine Area **Source: Council (2001)**

2.2. Sampling

2.2.1 Sample Collection

Mytilus galloprovincialis (Mediterranean mussel) and *Ulva lactuca* (sea lettuce) were collected by hand from the intertidal rocks during low tide over a period of 5 weeks (between 9/12/15 and 13/1/2016). They were collected during summer for Chapter 3 and 4 studies; monthly covering one full season cycle (between 14/7/16 to 29/06/17) for Chapter 5 and 6 studies. The bivalves (an average of 6 per 10 sites) collected from the rocks at the intertidal zones were of average uniform total weight (approximately 30 ± 7 g) to minimise within-site and site-site isotopic biased-variability. The bivalves and seaweeds were placed separately in individual clean ziplock plastic bags labelled with date and sample site location. They were immediately placed in a plastic cooler and taken to the laboratory where they were rinsed in distilled water and frozen (-18 °C) prior to analysis. Water samples collected from nearshore marine waters and their proximate water bodies were filtered in the laboratory on pre-combusted (500 °C, 4 h) 25mm diameter Whatman GF/F glass fibre filters under moderate vacuum within a few hours of collection for suspended particulate organic matter. Loaded filters were dried (50 °C, 48 h) and stored in under vacuum overnight. Grab measures of nutrient source organic materials such as sewage effluent, sea lion faeces, farm manure, compost, sheep faeces, and detrital matter (made up of decayed seagrasses, plant litter, animal faecal matter and sediments) collected within and immediate environment of the study sites were separately put in clean ziplock plastic bags labelled with date and sample site location.

2.2.2 Physical and Chemical Parameters

Physical and chemical parameters were measured in situ at the point of sampling using the YSI Professional 30 instrument (Pro 30) probe. The temperature was measured using the YSI Pro 30 calibrated against a mercury thermometer held in an ice-water bath in an open container to achieve a reference temperature of 0°C. Conductivity and special conductance were measured after the probe had been standardised compared to 0.5, 0.1 and 0.01 M standard potassium chloride solutions. Hydrogen ion concentration (pH) was measured with probe and glass combination electrode calibrated with standard buffer solutions at pH 4 and 7. Salinity was measured after the salinity refractometer probe had been injected into NIST (National Institute of Standards and Technology) reference fluid for proper standardisation. The dissolved oxygen probe was standardised against a zero oxygen standard solution (5 %

sodium sulphite solution) prepared by dissolving 1 gram of sodium sulphite (Na_2S0_3) and 1 mg crystals of cobalt chloride ($CoCl_2$) in 1 litre of deionised water.

2.2.3 Sample Preparation

M. galloprovincialis was dissected into different tissues (the abductor and digestive tissues of interest in this study). The dissected tissues and samples of *U. lactuca* were dried at 70 °C for 24 h. Once dry samples were homogenised with the aid of an MM400 bench-top Retsch ball mill, duplicate aliquots of 0.8 mg of homogenised tissues of the biological samples were weighed into separate 5 × 3.5 mm tin cups, and further dried under vacuum overnight. Solid samples such as seal lion faeces, farm manure, compost, sheep faeces, and detrital matter were also homogenised and weighed into separate 5 × 3.5 mm tin cups and further dried under vacuum overnight.

2.3 Isotope Ratio Analysis

2.3.1 Determination of Carbon and Nitrogen Isotopic Ratios in Samples by Elemental Analyser-Isotope Ratio Mass Spectrometry (EA-IRMS)

Duplicate aliquots of 0.8 mg of homogenized samples and the filter papers from suspended particulate organic matter in the water samples were packed in 5 x 3.5 mm and 12 x 6 mm tin capsules respectively. They were left to dry under vacuum overnight.

The samples with internal and certified pre-calibrated standards and blanks were fixed in an automatic carousel and combusted sequentially using a Carlo-Erba NA-1500 elemental analyser-isotope ratio mass spectrometry (EA-IRMS) (see Figure 11) for the determination of the carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$). The flash combustion is quantitative; no isotopic fractionation is involved.

Nitrogen and carbon isotopes were assayed by combustion of the samples, to produce N_2 and CO_2 (as reference gases), using helium (as a carrier gas). The pulse of oxygen is for the combustion. These gases are separated on a packed molecular sieve GC column and sent sequentially to the inlet of the Europa Scientific continuous flow mode “20/20 Hydra” (Europa Scientific, UK) isotope ratio mass spectrometer (CF-IRMS) which is connected to the Sercon System Controller which operates under Sercon Callisto Software. During the programmed run tests, individual samples are combusted at 1050 °C and any resulting nitrogen oxides reduced at 650 °C. Grade 5 Helium carries the gaseous reaction products at 75 ml/min.

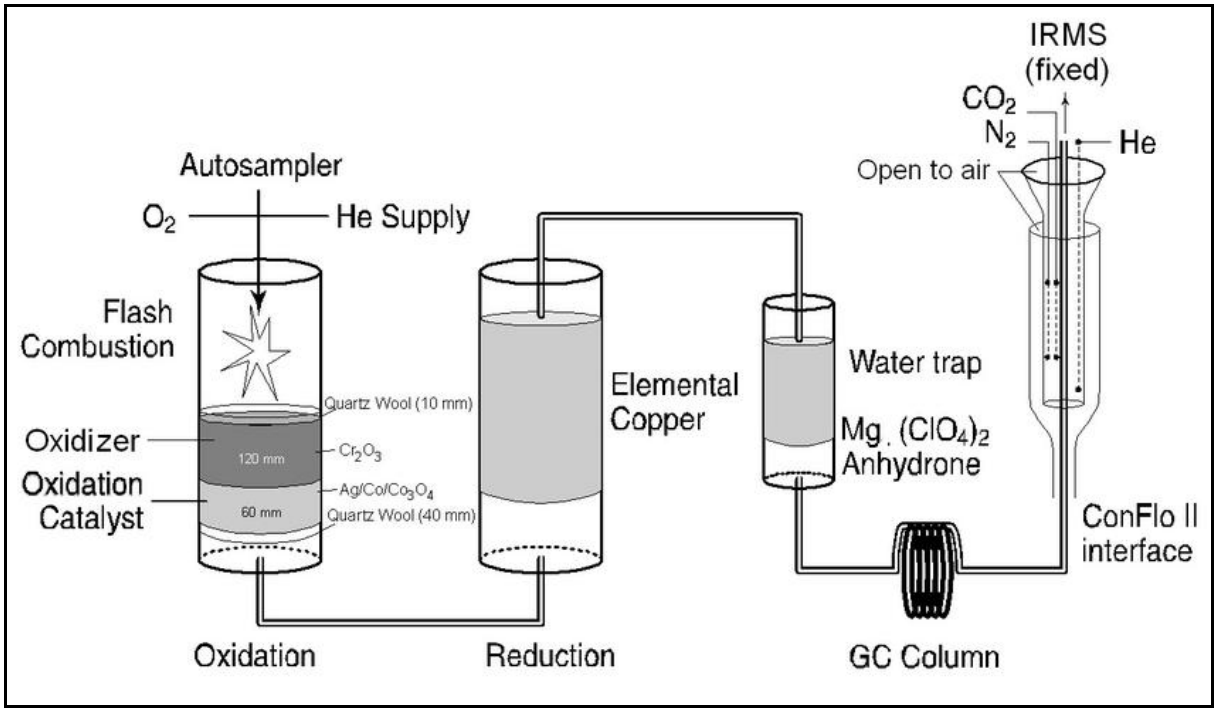


Figure 11 : Diagram of the Carlo Erba elemental analyser.

(Source: https://isotopes.usgs.gov/lab/services/RSIL_SOP_1832.pdf)

The flow of the working gases, temperature and pressure were closely monitored to avoid isotopic shifts. The Europa Scientific 20-20 ratio mass spectrometry has a universal triple collector, two wide cups with a narrow cup in the middle; it is capable of measuring mass/charge (m/z) 28, 29, 30 or with a magnet current change 44, 45, 46, simultaneously. The ion beams from these m/z values are as follows: m/z 28 = N_2 = $^{14}N^{14}N$; m/z 29 = N_2 = $^{14}N^{15}N$ primarily; m/z 30 = NO = $^{14}N^{16}O$ primarily, high levels of the latter is a sign of contamination or incomplete reduction; m/z 44 = CO_2 = $^{12}C^{16}O^{16}O$; m/z 45 = CO_2 = $^{13}C^{16}O^{16}O$ primarily; and m/z 46 = CO_2 = $^{12}C^{16}O^{18}O$ primarily.

The raw isotopic ratios obtained were normalised by three-point calibration to the international scales using two internationally accepted reference materials (USGS-40 and USGS-41) and internal laboratory standard (ethylenediaminetetra-acetic acid (EDTA-OAS) (see Figure 5 for their respective values) of known carbon and nitrogen isotopic signatures, assayed with the unknown samples. A linearity correction method was applied to ensure that the isotopic values obtained were not affected by the sample size. Accuracy and precision of the obtained isotopic results were assessed as described in IANZ (2004) and random

inclusion of two in-house standards (green mussel and copepod) to mimic the nature of the sample materials being analysed.

The stable isotope ratios of C and N expressed in δ notation were obtained (see Equation 1) and measured in parts per thousand/per mil (‰) against international standard reference materials (i.e. $\delta^{13}C$ and $\delta^{15}N$ were referenced to Pee Dee Belemnite (PDB) limestone carbonate and atmospheric N_2 respectively).

2.3.2 Accuracy and Precision

Accuracy and precision check of the measured isotopic results were ensured by employing the Root Mean Square Error (RMSE) model in Excel 2009. The differences between sequential duplicates of every 10^{th} measured sample was used expected as the "standard" based on IANZ (2004). The accepted RMSE values are ± 0.1 ‰ for $\delta^{13}C$ and ± 0.2 ‰ for $\delta^{15}N$.

2.3.3 Quality Assurance

Raw isotopic ratios obtained were normalised by three-point calibration to the international scales using two International Atomic Energy Agency reference materials (USGS-40 and USGS-41) and internal laboratory standard (ethylenediaminetetra-acetic acid [EDTA-OAS]) of known carbon and nitrogen isotopic signatures, assayed with the unknown samples. There was the random inclusion of two in-house standards (green mussel and copepod) to mimic the nature of the sample materials being analysed. The accepted mean of the international accepted reference materials with standard deviations is represented in Table 5.

Table 5: Reference materials, their certified values with uncertainties

Reference material	$\delta^{15}N_{AIR}$(‰)	$\delta^{13}C_{VPDB}$ (‰)
USGS-40	-4.52 ± 0.06	-26.24 ± 0.042
USGS-41	47.57 ± 0.1	37.76 ± 0.049
EDTA-OAS	-0.73 ± 0.12	-38.93 ± 0.20

2.3.4 Quality Control and Assurance

2.3.5 Blank Corrections in the Samples

The correction of the effects of blanks in the isotopic ratios of the measured samples was carried out to lessen the influence of sample contamination during sample preparation via

contributions from the blanks (i.e. tin cups used for packing samples, N_2 in the oxygen used for combustion). The isotopic ratio of the sample during the measurement is assumed as the summation of the sample and the blank. To resolve for the blank isotopic ratio contributions in the samples, a modified mathematical model based on Hayes (2004) was adopted as explained in Equation 5&6:

Using Σ to represent the sample prepared for mass spectroscopic analysis while x and b represent the sample and blank respectively,

Therefore,

$$m_\Sigma \delta_\Sigma = m_x \delta_x + m_b \delta_b \text{.....Equation 5}$$

Substituting mx = mΣ - mb and rearranging yields, the resultant equation is as follows;

$$\delta_\Sigma = \delta_x - m_b (\delta_x - \delta_b)/m_\Sigma \text{.....Equation 6}$$

An equation similar to y = a + bx was obtained.

Therefore, by plotting δ_Σ vs. $1/m_\Sigma$ yield of the accurate value of the isotopic ratios of the sample (i.e. blank-corrected value of the sample) (δ_x) was the intercept.

2.4 Nutrient and Chlorophyll a Concentration Analyses

2.4.1 Determination of Nitrates and Nitrites

Nitrate and nitrites were sequentially measured using a slightly modified spectrophotometric method described in (García-Robledo et al. 2014). The critical step for the accurate determination of nitrates in the water samples, the reduction of nitrate to nitrite. This was achieved by means of a 2 % w/v of vanadium III chloride solution prepared in 6 N HCl solution in the presence of a mixed reagent of sulphanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) solutions in equal proportions.

Sulphanilamide solution was prepared by dissolving 5.0 g of sulphanilamide in 50 ml of concentrated (12M) hydrochloric acid (HCl) diluted in about 300 ml of deionised water and after cooling made up to 500 ml with deionised water. N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) reagent was prepared by dissolving 0.5 g NED in 500 ml of deionised water (MilliQ). Nitrate stock solution (10 mM) was prepared by dissolving 1.011 g of KNO_3 (oven dried at 100 °C for 1 h) in 1liter of deionised water.

Nitrite solution (10 mM) was prepared by dissolving 0.690 g $NaNO_2$ in 1liter of deionised water. Reagent blank solutions were prepared using deionised water. The nitrate and nitrite solutions were made to 25 µM-standard solutions by adding 250 µl stock solution in a separate 100 ml Pyrex volumetric flasks.

Duplicate 8 ml of water samples and working serial dilutions (ranging from 0.1 to 25 µM) from nitrite standard solutions nitrite into individual 10 ml volumetric flasks. 4 ml of the 8 ml water samples containing each of the different working standard solutions in separate volumetric flasks, 400 µl of mixed reagent was added and shaken properly. The mixture was incubated at 25 °C for 20 min in the oven, transferred into a 1cm cuvette. The absorbance of the blank reagent (deionised water), resulting pink coloured solutions of the water samples and working standard solutions were measured at 540 nm via Novaspec II spectrophotometer.

800 µl of vanadium chloride reagent was then added to the remaining 4 ml of the mixture solution. The mixture was incubated at 60 °C for 25 minutes in a water bath. The mixture was then cooled to room temperature in a cold-water bath. The reagent blank and absorbance of the resulting individual pink coloured solutions were measured at 540 nm.

The nitrate and nitrate concentrations in the water samples were calculated using the derived linear regression equation from the standard curve from the parallel analysis of the sets of nitrite and nitrate working standards.

2.4.2 Dissolved Reactive Phosphate Measurement

Dissolved phosphate concentrations in the water samples were quantified as described in (Strickland and Parsons 1968). Mixed reagent A was prepared by mixing 100 ml of 3 % w/v ammonium molybdate solution with 250 ml of 2.4 M sulphuric acid solution.

Mixed reagent B was prepared by mixing 100 ml of 3 % w/v ascorbic acid solution and 50 ml potassium antimony tartrate solution. Mixed reagents A and B were freshly prepared daily. 10 mM of potassium phosphate stock solution was prepared in a dry, dark glass bottle hitherto washed in phosphate-free detergent, rinsed in 10 % HCl followed by further cleaning in deionized water to avoid phosphate contamination.

The mixed reagent was stored in the refrigerator up until use. 25 µM potassium phosphate working stock solution was prepared in a Pyrex 100ml volumetric flask from the stock solution. Working standard solutions of potassium phosphate ranging from 0 to 10 µM were then prepared in 10ml volumetric flasks. 10 ml of an aliquot of the water sample and working

standards were placed in separate 15 ml volumetric flasks, and 1ml of mixed reagent A and B was added.

The mixture was swirled and allowed 30 minutes for blue colour development, the absorbance of the blue molybdenum complex formed was measured at 880 nm in a 1cm-cylindrical cell using a Shimadzu model 2600 spectrophotometer. Deionised water was used as the reagent blank.

The dissolved phosphate concentrations in the water samples were determined mathematically via linear regression equation derived from the standard curve of the parallel analysis of sets of prepared phosphate working standards.

2.4.3 Chlorophyll a Pigmentation in the Water Samples

500 ml water samples were collected were filtered through separate Whatman GF/F glass fibre with a thin layer of $MgCO_3$ laid on. The wet filter was immediately ground up. After 1 hr of eluting in 10 ml of 90 % v/v acetone the sample was centrifuged to remove all solids from suspension, the resulting solution was decanted in a test tube and placed in a Turner10-AU fluorimeter for fluorescence measurement.

Two drops of 1.2 M hydrochloric acid was later added to the sample, and the fluorescence measurement was repeated. The concentration of chlorophyll-a was obtained from the Equation in 7, where F_o and F_A are the fluorescence before and after acid addition, v is the volume of acetone used for extraction and V is the sample volume. The values of 2.0830 and 1.0769 were the intercept and slope respectively, obtained from the routine instrumental calibration curve obtained with chlorophyll a standard (*Anacystis nidulans*) purchased from Sigma-Aldrich.

$$\text{Chlorophyll a concentration (ug/l)} = 2.0830 * 1.0769 * (F_O - F_A) * v / V \quad \text{.....Equation 7}$$

2.5 Biochemical Tracer Analysis

2.5.1 Faecal Sterol and Fatty Acid

Faecal sterol analysis in the gut of *Mytilus galloprovincialis* was conducted using a modified method as described in Chou and Liu (2004) and Nimz and Morgan (1993). The method comprised extraction of total lipids in the tissue, removal of the solvent, saponification of lipids, extraction of non-saponifiable materials with an organic solvent, and derivatisation

preceding to analysis. Seven (four from Smaills Beach and three from Allans Beach) marine bivalves were analysed for faecal sterols.

2.5.2 Chemical Reagents

All the chemicals and solvents used for analysis were of High-performance liquid chromatography (HPLC) grade with 99 % purity. Standard reference material (SRM) such as cholesterol, coprostanol, and 24-ethylcoprostanol (Coprostigmastanol) and cholestane (an internal standard) purchased from Merck KGaA, Germany (also known as Sigma-Aldrich). Cholestane was preferred internal standard because it is a sterol hydrocarbon, lacking the position 3 hydroxyl group typical for sterols.

A non-polar, saturated compound of similar chemical structure that elutes separately without interference within the GC with faecal sterols. Derivatising reagent made up 99 % N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% Trimethylchlorosilane (TMCS) and other solvents such as dichloromethane, benzene, n-hexane and methanol were also bought from Merck KGaA.

Anhydrous sodium sulphate and potassium hydroxide kept in a closed plastic container placed in a dissector containing drying agent and required quotas heated in an oven at 70 °C for 1 h prior to analysis to ensure organic-free condition.

2.5.3 Sterol and Fatty Acid Derivatization

5 g of the homogenised gut of the marine bivalve mixed with 0.5 g of anhydrous sodium sulphate and 5 µg of cholestane were Soxhlet extracted and saponified for 12 h in a 2:1 mixture 0.5 N methanolic KOH (15 % water) and benzene. After saponification, the sample was cooled to room temperature. Lipids were isolated by partitioning of the extracts by adding 10 ml of deionized water to regulate the mixture pH to 7, followed by further extraction with dichloromethane (3 × 20 ml). The volume of the dichloromethane extract sample reduced by means of a rotary evaporator. The rotatory evaporator flask was rinsed with hexane (3 × 2 ml), and the rinse solution collected together.

The rinsed mixture was transferred on top of a 1.0 cm i.d. glass column containing 2.5 g of 5 % deactivated alumina and 5.0 g activated silica gel for separation of sterols and fatty acids. The sterol and fatty acid fractions were eluted with 10 ml of dichloromethane/acetone (3:1). The sterol fraction was concentrated under a stream of dry nitrogen and made to 2 ml by adding dichloromethane just prior to GC analysis. Derivatization was performed with the

addition of 100 µl mix reagent made up of 99 % N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1 % Trimethylchlorosilane (TMCS) as described in Nimz and Morgan (1993).

Stock solutions of the sterols (coprostanol, cholesterol and 24-ethylcoprostanol) dissolved in dichloromethane at a concentration of 100 mg/l. The sterol standards diluted to concentrations ranging from 0.2 to 1.0 mg/l. These solutions were stored at - 4 °C prior to derivatization and eventual use as calibration standards.

2.5.4 Method Validation

Quality control (i.e. no evidence of contamination) and validation of the method used achieved with the use of deionized water subjected to ultraviolet treatment UV (to remove organic matter) as working water samples for each of the standard reference material (SRM). Blank extraction (i.e. dichloromethane) were performed routinely as feedback for contamination or interference with each of the faecal sterol standards.

2.5.5 GC-MS Instrumentation

Derivatized samples were analysed by an Agilent 5973N Mass Selective Detector (MSD) coupled with the 6890 Plus Series gas chromatograph (GC) system, equipped with an automatic sampler and a computer workstation. The injection port and GC-MS interface kept at 280 and 300 °C, respectively. Helium (He), the carrier gas was set to flow at the rate of 0.8 mL/min (average velocity = 59 cm/sec).

Selected-reaction monitoring scheme was performed using the EI mode (70 eV), at an anionization current of 150 Ma and nitrogen as the Q2 collision gas (1.5 ml/min) with 2.25 mL/min atomic He in the collision cell to reduce chemical noise from metastable He. The GC was fitted with a Zebron ZB-5MS Capillary GC Column (30m x 0.32mm x 0.25µm) purchased from Zebron phenomenex, USA. The GC oven temperature programme was set at 70 °C for 5 min; then ramped at 30 °C / min to 270 °C.

The MS ion source maintained at 280 °C and the quadrupoles at 150 °C. An aliquot of 1.0 µl of derivatized and silylated samples and standard reference material solutions introduced into the column in split mode. Data acquisition performed in the full scan mode (in the range m/z 35–550) to confirm the retention times of analytes and in selected ion monitoring (SIM) mode for quantification.

A dwell time of 100 ms selected, and the filament delay time was set as 8 min. Target sterols, standard reference material SRM transitions and their respective GC peaks and retention times observed and recorded. The resultant peak and the respective retention times for each of the standard reference material aided the confirmation of the known sterol configurations measured in the derivatized solution.

2.5.6 Unknown Organics

The identification of unknowns (compounds) achieved via the association of relative retention times with measured authentic standards on the Wiley (http://www.palisade.com) and National Institute of Standards and Technology (https://www.nist.gov) MS libraries with libraries of 390,000 and 129,000 spectra GC-MS measured organics. The Agilent 5973N MSD with control/data handled by Hewlett-Packard Enhanced Chem Station and Selected Ion Monitoring (SIM) of 30 atoms/molecules by 50 groups, with scanning rate (electronic) of 10 000 atomic mass units (amu), 1 scan per second performed the identification.

2.5.7 Limit of Detection and Quantification

The detection limit for each of the sterols was calculated based on the weight that gives a signal three times the peak-to-peak noise of the background signal while ten times was used to determine quantification limit as described in Uerpmann-Wittzack (2017) [91]. A linear regression model performed on the measured peak area ratios against the increasing weight ratios of sterols to internal standard to obtain the linearity of SIM responses and to plot calibration curves for the quantitative measurement of sterols.

The linearity of the SIM response used vary from 10 to 10,000 ng/l with coefficients of determination (R^2) of 0.90 ± 2. The relative standard deviations (RSDs) for the three faecal sterol standards were 15.3 %, 16.2 %, and 20.6 % for coprostanol, cholesterol and 24-ethylcoprostanol respectively, under triplicate experiments. Good limits of detection (LODs) (calculated S/N = 3 under SIM) in the range of 1.3 – 15 ng/l were achieved (Table 6). The recoveries were 89 %, 95 % and 97 % (spiking concentration: 100 ng/l) for coprostanol, cholesterol and 24-ethylcoprostanol respectively prepared under triplicate experiments.

Table 6: Recoveries, RSDs and limits of detection for the reference materials

Compound	Recovery (%)	RSD (%) n =3	LOD (ng/l)
Coprostanol	15.3	89	10
Cholesterol	16.2	95	1.3
24-ethylcoprostanol	20.6	97	3

2.6 Elemental Analysis

Trace metal concentrations in the whole tissues of *Mytilus galloprovincialis* were determined using the automated quadrupole Agilent 7900 Inductive Coupled Plasma-Mass Spectrometry (ICP-MS). The practical method used consists of digestion of the tissue and subsequent analysis by ICP-MS. For quality assurance and the avoidance of contamination, analytical chemical reagents and deionised water used were trace metal grades while all hardware such as polytetrafluoroethylene (PTFE) Teflon plastics and pipette tips were acid-washed for 24 hr in 50 % HCl and rinsed three times in Milli-Q® before drying in a laminar flow bench.

2.6.1 Digestion

The digestion of the tissue by microwave heating was performed according to (Ashoka et al. 2009). 0.2g of homogenate whole tissue of the mussel weighed into acid-cleaned tetra fluorine methoxil (TFM) digestion vessels in triplicates. Under a fume cupboard, 5 ml of 15 M quartz distilled nitric acid (65 % m/v) was added to each of the vessels and the samples allowed to sit for 45 minutes without heating for pre-digestion. 1 ml of ultra-clean hydrogen peroxide (30 % m/v) added subsequently to each of the sample solutions and again allowed to stand for 1 h to prevent excess venting.

The microwave vessel caps were secured firmly, placed in a MARS6 microwave oven reaction system. With the setting mode at animal tissue, Xpress digestion vessels, the temperature at 200 ^{o}C for 20 minutes, the samples allowed to digest in the microwave digestion unit. After digestion, the sample solutions allowed to cool for 30 minutes before transfer to clean Digitubes (50 ml) followed by drying down on the hot plate set 90 ^{o}C. The resulting dried residue was redissolved in 4.75 ml of 2 % HNO_3.

2.6.2 Quality Control and Assurance

Five blank samples of MQ water (0.2 ml) and 0.2 g of fish protein (DORM-4) certified reference material (CRM) (from the National Research Council, Canada) measured into digestion vessel. Blank, trial duplicate mussels and standard samples were digested at the same time as the samples to assess the average deviation of the elemental concentrations of the certified reference material for the trueness and precision of the method. The Relative Standard deviation in percentage (RSD %) of the blank and CRM samples was evaluated for the precision of the method calculated as stated in Equation 8. Table 7, Table 8 and Table 9 represent metal concentrations, standard deviations and RSDs, in the percentage recovery

(only in CRM) and detection limit of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb) in the blank, trial duplicates of mussel and CRM samples.

$$RSD\ (\%) = (SD/X) * 100 \ldots \text{Equation 8}$$

Where; RSD (%) = relative standard deviation in samples, SD = standard deviation (mg/kg) and X = mean (mg/kg)

Table 7: Mean, standard error and limit of detection of trace metal concentrations in blank samples

Metal	As (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)
Mean	0.12	0.01	0.02	< 0.01	< 0.01	0.01
SD	0.06	0.05	0.29	0.01	0.01	0.04
LOD	0.15	0.05	0.15	0.10	0.20	0.02

Table 8: Certified and observed mean values with standard deviation, recovery percentage, detection limit and relative standard deviations for each of the trace metal elements in the certified reference material

Metal	Certified value (mg/kg)	Observed value (mg/kg)	Recovery (%)	Detection limit (mg/kg)	SD	RSD (%)
As	6.87 ± 0.44	6.80 ± 0.15	99	0.15	0.15	2
Cd	0.30 ± 0.02	0.29 ± 0.01	97	0.05	0.29	2
Cr	1.87 ± 0.18	1.93 ± 0.08	103	0.15	0.08	4
Cu	15.7 ± 0.46	15.2 ± 0.29	97	0.10	0.29	2
Ni	1.34 ± 0.14	1.29 ± 0.09	99	0.20	0.09	7
Pb	0.40 ± 0.62	0.38 ± 0.01	94	0.20	0.13	3

Table 9: Observed mean values with standard deviation and relative standard deviations for each of the trace metal elements in the trial duplicate mussels

Variable	Metal Conc. (mg/kg)					
	As	Cd	Cr	Cu	Ni	Pb
Mean	8.18	0.97	0.94	4.82	4.33	1.00
SD	0.29	0.01	0.04	0.16	0.15	0.01
RSD (%)	5	2	5	5	5	1

2.6.3 Dilution

The digested samples were analysed for their trace metal content on the ICP-MS. In order to reduce the chance of exceeding the upper limits of detection of the instrument, 0.25 ml of each sample digest and blanks were diluted with 4.75 mL of 2 % v/v HNO_3. The final volume came up to 5 ml. the dilution factor equation is given in Equation 9. To address instrumental

drift and matrix effects, the samples were pre-spiked with 1 ml of 7 reference solution elements (^{9}Be, ^{45}Sc, ^{72}Ge, ^{103}Rh, ^{115}In, ^{159}Tb, ^{209}Bi). Calibration standards were prepared from a 10 mg/l stock solution of Agilent multi-element standard (NIST traceable) by means of serial dilutions. Calibration curves for the quantitative measurement of trace elements measured estimated graphically. Each analytical run contained three samples of DORM-4 fish protein certified reference material (CRM) for quality assurance purposes.

Dilution factor for each samples estimation was calculated using Equation 9:

Dilution factor = [25 ml / dry weight (g)]* 1000 (ml/g)…Equation 9

2.6.4 ICP-MS Instrumentation

The Agilent 7900 ICP-MS is equipped with standard nickel cones, micromist glass concentric nebulizer and ultra-high matrix introduction (UHMI) system that allows for the determination of samples containing up to 25 % of total dissolved solids through further dilution by the UHMI aerosol dilution technology. This ensures that less than 0.2 % TDS is introduced into the ICP torch.

The Agilent 7900 ICP-MS operates on an ICP-MS MassHunter software for automatic method acquisition. When aqueous samples are introduced into the ICP-MS, they are nebulised (converted to micro-mist), then channelled through a stream flow of argon gas (a rate between 13 to 18 litres per minute) in neutrally charged plasma (containing free ions) which dries, decomposes, vaporizes, atomizes and ionizes.

The ions passed into a mass spectrometer for separation based on their respective mass-to-charge ratio (m/z). The ion detector records the abundance of ions in the form of a spectrum where the magnitude of each peak is proportional to the concentration of the element been measured samples. During analysis, the octopole reaction system ORS4 operated in helium mode to reduce polyatomic interferences, improve detection limits for several elements and to eliminate the need for mathematical interference correction. The instrument settings are illustrated in Table 10.

Table 10: ICP-MS conditions used for trace metal analysis

Parameter	Setting
RF power (W)	1550
Plasma gas flow rate (L/min)	15
Auxiliary gas flow rate (L/min)	0.9
Carrier gas flow rate (L/min)	0.79
Makeup gas flow rate (L/min)	0.40
Nebuliser pump (rps)	2
Spray chamber temp (^{o}C)	-2
Torch injector internal diameter (mm)	2.5
Sample depth (mm)	9.0
Interface	Pt sampler cone, Ni skimmer cone
CeO^{+}/Ce^{+} (%)	0.5
Collision gas / flow rate(L/min)	He / 4.5
Octopole bias (V)	-20
Quadrupole bias(V)	-16
Internal standards	^{9}Be, ^{45}Sc, ^{72}Ge, ^{103}Rh, ^{115}In, ^{159}Tb, ^{209}Bi
Integration time varied over mass range (ms)	10 – 500 depending on expected concentration
Number of replicates per sample	3

2.7 Statistical Methodology

For the purpose of validating and interpreting the significance and correlation of the data collected, the data were subjected to various statistical analyses and tests. Apart from the conventional univariate analysis, which is perhaps the simplest form of descriptive statistical analysis (i.e. mean, range, standard deviations etc.); this thesis used other robust explanatory statistical models considered as bivariate and multivariate approaches. Some of these major approaches utilised for the interpretation of the data were such as linear regression, Pearson's correlation, analysis of variance (ANOVA), principal component analysis (PCA), Non-Metric Multidimensional Scaling (NMDS), Linear Bivariate Ordinary Least Squares (OLS), Kruskal-Wallis test and Kolmogorov-Smirnov Test (KS Test).

Statistical tests were performed using the Microsoft Excel 2010® software, PAST® software and IBM SPSS® software. The isotopic ratio data for the mixing models (Linear Mixed-Effect Model and MixSIAR) utilised in this thesis were processed using the R® Stats software Package. Outliers were detected using MATLAB® software.

2.7.1 Regression Analysis

A variable being examined can change linearly as a function of time, or it could be correlated to another variable. The rate of such change, m (the slope of the correlation), or the initial value of this correlation, c (intercept), may be important (Equation 10) which is described by the following equation:

$$y = mx + c \text{.....Equation 10}$$

Where;

y and x are dependent and independent variables respectively. The strength of the relationship between y and x is the called coefficient of determination (r^2). The coefficient of determination equal to unity or zero indicates maximum or null relationships respectively. The linear regression analysis was useful is the determination of the concentration of constituents (such as nutrients in water samples) in a mixture of analytes.

2.7.2 Pearson's Product Moment Correlation and Significance Test

To test for the significance of the correlation between two variables (p-values), a parametric correlation coefficient (i.e. Pearson's correlation) was used. The Pearson's product moment correlation (Pearson's correlation) was computed PAST® Palaeontological Statistics Software Version 3.2.1. The correlation coefficient (r) was calculated using Equation 11.

$$r^2 = (n\sum xy - \sum x. \sum y)^2 / (n\sum x^2 - (\sum x)^2 (n\sum y^2 - (\sum y)^2 \text{....Equation 11}$$

Calculation of the *p*-value was based on the hypothesis testing mechanism via comparing a calculated *p*-value (the probability of achieving the null hypothesis result given the by the data observed) with t-test statistics (set at 95 % significance with $\alpha = 0.05$) (Equation 12). The result was considered significant if the calculated p-value < α.

$$t = r * (\sqrt{n}-2)/ (\sqrt{1}-r^2) \text{.... Equation 12}$$

Where r is the correlation coefficient and n is the number of pairs of data

2.7.3 Root Mean Square (RMSE)

RMSE is the measure of the difference between predicted values in a model and the actual observed values. This is the average squared distance of a data point to a fitted line (Equation 13). RMSE is a good measure for accuracy and error check as it can detect the imperfection

of the fit of the estimator to the data. In this study, the measure of RMSE was used to determine how well the virtual model could predict the actual isotopic ratios in the samples measured.

$$RMSE = \sqrt{[1/n\ (\sum (F_i - O_i)^2]} \text{.....Equation 13}$$

Where F_i is the predicted values, O_i, is the observed isotopic ratio values and n, is the number of observations (samples).

2.7.4 Non-Metric Multidimensional Scaling (NMDS)

The NMDS was used to visualise the level of similarity of individual cases of a dataset. The NMDS analysis (see Chapter 3) was achieved using the rank orders (as distances) for ordination (non-metric) rather than Euclidean distances (metric) to categorise the different tissues of *Mytilus galloprovincialis* as to determine the mechanisms controlling differences in isotopic ratios in the different tissues. The Gower Similarity Coefficient Distance Index (GSCDI) with a stress value of 0.054 was considered for the NMDS analysis. The algorithm was processed via PAST® software attempts to place the data points in a two-dimensional coordinate system such that the ranked differences are preserved.

2.7.5 Linear Bivariate Ordinary Least Squares (OLS)

The OLS is a form of regression analysis, which assumes the *x* values are fixed, and finds the line that minimizes the squared errors in the *y* values of a dataset. This was used as an additional model to visualise the refinement in the groupings of the different tissues of the bivalve due to portioning of isotopes. The approach that forces the regression line through zero was applied for the confidence band. The details of this approach are discussed in Warton et al. (2006).

2.7.6 Analysis of Variance (ANOVA)

ANOVA is a statistical method to compare sample means of sample size (n) (i.e. the number of total samples in the data set), here we are referring to k (the number of groups) of a data set of more than two groups. The test was performed using IBM SPSS® software. ANOVA produces an F-test that measures the equality of two sample's variations to examine whether the difference between analysed data is significant or was a result of random variations. An F-test value larger than critical F value indicates that between sample differences are significant. The F test result is considered reliable if the normality assumption of the sample

population is met. In this thesis, any observed p-value equals to $\alpha = 0.05$ or less for a relationship between two variables is affirmed to represent a significant difference.

2.7.7 The Kruskal-Wallis test

The Kruskal-Wallis test (via IBM SPSS® software) (applied in Chapter 3) evaluates whether the population medians on a dependent variable are the same across all levels of a factor. To conduct the Kruskal-Wallis test, using the K independent samples procedure, cases must have scores on an independent or grouping variable and on a dependent variable. The independent or grouping variable divides individuals into two or more groups, and the dependent variable assesses individuals on at least an ordinal scale. Because the analysis for the Kruskal-Wallis test is conducted on ranked scores, the population distributions for the test variable (the scores that the ranks are based on) do not have to be of any particular form (e.g., normal). However, these distributions should be continuous and have an identical form. The Kruskal-Wallis test, a chi-square statistic, is used to evaluate differences in mean ranks to assess the null hypothesis that the medians are equal across the groups. The details of the procedure for the Kruskal-Wallis test is explained in Green and Salkind (2008).

2.7.8 The Kolmogorov-Smirnov Test (KS Test)

Using the Kolmogorov-Smirnov test, which is an assessment model used (Chapters 5) to test normality in the distribution of suspended particulate organic matter between the nearshore marine waters and their respective proximate water bodies Normal distribution was assumed for these data according to the Central Limit Theorem (Zar 2013) considering each pooled sample as an average. The Kolmogorov-Smirnov test (D) was calculated as follows using Equation 14:

$$D = \text{Max}\,[\text{abs}\,(S_1(Y)-S_2(Y))] \quad \text{.....Equation 14}$$

Where;

D is the maximum absolute (abs) deviation Kolmogorov statistic,

$S_1(Y)$ is the observed $\delta^{15}N$ or $\delta^{15}C$ cumulative distribution of suspended particulate organic matter at the interfaces (nearshore marine waters and proximate water bodies)

$S_2(Y)$ is the observed $\delta^{15}N$ or $\delta^{15}C$ cumulative distribution of suspended particulate organic matter at the interfaces (nearshore marine waters and proximate water bodies)

The Kolmogorov derived asymptotic distributions for two-sided p-value was also determined as $\mathbf{N} \rightarrow \infty$

The basic assumptions of this model are that the;

- Samples were randomly selected from the same sample population.
- Samples are mutually independent.
- Sample values are continuous variables.

Hypothesis

H_0 = There is no continuous distribution of δ^{15}N or δ^{13}C in the suspended particulate organic matter between the nearshore marine waters and respective proximate water body ($H_o \leq 0.05$)

H_1 = There is a continuous distribution of δ^{15}N or δ^{13}C in the suspended particulate organic matter between the nearshore marine waters and respective proximate water body ($H_o \geq 0.05$)

H_1 is accepted when the asymptotic significance p-value is greater than 0.05 and H_0 is rejected.

H_1 is rejected when the asymptotic significance p-value is lesser than or equal to 0.05 (95% level of confidence), and H_0 is accepted.

2.7.9 Principal Component Analysis (PCA)

PCA is the simplest of the true eigenvector-based multivariate explanatory statistical techniques. It reveals the internal structure of a dataset in a way that best explains the variance in the data. The multivariate dataset is visualised as a set of coordinates in high-dimensional data space (1 axis per variable) and supplies the user with a lower-dimensional picture, a graphical projection of the object when viewed from its most informative viewpoint. This was done using the first few principal components so that the dimensionality of the transformed data is reduced. The normalization of each attribute, a key technique used when making use of PCA consists of mean centering, subtracting each data value from its variable's measured mean so that its empirical mean (average) is zero and, possibly, normalizing each variable's variance to make it equal to 1. The results of a PCA are usually discussed in terms of component scores, sometimes called factor scores (the transformed

variable values corresponding to a particular data point), and loadings (the weight by which each original standardized variable should be multiplied to get the component score). The PCA was used in chapter 5 to give a vivid representation of the association between the physical and chemical parameters of the water samples collected during one seasonal cycle. The nature of the water, season and site was also clearly visualised by the PCA model.

The principal component analysis is expressed as described in Wold et al. (1987) (see Equation 15):

$$Z^1 = \Phi^{11}X^1 + \Phi^{21}X^2 + \Phi^{31}X^3 + ... + \Phi^{p1}X^p \quad \text{.....Equation 15}$$

Where,

- Z^1 is the first principal component
- Φ^{p1} is the loading vector comprising of loadings (Φ^1, Φ^2...) of the first principal component. The loadings are constrained to a sum of square equals to 1. This is because a large magnitude of loadings may lead to large variance. It also defines the direction of the principal component (Z^1) along which data varies the most. It results in a line in *p* dimensional space, which is closest to the *n* observations. Closeness is measured using an average squared Euclidean distance.
- $X^1...X^p$ are normalized predictors. Normalized predictors have mean equals to zero, and standard deviation equals to one

The First principal component consists of a linear combination of original predictor variables, which captures the maximum variance in the data set.

The second principal component (Z^2) has a linear combination of original predictors, which captures the remaining variance in the data set and is uncorrelated with Z^1

2.8 Detection of Outliers

Outliers are numerical values exhibiting unusual deviation from the mean or median values. They can overstate the coefficient of determination in regressions and hence produce erroneous results. While the general agreement is to allow censoring of proven outliers, the source of such unusual values requires careful examination before taking a decision to exclude them from the interpretation of the data. For the purpose of this research, numerical values that differ unexpectedly from the others due to equipment failure and human error

(titration error) were excluded from the dataset required using Rosner's Outlier Test. The generalized (Extreme Studentized Deviate) ESD many-outlier procedure used is described in (Rosner 1975). This method was used because it could point out enormous outliners in the data set. Their detection was achieved using MATLAB algorithm functions.

2.9 Mixing Models

Isotopic analysis aids the determination of the dietary outlines of a consumer by suggesting the particular resources used by a consumer. Mixing models typically used geometric techniques to estimate the proportional contribution of three or more food resources to a consumer's nourishment using δ values. When a consumer only uses two food resources, modest qualitative comparisons can be made using a single elemental tracer (Layman et al. 2012). Once the number of potential resources increases, the ability to precisely, identify the source of dietary contributions in a consumer becomes more challenging.

In unusual cases, supposing an animal shifts diets, the isotopic composition of animals' tissues begins changing to reflect that of the fresh diet. This depends on the integration of isotopic contributions over some time in the past. Thus, using tissues with different turnovers will give information about the past diet of an animal over different time intervals. The turnover rate of a tissue's constituents governs the time window of isotopic incorporation (Dalerum and Angerbjörn 2005; Newsome et al. 2007; Tieszen et al. 1983; Wolf and Del Rio 2000). Hence, stable isotopes can document diet shifts in animals. Isotope mass balance mixing models assume equilibrium. They accept the notion that any material consumed over time by an animal as a dietary component in a fixed combination reaches a steady state reflect or be integrated into the isotopic composition of its tissues. For the purpose of this research, to parse the relative contributions of allochthonous and autochthonous sources of productivity, nutrients and contaminants, it is necessary to use mixing models, which have increased in sophistication parameters specific to aquatic food web ecology, such as the per-trophic-level contribution of environmental water to the consumer.

They range from simple linear mixing models that do not necessarily incorporate complexities such as variability in isotope values, hierarchical variance structure, and discrimination factors, to fully Bayesian mixing models that permit more flexibility and may incorporate some or all of these features. In a comparison of a linear mixing model to a Bayesian mixing model, linear mixing models overestimated the terrestrial contribution by about 10 % compared to the Bayesian results and could not account for multiple sources of

uncertainty, rendering the uncertainty around the mean estimates incomparable for the two approaches (Cole and Solomon, 2012).

Therefore, a Bayesian approach, particularly one that can be adapted to the specific parameters relevant to aquatic food webs and uncertainty was effective for this research. Any of these mixing models may also incorporate C and/or N isotopes. Since the biogeochemical controls on each of these elements are different, there is the need to utilise the multiple tracers together to resolve contributions of potential nutrient and contaminant sources. However, Bayesian-based approaches like all mixing models, are not a quick fix or a substitute for poor sampling strategy; moreover, they are not particularly useful for asking questions about systems where complementary information is largely lacking. Therefore, other independent assessors such as measurement of molecular tracers and elemental analysis were used in this study to make accurate decisions.

2.9.1 Linear Isotope Mass Balance Mixing Model

The linear isotope mass-balance mixing models assume that the isotopic compositions of the tissues equal the weighted average of the isotopic composition of the diet's constituents. It was applied to two diet constituents. The mathematical expression of the model is specified in Equation 16

$$\delta X_{tissue} = p\,\delta X_A + (1- p)\,\delta X_B \quad \text{.....Equation 16}$$

Where δ can be ^{13}C or ^{15}N

p is the fraction of diet A,

XA and XB are the isotopic compositions of diet components A and B.

2.9.2 Linear Mixed-Effect Model (LMM)

The carbon and nitrogen isotopic signatures values obtained in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* were subjected to statistical evaluation via linear mixed-effect models. Using the R Statistical Software via the linear mixed-effects 4 (lme4) (Bates 2010) separate built-in linear mixed-effect models (see equation 2) were fitted for the carbon and nitrogen isotopic signature values in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* in order to perform a likelihood of ratio testing of interactions among the random and fixed effects. The random effects were the various sites while primary factors of interest such as contamination, tissue type and year were the fixed effects. The built-in fitted model used for the Linear Mixed-Effect Modelling analysis (LMM) is expressed in Equation 1:

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \beta_3 x_{3i} + \beta_4 x_{1i} x_{2i} + \beta_5 x_{1i} x_{3i} + \beta_6 x_{2i} x_{3i} + \alpha_{j(i)} + \varepsilon_i$$.....Equation 17

Where:

y_i= outcome for the i^{th} experimental unit (sample) from site j

β_0= coefficient for the intercept,

β_1= coefficient for the contamination effect,

β_2= coefficient for the year effect,

β_3= coefficient for the tissue effect,

β_4= coefficient for the interaction between contamination and year effects,

β_5= coefficient for the interaction between contamination and tissue effects,

β_6= coefficient for the interaction between year and tissue effects,

$\alpha_{j(i)}$= random effect for the i^{th} was obtained, where: $\alpha_{j(i)} \sim \text{Normal}(0, \sigma_\alpha^2)$,

ε_i= residual error term for the i^{th} unit, where: $\alpha_{j(i)} \sim \text{Normal}(0, \sigma_\varepsilon^2)$ and

x_{1i}, x_{2i} and x_{3i} are indicator variables for the contamination, year and tissue effects respectively for the i^{th} experimental unit where a value of 0 indicates the absence of a factor, and 1 indicates the presence.

2.9.3 MixSIAR Model Framework

MixSIAR is an inclusive, robust and flexible Bayesian tracer (e.g., stable isotope) mixing model framework that functions on the open-source R package was used to determine the source contributions (organic materials) in the tissue of the sentinel organism used. The advantage of MixSIAR over other mixing model software of the same function is the ability to include fixed and random effects as covariates explaining variability in mixture proportions and calculate relative support for multiple models via information criteria.

The MixSIAR model was structured to transform consumer measurements taken from *N* consumer (*Mytilus galloprovincialis*) with *J* isotope values into the proportional source contributions based on the notion that the consumers are deriving nourishment from a combination of *K* (nutrient source of enrichment) known sources. Considerations are given to further measurements on the sources themselves, working out N^{s}_{k} measurements on source *k*, and N^{c}_{k} measurements on trophic enrichment factors (TEFs) for source *k*.

Based on Parnell et al. (2013), the major components of the model are as follows:

- Y_{ij} represents the consumer isotope measurement on observation *i* (*i* = 1….*N*) for isotope value *j* (*j* = 1….*J*), where; Y_i is the *J* -vector of isotope values for consumer *i* and *Y* is the full set of consumer data.

- Y^{s}_{ijk} represents the source isotope measurement for observation *i* (*i* = 1…Nsk), isotope value j (j = 1….*J*) and source k (*k* = 1….*K*), where; Y^{s}_{ik} is the J -vector of source measurements for observation *i* on source *k* and Y^{s} is the full set of source data.

- Y^{c}_{ijk} represents the TEF isotope measurement (superscript *c* is the TEF representing a correction term for observation *i* (*i* = 1…. N^{c}), isotope value *j* (*j* = 1…*J*) and source *k* (*k* = 1….*K*), where; Y^{c}_{ik} as the *J* -vector of TEF measurements for observation i on source k and Y^{c} is for the full set of TEF data.

- ^{s}ijk is the source random effect for consumer i on isotope value *j* and source *k*, where; ^{s}ik is the *J* -vector of isotope source values for consumer *i* on source *k* and s_i to be the JK matrix of source values for consumer *i*.

- $^{c}_{ijk}$ is the TEF random effect for consumer *i* on isotope value *j* and source *k*, where; $^{c}_{ik}$ is the *J* -vector of TEF values for consumer *i* on source *k* and c_i as the *J* X *K* matrix of TEF values related to consumer *i*.

- P_{ik} is the dietary contribution of source *k* for consumer *i*, where; *Pi* is the *K*-vector of dietary proportions for consumer *i* (bearing in mind that the estimation of the dietary proportions of source *k* is the main motivation of the model)

- ϵ_{ijk} is the random noise term representing residual variation, where; *i* is the *J*-vector of residual terms for consumer *i* and set $\epsilon_i \sim N(0, \sum)$ with $\sum$ as a covariance matrix which gives an inverse-Wishart prior distribution.

Conditional independence assumptions between the consumer, source and TEF data sets were also considered for the modelling structure of the dietary proportions p based on an isometric log-ratio (ilr) approach suggested by (Egozcue et al. 2003).

CHAPTER 3

Spatial richness and variance of carbon and nitrogen isotopes in the tissues of *Mytilus galloprovincialis* from the coastal waters along Otago Peninsula, New Zealand

Overview

This chapter is a preliminary survey of the differences in carbon and nitrogen isotope ratios in *Mytilus galloprovincialis*. It examines the variances of isotope apportioning in the different tissue-type and possible mechanisms responsible for the robust differences in their isotope abundances. The influence of tissue type and physiology, tissue biochemical composition (i.e. lipid and protein content) and turnover rate were examined as one of the contributory factors for the observed variance of carbon and nitrogen isotope ratios in the sentinel organism across the sites studied. The focus of the study was to determine the tissues of choice for investigating the nutrient supply and contaminant dynamics of the coastal marine waters. The abductor and digestive were considered probable tissues of choice for the intended further long-term ecological study of the coastal marine waters.

3.1 Introduction

Stable Isotope Ratio Analysis (SIRA) is a widely used technique in ecological studies. It is often employed for exploring the impact and flow of organic materials in aquatic ecosystems, elucidating the trophic structures and pathway of energy in food webs (McCutchan et al. 2003). The stable isotopes of elements such as carbon, sulphur, hydrogen, oxygen and nitrogen are frequently used because they are elemental constituents of nutrients and organic materials (Ríos et al. 1998) and are essential in vital biochemical processes in living organisms (Hoefs 1997). Carbon isotope ratios in the tissues of an organism are useful in tracing and discriminating sources of organic materials and contaminants in aquatic ecosystems, while the nitrogen isotope ratios can indicate the ecological niche of an organism and sources of nutrients (Post, 2002).

However, the successful application of SIA technique to reveal organic materials as food resources and contaminants in an aquatic ecosystem depends on the use of a sentinel organism that has a life span long enough for substantial accumulation and assimilation of organic materials of interest to a high concentration in its tissues. In addition, such an organism must be able to undergo physiological or behavioural changes in response to its close association with the source of organic material of interest. The population of such an

organism must be large enough to sustain the collection required for the monitoring study as well as devoid of major adverse environmental impact (Glickman et al. 1991; Hamza-Chaffai 2014; Post et al. 2000).

Marine organisms such as bivalves can filter, accumulate and concentrate heterogeneous organic materials in their tissues resulting in biomagnification along the food chain (Bocchetti et al. 2008; Da Ros et al. 2007; Degger et al. 2011; Lawal-Are and Babaranti 2014; Pinnegar and Polunin 1999). They assist in the natural purification of water via filtration in suspension (water column) and sediment layers. They also play a major purpose in the nutrient dynamics of the water column and aquatic food web (Vaughn and Hakenkamp 2001).

They are used as indicator species for mass-balance nutrient monitoring (arising from inappropriate land-use practices and management) intended to ensure ecosystem-based management and conservation of nearshore coastal marine resources (Lester et al. 2010; Tallis et al. 2010; Wepener 2008). Their sedentary nature, longevity and their susceptibility to chemical changes in the environment make them particularly suitable sentinel organisms (Kopp et al. 2005).

Bivalves are important in marine aquaculture (Bayne 1993) and a source of protein for humans. They play an essential role in the cycling of matter and energy flow. They are a connecting link between the primary production and organic detritus aquatic trophic chain (Strayer 1983). Therefore, bivalves are sentinel organism for investigating the scale and ecological impact of nutrients and chemical contaminants from anthropogenic sources. Example of such chemical measurements on bivalves includes; lipid peroxides, polycyclic aromatic hydrocarbons, faecal sterols, trace metals. In addition, metallothionein measurement can provide information on oxygen strain (oxidative stress) from crude oil, sewage and metal contamination in nearshore marine waters and resident biota (Bocchetti and Regoli 2006; de Almeida et al. 2007; Domouhtsidou et al. 2004; Krieger et al. 1981; Lawal-Are and Babaranti 2014; Salas et al. 2006).

Nutrient supply from anthropogenic inputs via riverine and estuarine interface systems to nearshore coastal marine areas can result in episodic coastal eutrophication and influence primary production (Cloern et al. 2014a; Kennish 1996, 2002). Such influences can reflect as isotopic signatures in the tissues of nearshore marine resident biota such as algae (primary producer) and bivalves (primary consumer). Hence, the isotopic ratios recorded in bivalve

tissues are useful tools for tracing and discerning the sources of organic carbon and nitrogen in nearshore marine ecosystems. Spatial distribution of bivalve species are influenced by geomorphic characteristics and environmental factors (Bódis et al. 2011; Dutertre et al. 2013; Harriague et al. 2006; Mcdonald et al. 2015; Rufino et al. 2010)

Stable isotope ratios in *Mytilus galloprovincialis* was successfully employed to examine the impact of organic matter from sewage and industrial effluents (Dolenec et al. 2011; Rogers 2003b). It has been utilised to indicate environmental conditions (Kanduč et al. 2011), identify food sources (Machás and Santos 1999; Xu and Yang 2007) and clarify trophic structure (Baeta et al. 2009) in nearshore waters. Even though several of these studies dealt with isotopic intra-tissue variations in *Mytilus galloprovincialis,* they fail to place adequate consideration on the influence of diverse land-use types.

Studies have shown that carbon and nitrogen isotopic turnover rates and isotope enrichment in organisms are tissue specific and influenced by the lipid content of the tissue (Fry 2006; Thompson et al. 2000b). Fry and Arnold (1982) found out that rapid carbon turnover rate in the tissue of *Penaeus aztecus* (brown shrimp) was a function of growth and metabolic rates. Fry (1981) observed that diet switching could also alter isotope turnover rates and enrichment in organisms. He detected exceedingly variable isotope turnover rates and isotopic enrichment in larger estuarine shrimp, which switch to an offshore diet due to migration. Guelinckx et al. (2007), Logan et al. (2006) and Grey (2000) through their findings, established that that diet-switching could alter isotope turnover rates and enrichment in organisms. Hobson and Clark (1992) recorded exponential turnover rates of carbon in the tissues of adult Japanese quail (*Coturnix japonica*) by switching from a wheat-based (C3) to a corn-based (C4) diet.

Previous studies carried out by Horn (2001) to mark out sewage effluent in the coastal waters using *Mytilus galloprovincialis* and *Ulva lactuca* as biotracer species revealed the diversity of organic material in the coastal waters. Tomahawk, St. Clair and St. Kilda Beaches had mean δ^{13}C of -22.1 ± 0.5 ‰, -23.6 ± 0.2 ‰ and -23.2 ± 0.6 ‰ respectively. Their corresponding δ^{15}N ± standard errors are 3.7 ± 1.0 ‰, 4.0 ± 0.9 ‰ and 3.3 ± 0.8 ‰. The carbon and nitrogen isotope is indicative of sewage contamination (with storm water influence at St. Clair due to reduced salinity) of coastal waters relative to Akatore Creek's isotope ratio values (mean δ^{13}C and δ^{15}N values of -17.3 ± 2.1 ‰ and 5.8 ± 1.7 ‰ respectively). Sandfly

Bay Beach displayed a shift towards seal faecal matter (mean δ^{13}C and δ^{15}N values of -22.6 ± 1.8 ‰ and 9.9 ± 2.4 ‰ respectively).

Bivalves are filter feeders of suspended particulate organic matter. They probably ingest and assimilate varied organic materials (i.e. heterogeneous organic compounds containing carbon and nitrogen) during the feeding process from neighbouring water bodies. Their different tissues are likely to have different isotope values that may reflect different turnover rates that can indicate dietary inputs at different timescales. This is particularly useful in situations where there are multiple organic inputs, some of which may be sporadic and transient.

Hence, an evaluation of the isotope variability at the tissue level of *Mytilus galloprovincialis* and the information on the possible mechanisms responsible for isotope disparities among the different tissues of the sentinel organism will be desirable. This will give further information on the diet and trophic level of *Mytilus galloprovincialis* depending on the choice of tissue type. This will provide further insight into the consequences of land-use influence on the organic materials dynamics, contaminant fluxes and subsequent assimilation in the resident biota of the coastal marine waters.

Thus, the distribution and variability of carbon and nitrogen isotope ratios in the tissues (digestive, mantle, byssus thread, abductor, gills and foot) of *Mytilus galloprovincialis* collected from ten different sites along Otago's Coastline; New Zealand was studied to provide answers to the following questions:

(a) Are there carbon and nitrogen isotope differences between the tissues of *Mytilus galloprovincialis*?

(b) What mechanisms are responsible for the differences in C and N isotope ratios in *Mytilus galloprovincialis*?

(c) What are the underlying implications of the differences in carbon and nitrogen isotope ratios in *Mytilus galloprovincialis*?

(d) Could these isotope differences suggest or reveal tissues of choice that could be pointers for studying nutrient supply and contaminant fluxes in the coastal marine waters?

Outcomes from this study will be used for further ecological studies on spatio-temporal on tracing organic materials (i.e. sewage-derived and terrigenous) and contaminants in the coastal marine waters and insight into their flow and fate. This will provide the needed

information for effective decision making on land-use preservation and management intended at ensuring adequate preservation of the coastal marine waters and resources.

3.2 Materials and Methods

3.2.1 Study Site

The marine bivalves collected from 10 beaches along nearly 48 kilometres of the Otago coastline comprised of several sandy beaches and irregular cliff headlands. The location of each of the sampling sites recorded with the aid of a handheld GPS tracking device and shown in Table 11 and Figure 12. The sites comprise of ten beaches along the Otago's Coastal Marine Area. The coastal marine waters have tidal channels such as rivers and estuaries. The coastline is impacted by multiple stressors ranging from long-term municipal wastewater discharge (predominantly human sewage), urban and rural stormwater (Council 2012), quarrying, forestry and lumbering activities, agricultural pollutants from the application of fertilisers for pasture growth, animal organic wastes from pastoral farm and marine animals. Previous environmental studies by Greening et al. (2007), Loutit (1985) and North et al. (2006) reported a wide range anthropogenic stressors such as faecal bacteria, sewage effluent and land-fill leachate impacting the coastline.

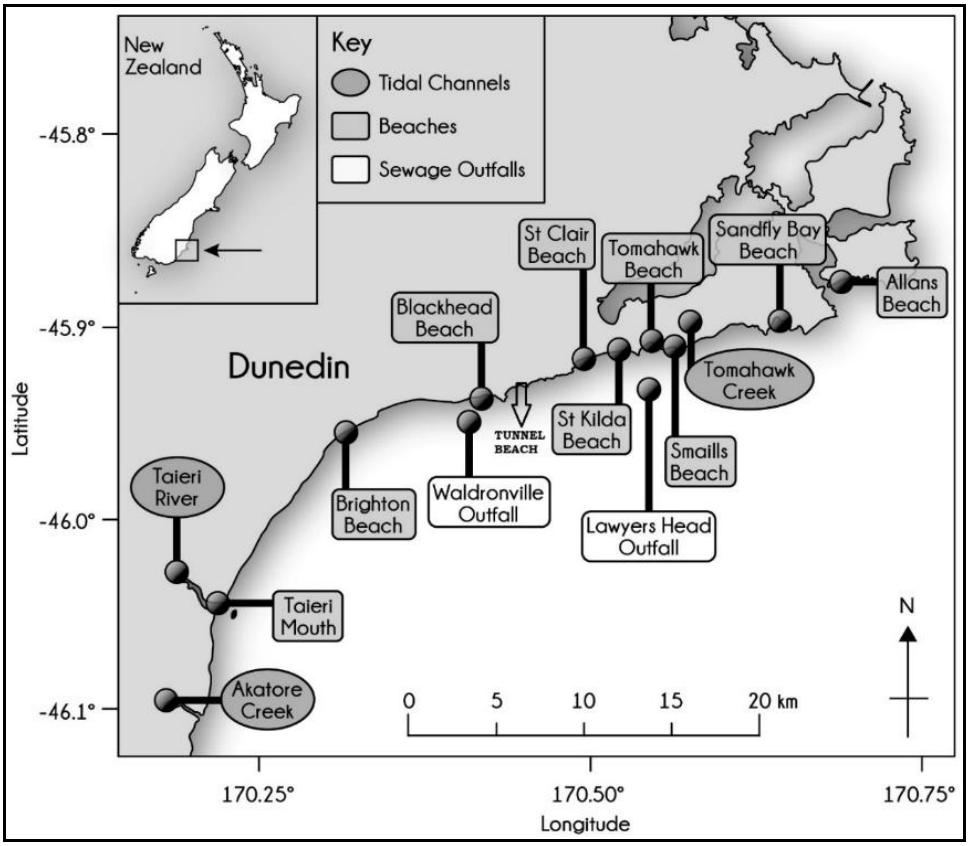


Figure 12: Schematic representation of Otago's Coastline (Dunedin, New Zealand) highlighting the position of various beaches with associated tidal channels and ocean outfalls.

Table 11: Location names with their respective coordinates, land-use impact and contamination status of the study sites

Site name	Latitude	Longitude	Land-use type	Contamination*
Allans	45.857	170.679	Farming and rural	Uncontaminated
Blackhead	40.168	176.827	Quarry and rural	Uncontaminated
Brighton	45.947	170.335	Residential and partially commercial	Uncontaminated
St. Clair	45.910	170.501	Residential and commercial	Contaminated
St. Kilda	45.908	170.517	Residential and commercial	Contaminated
Sandfly Bay	40.925	173.055	Farming and rural	Uncontaminated
Smaills	46.019	169.089	Residential, commercial and farming	Contaminated
Tomahawk	45.907	170.540	Residential, commercial and farming	Contaminated
Tunnel	45.921	170.459	Farming and rural	Uncontaminated
Taieri	46.051	170.190	Intensive farming and partially residential	Uncontaminated

***contamination status categorisation (due to the impact of municipal sewage effluent) was based on the reports written by Moore (2015), Horn (2001) and Ryder (2000).**

3.2.2 Sample Collection and Preparation

Mytilus galloprovincialis collected by hand from the intertidal rocks. The collection conducted during low tide over a period of 5 weeks (between 9/12/15 and 13/1/2016) during summer. The bivalves (an average of 4 per site) collected from the rocks at the intertidal zones were of average uniform total weight (approximately 30 ± 7 g) to minimise within-site and site-site isotopic biased-variability. The bivalves placed into individual clean ziplock plastic bags labelled with date and sample site location. They were immediately placed in a plastic cooler and taken to the laboratory where they were rinsed in distilled water and frozen (-18 °C) prior to analysis.

3.2.3 Determination of Stable Isotopic Ratios in the Tissues of *Mytilus galloprovincialis*

The bivalves dissected into different tissues (Figure 13), dried at 70 °C for 24 hours and homogenised separated into a fine powder with the aid of an MM400 bench-top Retsch ball mill. Duplicate aliquots of 0.8 mg of homogenized bivalve tissues weighed into separate 5 x 3.5 mm tin capsules. They were left to dry under vacuum overnight. The samples with standards and blanks were loaded on an automatic carousel and combusted consecutively using an elemental analyser (Carlo Erba NA1100) interfaced to an isotope ratio mass spectrometry (Sercon 20-20) for the determination of the carbon and nitrogen isotope ratios (δ^{13}C and δ^{15}N).

Figure 13: Dissected tissues of *Mytilus galloprovincialis*

Raw isotopic ratios were normalised by three-point calibration to the international scales using two international reference materials (USGS-40 and USGS-41) and an internal laboratory standard (ethylenediaminetetraacetic acid (EDTA-OAS, $\delta^{13}C$ = -38.92 ± 0.04 ‰ ; $\delta^{15}N$ = -0.70 ± 0.17 ‰). Linearity correction applied to ensure that the isotopic values obtained not affected by the sample size. Accuracy and precision (± 0.2 ‰ for $\delta^{15}N$ and ± 0.1 ‰ for $\delta^{13}C$) of the obtained isotopic results were assessed by employing the Root Mean Square Error (RMSE) differences between sequential duplicates of every 10^{th} sample (IANZ 2004) and random inclusion of two in-house standards (green mussel and copepod) to mimic the nature of the sample materials being analyzed. The ratios of stable isotopes ^{13}C:^{12}C and ^{15}N:^{14}N expressed in δ notation obtained (using Equation 1)

3.2.4 Statistical Analysis

The carbon and nitrogen isotope ratios in the tissues of *Mytilus galloprovincialis* were subjected to statistical evaluation. The mean, standard deviations and range values were

evaluated. The $\delta^{13}C$ and $\delta^{15}N$ values of the tissue were assessed by Pearson Product-Moment Correlation Coefficient (PPMCC) to examine the association among the various tissues.

For a better understanding on the relationships among the different tissues of *Mytilus galloprovincialis*, the carbon and nitrogen isotope ratios from the different tissues of the bivalve were to non-metric multidimensional scaling (NMDS) and linear bivariate analyses of the ordinary least squares using PAST software (Hammer et al. 2001). The NMDS analysis was achieved using the rank orders (as distances) for ordination (non-metric) rather than Euclidean distances (metric). The Gower similarity coefficient index with stress of 0.054 considered for the NMDS analysis. A factorial ANOVA was used to compare the means of $\delta^{13}C$ and $\delta^{15}N$ values in the bivalves to ascertain whether the observed variance for the two main effects (i.e. tissue and site) can be resolved. The factorial ANOVA was appropriate because it can appraise the main effects and the interaction effect. A single factor analysis of variance (ANOVA) of the mean distribution of $\delta^{13}C$ and $\delta^{15}N$ values across tissues and sites was conducted to test if the mean differences among main effects (i.e. the tissue or site) to ascertain were significant in relation to the $\delta^{13}C$ and $\delta^{15}N$ values (responses) in the bivalve. The ANOVA's were tested against the null hypothesis that there exist no significant differences in the mean $\delta^{13}C$ and $\delta^{15}N$ between the tissues and sites centered on the notion that the marine bivalves were randomly selected from the same sample population. The Kruskal-Wallis test was used to examine the over bearing effect of $\delta^{13}C$ and $\delta^{15}N$ values obtained in the tissues of the bivalve across all the sites. This was done to test the notion that there will be no overbearing variance in the C and N isotope signals in the bivalve since they were randomly selected from the same sample population.

3.3 Results

3.3.1 Variations of the $\delta^{13}C$ and $\delta^{15}N$ in *Mytilus galloprovincialis*

The summary of the mean, range and standard deviation values of the carbon and nitrogen isotope ratios of *Mytilus galloprovincialis* based on tissue and site compares are in Table 12 and 13.

A tissue-site appraisal of the isotopic ratios in the bivalve studied revealed the highest $\delta^{13}C$ mean value of -16.75 ± 0.09 ‰ (n = 2) was recorded at Tunnel Beach in the byssus thread and the lowest mean value recorded was in the digestive tissue at Sandfly Bay Beach (-21.23 ± 0.02 ‰, n = 2). The abductor tissues had the highest $\delta^{15}N$ value of 9.79 ± 0.12 ‰, (n = 2) at

Sandfly Bay Beach while the lowest value was recorded in the digestive tissue at Taieri Beach (7.35 ± 1.14 ‰, n = 2) (Table 12).

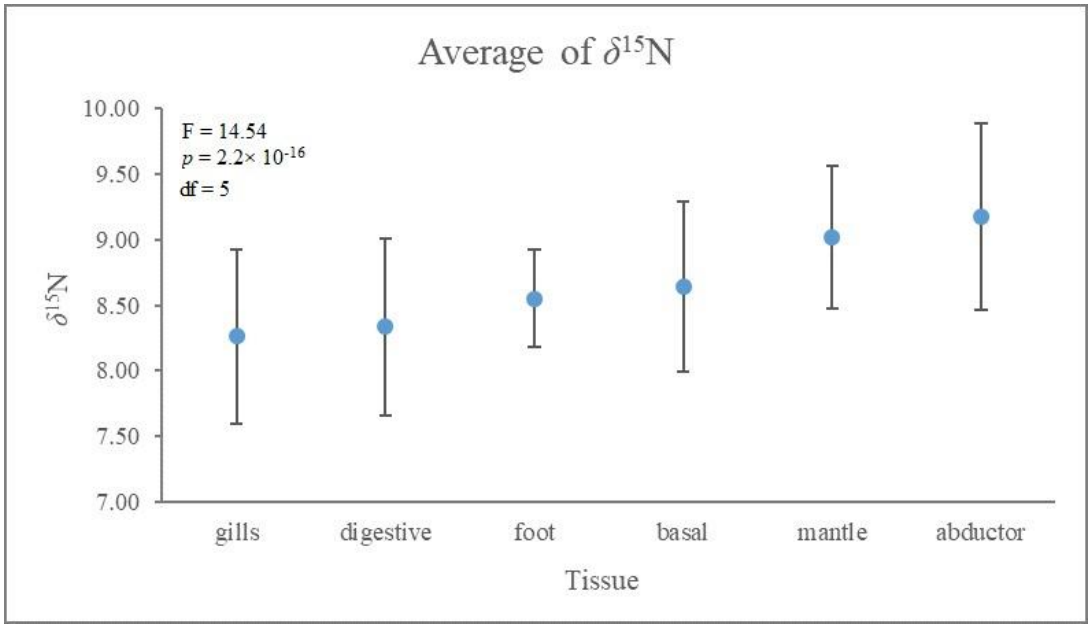
A comparison of grand mean in all the tissues across all the site comparison revealed the highest δ^{15}N value of 9.18 ± 0.71 ‰ (n = 20) for the abductor tissue and the lowest value in the gills (8.26 ± 0.67 ‰, n = 20) (Figure 14A). The lowest δ^{13}C grand average of -19.82 ± 0.72 ‰ (n =20) was detected in the digestive, and highest grand average value of -17.50 ± 0.61 ‰ (n = 20) was recorded in the byssus (Figure 14B).

A site comparison indicated that Tomahawk Beach had the highest δ^{15}N value of 9.13 ± 0.48 ‰ (n = 6) and lowest of 8.05 ± 0.62 ‰ at Tunnel (Figure 14C). The highest δ^{13}C value of -18.48 ± 0.70 ‰ (n = 6) at St. Kilda and lowest of -19.57 ± 1.39 ‰ at Sandfly Bay (Figure 14D).

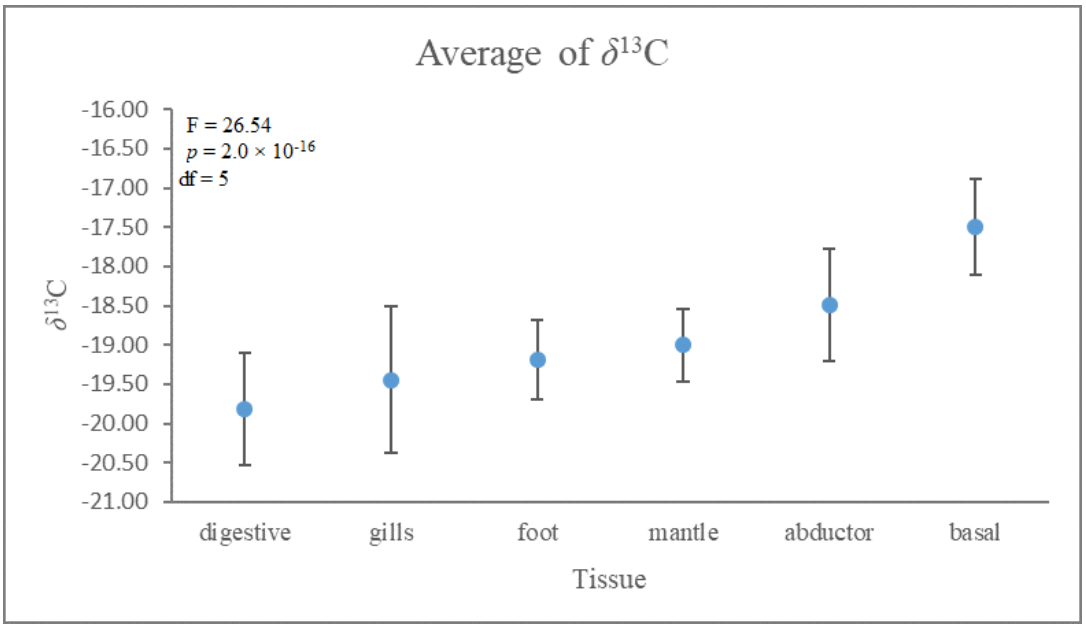
The range of δ^{15}N values for each of the diverse tissues across all sites varied between varied between 0.79 ‰ and 1.94 ‰ (n = 20) in the mantle and abductor tissues respectively. The intra-tissue δ^{13}C range values across the diverse tissues and site fluctuated from 1.22 ‰ to 2.75 ‰ (n = 20) in the foot and gills, respectively (Table 13).

The δ^{13}C and δ^{15}N values in the tissues of *Mytilus galloprovincialis* displayed similar outlines based on ^{15}N and ^{13}C integration with the exception of the byssus, which had a different orientation in ^{13}C integration cf. to other tissues (Figure 15A-B).

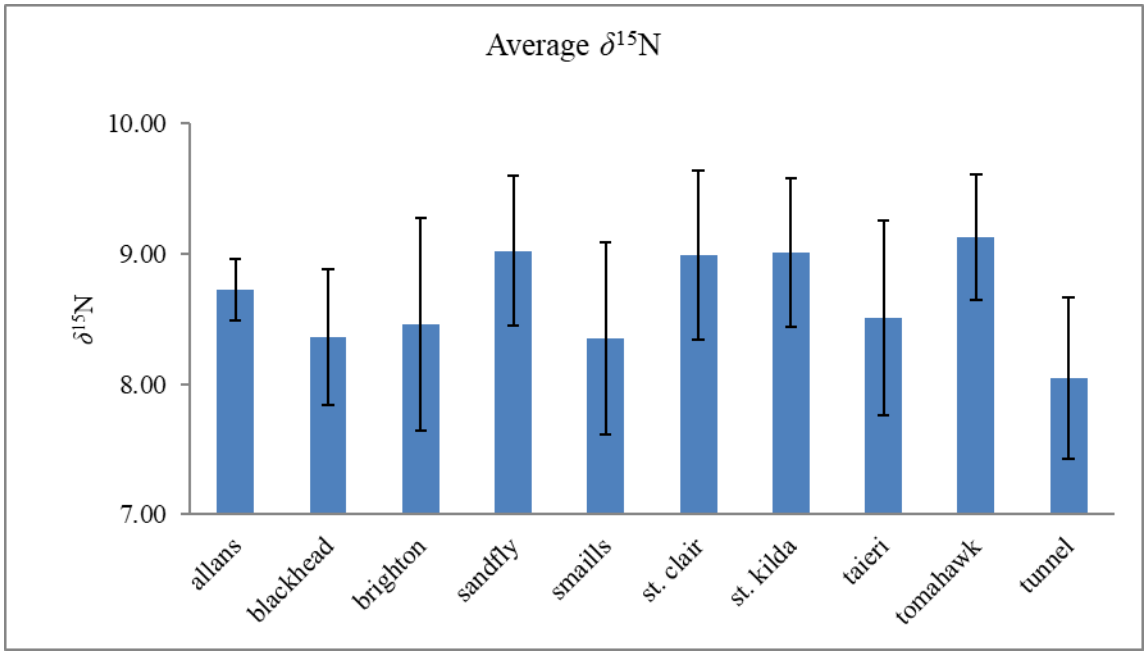
A.


Average of δ^{15}N
F = 14.54
p = 2.2× 10^{-16}
df = 5
δ^{15}N
10.00
9.50
9.00
8.50
8.00
7.50
7.00
gills
digestive
foot
basal
mantle
abductor
Tissue

B.


Average of δ^{13}C
F = 26.54
p = 2.0 × 10^{-16}
df = 5
δ^{13}C
-16.00
-16.50
-17.00
-17.50
-18.00
-18.50
-19.00
-19.50
-20.00
-20.50
-21.00
digestive
gills
foot
mantle
abductor
basal
Tissue

C.



D.

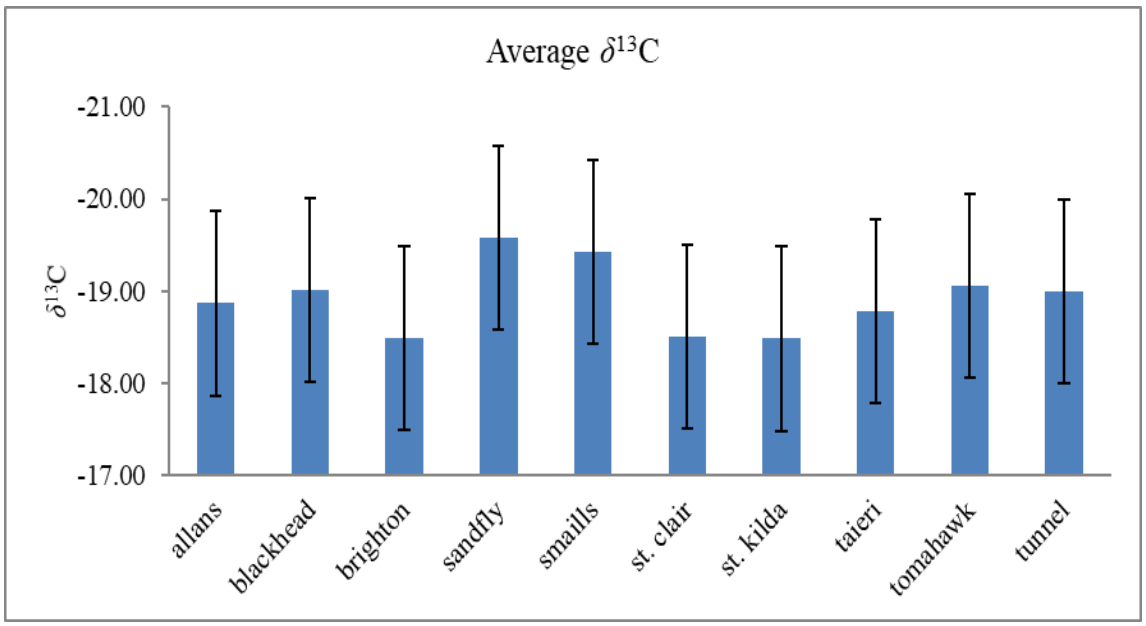
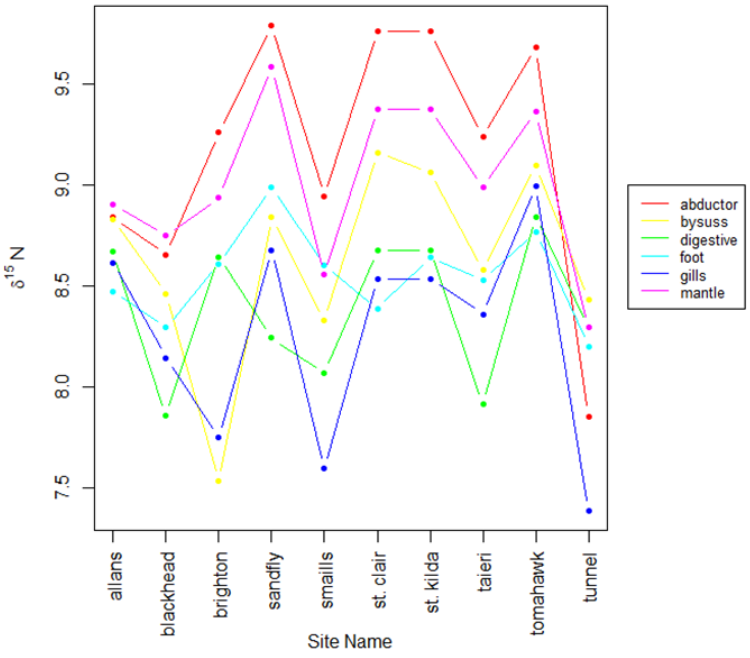
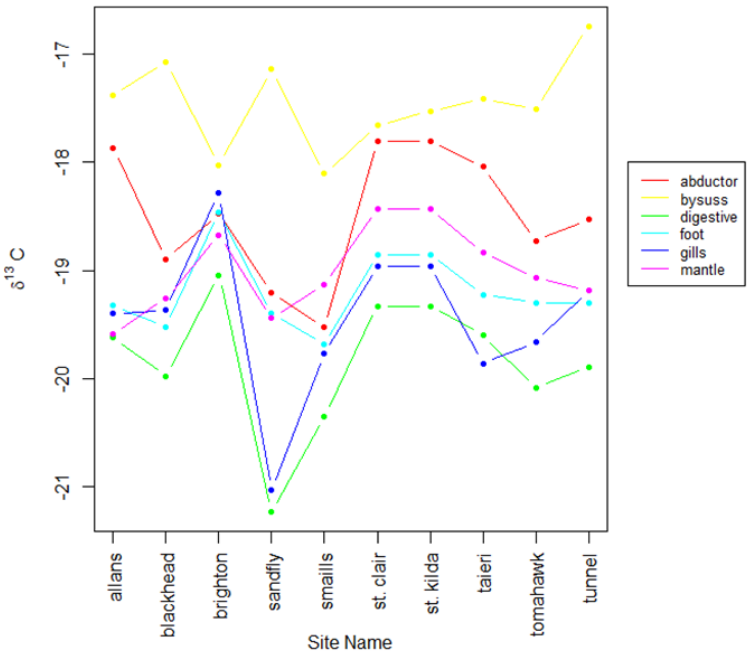


Figure 14A-D: Comparison of the averages of nitrogen and carbon isotope signatures (with standard deviation bars) in *Mytilus galloprovincialis* based tissues and sites. Significant differences of $p < 0.001$ (with level of significance set at 5 %, $\alpha = 0.05$) inserted were obtained for the analyses of the variance of the δ^{15}N and δ^{13}C values across tissues and site. C and N average values (with standard deviation bars) for the different sites were the isotope values of all the tissue types.



A.



B.

Figure 15A-B: Graphical representation of the patterns of the nitrogen and carbon isotope tissue-site compares. The byssus consistently displayed to positive δ^{13}C response across the ten sites studied.

Table 12: Mean carbon and nitrogen isotopes in the different tissues of *Mytilus galloprovincialis* from the ten beaches along Otago coastline (ABD-abductor, BYS- byssus, DGS-digestive, FOT-foot, GLS-gills and MNT-mantle).

Site	Tissue											
	ABD	BYS	DGS	FOT	GLS	MNT	ABD	BYS	DGS	FOT	GLS	MNT
	Average of δ^{15}N (n = 4)						Average of δ^{13}C (n = 4)					
Allans	8.84	8.83	8.67	8.48	8.62	8.90	-17.87	-17.39	-19.62	-19.33	-19.40	-19.59
Blackhead	8.66	8.46	7.86	8.30	8.15	8.75	-18.89	-17.08	-19.98	-19.52	-19.37	-19.26
Brighton	9.26	7.54	8.65	8.61	7.75	8.94	-18.48	-18.02	-19.04	-18.47	-18.28	-18.67
Sandfly	9.79	8.84	8.25	8.99	8.68	9.59	-19.20	-17.14	-21.23	-19.40	-21.03	-19.44
Smaills	8.95	8.33	8.07	8.61	7.60	8.56	-19.53	-18.10	-20.35	-19.68	-19.77	-19.13
St. Clair	9.76	9.16	8.68	8.39	8.54	9.38	-17.80	-17.66	-19.33	-18.85	-18.96	-18.43
St. Kilda	9.76	9.07	8.68	8.64	8.54	9.38	-17.80	-17.53	-19.33	-18.85	-18.96	-18.43
Taieri	9.24	8.58	7.35	8.53	8.36	8.99	-18.04	-17.41	-19.32	-19.22	-19.86	-18.83
Tomahawk	9.68	9.10	8.85	8.77	9.00	9.36	-18.73	-17.51	-20.08	-19.30	-19.66	-19.07
Tunnel	7.86	8.44	8.30	8.20	7.39	8.30	-18.53	-16.75	-19.89	-19.30	-19.18	-19.18
	Std. Dev. of δ^{15}N (n = 4)						Std. Dev. of δ^{13}C (n = 4)					
Allans	0.11	0.15	0.12	0.04	0.06	0.44	0.55	0.02	0.62	0.01	0.01	0.83
Blackhead	0.57	0.21	0.38	0.14	0.75	0.48	0.60	0.66	0.08	0.66	0.49	0.04
Brighton	0.30	1.29	0.42	0.06	0.31	0.18	0.52	0.50	0.13	1.00	0.04	0.14
Sandfly	0.12	0.17	0.27	0.23	0.38	0.10	0.07	0.13	0.03	0.12	0.40	0.09
Smaills	0.54	0.50	0.68	0.40	0.95	0.80	0.31	1.01	0.15	0.21	1.28	0.22
St. Clair	0.35	0.28	0.49	0.72	0.46	0.37	0.40	0.11	0.05	0.18	0.46	0.14
St. Kilda	0.35	0.37	0.49	0.27	0.46	0.37	0.40	0.26	0.05	0.18	0.46	0.14
Taieri	0.17	0.15	1.14	0.12	0.14	0.20	0.14	1.06	0.92	0.18	0.07	0.11
Tomahawk	0.56	0.37	0.44	0.36	0.16	0.39	0.05	0.08	0.18	0.32	0.07	0.10
Tunnel	0.76	0.11	0.67	0.43	0.55	0.49	0.93	0.09	0.59	0.28	1.57	0.13

Std. Dev. stands for standard deviation

Table 13: Grand average of the means and range values of carbon and nitrogen isotope ratios in the different tissues of *Mytilus galloprovincialis*

Variable	Tissue					
	Abductor	**Byssus**	**Foot**	**Gills**	**Mantle**	**Digestive**
δ^{15}N						
Range (n=2)	1.94 ‰	1.62 ‰	0.79 ‰	1.61 ‰	1.29 ‰	1.50 ‰
Gr. Av. ± SD (n = 10)	9.18 ± 0.71 ‰	8.64 ± 0.65 ‰	8.55 ± 0.37 ‰	8.26 ± 0.67 ‰	9.01 ± 0.54 ‰	8.33 ± 0.68 ‰
δ^{13}C						
Range (n=2)	1.73 ‰	1.35 ‰	1.22 ‰	2.75 ‰	1.16 ‰	2.19 ‰
Gr. Av. ± SD (n =10)	-18.49 ± 0.72 ‰	-17.50 ± 0.61 ‰	-19.19 ± 0.51 ‰	-19.45 ± 0.93 ‰	-19.00 ± 0.46 ‰	-19.82 ± 0.72 ‰

Gr. Av. stands for the grand average of the means

3.3.2 Relationship between the δ^{13}C and δ^{15}N signatures in *Mytilus galloprovincialis*

A pairwise association contrast of the inter-tissue δ^{15}N values among the different tissues of *Mytilus galloprovincialis* revealed that the abductor had correlation values of 0.78, 0.74 and 0.70 with foot, gills and mantle. The byssus had correlation values of 0.86, 0.79 and 0.52 with gills, mantle and digestive tissues, respectively. The mantle had a correlation value of 0.93 with mantle (Table 14).

A pairwise connection contrast of the inter-tissue δ^{13}C values among the different tissues of *Mytilus galloprovincialis* revealed that the abductor had correlation values of 0.65, 0.50, 0.70 and 0.75 with foot, gills, and mantle and digestive tissues respectively. The foot exhibited relationship values of 0.70, 0.60 and 0.73 with gills, mantle and digestive tissues accordingly. The digestive tissue displayed association values of 0.85 and 0.80 with gills and mantle, respectively (Table 15). The byssus tissue showed a robust relationship with other the internal tissues in the bivalve for the δ^{15}N contrasts, whereas it exhibited weak relationships with other internal tissues (Table 14 & 15). This is indicative of the discrete controlling ^{13}C and ^{15}N integration in *Mytilus galloprovincialis* in the byssus leading to ^{13}C discrimination.

Table 14: δ^{15}N-contrasts of the correlation (R^2) in the tissues of *Mytilus galloprovincialis* collected from the ten beaches along Otago's coastline

Tissue	Abductor	Byssus	Foot	Gills	Mantle	Digestive
Abductor	1.00					
Byssus	0.58	1.00				
Foot	0.78	0.45	1.00			
Gills	0.74	0.86	0.58	1.00		
Mantle	0.70	0.79	0.49	0.93	1.00	
Digestive	0.25	0.52	0.37	0.47	0.36	1.00

Table 15: δ^{13}C-contrasts of the correlation (R^2) in the tissues of *Mytilus galloprovincialis* collected from the intertidal rocks of ten beaches along Otago's coastline

Tissue	Abductor	Byssus	Foot	Gills	Mantle	Digestive
Abductor	1.00					
Byssus	-0.11	1.00				
Foot	0.65	-0.15	1.00			
Gills	0.50	0.01	0.70	1.00		
Mantle	0.70	-0.30	0.60	0.46	1.00	
Digestive	0.75	-0.05	0.73	0.85	0.80	1.00

3.3.3 Bivariate Analysis

The NMDS and linear bivariate analysis of the ordinary least squares of mean carbon and nitrogen isotope ratios gave a vivid visual illustration of the assemblages of the different tissues of *Mytilus galloprovincialis* (Figure 16 & 17). For the linear bivariate analysis, an intercept of zero with a slope of -2.12 was applied during the analysis.

The refinement groupings exhibited in the six different tissues of the bivalve was according to the portioning of isotopes of carbon and nitrogen isotope in the bivalve (Figure 16). This might be due to the lipid and protein content, metabolic rate and the functioning (physiology) of the tissue, which in turn may determine the turnover rates of organic materials in each of the bivalve's tissues studied.

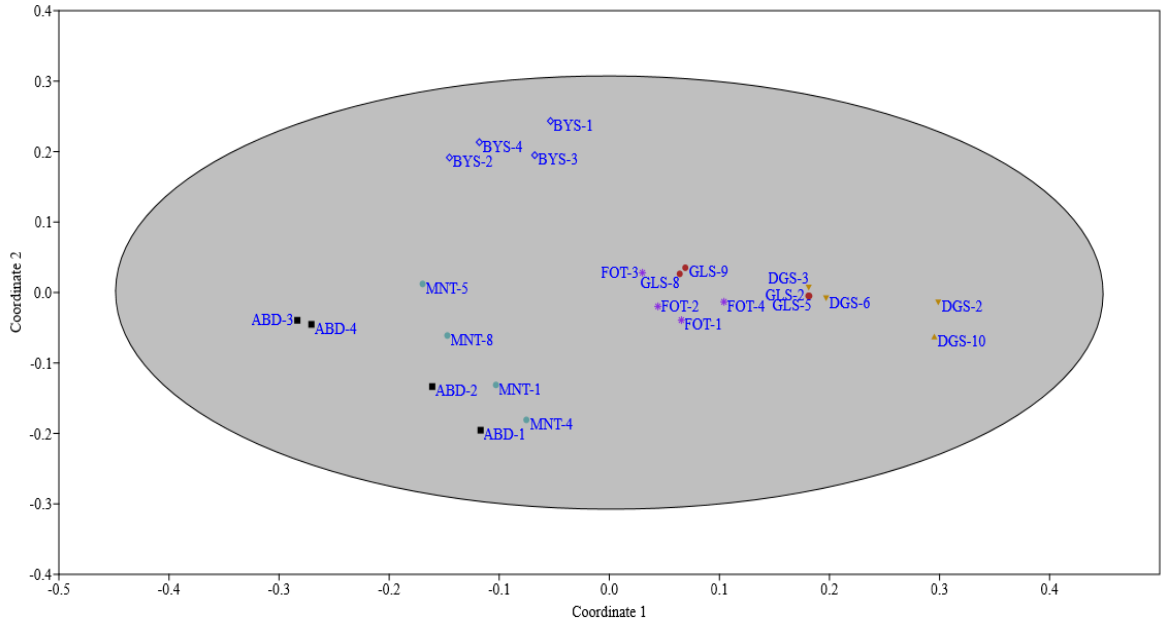


Figure 16: NMDS (with an ellipse at 95 % confidence) tissue-categorisation in *Mytilus galloprovincialis* (ABD-abductor, BYS-byssus, DGS-digestive, FOT-foot, GLS-gills, MNT-mantle).

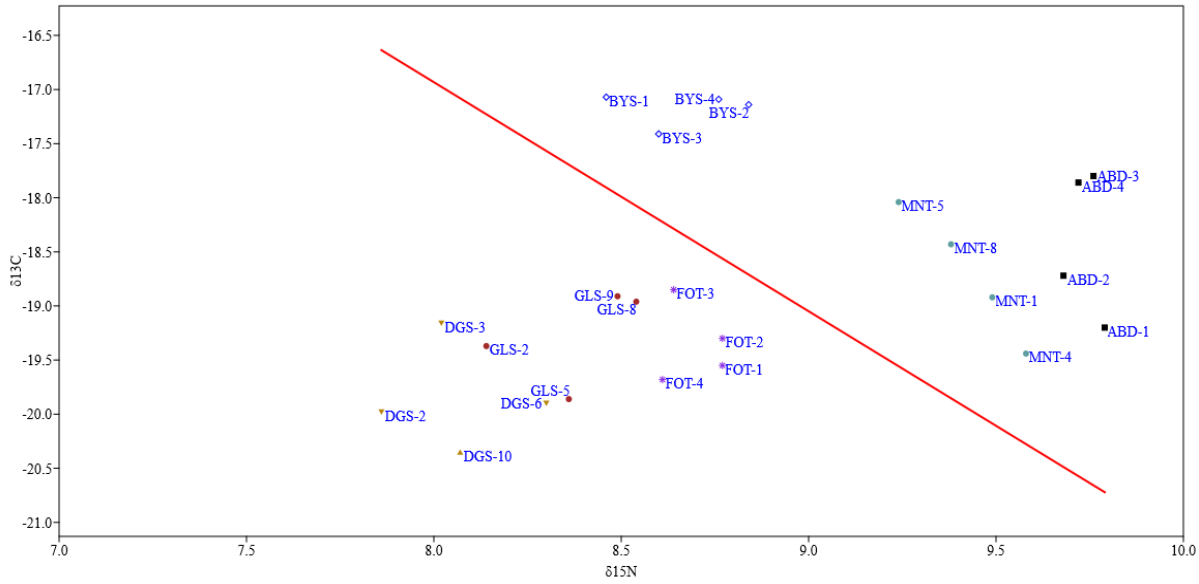


Figure 17: Scatter plots of the linear bivariate ordinary least squares of mean carbon and nitrogen isotopic signatures in the tissues of *Mytilus galloprovincialis*. Red line separation (designating an intercept of zero with a slope of -2.12) allows observed groupings of isotopic variations in the tissues of *Mytilus galloprovincialis* based on isotopic fractionation controlled by tissue lipid composition and metabolism (ABD-abductor DGS-digestive, FOT-foot, GLS-gills, MNT-mantle) and isotope distribution possibly controlled by tissue lipid composition (BYS-byssus).

3.3.4 δ^{13}C and δ^{15}N Mean and Median Analyses in *Mytilus galloprovincialis*

The δ^{13}C and δ^{15}N mean values across tissues and sites were found to be significant having p values < 0.001 (with level of significance set at 5 %, $\alpha = 0.05$) (Figure 14A-D). The factorial analysis of variance (ANOVA) of mean δ^{15}N and δ^{13}C values for tissue and site responses in the bivalve revealed significant differences ($p = 5.72\times10^{-4}$, n = 45 for δ^{15}N and 1.95×10^{-7}, n = 45 for δ^{13}C) indicating unequal means of δ^{13}C and δ^{15}N values in the tissue and site response (Table 16).

In general, the tissue and site contrast for δ^{15}N and δ^{13}C values were significant, suggesting that there is evidence for both tissue type and site sampled having some effect on the δ^{15}N and δ^{13}C values. Significant differences were observed for δ^{15}N values δ^{15}N ($p = 0.001$), and δ^{13}C values ($p = 4.96$) to test for the same median. The chi-squared rank value of 20.55 for δ^{15}N and 37.40 for δ^{13}C (Table 17). This shows that there existed an overbearing effect (arising from the unequal median) among the δ^{15}N and δ^{13}C values in the bivalve over one

another. This effect might result from the varying distinct tissue turnover rates in *Mytilus galloprovincialis*.

Table 16; Factorial Analysis of variance (ANOVA) of carbon and nitrogen isotope ratios in *Mytilus galloprovincialis* collected from the intertidal rocks of ten beaches along Otago coastline.

	δ^{15}N				δ^{13}C			
Variation	**SS**	**df**	**F value**	***p-value***	**SS**	**df**	**F value**	***p-value***
Site	14.05	9	7.12	8.65×10^{-9}	5	9	6.12	1.72×10^{-7}
Tissue	0.54	5	0.49	0.78	7.06	5	15.76	7.75×10^{-13}
Site × Tissue	20.09	45	1.91	5.72×10^{-4}	13.34	45	2.96	1.95×10^{-7}
Residuals	39.04				43.17			

(The level of significance was set at 5 %, α = 0.05)

Table 17: F-test and median analyses of intra-tissue interactions of carbon and nitrogen isotope variations in *Mytilus galloprovincialis* collected from the intertidal rocks of ten beaches along Otago coastline.

Variable	δ^{15}N	δ^{13}C
H (χ^2)	20.55	37.40
H_C (tie corrected)	20.56	37.41
***p-value* (same median)**	0.001	4.96×10^{-7}

The level of significance was set at 5 %, α = 0.05 considered significant

3.4 Discussion

In this study, the δ^{15}N abundance pattern in the *Mytilus galloprovincialis* was in the following descending order: abductor > mantle > byssus > foot > digestive > gills. The δ^{13}C abundance pattern was found to be byssus > abductor > mantle > foot > gills > digestive. The robust differences in δ^{15}N and δ^{13}C values in the abductor tissue compared to the digestive tissue is in agreement with previous studies carried out by Deudero et al. (2009a); Lorrain et al. (2002); Machás and Santos (1999); Page and Lastra (2003)

The isotope variability in the carbon and nitrogen isotope ratios of the different tissues of *Mytilus galloprovincialis* is because of the partitioning of isotopes of carbon and nitrogen among the tissues influenced by the metabolism (utilisation) of organic materials by the bivalve and prevailing environmental factors. The abductor tissues had elevated δ^{15}N across

all the sampled sites except at Allans, Tunnel and Blackhead Beaches. The digestive tissues were lower in δ^{15}N across all the sites except at Allans and Tunnel Beaches.

The byssus thread exhibited higher δ^{13}C across all the sites except Allan's Beach. The observed inconsistencies of carbon and nitrogen isotopic compositions in the different tissues of *Mytilus galloprovincialis* across the study sites might be attributed to the physiology and metabolic efficiencies which could consecutively influence the ingestion, turnover rates and absorption of organic materials in the different tissues of *Mytilus galloprovincialis* (Bayne et al. 1988).

The absence of carbon isotopic enrichment correlation of the byssus thread tissue with other tissues can be attributed to the fact that the organ is primarily used for mechanical function (Brazee and Carrington 2006; Carrington 2002) rather than metabolic activities as other tissues. Other ascribed factors for this nonconformity are; the nature of the tissue as collagenous (having complex protein molecules) and low lipid composition of less than 0.05 % of its dry mass and phospholipids (< 65 %) (Hennebicq et al. 2013). It also has low decay (4 – 6 weeks) and high-energy consuming regeneration rates (nearly 8 – 15 % of the total energy consumption of the bivalve) (Brazee and Carrington 2006; Carrington 2002; Hawkins and Bayne 1985) and environmental conditions such as temperature which can affect its formation (Young, 1985) and the 'metabolic inert' nature of the tissue.

Tissues with low turnover rates tend to integrate organic materials over a long period. For instance, higher values of δ^{15}N and δ^{13}C noted in the abductor tissue with low metabolic rate while lower values were recorded in the digestive and gill tissues with high metabolic rates. The lipid content in the tissue of the bivalve is also an influential factor in the accumulation of organic materials. Studies had shown that the differences in the lipid in the tissue of mussels might account for about 18 – 32 % shift in the accumulation rate of chemical substances and materials (Hansen et al. 1978). In addition, it appears that the tissue-specific δ^{15}N and δ^{13}C integration can be attributed to differences in the biochemical composition, nitrogen and carbon turnover rate and diet-tissue fractionation (Yokoyama and Ishihi 2006)

Therefore, tissue such as the digestive mass with high lipid content has a tendency to accumulate material with lower ^{15}N and ^{13}C than the abductor, which has low lipid content. The high lipid content of the digestive tissue comes from its lipid-rich stomach contents and the significant role of storing metabolic reserves (e.g. lipids) required for gamete formation and coping with environmental stress caused by chemical contaminants (Bayne 1976;

Gosling 2003). The mantle and foot tissues of *Perna canaliculus*, a marine bivalve had been reported to have higher concentrations of lipid (approximately 5.4 g/100 g of total lipid extract) than the abductor tissue (3.9 g / 100 g of total lipid extract) (Miller et al. 2014).

The overlapping mean δ^{15}N signatures of (□8.3 ‰) in the digestive and gills indicated that the diet of the bivalve might be from an isotopically uniform nitrogen source. However, we postulated that the gills might provide a transient snapshot of the nutrient and contaminant fluxes in the ambient water column while the digestive tissue will provide a time-integrated signal. Even though we found a similar trend in the distribution of δ^{15}N in these two tissues, the trend was not repeated in the distribution of δ^{13}C. This is attributed to the retention of phytoplankton (i.e. during feeding processes) and other particulate materials on the gills contributing to the bulk δ^{15}N measured on the gills.

The gills and digestive tissues of shellfish and finfish can concentrate organic substances in higher concentrations than present in ambient water (Björk 1995; Cheung et al. 2001; Laughlin et al. 1986; Rainbow 1985). The stable isotope ratios in the digestive tissues can thus provide dietary indicators based on comparatively long-term dietary assimilated and not just on the spot ingested food materials. The byssus thread likewise the hair, feathers and dentine of teeth, are 'metabolically inert'. As soon as they are formed, deposited in an organism they represent the isotope signature of a consumer's diet at the time of deposition. If their rate of tissue deposition is known, they can suggest a timeline of the consumer's dietary history (Layman et al. 2012).

The observed tissue isotope range values from 0.79 ‰ and 1.94 ‰ for δ^{15}N, and for 1.22 ‰ to 2.75 ‰ δ^{13}C in the bivalve (based on the between tissues and site comparisons) could be an influence in the significant differences (i.e. factorial ANOVA) recorded for the δ^{15}N, and δ^{13}C mean signal in the bivalve. This is attributed to the effects of different tissue uptakes (varying tissue turnover rates) at different sites or to the effects of different sites on different tissue uptakes. This could point to the linkage between the scale of primary production and utilisation of organic nutrients by the bivalve.

The byssus thread could probably be an indicative tool for determining exposure to environmental or industrial pollutants and other organic contaminants in the nearshore marine waters. It may aid as a paleontological tool in studies centred on climate change, land erosion, and marine deposits due to its ability to collect organic materials from eroded sediment and weathered rock materials.

3.5 Conclusion

In general, internal tissues showed a robust positive correlation in their carbon and nitrogen isotopic ratios contrasts with one another while the only assessed external tissue (i.e. byssus thread) showed non-conforming correlations in carbon isotopic ratios with each of the other tissues. The different tissues appeared to exhibit strong inclination to the availability of organic materials (nitrogen and carbon sources) in their respective localities. The robust differences recorded between the abductor and digestive tissues of the bivalve sampled in this study suggested that these tissues could be better choices as pointers for surveying the temporal and spatial study of tracing the nutrient supply, food web dynamics, energy and organic material flow in the coastal marine waters. Though, the gills of the bivalve could be considered as an alternate tissue for the digestive tissue in this undertaking due to its physiological role (i.e. physical and metabolic activities such as feeding) in filtering of suspended particles in the water column (Tran et al. 2002) and breathing (Mora et al. 1999).

4.0 Future Recommendations

Isotopic differences among tissues reflect their turnover rates; tissues having long turnover time can represent long-term nutrient incorporation, while those having short turnover time can represent short-term nutrient incorporation. Therefore, there must be caution at the experimental design stage on the choice of tissue type for isotope analysis to address the ecological question of interest.

The tissues of the bivalve provided the opportunity as indicators for tracing nutrient supply and contaminant fluxes in the coastal marine ecosystem. The variances in the stable isotope compositions of *Mytilus galloprovincialis* with consideration to environmental indicators such as food sources and exposure to various organic contaminants were used to assess the impact of long-term discharged sewage effluent on the coastal marine waters (Babaranti *et al.*, 2019).

Nevertheless, the measurements of other chemical tracers (biomarkers) with the isotopic ratios of suspended particulate materials in the water column, water indicators (i.e. physical biological and chemical) and hydrographical features of the coastal waters must be monitored on long-term scale to better understand the nutrient supply and contaminant dynamics of the coastal marine waters. In addition, authentication of the isotopic fractionation in the various tissues of the bivalve under various sample preparation techniques may also be essential in this regard.

This chapter has been submitted and is under review in:

Babaranti Oluwuyi, Frew Russell & McComb Kiri (2019). Spatial richness and variance of isotopes of carbon and nitrogen in *Mytilus galloprovincialis* from Otago Coastal Waters, New Zealand. *New Zealand Journal of Ecology*.

Contributions of Authors:

Babaranti Oluwuyi conducted the majority of the experimental work and drafted the manuscript. McComb Kiri provided advice and comments on the manuscript draft. Russell Frew supervised the project and provided comments on the manuscript.

CHAPTER 4

Isotopic signatures in *Mytilus galloprovincialis* and *Ulva lactuca* as indicators for assessing discharged sewage effluent in coastal waters along Otago Peninsula, New Zealand

Overview

The injection of nutrient rich effluent such as sewage derived organic matter can perturb the marine ecosystem. It can enter the marine food web through absorption of dissolved inorganic nutrients by primary producers such as algae. The filter feeders, located at next trophic niche, can consume sewage contaminated detrital organic matter or ingest sewage particulate organic matter directly. The implication is the contamination of the coastal marine food web by sewage derived organic matter making coastal fisheries unhealthy for consumption. In this chapter, the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* were used as biotracers for assessing the impact of long-term discharged sewage effluent on the nearshore marine waters. Two scenarios were evaluated before the upgrade, after upgrade and modification of the disposal method of the sewage treatment facility. The study was carried out to appraise if the upgrade of the sewage plant had brought significant changes to the previously sewage contaminated sites.

4.1 Introduction

Sewage, a major organic component of domestic and municipal wastewater causes increased secondary productivity, eutrophication, heavy metal contamination, reduced oxygen levels and biodiversity (Chary et al. 2008; Cheevaporn and Menasveta 2003; Hillebrand and Sommer 2000; Jarvie et al. 2006; Morillo et al. 2004). This can lead to ecological disturbances in the natural aquatic ecosystems (Browne et al. 2011; Deegan and Buchsbaum 2005; Diaz et al. 2008; Hargrave et al. 2008). Thus, it is imperative we ensure that the quality of treated wastewater effluent from municipal treatment plants meets the stipulated safe levels approved by the statutory and regulatory authorities before discharged into the receiving water bodies (Ellis 2004; Teklehaimanot et al. 2014).

Inadequately treated effluent discharged into the marine area poses environmental and health hazards to the resident biota in the adjacent coastal waters. Between 1908 and 1950s raw sewage was discharged directly into the Pacific Ocean at Lawyers Head (Council 2001) by the Dunedin Water Pollution Control Plant (now called Tahuna Wastewater Treatment Plant).

Presently, the Green Island Wastewater Plant (GIWWTP) and Tahanu Wastewater Treatment Plant (TWWTP) serving over 120,000 people in Dunedin, New Zealand discharge adequately treated wastewater effluent from Waldronville and Lawyers Head respectively (Figure 10) into the Pacific Ocean from two ocean outfalls pipes that were extended from 550 m to 1100 m in 2009. Between 2010 and 2013, there was an upgrading of the wastewater treatment facilities to handle both primary and secondary wastewater treatment processes. The advancement in the wastewater management involved the construction of a new pump station to the increase flow rate of the wastewater treatment plants. This was done to improve the quality of effluent and shoreline water quality, reduce organic matter content and ensure public health protection, which was a major concern at that time (Bouman and Archer 2014).

Before the extension of the ocean outfalls pipes, discharged wastewater effluent contaminated parts of the Otago coastal marine area, which has more than 80 protected wildlife areas, which accommodated marine mammals and birds along its landward edge (Council 2001; Gormley et al. 2012; Rayment et al. 2010).

The various bacteriological studies (a measurement of *E. coli* conducted on shellfish, sediments and seawater) had ascribed the contamination of the portions of Otago coastline to the wastewater effluent discharge from the TWWTP (Lewis et al. 2010; Nicholson et al. 1989; RCL 2000). One of such studies reported that over 50 % of shell fish collected from the sites close to the sewage outfalls at Lawyers Head had elevated level of faecal coliforms and enteric viruses of >230 and <4600 / 100 g flesh, well above the European Union Class A standard for shellfish flesh. Five downstream sites closest to the sewage outfall were found to be heavily contaminated by faecal coliforms and enteric viruses while two upstream sites were considered uncontaminated (Greening et al. 2007). However, these studies were only spot tests (Doré et al. 2000; Ebner et al. 2009) and appropriate for assessing water quality standards (Abbasi and Abbasi 2011; Dede et al. 2013). They failed to account for other possible sources of contamination and provide no estimate of the amount of sewage-derived organic matter in the nearshore marine flora and fauna.

Horn (2001) conducted an isotopic monitoring study on seawater samples, and the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* across various beaches along the Otago Peninsula to trace the pattern and distribution of sewage effluent discharged from Lawyers Head. He observed that the nitrogen isotopic ratios of the digestive tissues of the endmember mussels vary considerably. He reported that major sewage contamination occurred at Lawyers Head

and Tomahawk while there was minor contamination at St. Kilda and Smaills. He estimated that more than 60 % of the mussels and seaweeds sampled at Lawyers Head and Tomahawk Beach had their isotopic ratios affected by discharged sewage effluent.

North et al. (2006) carried out an isotopic study aimed at investigating the possibility of landfill leachates as a source of contamination from the solid waste disposal site. The study involved the collection and analyses of surface water samples from Kaikorai wetland areas made up of stream and estuary waters from Green Island Landfill (GILF) over an 8-month period. They revealed that landfill leachates could also be a possible source of contamination to the Kaikorai downstream, which eventually has a runoff into the Otago coastline at Waldronville.

Consequently, there is the prospect of using stable isotopic ratios in the tissues of the sentinel organism to assess the impact sewage-derived organic matter and other terrigenous materials on the nearshore marine ecosystem. Stable isotopic signatures in the tissues of organisms had been found to be associated with an organism diet over time and space (Bump et al. 2007; Rogers 2003).

Stable isotopic analysis on the tissues of marine flora and fauna have been used as an indicative tool to assess the impact of sewage-derived organic matter (SDOM) on the food web structure of nearshore marine ecosystems. It has also been utilised to investigate the recovery of marine flora and fauna at sites previously disrupted by sewage effluent discharges (Barr et al. 2013; Michener and Kaufman 2008; Savage 2005).

Sewage derived organic matter had been known to significantly alter the stable carbon and nitrogen isotopic signatures (expressed as the δ^{13}C and δ^{15}N) of marine flora (i.e. macro-algae) and fauna (e.g. filter-feeders) (Bedard-Haughn et al. 2003; Dudley and Shima 2010). Such flora and fauna exhibit distinct isotopic signatures in their tissues as a reflection of the integration, assimilation and utilisation of the sewage-derived organic matter in their immediate environment over time. The carbon and nitrogen isotopic signatures in *M. galloprovincialis* are the comparisons of ratios of the heavy to light isotope of the element as calculated in Equation 1.

Consumer organisms have been known to exhibit isotopic signatures which can either be similar or vary from their diets with an average fractionation trophic enrichment of +0.4 to +1 ‰ for δ^{13}C 1987 and +3 to +4 ‰ for δ^{15}N (Kline 1999; Post 2002).

In assessing the impact of sewage-derived organic matter in a nearshore marine ecosystem, the disparities in the carbon and nitrogen isotopic ratios in the tissues of endmembers can become useful monitoring tools for providing more information on the source and magnitude of sewage contribution to the diet of resident biota (Peterson 1999). Hence, for the purpose of this study, stable isotopic ratios in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* were selected as indicators to be used. Mussels are sedentary and long-lived (Alfaro et al. 2001; Nordsieck 2006; SITO 2006) characteristic features which make them suitable sentinel organisms as time-averaged integrators of sewage exposure for the sea lettuces which are short-lived by nature (Cabana and Rasmussen 1996; Dudley and Shima 2010; Post 2002).

Furthermore, filter feeders such as bivalves (i.e. mussels) can directly ingest (via the gills) and assimilate sewage particulate organic matter (containing carbon and nitrogen) along with their diet (phytoplankton and detritus) from the water column into the tissues and reassigned such higher up the food chain (biomagnification) (Ouédraogo et al. 2015; Pan and Wen-Xiong 2004).

In choosing the most appropriate tissues for the stable isotope study, consideration was given to the previous studies conducted by other workers on carbon and nitrogen isotopic turnover and enrichment in the different tissues of organisms. Most of the studies showed that carbon and nitrogen isotopic turnover rates and isotopic enrichment in organisms are tissue-specific and influenced by lipid content in the tissue (Lorrain et al. 2002; Thompson et al. 2000a).

Thus, a preliminary stable isotope study to investigate the variance of isotopic ratios of carbon and nitrogen in the different tissues of *Mytilus galloprovincialis* was carried out to determine the tissues of choice to be utilised for this study. The results obtained were compared with other isotopic studies, using the set up for power analysis as described in Preacher and Coffman (2006) the required sample size was determined. This was done to control the number of samples to be collected to avoid the unnecessary sacrifice of biological samples and ensure the reliability of the results. The abductor tissue was noted to have the highest isotopic carbon and nitrogen turnover, whereas the digestive tissue has the lowest isotopic tissue turnover. This observation was in accordance with other workers (Deudero et al. 2009b; Gaston and Suthers 2004). The abductor and digestive tissues of the blue mussel were found to be probable indicators for assessing the impact of sewage-derived organic matter on the nearshore marine fauna in the nearshore marine waters.

To the best of our knowledge, no stable isotopic studies had been carried out to evaluate the impact of the modifications in the sewage treatment and disposal on the nearshore marine waters and resident biota along the Otago Peninsula. Hence, this study is attempted to utilise the isotopic ratios of sentinel organisms at these sites to evaluate the status of sewage derived organic materials in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* of the nearshore marine waters. Therefore, this study will address the following research questions:

1. Are the variabilities in the $\delta^{13}C$ and $\delta^{15}N$ in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* reliable indicators of assessing sewage contamination in the nearshore marine waters along the Otago Peninsula?

2. Has the improvement in the sewage treatment processes and disposal at the TWWTP brought significant changes in the carbon and nitrogen isotopic signatures of *Mytilus galloprovincialis* and *Ulva lactuca* collected from the nearshore marine ecosystem along the Otago Peninsula?

3. Are there other possible terrestrial-based organic materials contributing carbonaceous and nitrogenous materials to the nearshore waters, which may sway the carbon and nitrogen isotopic ratios of *Mytilus galloprovincialis* and *Ulva lactuca*?

4. How much of terrestrial-based organic materials are integrated into the tissues (i.e. digestive) of *Mytilus galloprovincialis* collected from the nearshore marine waters along the Otago Peninsula?

To answer these questions, the Linear Mixed Effects Model analysis (LMM) (see Equation 8), an extension of regression analysis, was used. It was used to compare the stable carbon and nitrogen isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) in the tissues of *Mytilus galloprovincialis*, and *Ulva lactuca* collected in 2001 (before upgrade of the sewage treatment plant) and 2015/16 (after upgrade of the sewage treatment plant). Endmembers were measured to determine if there had been changes in the isotopic signatures $\delta^{13}C$ and $\delta^{15}N$ in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* collected from the study sites categorized into control sites (uncontaminated sites) and previously sewage contaminated sites comprising of eight beaches and three tidal channels.

The groupings of these sites were based on proximity to the sewage outfall, the written reports of the 2000 ORC Resource Consent 97530 97530 (RCL 2000) and 2007 FRST Programme C03X0301(Greening et al. 2007). The nature of possible sources of carbon and

nitrogen into the nearshore marine waters were identified. They were distinguished by the characteristic isotopic ratios of the particulate organic matter collected from the tidal channels flowing into the coastal marine waters.

A mass balance linear mixing model was used in estimating the diet source contributions of carbon and nitrogen materials in the tissue of marine biota collected from the study sites along the Otago Peninsula. The two main sources considered were marine particulate organic matter and sewage effluent particulate organic matter. This was done to quantify the contribution of sewage material in the marine biota.

The findings from this study showed the potential deployment of other analytical techniques and further isotopic mixing model to be used as a tool to elucidate the flow and fate of various sources of organic materials in the nearshore marine waters. This will provide a better understanding of the impact of anthropogenic organic carbon and nitrogenous materials on the nearshore marine waters and its consequences on the functioning of the coastal marine ecosystem.

Insights into the influence of human-induced stressors on the dynamics of organic materials in the nearshore marine ecosystem will ensure proper management, conservation and preservation of the coastal marine aquaculture resources through a comprehensive ecosystem based management with a focus on proper land-use management.

4.2 Material and Methods

4.2.1 Sampling Sites

Mytilus galloprovincialis and *Ulva lactuca* were collected from the eight beaches along the Otago coastline at low tide before the upgrade of the treatment plants from 4/05/01 to 10/08/01 (weekly) and after the upgrade from 9/12/15 to 13/4/2016 (weekly). Samples were collected from the rocks in the intertidal zone. The marine bivalves were of average uniform sizes to avoid sampling bias, which might ensue from in-site and site-site isotopic variability. The study sites cover about 48 kilometres within the Otago Marine Area. Water samples were collected from the three tidal channels having free connection to the nearshore marine waters (see Figure 11). At each of the sampling sites, the date and time of sampling, prevailing weather conditions, swell, wind direction, as well as possible visible sources of environmental concern, were noted and recorded. The location of each of the sampling sites was recorded with the aid of a handheld GPS tracking device. It is illustrated in Table 18.

Table 18: Sampling site with assigned code, name and coordinates

Site	Latitude	Longitude
Allans	45.857	170.679
Blackhead	40.168	176.827
Brighton	45.948	170.335
St. Clair	45.910	170.501
St. Kilda	45.908	170.517
Sandfly Bay	40.925	173.055
Smaills	46.019	169.089
Tomahawk	45.907	170.540
Tomahawk Creek	46.019	169.089
Atakore Creek	46.109	170.177
Taeiri Mouth	46.051	170.190

4.2.2 Sample Preparation and Analysis

Mytilus galloprovincialis and *Ulva lactuca* were individually placed into clean ziplock plastic bags labelled with date and sample site location. They were immediately placed in a plastic cooler for onward transport to the laboratory where they were rinsed in distilled water and frozen prior to analysis. *Mytilus galloprovincialis* was dissected into different tissues (the abductor and digestive tissues of interest in this study). The dissected tissues and samples of *Ulva lactuca* were dried at 70 ^{o}C for 24 h. Once dry samples were homogenised with the aid of an MM400 bench-top Retsch ball mill, duplicate aliquots of 0.8 mg of homogenized tissues of the biological samples were measured into separate 5 x 3.5 mm tin cups. They were dried under a vacuum overnight.

Water samples collected from the eight beaches and three tidal channels were filtered through 25 mm GF/F grade to collect suspended particulate organic matter. Sewage effluent and seal faecal matter were collected from the source. They were processed for isotopic analysis. The samples with internal and certified pre-calibrated standards and blanks were placed in an auto sampler carousel and combusted using the Carlo Erba NA1500. The elemental analysis-isotope ratio mass spectrometry (EA-IRMS) operates in a continuous flow mode for the determination of the carbon and nitrogen isotope ratios (δ^{13}C and δ^{15}N). Nitrogen and carbon isotopes were assayed by combustion of the samples in the chromium oxide combustion column of the elemental analyser to produce N_2 and CO_2, using helium carrier gas.

These gases were resolved in a packed molecular sieve GC column and sent sequentially to the inlet of Europa Scientific continuous flow mode “20/20 Hydra” (Europa Scientific, UK)

isotope ratio mass spectrometer (CF-IRMS) interfaced to the Sercon System Controller which runs on Sercon Callisto Software. During the programmed run tests, the samples were combusted at 1050 °C, and NO_x species were reduced to N_2 at 650 °C within the elemental analyser using its normal reaction scheme.

Raw isotopic ratios obtained were normalised by three-point calibration to the international scales using two IAEA (International Atomic Energy Agency) reference materials (USGS-40 and USGS-41) and internal laboratory standard (ethylenediaminetetra-acetic acid (EDTA-OAS, $\delta^{13}C$ = -38.92 ‰ ± 0.04; $\delta^{15}N$ = -0.70 ‰ ± 0.17) of known carbon and nitrogen isotopic signatures, assayed with the unknown samples. Isotopic ratios in the tissues of sentinel organisms were determined using Equation 1.

A linearity correction method was applied to ensure that the isotopic values obtained were not affected by instrumental drift. Accuracy and precision of the obtained isotopic results were assessed by employing the root mean square error RMSE (see Equation 13) of the differences between sequential duplicates of every 10th sample (IANZ 2004) and random inclusion of two in-house standards (green mussel and copepod) to mimic the nature of the sample materials being analysed.

4.2.3 Statistical Analysis

The carbon and nitrogen isotopic signatures values obtained in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* were subjected to statistical evaluation via linear mixed-effect models. The average values of carbon and nitrogen isotopic ratios with standard errors are represented in table 4. Using the R Statistical Software via the linear mixed-effects 4 (lme4) (Bates 2010) separate built-in linear mixed-effect models (see Equation 2) were fitted for the carbon and nitrogen isotopic signature values in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* in order to perform a likelihood of ratio testing of interactions among the random and fixed effects. The random effects were the various sites while primary factors of interest such as contamination, tissue type and year were the fixed effects.

4.3 Results

4.3.1 Variations in the Carbon and Nitrogen Isotopic Ratios in *Mytilus galloprovincialis* and *Ulva lactuca*

The mean carbon and nitrogen isotopic ratios in *Mytilus galloprovincialis* and *Ulva lactuca* sampled in 2001 and 2015 are illustrated in Table 19. The disparity in the mean values of carbon and nitrogen isotopic ratios in *Mytilus galloprovincialis* and *Ulva lactuca* collected in 2001 and 2015 along the Otago Peninsula are represented in Table 20.

At the uncontaminated sites, the mean δ^{15}N values recorded in the abductor tissues of *Mytilus galloprovincialis* contrasted between 0.21 ‰ and 1.71 ‰ larger at Blackhead and Allans correspondingly whereas disparity values ranged between 2.52 ‰ and 0.40 ‰ lower at Brighton and Sandfly Bay accordingly than previously noted in 2001. The noticeable differences for the means of δ^{13}C fluctuated from 0.38 to 1.61 ‰ larger at Black Head and Brighton accordingly (Table 20).

Table 19 : Mean isotopic carbon and nitrogen signatures (with their respective standard errors) in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* collected from uncontaminated and previously contaminated sites along Otago coastline, New Zealand in 2001 and 2015.

	2001		2015	
Site	**$\delta^{15}N_{AIR}$ (‰) ± SE**	**$\delta^{13}C_{VPDB}$(‰) ± SE**	**$\delta^{15}N_{AIR}$ (‰) ± SE**	**$\delta^{13}C_{VPDB}$(‰) ± SE**
Uncontaminated sites		**(*Mytilus galloprovincialis*)**		
		Abductor		
Allans	8.78 ± 0.25	-18.00 ± 0.29	9.02 ± 0.13	-18.08 ± 0.40
Blackhead	7.73 ± 0.03	-20.42 ± 0.19	8.65 ± 0.29	-18.89 ± 0.30
Brighton	11.21 ± 0.56	-17.91 ± 0.23	9.26 ± 0.27	-18.48 ± 0.15
Sandfly Bay	10.19 ± 0.10	-20.82 ± 0.91	9.79 ± 0.09	-19.20 ± 0.04
		Digestive		
Allans	7.20 ± 0.17	-20.49 ± 0.10	8.91 ± 0.18	-20.08 ± 0.09
Blackhead	7.65 ± 0.31	-20.36 ± 0.17	7.86 ± 0.19	-19.98 ± 0.04
Brighton	10.54 ± 0.98	-20.77 ± 0.21	8.02 ± 0.34	-19.16 ± 0.07
Sandfly Bay	7.06 ± 0.25	-20.62 ± 0.11	8.24 ± 0.13	-21.23 ± 0.02
Previously contaminated sites		**(*Mytilus galloprovincialis*)**		
		Abductor		
St. Clair	10.04 ± 0.18	-18.08 ± 0.17	9.72 ± 0.09	-17.86 ± 0.04
St. Kilda	8.64 ± 0.11	-17.33 ± 0.41	9.76 ± 0.17	- 17.80 ± 0.20
Smaills	9.34 ± 0.13	-18.29 ± 0.32	8.95 ± 0.15	-19.53 ± 0.26
Tomahawk	7.33 ± 0.08	-18. 49 ± 0.50	9.68 ± 0.28	-18.72 ± 0.03
		Digestive		
St. Clair	7.08 ± 0.27	-20.81 ± 0.30	8.45 ± 0.07	-19.14 ± 0.14
St. Kilda	6.34 ± 0.22	-17.19 ± 0.24	8.68 ± 025	-19.33 ± 0.03
Smaills	7.20 ± 0.24	-20.65 ± 0.47	8.07 ± 0.15	-20.35 ± 0.07
Tomahawk	4.83 ± 0.31	-22.81 ± 0.44	8.84 ± 0.22	-20.08 ± 0.09
Uncontaminated site		**(*Ulva lactuca*)**		
Allans	9.12 ± 0.38	-10.41 ± 0.36	9.02 ± 0.11	-18.08 ± 0.01
Blackhead	8.05 ± 0.05	-16.63 ± 0.10	8.39 ± 0.00	-20.08 ± 0.06
Brighton	7.54 ± 0.05	-16.42 ± 0.19	8.55 ± 0.22	-19.67 ± 0.03
Sandfly Bay	9.18 ± 0.10	-14.63 ± 0.34	9.37 ± 0.02	-16.75 ± 0.08
Previously contaminated sites		**(*Ulva lactuca*)**		
St. Clair	6.88 ± 0.06	-14.47 ± 0.72	8.81 ± 0.29	-20.39 ± 0.03
St. Kilda	5.34 ± 0.67	-16.29 ± 0.30	9.10 ± 0.08	-21.63 ± 0.04
Smaills	10.46 ± 0.15	-12.38 ± 0.15	9.10 ± 0.16	-21.63 ± 0.01
Tomahawk	-3.07 ± 0.45	-15.74 ± 0.25	8.52 ± 0.01	-19.51 ± 0.03

Table 20: Noticeable differences (disparity values) between the mean carbon and nitrogen isotopic ratios in *Mytilus galloprovincialis* and *Ulva lactuca* collected in 2001 (before the upgrade of TWWTP) and 2015 (after the upgrade of TWWTP) from the nearshore marine waters along Otago Peninsula.

	Mytilus galloprovincialis				*Ulva lactuca*	
	Abductor		Digestive		seaweed	
Site	δ^{15}N (‰)	δ^{13}C (‰)	δ^{15}N (‰)	δ^{13}C (‰)	δ^{15}N (‰)	δ^{13}C (‰)
Uncontaminated						
Allans	+0.24	-0.08	+1.71	+0.41	-0.10	-7.67
Blackhead	+0.92	+1.53	+0.21	+0.38	+0.34	-3.45
Brighton	-1.95	-0.57	-2.52	+1.61	+1.01	-3.25
Sandfly Bay	-0.40	+1.62	+1.18	-0.61	+0.19	-2.12
Previously contaminated						
St. Clair	-0.32	+0.22	+1.37	-1.06	+1.93	-5.92
St. Kilda	+1.12	-0.47	+2.34	-2.00	+3.76	-5.34
Smaills	-0.39	-1.24	+0.87	-2.06	-1.36	-9.25
Tomahawk	+2.35	-0.23	+4.01	-1.59	+11.59	-3.77

The mean δ^{15}N values recorded in the abductor tissues of *Mytilus galloprovincialis* had disparity values fluctuated between 1.12 ‰ and 2.35 ‰ larger at St. Kilda and Tomahawk respectively whereas disparity values varied between 0.32 ‰ and 0.39 ‰ lower at St. Clair and Smaills accordingly than previously noted in 2001. The disparity values for the means of δ^{13}C fluctuated from 0.23 to 1.24 ‰ lower across the three previously contaminated sites except at St. Clair, where it was 0.22 ‰ higher than noted in 2001. In the digestive tissues, the recorded disparity values in the means δ^{15}N values in 2015 ranged between 1.37 to 4.01 ‰ larger across all the previously contaminated sites. The recorded disparity values for the means of δ^{13}C varied between 1.06 to 2.06 ‰ lesser across all the previously contaminated sites (Table 15). In the tissues of *Ulva lactuca*, the recorded disparity values for δ^{15}N means varied between 1.93 to 11.59 ‰ higher across three of the sites and 1.36 ‰ lesser at Smaills than observed in 2001. Contrasts between the endmember's nitrogen and carbon isotopic ratios recorded in the abductor and digestive tissues of the mussels sampled in 2015, revealed that the weighted mean δ^{15}N values differed from 0.11 to 1.55 ‰ larger in the abductor tissues than in the digestive tissues whereas the mean δ^{13}C values varied from 0.68 to 2.03 ‰ larger at the uncontaminated sites. At the previously contaminated sites, weighted mean

δ^{15}N values fluctuated between 0.84 and 1.27 ‰ larger in the abductor tissue than digestive tissue while the weighted mean δ^{13}C values varied between 0.82 and 1.82 ‰ larger.

4.3.2 Particulate Organic Matter (POM) in Samples

The mean δ^{15}N values for POM from filtered water samples ranged from 5.70 ± 0.20 ‰ to 10.06 ± 0.05 ‰ recorded at Akatore and Tomahawk Creeks, respectively. The mean δ^{13}C values for the POM ranged from -27.18 ± 0.01 ‰ to -21.57 ± 0.61 ‰ documented at Tomahawk and Akatore Creeks correspondingly (Table 21). The isotopic signatures of POM from these varying aquatic systems indicated the probable sources and type of anthropogenic materials been transported into them for onward transport to the coastal marine waters. The reported higher mean carbon isotopic (δ^{13}C) and lower nitrogen isotopic (δ^{15}N) values of Akatore Creek, and Taieri Mouth indicated marine based organic matter while that of Tomahawk Creek seemed terrestrial due to the higher δ^{15}N and lower δ^{13}C isotopic ratios.

Table 21: Mean with standard error values of nitrogen and carbon isotopic signatures values from the particulate organic matter in filtered water, sewage effluent and seal faeces samples collected from the coastal marine waters along Otago coastline in 2001(bracket) and 2015.

Site	$\delta^{15}N_{AIR}$ (‰) ± SE	$\delta^{13}C_{PVB}$ (‰) ± SE
Akatore Creek	5.70 ± 0.20 (5.80 ± 1.70)	-21.57 ± 0.61(-17.30 ± 2.10)
Taieri Mouth	5.80 ± 0.16 (5.40 ± 2.60)	-24.03 ± 0.73 (-24.30 ± 1.50)
Tomahawk Creek	10.06 ± 0.06 (8.70 ± 0.50)	-27.18 ± 0.01 (-27.00 ±0.30)
Effluent POM	3.10 ± 0.50	-25.80 ± 0.10
Seal faeces POM	14.20 ± 0.60	-21.90 ± 0.30

4.3.3 Sewage, Marine and Terrigenous Particulate Matter in *Mytilus galloprovincialis*

The nitrogen and carbon isotopic signatures analyses on digestive tissues of *Mytilus galloprovincialis* collected in 2001 before the upgrade of the sewage treatment plant revealed that 90 % of *Mytilus galloprovincialis* collected from Tomahawk were heavily isotopically lower in δ^{15}N and δ^{13}C. It was observed that 60 % of nitrogen and 40 % of carbon in the digestive tissue were derived from sewage particulate matter while between 30 % and 60 % of the mussels from St. Kilda, Smaills and St. Clair showed signs of mild sewage contamination. This suggests that sewage effluent seemed to provide a source of sustenance

for the ingested phytoplankton biomass or direct ingestion of sewage particulate organic matter by *Mytilus galloprovincialis* appraised at the previously contaminated sites so much to influence the marine bivalve isotopic ratios greatly.

Ulva lactuca collected in 2001 from Tomahawk has negative mean δ^{15}N, an indication of sewage effluent alteration, and 40 % of *Ulva lactuca* sampled St. Clair Beach also indicated influence by sewage effluent. In 2015, *Mytilus galloprovincialis* collected and analysed showed no sign of sewage effluent contamination.

The contrast between the carbon and nitrogen isotopic ratios in the digestive tissues of the mussels from all sites evidently seemed clustered close to one to one another (Figure 18) in 2015, a direct contrast to 2001 observation (Figure 19). This is attributed to the mussels feeding on a single major food source. It was observed that the mussels tend to obtain nutrition from the marine particulate organic matter as most of the mussels sampled at the previously contaminated and uncontaminated sites assumed trophic shift (enrichment factor) of roughly 3 ‰ for δ^{15}N and 1 ‰ for the δ^{13}C cf. to the marine particulate organic matter. The marine bivalve might also be deriving sustenance from other terrestrial organic materials incursions to the nearshore waters.

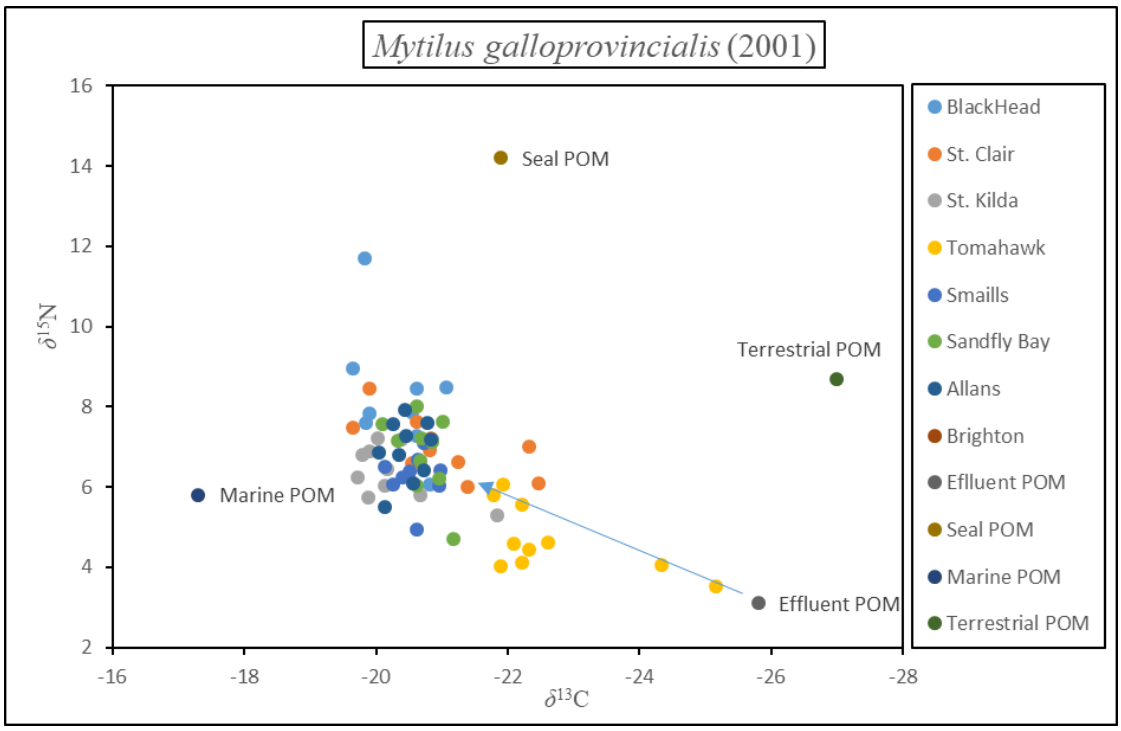


Figure 18: Comparison of carbon and nitrogen stable isotopic ratios in the digestive tissues of *Mytilus galloprovincialis* and particulate materials collected in 2001 along the Otago coastline, New Zealand [data from Horn (2001)].

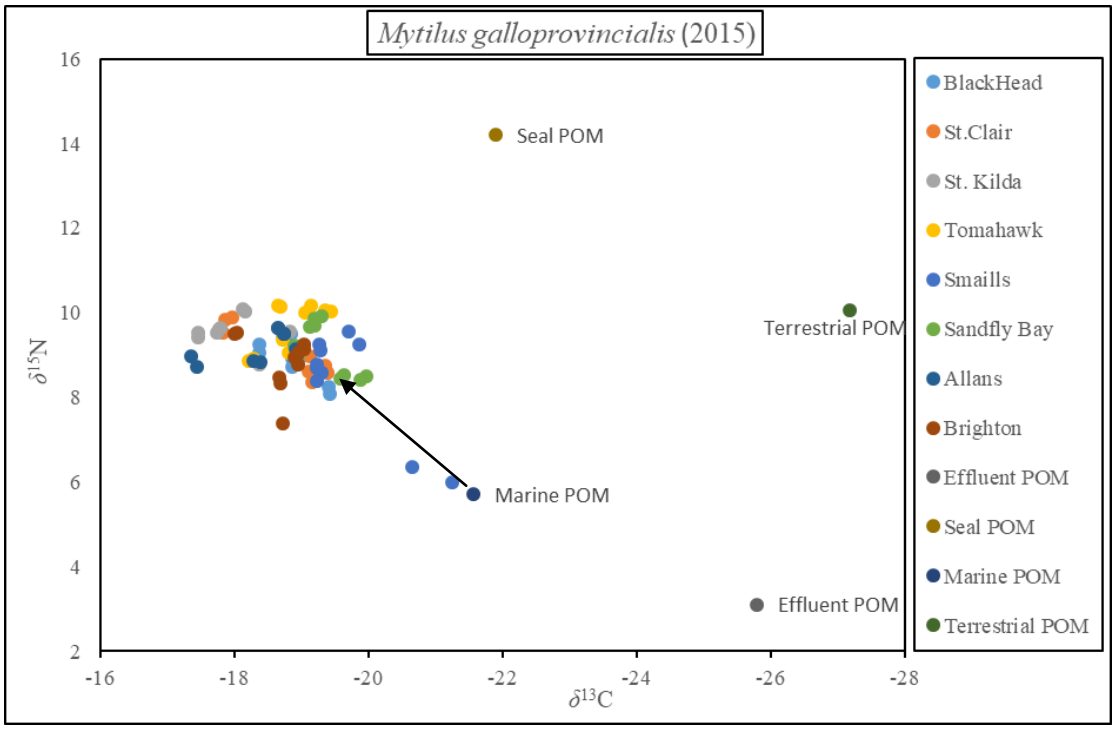


Figure 19: Comparison of carbon and nitrogen stable isotopic ratios in the digestive tissues of *Mytilus galloprovincialis* and particulate materials collected in 2015 along the Otago coastline, New Zealand.

The contrasts of carbon and nitrogen ratios in the tissues of *Ulva lactuca* in the 2015 sampling were much more predictable as opposed to the trend observed in 2001. Of particular note are the results from at Tomahawk where 2001 nutrient enrichment was predominantly sewage-derived organic matter (Figure 20) whereas all sites exhibited the marine source predominantly in 2015 (Figure 21). In 2015, though no evident sewage effluent influence but the isotope values plotted inclined toward seal faecal matter and terrigenous-based matter sources at all other sites sampled (Figure 21).

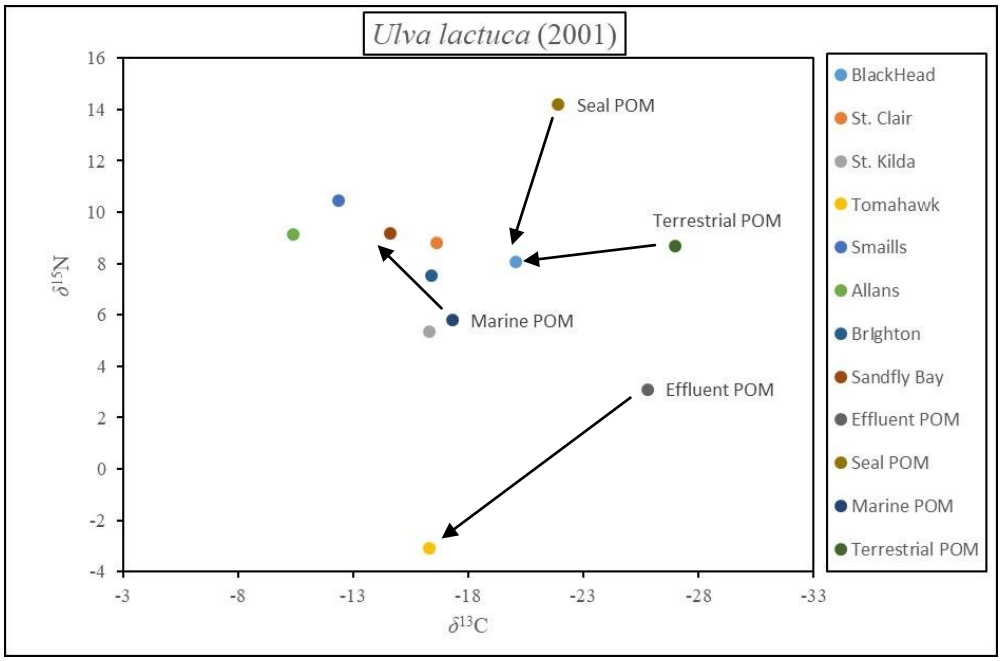


Figure 20: Comparison of carbon and nitrogen stable isotopic ratios in the tissues of *Ulva lactuca* and particulate materials collected in 2001 along the Otago coastline, New Zealand.

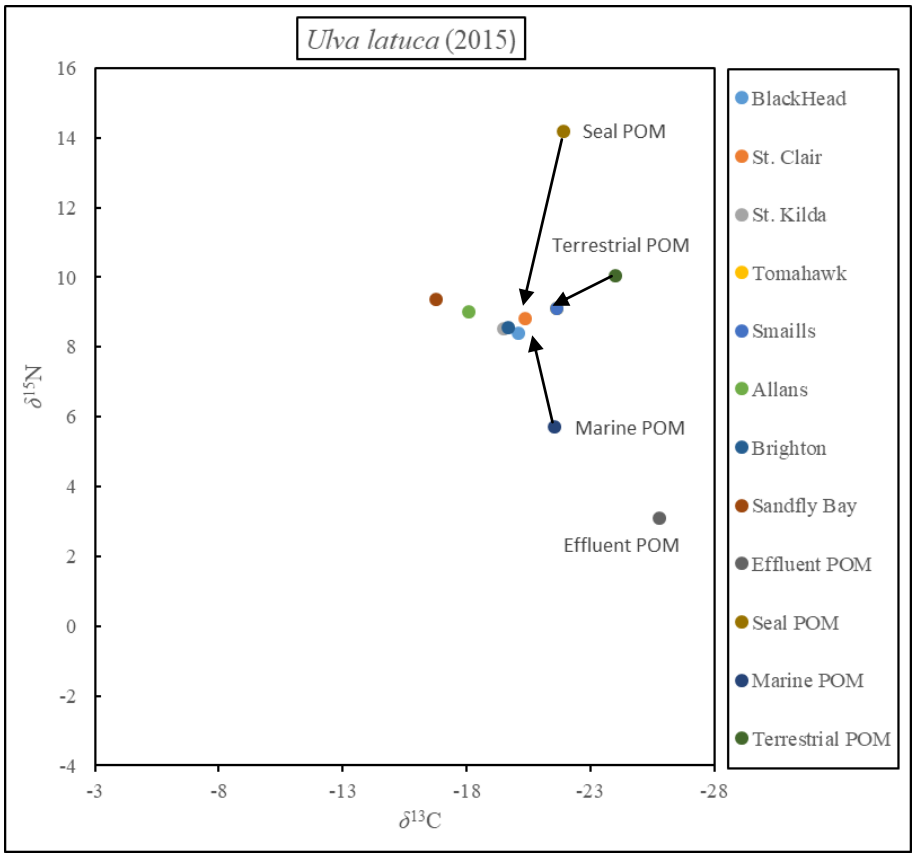


Figure 21: Comparison of carbon and nitrogen stable isotopic ratios in the tissues of *Ulva lactuca* and particulate materials collected in 2015 along the Otago coastline, New Zealand.

4.3.4 Linear Isotopic Mixing Model

Estimating the relative contributions of carbon and nitrogen organic materials from terrestrial sources in the digestive tissues of *Mytilus galloprovincialis* sampled in 2015 to quantify the magnitude of suspended particulate organic matter (i.e. terrigenous materials) in the biological samples. A simple modified two-end member linear isotopic mixing model by Waldron et al. (2001) as expressed in Equation 18 and 19 below was used :

$$\delta^{15}N_{\text{mussel}} = (\delta^{15}N_{POM})\,X + Y(\delta^{15}N_{control}) \quad \text{.....Equation 18}$$

$$\delta^{13}C_{\text{mussel}} = (\delta^{13}C_{POM})\,X + Y(\delta^{13}C_{control}) \quad \text{.....Equation 19}$$

X= contribution from marine/phytoplankton source and Y= (1-X) which is the terrigenous materials (multiplied by 100).

Using the mean isotopic ratios of the POM from filtered water samples collected from Tomahawk Creek ($\delta^{15}N$ = 10.06 ‰ and $\delta^{13}C$ = -27.18 ‰) as the terrestrial source. The mean isotopic ratios of mussels collected from Blackhead (uncontaminated site) (i.e. $\delta^{15}N$ = 7.86 ‰ and $\delta^{13}C$ = -19.98 ‰) as the control end member while $\delta^{15}N_{mussel}$ and $\delta^{13}C_{mussel}$ were for the carbon and nitrogen isotopic ratios of mussels sampled from the previously sewage contaminated sites. We assumed a two-source nutrient enrichment isotopic mixing model for the mussels coming from marine and terrigenous sources.

Based on this assumption, the estimated$\delta^{15}N$-percentage contribution of the marine to terrestrial contribution in the digestive tissues of the mussels collected from each of the previously contaminated sites were as follows; St. Clair (37 % : 63 %), St. Kilda (49 % : 51 %), Smaills (87 % : 13 %) and Tomahawk (52 % : 48 %). The $\delta^{13}C$ - percentage contribution was principally from marine particulate matter for most of the sites. Smaills and Tomahawk had 4 % and 1 % of $\delta^{13}C$ terrestrial particulate matter contribution accordingly.

No influence of terrestrial particulate matter was detected at St. Kilda and St. Clair. Attempt to estimate the contribution of the sewage effluent in the digestive tissue of *Mytilus galloprovincialis* returned negative values for both C and N.

4.3.5 Analysis of Variance between Carbon and Nitrogen Isotopic Signatures in *Mytilus galloprovincialis* and *Ulva lactuca*

The mean carbon and nitrogen isotopic ratio values in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* for 2001 and 2015 were subjected to statistical analysis using the linear

mixed effect models (LMM) to determine if there were significant interactions between the carbon and nitrogen isotopic ratios reported in 2001 and 2015 within and across the study sites. The LMM determined the ratio testing values for the relationship among the variability of carbon and nitrogen isotopic ratios recorded in *Mytilus galloprovincialis* and *Ulva lactuca* across the endmember sites (uncontaminated and previously contaminated).

The outcomes of the interactions are represented in Table 22. Significant differences ($p = < 0.0001$, $n = 169$ (abductor); ($p = 0.0022$, $n = 169$ (digestive) were recorded between 2001 and 2015 nitrogen isotopic ratios in the digestive tissues of *Mytilus galloprovincialis* and tissues of *Ulva lactuca* ($p = < 0.0001$, $n = 30$) collected at the previously contaminated sites. There were no significant differences in the 2015 nitrogen isotopic ratios in the digestive and abductor tissues of *Mytilus galloprovincialis* ($p = 0.383$, $n = 8$ (abductor); ($p = 0.860$, $n = 8$ (digestive). No significant differences was observed in the 2015 carbon isotopic ratios in the digestive and abductor tissues of *Mytilus galloprovincialis* ($p = 0.249$, $n = 6$ (abductor); ($p = 0.84$, $n = 6$ (digestive). No significant differences were observed in $\delta^{15}N$ ($p = 0.848$, $n = 6$) and $\delta^{13}C$ ($p = 0.002$, $n = 6$) in the tissues of *Ulva lactuca* sampled at the uncontaminated and previously contaminated sites in 2015 (Table 22).

Table 22: : Linear Mixed-effects Model (LMM) analysis result of the stable carbon and nitrogen isotope signatures in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* collected in 2001 and 2015 from the various study sites along Otago coastline.

Site contrast	Year	Variable	Tissue	df (n-1)	t ratio	p-value*
Previously contaminated	2001-2015	δ^{15}N	Abductor	169	-3.61	0.0022
Uncontaminated	2001-2015	δ^{15}N	Abductor	169	1.71	0.3202
Previously contaminated	2001-2015	δ^{15}N	Digestive	169	-9.26	<0.0001
Uncontaminated	2001-2015	δ^{15}N	Digestive	169	-3.75	0.0014
Previously contaminated	2001-2015	δ^{15}N	Seaweed	30	-8.09	<0.0001
Uncontaminated	2001-2015	δ^{15}N	Seaweed	30	1.65	0.1102
Previously contaminated	2001-2015	δ^{13}C	Abductor	177	0.52	0.9546
Uncontaminated	2001-2015	δ^{13}C	Abductor	177	0.79	0.8595
Previously contaminated	2001-2015	δ^{13}C	Digestive	177	-6.84	<0.0001
Uncontaminated	2001-2015	δ^{13}C	Digestive	177	-4.98	<0.0001
Previously contaminated	2001-2015	δ^{13}C	Seaweed	30	15.69	<0.0001
Uncontaminated	2001-2015	δ^{13}C	Seaweed	30	34.19	<0.0001
Uncontaminated-Previously contaminated	2001	δ^{15}N	Abductor	6	1.35	0.0170
Uncontaminated-Previously contaminated	2001	δ^{15}N	Digestive	6	2.50	0.0401
Uncontaminated-Previously contaminated	2001	δ^{13}C	Abductor	6	-0.89	0.4067
Uncontaminated-Previously contaminated	2001	δ^{13}C	Digestive	6	1.70	0.1404
Uncontaminated-Previously contaminated	2001	δ^{15}N	Seaweed	6	3.59	0.0115
Uncontaminated-Previously contaminated	2001	δ^{13}C	Seaweed	6	0.43	0.6838
Uncontaminated-Previously contaminated	2015	δ^{15}N	Abductor	8	-0.92	0.3831
Uncontaminated-Previously contaminated	2015	δ^{15}N	Digestive	8	0.18	0.8587
Uncontaminated-Previously contaminated	2015	δ^{13}C	Abductor	6	-1.28	0.2476
Uncontaminated-Previously contaminated	2015	δ^{13}C	Digestive	6	0.22	0.8357
Uncontaminated-Previously contaminated	2015	δ^{15}N	Seaweed	6	0.20	0.8469
Uncontaminated-Previously contaminated	2015	δ^{13}C	Seaweed	6	0.68	0.5235

(*The level of significance was set at 5 %. α = 0.05 is significant)

4.4 Discussion

At the time of the first sampling event (2001), two of the study sites (Smaills and Tomahawk Beaches) were known to be contaminated with sewage-derived matter and exhibited high faecal coliform counts (Greening et al. 2007; Lewis et al. 2010). In 2001, the carbon and nitrogen isotopic ratios in the tissues of *Mytilus galloprovincialis*, and *Ulva lactuca* also show that those two sites (Tomahawk Beach and Smaills Beach) were impacted by discharged sewage effluent evidenced in the lower δ^{13}C and δ^{15}N values.

The tissues of *Ulva lactuca* sampled at Tomahawk (closest site to the outfall) and St. Kilda had lower δ^{15}N values than the other sites suggestive of uptake of sewage effluent.

Sewage effluent and other anthropogenic inputs had been reported to modify the carbon and nitrogen isotopic ratios of macro-algae (Fry 2002; Fry et al. 2003; Gartner et al. 2002; Rogers 2003b; Savage 2005; Savage and Elmgren 2004). This was responsible for the excessive growth of microalgae in nearshore marine waters (Baker et al. 2007; Connolly et al. 2013; Morand and Merceron 2005; Yang et al. 2008).

In 2015, the tissues of *Ulva lactuca* sampled at Tomahawk and St. Kilda was found to have δ^{15}N values elevated by 11.59 ‰ and 3.76 ‰ respectively above those recorded in 2001 indicating a remarkable recovery from sewage influence. There was good agreement between the 2001 and 2015 mean δ^{15}N values in the tissues of *Ulva lactuca* between the recorded values at the uncontaminated sites (i.e. reference sites) which varied between 8 ‰ and 9 ‰ ($p = 0.112$, $n = 30$) suggestive of the absence of sewage organic matter as a nutrient source. However, the recorded δ^{13}C mean values between 2001 and 2015 in the tissues of *Ulva lactuca* was a departure from the observed trend in δ^{15}N at the reference sites varying from -20 ‰ to -10 ‰ ($p < 0.0001$, $n = 30$).

This abnormality in the values of δ^{13}C is attributed to the discrepancies in ^{13}C discrimination during photosynthesis and respiration by the seaweeds. The modifications in the treatment and disposal of sewage effluent appear to have had a profound positive effect on the environmental conditions at the previously contaminated sites. The extension of the outfall pipes would have assisted in limiting the influence of periodic flood currents associated with the tidal and wave actions that usually cascade sewage effluent towards the previously contaminated sites. The digestive tissues of *Mytilus galloprovincialis* provided a good pointer to the declined influence of the sewage-derived exhibiting a trophic enrichment (δ^{13}C ~1 ‰ and δ^{13}N ~3 ‰) towards the marine POM.

Discriminating between the 2010 and 2015 carbon and nitrogen isotopic ratios in the tissues of *Mytilus galloprovincialis* from reference and previously contaminated sites revealed highly significant differences in both abductor and digestive tissues. In 2015, the comparison of the carbon and nitrogen isotopic ratios in the tissues (i.e. abductor and digestive) of *Mytilus galloprovincialis* from reference and previously contaminated sites revealed no significant differences. The carbon and nitrogen isotopic values of the suspended particulate organic matter from filtered water samples of the riverine and estuarine systems with an open

connection to the ocean reflected the diversified nature of the pool of organic materials incursions to the nearshore coastal waters. These sources range from natural to human-induced activities, e.g. atmospheric deposition, organic waste materials from the farm (manure) and grazing marine animals (sea lions and birds), fertilisers and possibly ground-water leachates arising from the nitrification of ammonium from animal organic waste residues underground. Land-based nitrogen sources from tidal channels seemed to sway the nearshore marine waters while carbon sources were predominantly marine origin.

The macro-algae communities (such as *Macrocystis sp*.) that are washed onshore and into the tidal channels contribute a substantial amount of carbon and nutrients to the nearshore waters and freshwater systems along the coastline (Duggins 1988; Hepburn et al. 2007; Michelou et al. 2013). Other likely sources are sewage-derived matter influxes from pastoral animals, farm organic manure, detrital matter (i.e. decomposition of plant materials, seagrasses in estuaries), rural runoffs and watershed catchment area.

4.5 Conclusion

Carbon and nitrogen isotopic ratios in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* were found to be suitable indicators for investigating the impact of sewage-derived organic matter and possible terrigenous materials on the nearshore marine waters. *Mytilus galloprovincialis* and *Ulva lactuca* were disrupted by sewage effluent at the two previously contaminated beaches in 2001. A repeat survey in 2105 showed dramatic changes in the isotope ratios of these sentinel organisms from the values reported 2001, suggestive of the modifications in the treatment and disposal of sewage effluent at the outfalls positioned at the previously contaminated sites. The isotopic ratios of the sentinel organisms from previously and uncontaminated sites were indistinguishable in 2015, which was a departure from the survey conducted in 2001. Mixing models reveal mussels at the two previously contaminated beaches not only get most of their nutrition from marine POM but also a subsidy from terrestrial sources.

However, the δ^{15}N values (SPOM) of filtered water samples from Akatore Creek and Taieri Mouth Beach showed that the rivers receive atmospheric deposition while the SPOM value from Tomahawk creek maybe from δ^{15}N-sewage possibly from animal organic waste, organic fertilisers or ground water from leaching septic system arising from nitrification of ammonium (Aravena et al. 1993; Steffy and Kilham 2004).

The reported varying SPOM isotopic ratios indicated that the Otago coastal marine waters might be influenced by a variety of terrestrial-based organic matter arising from human activities. The two-source mixing model linear mixing model may perhaps not be well apt to quantify the contributions of the different sources of N and C in the tissues of the sentinel organisms. The model assumes that nutrient sources (marine and terrestrial sources) have stoichiometric identical; they contain exactly the same relative carbon and nitrogen contents, and they are assimilated with equal efficiency. The results from these assumptions were based on the fact that carbon and nitrogen isotopes are totally homogenized in the consumer's body prior to tissue synthesis.

5.0 Implication for Future Studies

The sentinel organisms provided an opportunity for further temporal and spatial scale isotopic studies juxtaposed with complex conceptual isotopic mixing models capable of incorporate differences in the food's stoichiometry, assimilation efficiency and homogeneity assumption. The intended mixing models must be able to de-convolute multiple nutrient sources using stable isotope data to assess their proportional contributions. The application of biochemical tracers to trace the sources, flow, cycling and providence of nutrients and organic materials in the nearshore marine waters should also be of great consideration.

The survey of the environmental conditions, biological activity and hydrodynamics of the nearshore waters will also provide additional insight into the underlying mechanisms responsible for the cross-boundary (i.e. land–ocean coupling) transfer of nutrients and organic materials to the nearshore waters and their eventual sequestered to the nearshore marine food web.

The published results of this chapter are in:

Barbaranti Oluwuyi, Stephen Horn, Tim Jowett and Russell Frew (2019). Isotopic signatures in *Mytilus galloprovincialis* and *Ulva latuca* as bioindicators for assessing discharged sewage effluent in coastal waters along Otago Peninsula, New Zealand. *Geology, Ecology, and Landscapes*, 3(1), 53-64.

Oluwuyi Barbaranti conducted the majority of the experimental work and drafted the manuscript. Stephen Horn conducted the 2001 sampling and analysis. Tim Jowett provided advice and comments on the manuscript draft. Russell Frew supervised the project and provided comments on the manuscript.

CHAPTER 5

Stable isotopic inquest: Insight into the flow, fate of nutrients and organic materials at the intertidal mixing zone of Otago coastal waters, New Zealand

Overview

This chapter examines the potential transfer, fate and influence of terrestrial-based organic materials (i.e. sewage-derived organic matter) as nutrients and contaminants on the coastal marine waters. These materials may be quotas of sustenance for coastal marine fisheries (i.e. bivalve). This chapter serves as a follow-up study to investigate the possible influence of sewage-derived organic materials (from terrestrial) on the nearshore marine waters from proximate water bodies. The limitations of the two-source stable isotope mass balance model (used in the previous study) could account for the various potential N-source material contributions from the terrestrial source. Hence, the carbon and nitrogen stable isotopes of the resident organisms and probable terrestrial-based organic materials were built-in into a Bayesian constrained isotope mass balance mixing models (MixSIAR) to quantify their contributions in the tissues of marine bivalves collected from the coastal marine waters. Two coastal marine waters (which were portions of the ten beaches previously studied) influenced by tidal channels with contrasting hydro-chemical dynamics and varying degree of pastoral farming were selected for this study. Allans and Smaills Beaches impacted by Hoopers Inlet (tidal estuary) and Tomahawk Creek (freshwater stream) were designated as study sites. The Bayesian mixing model was used to conceptualise and assess different scenarios to quantify sewage-derived organic matter and other organic materials of interest sequestered in *Mytilus galloprovincialis*.

The main goals of this chapter are to:

- Determine the source contributions of terrestrial-based sewage organic materials in *Mytilus galloprovincialis*.
- Assess the potential influence of land–ocean connectivity on trophic subsidies in coastal marine food webs.

This was done to test the notion if there is an exchange of organic materials across ecosystem boundary, i.e. terrestrial to ocean margin and that these materials can serve as sustenance subsidy for the coastal marine fisheries.

5.1 Introduction

Estuaries are regarded as interfaces between riverine and marine systems, principal locations of interaction between man and the ocean (Simenstad 1983). They support ecosystem services such as the movement of organic materials and nutrient cycling (Savage et al. 2012; Thrush et al. 2013). They are highly productive (McLusky and Elliott 2004; Nixon et al. 1986), nutrient traps (Correll 1978), breeding and nursery grounds for fishes inhabiting the open ocean (Beck et al. 2003; Biggs and Cronin 1981; Peterson 2003; Smith and Parrish 2002). They are affected by tides, winds, and riverine overspill that induce significant fluctuations in their environmental conditions at cyclical and time-based scales (Ciborowski et al. 2008; De Jonge and Van Beusekom 1995; Officer and Kester 1991).

In most estuaries, the major trepidation is eutrophication caused by nutrient loading is linked to increasing human activities. Consequential increase in the level of nutrients and contaminants come from point sources such as municipal wastewater treatment plants, agricultural and industrial areas. This has brought about an increase in nutrient and organic matter cycling, alteration of ecosystem functioning due to accelerated growth rates of algae in spring and summer essentially regulated by the availability of nitrogen, phosphorus or both (Allan and Castillo 2007; Galloway et al. 2003; Hanisak 1983; Pedersen et al. 2010; Webb 1981). The environmental trepidations of such rapid algal growth are; decrease in water transparency, production of harmful algal toxins, asphyxiation of benthic biota (i.e. shellfish) and modification of sediment and water column chemistry (Cloern 2001; Heip 1995; Sfriso et al. 1992).

The exchanges (predominantly in estuaries) between terrestrial and marine inputs influence nutrient and organic matter cycling, primary and secondary production in nearshore marine systems (Cloern et al. 2014b; Levin et al. 2001; Polis et al. 1997; Talley et al. 2006). Studies have indicated that nutrients, organic matter and materials in estuaries can originate from internal sources (autochthonous) (Rabalais et al. 2009) or external sources (allochthonous) from neighbouring adjacent rivers, streams and upland vegetation (Kelly and Levin 2012; Thornton and McManus 1994). Nutrient and organic matter cycling are controlled by biomass production and heterotrophic bacterial respiration (Findlay et al. 1992; Fuhrman 1992; Williams 2000). Consequently, the net ecosystem production (NEP) which is the ratio of primary production and respiration (P/R) could be a good cursor for evaluating the relative significance of internal and external sources of organic carbon inputs and metabolic shifts.

The ratio may also reveal the sources of organic carbon, state of energy flow, efficiency and utilisation in coastal ecosystems.

The unique and dynamic nature of estuaries with the influence of fluxes of nutrients and organic materials from riverine inputs (Hedges et al. 1997) and transport of such to the coastal zones (mixing zones) of the ocean possibly hinder the definite identification and quantification of sources and sink of nutrients, organic matter and materials in nearshore coastal marine ecosystems. Marine and freshwater influxes into the estuaries are chiefly responsible for the episodic environmental fluctuations that produce changes in the physical, chemical and biological properties of estuarine waters. The bi-directional tidal flow drives the movement of nutrients and organic matter between and within the estuary-ocean interface. The hydrologic equilibrium between the ebb advection of freshwater and flood salt-water incursion in estuaries can cause major fluctuations in water indicators. They affect temperature and salinity, particulate organic matter composition and chlorophyll-a concentration (Cloern et al. 1989; De Jonge and Van Beusekom 1995; Grossart et al. 2004; Guinder et al. 2009; Magni et al. 2002; van Leussen et al. 1996; Velegrakis et al. 1997).

The quantity and flux of terrigenous materials transported from tidal channels to nearshore coastal marine ecosystems are suggestively controlled by the changes in the seasonal environmental conditions and other factors such as tidal frequency, level of primary production in adjacent freshwater or estuary (Ahad et al. 2008). The flushing time in estuaries is also an essential factor for determining critical estuarine processes such as the retention time for nutrients, wastes, sediment, and organic matter, tidal circulation and mixing processes of nutrients and contaminants (Montagna et al. 2012).

Carbon stable isotope ratios can be used to elucidate the impact of nutrients and organic matter on the primary production and trophic relationships in nearshore inlet waters as they can provide a snapshot of the biogeochemical cycling of carbon. The nitrogen stable isotope signatures can be utilised to trace sources of nutrients and trophic position of the resident biota. For instance, several workers such as Evgenidou and Valiela (2002), Shriver et al. (2002), Weiss et al. (2002) and Carmichael et al. (2012) have successfully used N-stable isotope ratios to trace N from sources on land to suspended particles in the water column (food sources) and eventually to tissues of bivalves feeding in estuaries. They were able to link land-derived sources of nutrient enrichment to specific episodic symptoms of eutrophication that in turn had an effect on the growth and survival of different bivalve

species. In their studies, they compared the growth and survival of transplanted of native bivalves to eutrophic-driven changes in food supply and environmental conditions.

There are various ecological conceptual models to elaborate the complex relationships between habitat dynamics, ecosystem processes and biodiversity in aquatic ecosystems. These models are the floodplain concept (FPC) for river-floodplains, riverine productivity Model (Thorp and Delong 1994) and river continuum concept (RCC) for large rivers (Sedell et al. 1989; Vannote et al. 1980) and outwelling hypothesis (OWH) for tidal marshes (Odum 2002). These models have been extensively used in the narrative for elucidating the sources and transfer of nutrients and organic matter in aquatic biomes. The first three models are essentially relevant to river-riparian systems, whereas the latter mentioned referred to river-marine systems.

The outwelling hypothesis postulates that eroded coastal wetlands have the potential to discharge substantial amounts of organic nutrients and matter into adjacent bays, inlets and estuaries (Odum 1980). This is based on the notion that eroded tidal, coastal wetland systems such as marsh-estuarine systems are capable of producing more organic materials than can be degraded or stored within them, and that the excess organic materials can probably be exported to the coastal ocean where they support near coastal ocean productivity.

Outwelling increases the productivity of coastal fisheries and plants (i.e. algae), nourishes plankton communities and causes abrupt increases in biological and chemical activities (Wolanski and Elliott 2015). Exported excess freshwater nutrients and organic matter from declining coastal wetlands to watershed basin and eventually into nearby coastal waters could promote high primary productivity ensuing coastal hypoxia (Dagg et al. 2007; Das et al. 2011; Rabalais et al. 2007; Turner et al. 2008). The exploitation of stable isotopic ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in marine biota can elucidate the impact of nutrients and organic matter on primary production and trophic relationships in coastal marine waters as they can provide a tracer of the biogeochemical cycling of carbon and nitrogen.

Hence, this study is intended to exploit stable isotopic ratios of elements of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in evaluating the relationship between the fluxes and cycling of nutrients and organic materials in the nearshore tidal channels and their receiving nearshore marine waters. The carbon and nitrogen stable isotopic ratios in resident biota and suspended particulate organic matter in the water column will be used to elucidate the exchanges of nutrient and organic materials in the coastal waters and resident biota. They are powerful

ecological indicators due to their relatively well-preserved natural state in the water column, stability during biochemical reactions, their plausible transmission and interment in tissues of the resident nearshore marine biota (i.e. *Mytilus galloprovincialis*). The survey of the variance of carbon and nitrogen isotopic ratios in the tissues of *Mytilus galloprovincialis* sampled at the nearshore marine waters revealed that the digestive tissue is most appropriate for tracing nutrients and contaminants in the nearshore marine waters

Previous study of assessing the impact of long-term discharged sewage effluent on the nearshore coastal waters, the digestive tissues was found to have exhibited a trophic enrichment factor of 3 ‰ (δ^{15}N) and 1 ‰ (δ^{13}C) when compared to the isotope values for marine particulate organic matter (POM), suggestive of a dietary change away from the sewage derived organic matter (Babaranti et al. 2019).

Suspended POM collected from freshwater and estuarine water samples were suggestive of other possible nitrogen sources from human-driven activities such as pastoral farming, application of fertilisers, nitrification of ammonium from semi-urban septic tanks, and animal organic waste residues. Other land-based organic materials that could be of interest was not be accounted for due to the limitation of the isotope linear mixing model employed. Though, emphasise was not placed on the prevailing environmental conditions of the coastal marine waters.

Hence, the use of the carbon and nitrogen isotopic ratios of *Mytilus galloprovincialis* appears more propitious in elucidating the transfer of nutrients and organic materials at the exchanges of the coastal marine waters. However, it may be constrained by the unpredictability of the stable isotopic ratios of *Mytilus galloprovincialis* influenced by the high spatial and temporal heterogeneity in the quality and quantity of the primary producer (food source). There can also be overlapping of stable isotopic signatures of source end members (Canuel et al. 1995; Cloern et al. 2002; Phillips and Gregg 2003).

These limitations mitigated by prolonged sampling period and the use of robust isotope mass balance mixing models. In addition, the hydrological processes and environmental indicators in the coastal marine waters that may influence the biological and chemical processes occurring at the exchange interfaces between and within the coastal marine waters will be considered.

Consequently, the objectives of this study are to:

1. Examine the hydro-chemical dynamics of the tidal channels and the receiving coastal marine waters to determine if anthropogenic elements influence the coastal marine waters.

2. Pinpoint sewage derived organic materials of interest from land-use activities in *Mytilus galloprovincialis* to indicate nutrient supply and contaminant flux in the coastal marine waters.

3. Determine the contributions of land-based organic materials (particularly sewage-derived organic matter) assimilated alongside the diet of a resident organism to indicate the degree of nutrient supply and organic contamination in the receiving nearshore marine waters.

4. Examine the mechanisms involved in the transfer of terrestrial-based organic materials from the tidal channels to the receiving coastal marine waters and their eventual sequestration in resident organisms.

The above objectives are to address these fundamental hypothetical questions:

- Do the prevailing environmental factors of the coastal marine waters and proximate tidal channels indicate anthropogenic (human-driven) influence?
- Are there significant quantities of terrestrial-based organic materials (i.e. sewage-derived organic matter) being transferred from proximate tidal channels to coastal marine waters?
- What quantities are these terrestrial-based organic materials sequestered in the resident biota collected from the coastal marine waters?
- What are the essential processes responsible for the trans-boundary conveyance and distribution of organic nutrients and contaminants across land-sea margin?

In the attainment of the listed objectives to address the relevant hypothetical questions, the study will have provided information on the significance of proximate water bodies (i.e. tidal estuaries) in the transboundary conveyance and distribution of organic nutrients and contaminants arising from human activities to the receiving nearshore marine ecosystem. It

will also assist in clarifying the consequence of organic nutrients and contaminants from proximate water bodies as food resource subvention to the nearshore marine fisheries.

The outcomes from the study obtained will enhance better understanding of the nutrient supply and contamination dynamics of the nearshore marine waters, the itinerary of nutrients and organic materials in land-ocean margins, and role of tidal estuaries in biogeochemistry of nutrients and organic matter recycling, energy flow and budget in coastal ecosystems. This will give enhanced insight into the organic nutrient supply and contaminant dynamics of the coastal waters.

5.2 Materials and Methods

5.2.1 Study Area

Two coastal marine waters (Allans and Smaills Beaches) influenced by tidal channels of distinct hydrographical features were studied (Figure 22). They are remotely situated approximately 10 kilometres from each other along the Otago Peninsula, Dunedin on the east coast of the South Island of New Zealand. Allans and Smaills Beaches are positioned on 45.857 S; 170.679 E and 46.019 S; 169.089 E (Table 23) and influenced by freshwater incursions via tidal channels; Hoopers Inlet and Tomahawk Creek respectively.

Table 23 : Sampling site names and their coordinates.

Site name	Latitude	Longitude
Allans	45.857	170.679
Hoopers Inlet	45.861	170.669
Smaills	46.019	169.089
Tomahawk Creek	46.019	169.089

Allans Beach amasses freshwater incursions from Hoopers Inlet (a tidal mudflat saltmarsh habitat) located at the east end of the beach is 1800 m wide and 3000 m long with an area of 3.70 km^2 (Albrecht and Vennell 2007). It is a shallow tidal-dominated estuary lagoon which is 65 – 75 % exposed (Albrecht and Vennell 2007) and susceptible to closure, most probably during periods of low wave activity during which sand is deposited by wave action at the inlet's mouth. The tidal flushing time of water in the estuary varies between 5 - 6 h. The Hoopers Inlet has a catchment area of 33 km^2 (Konlechner 2013) with three small streams (such as the Battery, Stewarts and Weipers creeks) discharging directly into the inlet. The

inlet has a significant estuarine value habitat for birds and nursery for flat fish and recognized as one of the 'coastal protection areas' in the Otago Regional Plan.

Smaills Beach receives incessant freshwater inflows from Tomahawk Creek, a freshwater stream fed by precipitation from three smaller tributaries and flood plains of surrounding watershed catchment basin (with an estimated area of 2 km^2) during heavy rainfall comprising of pasture used for sheep and cattle grazing. Intensive pastoral farming (i.e. cattle and sheep grazing), urban storm-water systems and seepage from septic tanks of semi-urban settlements influence adjoining draining freshwater plumes into the nearshore waters of Smaills Beach. Rural runoff, forestry activities, long-term intensive pastoral farming and dredged waterway on farm, stock effluent runoff, influence the draining freshwater plumes of Allans Beach. In summer and autumn months, the stream (i.e. Tomahawk Creek) flow rate was typified by long periods of low flow rate < 10 m^3 s^{-1} (with a drainage area of 2 km^2) while high flow rate generally occur in winter and spring with stream discharge reaching >100 m^3 s^{-1} during storm events.

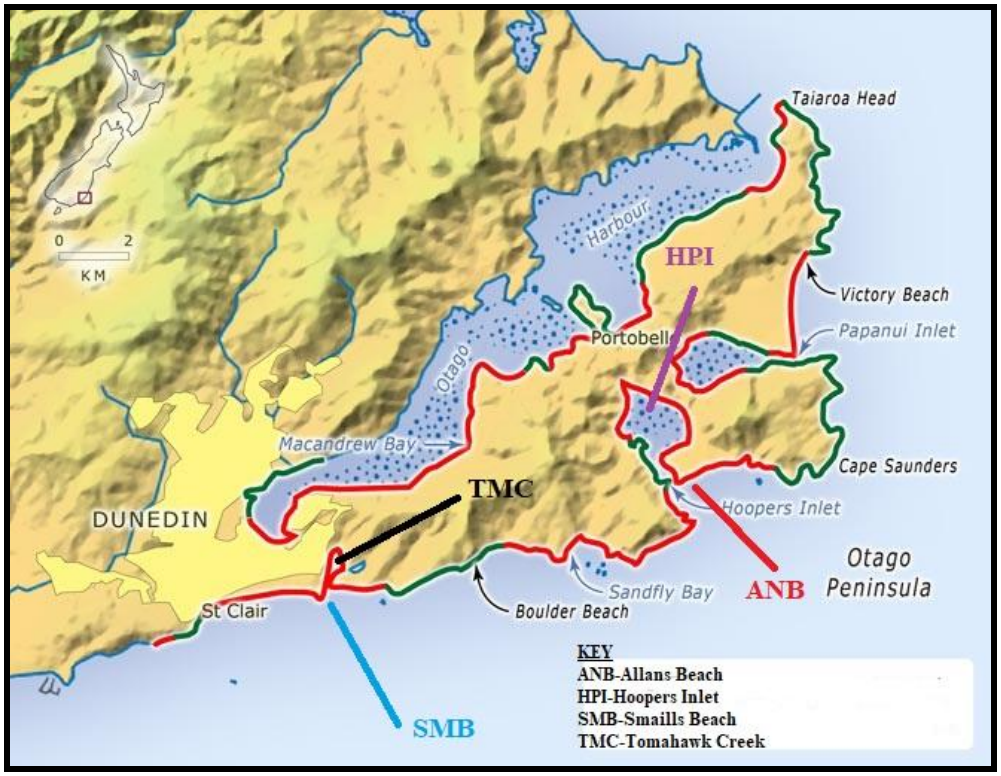


Figure 22: Coastal marine waters (ANB-Allans Beach; SMB-Smaills Beach) and their respective proximate tidal channels (HPI-Hoopers Inlet; TMC-Tomahawk Creek) studied

5.2.2 Sampling Procedure and Sample Preparation

As described in section 2.2.1- 2.2.2 of Chapter 2

5.2.3 Determination of Carbon and Nitrogen Isotopic Ratios in Samples

As explained in section 2.3.1 of Chapter 2

5.2.4 Nitrates and Nitrites Determination

As elucidated in 2.4.1 of Chapter 2

5.2.5 Reactive Phosphate Measurement

As stated in 2.4.2 of Chapter 2

5.2.6 Chlorophyll a in Water Samples

As described in 2.4.3 of Chapter 2

5.3 Results

5.2.7 Data Analysis

The parameters of water quality indicators were normalised to individual *Z*-scores (via standard deviation) and subjected to principal component analysis (PCA) to determine the relationships among water quality indicators. The statistical analytical tool was used to compare and visualize the Euclidean distances and relatedness among the physical and chemical parameters of the water samples analysed to ascertain the accountable influence for their connections.

Partial correlation analysis was used to compute the strength, degree and direction of the linear association among the water indicator variables via the pairwise comparison of the normalised variables (z-scores) using Euclidean variance-correlation distance with the assumptions that variables were normally distributed and continuous.

Kolmogorov–Smirnov test (KS test), a nonparametric was used to test for the equal (normal) distribution of suspended particulate organic matter between coastal marine waters and their corresponding proximate water bodies. The KS test model was set to compare the distance between the empirical isotopic distribution of suspended particulate organic matter in the water samples at the each of the interfaces via the intersection of asymptote curve lines as limiting approximations of the probability of equal distribution. This was done to confirm if the water samples at each of the interfaces are homogenous (adequately mixed) or heterogeneous (inadequately mixed).

The Moses Extreme Reactions test was employed to determine the magnitude of the interactions between the distribution of suspended particulate organic matter (SPOM) in the

coastal marine waters and proximate tidal channels. This was to confirm if the SPOM in the water samples were from a larger SPOM homogenous or heterogeneous population of the water samples at the interfaces.

To determine the source contributions of the N-source land organic materials in *Mytilus galloprovincialis* collected at the intertidal mixing zone, a robust isotope mixing model in R (MixSIAR) constructed on Bayesian model built upon a Gaussian likelihood with a mixture Dirichlet-distributed prior to the mean δ^{15}N and δ^{13}C isotopic ratios of potential nutrient sources and a consumer was incorporated.

5.3.1 Water Quality Indicators

Monitored physical, chemical and biological indicators of the waters from each of the study sites are represented in Table 24. The physical parameters appeared variable based on the prevailing environmental conditions of the respective seasonal cycle. Allans, Hoopers and Smaills were typically saline. Tomahawk Creek, a freshwater stream had slightly lower salinity in summer than winter, summer and autumn attributed to episodic influx of freshwater from intermittent precipitation.

The average water temperatures across all the sites varied between 8.13 °C and 16.70 °C (Hoopers Inlet). Water temperatures were lower in freshwater stream comparatively to the estuary (Hoopers Inlet) and coastal marine waters. This can be attributed to the fact that estuaries and oceanic waters possess can absorb and conserve heat better than freshwater (whose temperature is greatly influenced by precipitation and seasonal fluctuations).

Hoopers Inlet, a shallow estuary, was highly saline throughout the season (ranging from 31.2 to 34.4) with winter period having the lowest salinity measurement. Dissolved oxygen levels were higher in winter, spring and summer (varying from 4.7 to 12.7 mg/l) but significantly lower in autumn across all the sites studied. Nitrate levels were predominantly higher (ranged between 8.7 to 17.4 µM) at Tomahawk Creek than all the other study sites. Hoopers Inlet had lower nitrate concentrations comparable to the two nearshore marine waters at the intertidal mixing zones (i.e. Allans and Smaills) ranging from 0.5 in summer to 1.1 µM in winter. Nitrate concentrations were slightly higher at the intertidal mixing zones of Allans (4.0 µM) in winter and Smaills in autumn (6.8 µM).

Nitrite concentrations were < 1 µM across all the sites appraised. Chlorophyll a concentration was higher in summer at the tidal channels and respective proximate nearshore oceanic

waters, which ranged between 1.05 and 1.70 µg/l; than observed in winter and spring, which varied from 0.42 to 1.30 µg/l. The pH of the estuarine and nearshore oceanic waters was observed to be nearly 8 while freshwater samples were approximately 7. Phosphate concentrations were higher in freshwater (varied between 0.66 and 1.73 µM) than estuarine and nearshore oceanic waters samples an indication of runoffs via watershed catchment area. The nitrate levels in the riverine system (TMC) were consistently higher (varied between 8.69 and 17.38 µM) throughout the study period and impacted, the nearshore marine waters at Smaills Beach.

Table 24: Mean values and standard errors (underneath in italics) of the water quality indicators at the coastal waters of Allans Beach, Smaills Beach and waterbodies of Hoopers Inlet and Tomahawk Creek from 22/7/16 to 29/4/17 covering one seasonal cycle.

Site	pH	Sal.	Cond. µS/m	Temp. °C	D/O mg/l	NO_3 µM	NO_2 µM	PO_4 µM	Chl. a µg/l
				Winter					
Allans	8.10	32.58	37.24	8.93	12.68	3.97	0.25	0.86	0.45
	0.17	*0.94*	*5.67*	*0.79*	*0.42*	*1.30*	*0.04*	*0.14*	*0.12*
Hoopers	7.86	31.23	37.67	8.13	15.25	1.14	0.15	0.82	0.66
	0.26	*2.26*	*5.50*	*1.20*	*2.45*	*0.91*	*0.01*	*0.24*	*0.17*
Smaills	8.06	31.73	36.76	9.83	12.04	5.37	0.30	0.95	0.59
	0.03	*0.23*	*2.46*	*0.49*	*0.45*	*0.68*	*0.14*	*0.16*	*0.07*
Tomahawk	7.53	0.83	0.97	6.98	10.39	8.69	0.41	1.00	0.66
	0.11	*0.27*	*0.23*	*1.00*	*0.98*	*4.80*	*0.29*	*0.15*	*0.10*
				Spring					
Allans	7.97	34.38	47.77	12.60	11.68	2.38	0.36	0.88	0.42
	0.08	*0.23*	*4.77*	*0.20*	*0.56*	*0.36*	*0.15*	*0.25*	*0.11*
Hoopers	7.99	33.03	40.56	14.83	10.93	0.79	0.31	0.75	0.66
	0.12	*0.79*	*1.35*	*1.36*	*0.55*	*0.24*	*0.13*	*0.18*	*0.18*
Smaills	7.87	30.40	36.20	11.75	11.75	4.74	0.40	0.83	1.30
	0.12	*2.59*	*2.24*	*0.47*	*0.58*	*1.51*	*0.16*	*0.23*	*0.57*
Tomahawk	7.28	0.40	0.54	11.10	8.67	17.38	0.29	1.73	0.92
	0.11	*0.07*	*0.10*	*1.10*	*1.65*	*2.80*	*0.17*	*0.56*	*0.23*
				Summer					
Allans	7.88	31.38	41.25	15.85	9.82	1.10	0.19	0.37	1.07
	0.03	*3.13*	*5.04*	*0.80*	*2.63*	*0.29*	*0.03*	*0.08*	*0.15*
Hoopers	7.90	32.43	43.97	16.70	8.30	0.49	0.19	0.27	1.72
	0.04	*0.98*	*2.33*	*1.00*	*2.17*	*0.24*	*0.02*	*0.08*	0.38
Smaills	7.84	32.38	38.30	14.15	9.68	2.25	0.20	0.30	1.05
	0.05	*1.02*	*1.27*	*0.35*	*1.74*	*0.64*	*0.01*	*0.17*	*0.25*
Tomahawk	7.10	1.25	1.77	16.08	9.23	12.23	0.36	0.72	1.40
	0.07	*0.92*	*1.27*	*1.27*	*1.89*	*1.16*	*0.18*	*0.11*	*1.03*
				Autumn					
Allans	8.02	33.50	42.20	13.75	4.12	1.87	0.24	0.31	0.50
	0.01	*0.66*	*0.42*	*0.75*	*0.17*	*0.75*	*0.10*	*0.10*	*0.14*
Hoopers	7.89	32.93	43.83	14.30	3.43	0.94	0.15	0.37	1.37
	0.07	*1.51*	*3.25*	*1.42*	*0.47*	*0.52*	*0.02*	*0.16*	*0.57*
Smaills	7.95	31.45	39.55	12.80	3.86	6.76	0.32	0.37	0.52
	0.01	*1.50*	*2.74*	*0.41*	*0.21*	*4.92*	*0.13*	*0.12*	*0.07*
Tomahawk	7.36	1.03	1.78	11.98	4.74	16.19	0.52	0.66	0.28
	0.06	*0.42*	*0.71*	*0.47*	*0.26*	*2.95*	*0.12*	*0.09*	*0.11*

5.3.2 Principal Component Analysis (PCA) and Stacked Plot

Principal Component Analysis (PCA) is an analytical method that allows the reduction of the dimensionality of variations in data set while maintaining the characteristics of variables, which contribute most to the variations. The purpose of using the PCA in this instance is to evaluate the water quality parameters of the coastal marine waters and their respective proximate water bodies. This to assist the interpretation and extraction of the most important parameters for the assessing the mechanism responsible for observed variations in water quality of the coastal marine waters and their respective proximate water bodies. Since measured numerous parameters are likely interrelated, there is the need to determine the ecological drivers of the observed variations in the water quality parameters are characterised based on seasonal factors or anthropogenic influences or both.

The principal component analysis allows for the categorization of the various measured water quality indicator parameters into interconnected units of association for proper interpretation of the predominant environmental processes at the nearshore marine waters and their respective proximate water bodies. The main purpose of using the PCA was to assess the relationship among the physico-chemical parameters of the water samples collected at each of the sites.

The component 1 and 2 of the PCA accounted for 39.4 % and 21.8 % (i.e. Eigenvalues) of the observed variance, respectively (Figure 23). The PCA structure of the component 1&2 distinctively registered the water samples on seasonality dictated by the prevailing environmental conditions and characteristic feature of the aquatic system. The freshwater samples were distinguished from the oceanic and estuarine waters by variations in phosphate, nitrate and nitrite concentrations. The spring and winter water samples were clearly distinguished from autumn and summer water samples as a result of variations in salinity, conductivity, dissolved oxygen, phosphate and nitrite concentrations.

The PCA loadings for component 1 of the high dimension having water samples collected in spring and winter from the estuarine (Hoopers Inlet) nearshore oceanic and freshwater bodies categorised by high pH, salinity, dissolved oxygen, conductivity, phosphate and moderate chlorophyll-a concentrations.

The component 1 of the low dimension of PCA was characterised by lower pH, salinity with high nutrient (nitrate, nitrite and phosphate) and moderate chlorophyll-a concentrations. The PCA loadings for component 2 of the high dimension indicated waters samples collected in

summer and autumn were inter-related by moderate salinity, conductivity and moderate dissolved oxygen and phosphate concentrations.

The component 2 of the low dimension of PCA were characterised by high temperature and chlorophyll a concentration. Overall, the freshwater samples were nutrient-rich, and the nearshore oceanic waters had freshwater incursions. Coastal oceanic water samples collected and tested after heavy flood pulse (i.e. SMB-SP2, SMB-AU4 and HPI-WN4 in Figure 23) displayed distinctive feature from compared to previously tested water samples from the same site-season category.

In general, the PCA analysis displayed the differences in physical and chemical parameters of the water samples analysed from each of the sites; these differences were subtle and not so evident to separate water samples analysed from the estuary and coastal marine sites according to their environmental consistency. However, the noticeable differences agreed well with the predominant seasonal conditions and nature of the site (habitat type) studied rather than anthropogenic influences. Therefore, a stacked graph of the normalised individual Z-score values (via standard deviation) of the water quality indicator parameters based on site-season category was developed to inspect the degree of influence and prominence of each of the water indicators (see Figure 24).

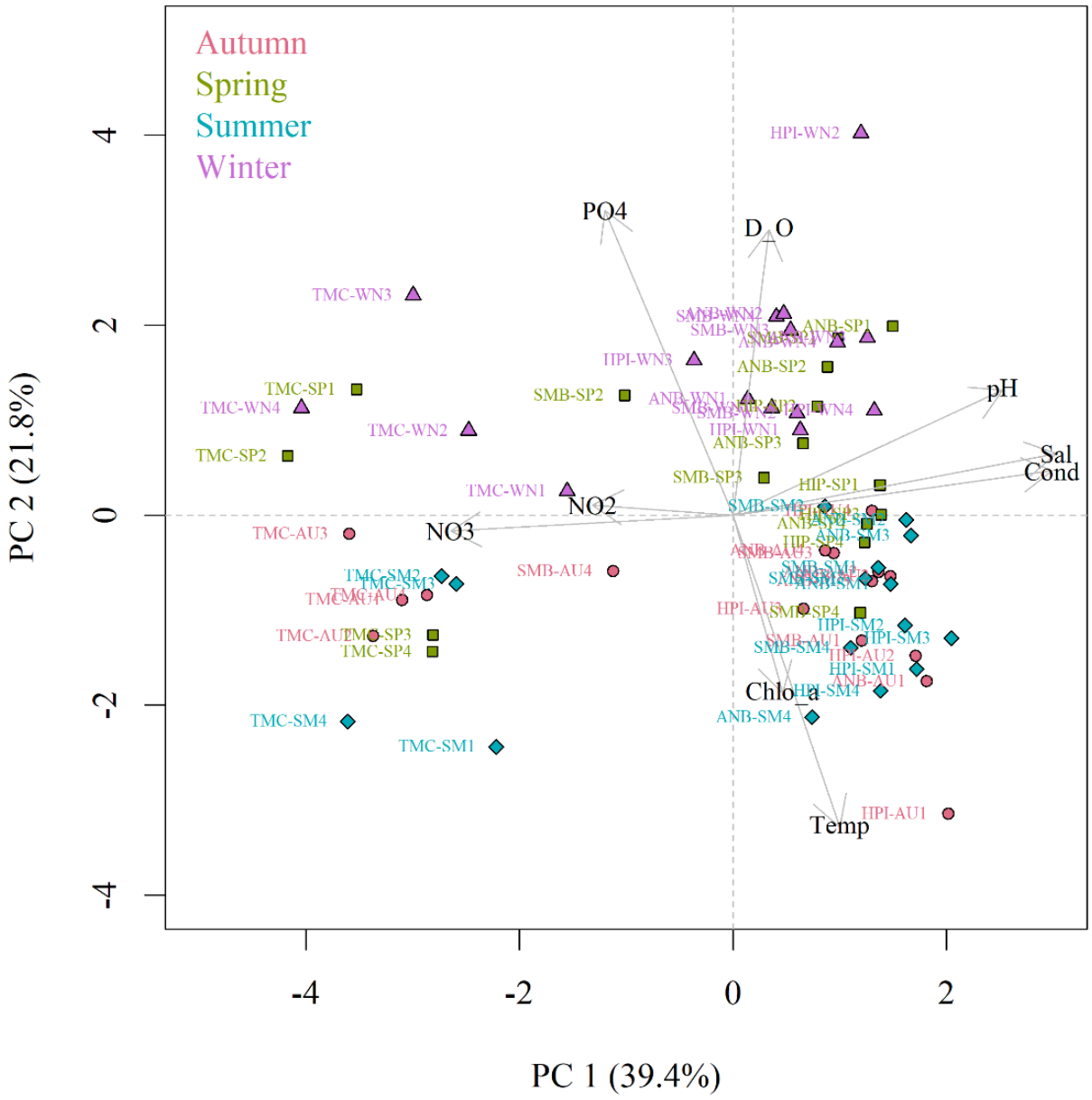


Figure 23: PCA Scatter bi-plot of water quality indicators (pH, Salinity, and site-season. Site and season codes indicated are ANB (Allans Beach), HPI (Hoopers Inlet), SMB (Smaills Beach), TMC (Tomahawk Creek), AU1-AU4 (autumn), SM1-SM4 (summer), SP1-SP4 (spring) and WN1-WN4 (winter).

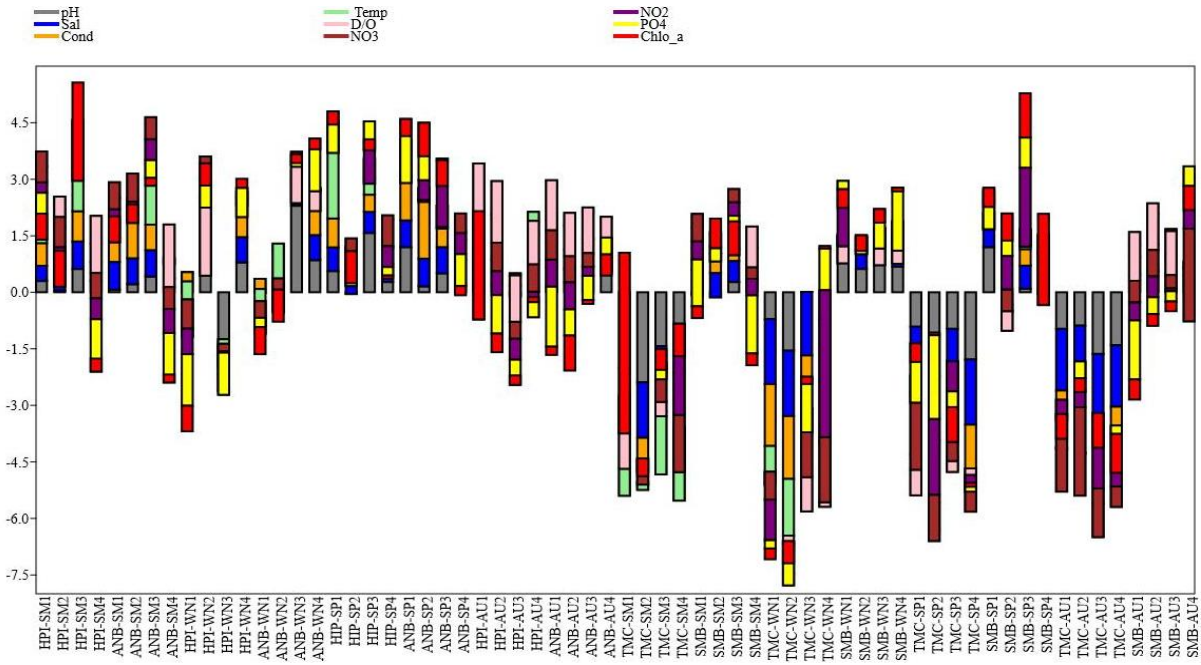


Figure 24: Stacked graph of the normalised individual Z-score values (via standard deviation) of the water quality indicators. Site and season codes indicated are ANB (Allans), HPI (Hoopers), SMB (Smaills), TMC (Tomahawk), AU1-AU4 (autumn), SM1-SM4 (summer), SP1-SP4 (spring) and WN1-WN4 (winter).

The pH, salinity, nutrient (nitrate and phosphate), dissolved oxygen appeared to be the main indicators in the hydrological conditions of the coastal marine waters and proximate water bodies. Temperature seemed a pertinent indicator of the coastal waters in summer, winter and spring. Chlorophyll a concentration, an indicator of the measure of biological productivity of the coastal marine waters was pertinent in summer and spring and less prominent in winter and autumn (Figure 24). Nevertheless, moderate chlorophyll a concentrations were recorded in winter attributed to favourable nutrients and water chemistry (pH, salinity and conductivity) moderate temperature and sunlight, stable/mixing conditions and low turbidity. This agrees with the observations of various coastal marine and estuarine monitoring studies described in Frolander (1964), Davies-Colley (2013) and Dudley (2018).

5.3.3 Pearson Correlation Coefficient (R)

The normalised Z-score (via standard deviation) of the water quality indicator were subjected to Pearson correlation coefficient to determine the association between each of the water quality indicator parameters. Changes in the chemical and physical characteristics of water are one of the signs of organic contamination, examining the association between each

of the water quality indicators could provide information on the health status of the water bodies. The outcomes of their interactions are represented in Table 25.

Hydrogen ion concentration had a correlation value of 0.79 (n = 63) with salinity and 0.76 (n = 63) with conductivity. Salinity was closely related to conductivity (0.96, n = 63) but had a negative association with nitrate concentration (-0.75, n = 63). Conductivity was negatively linked to nitrate concentration (-0.72, n = 63). Temperature was negatively associated with phosphate concentration (-0.51, n = 63). Nitrate concentration was significantly connected to nitrite concentration (0.34, n = 63).

Table 25: Pearson correlation coefficient (R) of each of the pairwise comparison of water quality indicators in the water samples (n = 63) collected conducted at 95 % confidence level (i.e. of 0.005). The bold figures denote significant differences.

	pH	Sal	Cond	Temp	D/O	NO_3	NO_2	PO_4	Chlo. a
pH									
Sal	0.79								
Cond	0.76	0.96							
Temp	-0.03	0.20	0.26						
D/O	0.20	0.13	0.09	-0.38					
NO_3	-0.50	-0.75	-0.72	-0.21	-0.13				
NO_2	-0.21	-0.25	-0.26	-0.07	-0.12	0.34			
PO_4	-0.16	-0.24	-0.18	-0.51	0.44	0.26	0.18		
Chlo. a	-0.08	0.05	0.06	0.32	0.09	-0.08	-0.13	-0.30	

Overall, conductivity, dictated by the amount of total dissolved solids in water exhibited a robust association with salinity and pH.

5.4 Spatial and Temporal Variations of Carbon and Nitrogen Isotope Ratios in Samples

5.4.1 Water Samples

The mean nitrogen isotope signatures in the suspended particulate organic matter obtained from filtered water samples collected at the intertidal mixing zones of the nearshore waters ranged between 4.5 ‰ in Allans during autumn and 6.7 ‰ in Smaills during summer. The SPOM mean δ^{15}N values tend to be uniform in winter (roughly 5.7 ‰) but higher in spring and summer (approx. 6.3 ‰). The mean δ^{15}N values tend to be higher at Tomahawk Creek (ranged from 8.2 to 12.4 ‰) than recorded at the intertidal mixing zones and Hoopers Inlet

(estuary) (varied from 4.7 and 8.2 ‰) across all the seasons (Table 26). At Allans Beach, the mean δ^{13}C values in SPOM at the coastal marine waters fluctuated from -23.3 ‰ in spring to -19.8 ‰ in summer. The mean δ^{13}C values in SPOM values recorded at the adjacent waters varied between -23.3 ‰ at Tomahawk Creek in winter and -19.5 ‰ at Hooper Inlet in winter. The mean particulate organic carbon (POC) content on the filter paper was higher (ranging from 184 µg/l at Tomahawk Creek in autumn to 513 µg/l at Smaills in spring) than mean particulate organic nitrogen (PON) (varying between 19.1 µg/l in autumn at Tomahawk Creek and 61.9 µg/l in spring at Smaills). Lower PON content was recorded for water filtrate samples suggestive of the importance of terrestrial input since terrestrial organic matter is relatively depleted in nitrogen. The autumn samples were lower in particulate organic nitrogen (PON) than all previous season an indication of more terrestrial influence. The runoff flush induces a shift in the SPOM isotopic composition of the coastal marine waters, making the terrestrial organic matter a component of intertidal mixing zone particle fluxes during the storm season experienced in autumn and winter. The elemental carbon to nitrogen mole ratio (C/N) at Tomahawk Creek, which ranged between 9.9 and 11.7 for all seasons and Allans Beach (10.5) in winter, indicated terrestrial input (sedimentary organic matter).

Mostly, the coastal marine waters experienced sedimentary organic matter deposition alongside the high influence of marine particulate matter. Tomahawk seemed to be a source for nutrients (particulate organic materials from pastoral farms and other detrital organic materials) from watershed systems by rainfall to the nearshore waters at Smaills Beach. Elevated δ^{15}N values in Tomahawk SPOM recorded in winter, summer and spring might have resulted from the loss of isotopically light ^{14}N and retention of isotopically enriched ^{15}N particulate organic pool in the water column via heterotrophic nitrogen fixation and denitrification upon the availability of oxidisable carbon source of kelp plants deposited into Tomahawk stream from Smaills Beach. Remineralisation was observed in the SPOM sampled in autumn across all the sites but more pronounced in Tomahawk SPOM. The Tomahawk SPOM was observed to decrease by 4.16 ‰ cf. to summer due to which can be attributed to microbial modification via putrefaction resulting in remineralisation in the water column. This was apparent in the corresponding increases in nitrate concentrations across the sites in Autumn. Studies have shown that remineralisation (i.e. nitrogen fixation) in shallow coastal marine waters (water depth ranging between 0.5 and 50 m) are moderated mainly by heterotrophic bacteria resulting in the release gradients of nutrients to the overlying water column or adsorption and burial in deeper sediment layer. The concentrations of inorganic

nitrogen species (i.e. NO_3 and NH_4) from such process in the water column of shallow coastal marine ecosystems are influenced by inputs arising from fluvial discharges and those resulting from exchange across the sediment-water interface (Howarth et al. 1988; Jorgensen et al. 1989; La Roche 1983; Lomstein and Blackburn 1992).

Table 26: Average values (with standard errors) of the carbon and nitrogen isotopic ratio, concentration with mole ratio of SPOM from Allans Beach, Smaills Beach and water bodies of Hoopers Inlet and Tomahawk Creek from 22/7/16 to 29/4/17 covering the four different seasonal periods

Site	$\delta^{15}N_{AIR}$ (‰)	PON (µg/l)	Season	$\delta^{13}C_{VPDB}$ (‰)	POC (µg/l)	C / N
			Winter			
Allans	5.73 ± 0.37	40.62 ± 05.61		-21.02 ± 1.00	369.34 ± 54.35	10.52 ± 0.72
Hoopers	5.72 ± 0.55	54.78 ± 10.88		-19.50 ± 0.58	407.71 ± 66.19	9.18 ± 0.66
Smaills	5.70 ± 0.14	47.00 ± 04.07		-22.26 ± 0.51	391.33 ± 41.73	9.59 ± 0.29
Tomahawk	12.13 ± 0.47	37.34 ± 04.25		-28.15 ± 0.17	319.16 ± 38.73	9.89 ± 0.13
			Spring			
Allans	6.32 ± 0.93	37.90 ± 2.55		-19.81 ± 0.73	300.96 ± 17.43	9.49 ± 0.53
Hoopers	8.13 ± 0.67	37.71 ± 2.27		-19.84 ± 0.12	263.15 ± 14.13	8.20 ± 0.16
Smaills	6.47 ± 0.41	61.86 ± 9.42		-23.28 ± 0.97	512.95 ± 23.89	9.23 ± 0.99
Tomahawk	10.06 ± 0.29	29.65 ± 3.47		-27.28 ± 0.26	266.77 ± 47.07	10.06 ± 0.51
			Summer			
Allans	6.20 ± 0.17	48.55 ± 4.24		-21.05 ± 0.20	321.94 ± 23.45	7.83 ± 0.20
Hoopers	4.95 ± 0.41	59.82 ± 2.84		-21.16 ± 0.79	359.44 ± 20.51	6.98 ± 0.19
Smaills	6.68 ± 0.11	46.66 ± 0.57		-21.13 ± 0.38	313.04 ± 0.41	8.22 ± 0.79
Tomahawk	12.41 ± 0.72	20.27 ± 0.98		-28.10 ± 0.27	202.31 ± 8.26	11.69 ± 0.32
			Autumn			
Allans	4.49 ± 0.40	33.32 ± 2.42		-21.48 ± 0.64	251.41 ± 13.41	9.04 ± 0.53
Hoopers	4.67 ± 0.31	30.85 ± 2.71		-23.03 ± 0.56	236.76 ± 16.94	9.17 ± 0.30
Smaills	6.50 ± 0.36	25.40 ± 1.85		-23.21 ± 0.47	210.56 ± 12.26	9.84 ± 0.26
Tomahawk	8.22 ± 0.72	19.13 ± 1.10		-26.77 ± 0.40	184.69 ± 09.56	11.42 ± 0.36

5.4.2 *Mytilus galloprovincialis*

Mean $\delta^{15}N$ values in the abductor tissue of *Mytilus galloprovincialis* ranged between 8.27 ‰ (Allans), and 9.00 ‰ (Smaills) recorded in spring. Mean $\delta^{13}C$ values fluctuated from -20.67 ‰ (Smaills) and -19.24 ‰ (Allans) recorded in summer and spring accordingly. In the

digestive tissue, mean δ^{15}N values for *Mytilus galloprovincialis* varied from 7.45 ‰ (Allans), and 8.41 ‰ (Smaills) observed in spring and summer accordingly. Mean δ^{13}C values fluctuated from -20.95 ‰ (Allans) and -19.28 ‰ (Smaills) in summer and autumn, respectively. The C/N mole ratio values were predominantly higher in the digestive tissues than the abductor tissues. Mean C/N mole ratio values in the abductor tissue varied 4.07 (Smaills) and 4.48 (Allans) in winter. Mean C/N mole ratio values in the digestive tissue varied 5.07 (Allans) and 5.58 (Allans) in summer and autumn correspondingly (Table 27).

Table 27: Mean and standard error values of the nitrogen and carbon isotopic ratios in the tissues of *Mytilus galloprovincialis* collected at the intertidal mixing zones of Allans Beach and Smaills Beach from 22/7/16 to 29/4/17 covering the four different seasonal periods

		Abductor			Digestive		
Site	**Season**	**$\delta^{15}N_{AIR}$ (‰)**	**$\delta^{13}C_{VPDB}$ (‰)**	**C:N-m/ratio**	**$\delta^{15}N_{AIR}$ (‰)**	**$\delta^{13}C_{VPDB}$ (‰)**	**C:N-m.ratio**
Allans	**winter**	8.49 ± 0.06	-19.25 ± 0.11	4.48 ± 0.13	7.80 ± 0.08	-20.51 ± 0.25	5.30 ± 0.22
Smaills	**winter**	8.80 ± 0.08	-19.05 ± 0.19	4.07 ± 0.09	8.06 ± 0.09	-20.54 ± 0.22	5.21 ± 0.13
Allans	**spring**	8.27 ± 0.09	-19.24 ± 0.16	4.27 ± 0.07	7.45 ± 0.14	-20.30 ± 0.28	5.21 ± 0.12
Smaills	**spring**	9.00 ± 0.12	-20.32 ± 0.40	4.38 ± 0.14	7.99 ± 0.13	-20.58 ± 0.48	5.13 ± 0.12
Allans	**summer**	8.95 ± 0.08	-19.84 ± 0.15	4.37 ± 0.09	8.18 ± 0.08	-20.95 ± 0.22	5.07 ± 0.10
Smaills	**summer**	8.54 ± 0.17	-20.67 ± 0.11	4.45 ± 0.13	8.41 ± 0.20	-21.58 ± 0.14	5.53 ± 0.21
Allans	**autumn**	8.69 ± 0.13	-19.82 ± 0.15	4.42 ± 0.11	8.17 ± 0.09	-20.81 ± 0.40	5.58 ± 0.14
Smaills	**autumn**	8.48 ± 0.09	-19.50 ± 0.16	4.25 ± 0.07	7.92 ± 0.09	-19.28 ± 0.48	5.17 ± 0.13

5.4.3 *Ulva lactuca*

The mean δ^{15}N values in the tissues of *Ulva lactuca* fluctuated from 8.23 ‰ (Smaills) to 12.68 ‰ (Allans) during winter and autumn, respectively. The mean δ^{13}C values varied from -19.48 ‰ to -11.78 ‰ in Allans during summer and winter accordingly at the coastal marine waters. At the estuary (Hoopers Inlet), the mean δ^{15}N values in the tissues of *Ulva lactuca* fluctuated from 7.67 ‰ to 8.74 ‰ during winter and spring respectively. The mean δ^{13}C values varied from -16.10 ‰ to -12.94 ‰ in during winter and spring accordingly. Mean C:N mole ratio values in *Ulva lactuca* ranged from 9.67 (Allans) to 24.73 (Hoopers; Inlet) during spring and summer accordingly (Table 28). No growth of *Ulva lactuca* was observed at the estuary during autumn. Mean C/N mole ratio values in the *Ulva lactuca* tissues fluctuated between 9.67 at Allans Beach in spring and 24.73 at Hoopers Inlet in summer. There was an

increase of nearly 3 ‰ in the δ^{15}N values in *Ulva lactuca* sampled in Smaills during spring, summer and autumn c.f. recorded δ^{15}N values (winter) in *Ulva lactuca* from the same site. Similar δ^{15}N enrichment was observed in *Ulva lactuca* from Hoopers Inlet in autumn c.f. recorded δ^{15}N values (winter). However, similar δ^{15}N values were recorded in *Ulva lactuca* analysed in autumn from Smaills and Hoopers Inlet. The δ^{15}N values recorded in *Ulva lactuca* during autumn was found to be nearly 3 ‰ greater than the national baseline indicator of nitrogen-loading for coastal waters and indicated possible contributions from terrestrially-derived nitrogen to coastal marine waters as suggested by Barr et al. (2013a). Another indicator for the alteration of δ^{15}N *Ulva lactuca* in autumn is the losses of ^{14}N due to coupled-uncoupled denitrification arising from N-recycling (remineralisation) in the water column.

This is may be indicative of nutrient inputs from the watershed systems to the coastal marine waters. The incidence of high rainfall during any of these seasons will likely increase stream flow levels and assist in conveying nutrients from the watershed system to the coastal marine waters. These nutrients are possibly organic materials from pastoral farms and other detrital materials that may cause an increase in total nitrogen attributed and ammonia (from the decomposition of organic matter) during the latter part of the summer and early fall thereby making ammonia readily available as a nutrient for *Ulva lactuca*. This observation is in accordance with Pruell et al. (2006).

Tyler et al. (2005) had reported that *Ulva lactuca* accumulates ammonia favourably relative to nitrate and urea. Consequently, the elevated δ^{15}N enrichment in *Ulva lactuca* is indicative of increase ammonia uptake. The absence of *Ulva lactuca* in the estuary during autumn is indicative of insufficient of dissolved nutrients to promote its rapid growth. Sea lettuce readily accumulates dissolved nutrients to grow rapidly (Cohen and Neori 1991).

Table 28: Mean and standard error values of the nitrogen and carbon isotopic ratios with mole ratio in the tissues of *Ulva lactuca* collected at the intertidal mixing zones of Allans Beach, Hoopers Inlet and Smaills Beach from 22/7/16 to 29/4/17 covering the four different seasonal periods.

Site	$\delta^{15}N_{AIR}$ (‰)	Season	$\delta^{13}C_{VPDB}$ (‰)	C:N-m.ratio
		Winter		
Allans	8.60 ± 0.33		-19.48 ± 0.27	17.61 ± 1.18
Smaills	8.23 ± 0.08		-18.62 ± 0.34	16.76 ± 1.28
Hoopers Inlet	7.67 ± 0.15		-16.10 ± 0.95	12.89 ± 0.51
		Spring		
Allans	9.84 ± 0.44		-16.05 ± 0.66	9.67 ± 0.20
Smaills	11.20 ± 0.25		-16.19 ± 0.49	11.14 ± 0.17
Hoopers Inlet	8.74 ± 015		-12.94 ± 0.39	17.58 ± 0.31
		Summer		
Allans	8.80 ± 0.07		-11.78 ± 0.05	12.77 ± 0.06
Smaills	12.23 ± 0.05		-15.48 ± 0.01	10.86 ± 0.02
Hoopers Inlet	8.15 ± 0.09		-15.92 ± 0.07	24.73 ± 0.22
		Autumn		
Allans	12.58 ± 0.06		-14.55 ± 0.06	10.60 ± 0.06
Smaills	12.56 ± 0.03		-15.60 ± 0.03	10.79 ± 0.03
Hoopers Inlet	-		-	-

5.4.4 Terrestrial-derived Organic Materials

The mean $\delta^{15}N$ of potential nutrient source organic materials varied between 3.07 ‰ (sewage effluent) and 14.20 ‰ (seal faeces) while the mean $\delta^{13}C$ fluctuated between -30.62 ‰ (cow faeces) and -12.83 ‰ and (detrital matter). The mean C/N molar ratio in the nutrient source organic materials ranged from 4.66 (farm manure) to 25.32 (detrital matter) (Table 29). The detrital matter collected in the estuary (Hoopers Inlet) had a distinctive C and N isotope values of a C_4 plant (Hamilton and Lewis, 1992).

Table 29: Mean and standard deviation values of the nitrogen and carbon isotopic ratios and mole ratios of N-source organic materials collected within the coastal marine waters, adjacent water bodies and immediate surroundings

N-source organic material	δ^{15}N	δ^{13}C	C:N-m/ratio
Compost	1.09 ± 0.20	-28.04 ± 0.11	18.66 ± 0.37
Cow faeces	2.57 ± 0.84	-30.62 ± 0.23	24.11 ± 0.38
Detrital matter	5.41 ± 0.21	-12.83 ± 0.03	33.72 ± 0.78
Farm manure	5.93 ± 0.15	-21.47 ± 0.63	4.66 ± 0.28
Sheep faeces	5.90 ± 0.31	-30.41 ± 0.10	25.32 ± 0.69
Sea lion faeces	14.20 ± 0.60	-21.90 ± 0.30	6.00 ± 0.36
Sewage effluent	3.07 ± 0.79	-25.80 ± 0.12	5.01 ± 0.40

5.5 Distribution of Suspended Particulate Organic Matter

5.5.1 The Kolmogorov-Smirnov Test (KS Test)

Using the Kolmogorov-Smirnov test, which is an assessment model used to test normality in the distribution of suspended particulate organic matter between the nearshore marine waters and their respective proximate water bodies. Normal distribution (the Normal P-plot validation test for normality of δ^{13}C and δ^{15}N SPOM are expressed in the Appendix) was assumed for these data according to the Central Limit Theorem (Zar 2013) where each of the pooled sample means is considered as an average. The average of the standard deviations of the sample means was also determined by the model and used to determine the *Z* score that was used to determine the characteristics of the population sample based on Hypothesis #1 and # 2 stated below. The model was chosen because it can handle both discrete and continuous variables. The outcomes of the model are presented in Table 30.

The KS test model established that there was continuous distribution of δ^{15}N and δ^{13}C in the suspended particulate organic matter collected from the estuary (Hoopers Inlet) and marine coastal waters (ocean) (Figure 25A-B) while from the freshwater to the marine coastal waters (ocean) gradient exhibited discontinuous distribution of δ^{15}N and δ^{13}C in the suspended particulate organic matter (Figure 25C-D).

Hypothesis # 1

H_0 = There is no continuous distribution of $\delta^{15}N$ or $\delta^{13}C$ in the suspended particulate organic matter between the nearshore marine waters and respective proximate water body ($\alpha \leq 0.05$)

Hypothesis # 2

H_1 = There is a continuous distribution of $\delta^{15}N$ or $\delta^{13}C$ in the suspended particulate organic matter between the nearshore marine waters and respective proximate water body ($\alpha \geq 0.05$)

Acceptance and rejection of Hypothesis

H_1 is accepted when the asymptotic significance *p* value is greater than 0.05 and H_0 is rejected.

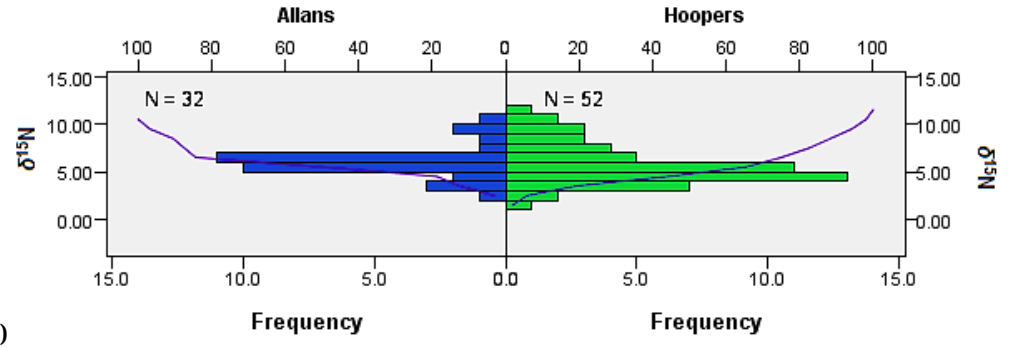
H_1 is rejected when the asymptotic significance *p* value is lesser than or equal to 0.05 (95% level of confidence), and H_0 is accepted.

Fundamentally, the extent of the intercept of the asymptote curves that describes the behaviour of the graphs of the independent variables are used to compute the *p* values.

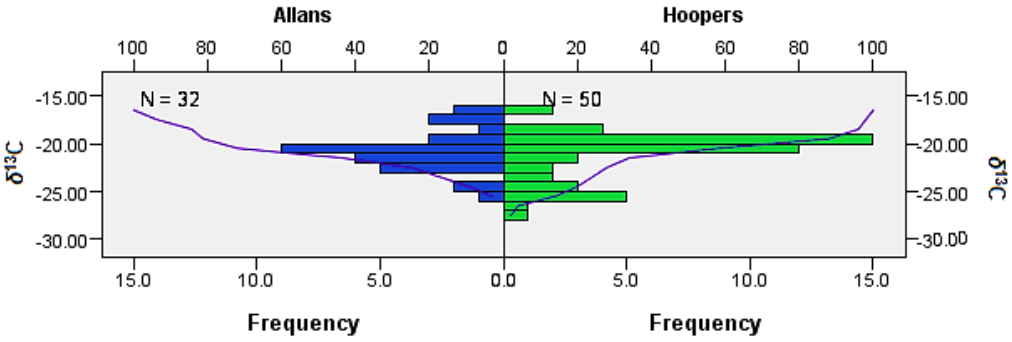
Table 30: Kolmogorov-Smirnov test for the continuous distribution of suspended particulate organic matter between the coastal waters and their respective adjacent water bodies (i.e. asymptotic distribution for two-sided p-value at 95% with confidence limit $\alpha \leq 0.05$)

Variable	Site contrast	System	N	Abs. value	D-value	*p*-value
$\delta^{15}N$	HPI-ANB	estuary-ocean	84	0.296	1.316	0.063
$\delta^{13}C$	HPI-ANB	estuary-ocean	82	0.214	0.944	0.335
$\delta^{15}N$	TMC-SMB	freshwater-ocean	79	0.790	3.447	0.000
$\delta^{13}C$	TMC-SMB	freshwater-ocean	79	0.947	4.134	0.000

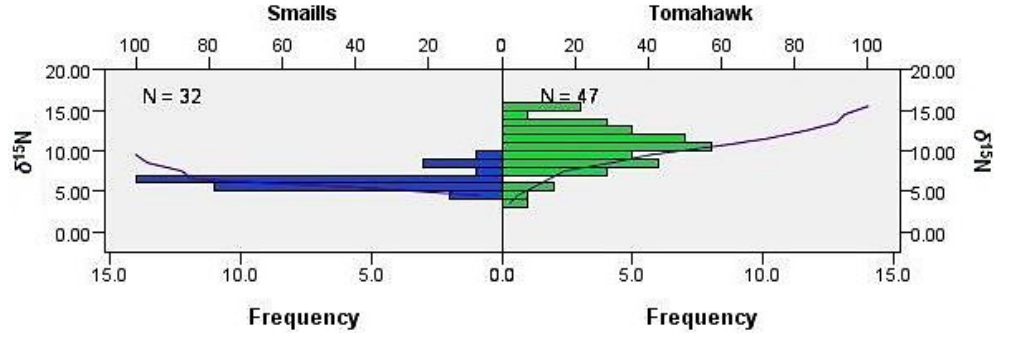
The asymptote curves for the distribution of $\delta^{15}N$ and $\delta^{13}C$ in the suspended particulate organic matter at the estuary-ocean interface exhibited an almost similar framework (Figure 25A-B) while those of freshwater-ocean interface had a dissimilar framework (Figure 25C-D).



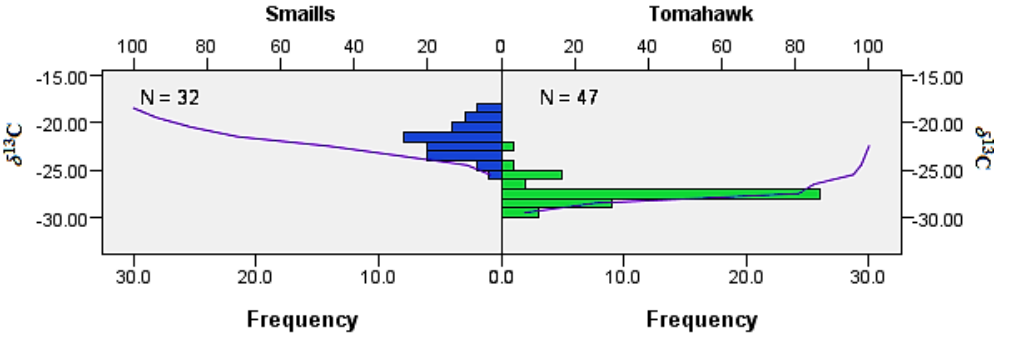
(A)



(B)



(C)



(D)

Figure 25A-D: Kolmogorov-Smirnov test pyramid chart showing back-to-back histograms of the categories of site contrast of the carbon and nitrogen isotopic ratios in the suspended particulate organic matter and the number of samples in each of the coastal waters and their respective adjacent water bodies.

5.5.2 The Independent-Samples Median Chi Square Test and Independent-Samples Moses Test of Extreme Reaction

The independent-samples median Chi square test (Table 31) and independent-samples Moses test of extreme reaction (Table 32) were conducted to determine the extent of the dispersion in the distribution of δ^{15}N- and δ^{13}C-SPOM in the suspended particulate organic matter collected between adjacent water bodies and the coastal marine waters (ocean). The loadings for both tests are represented in Figure 26A-D and 27A-D. These two tests were found appropriate for evaluating the power and degree of the observed dispersion and variance (via K-S test) in the normal distribution of δ^{15}N and δ^{13}C of the suspended particulate organic matter collected between adjacent water bodies and the coastal marine waters (ocean).

Table 31: Independent-samples median Chi-square test of the comparison of suspended particulate organic matter between the coastal marine waters and their respective adjacent water bodies

Variable	Site contrast	Interface	n	Median	X^2	*p*-value
δ^{15}**N**	HPI-ANB	estuary-ocean	84	5.62	4.089	0.043
δ^{13}**C**	HPI-ANB	estuary-ocean	82	-20.59	1.281	0.258
δ^{15}**N**	TMC-SMB	river-ocean	79	8.49	37.16	0.000
δ^{13}**C**	TMC-SMB	river-ocean	79	-26.23	51.81	0.000

Table 32: Independent-samples Moses test of the extreme reaction of the comparison of suspended particulate organic matter between the coastal marine waters and their respective adjacent water bodies

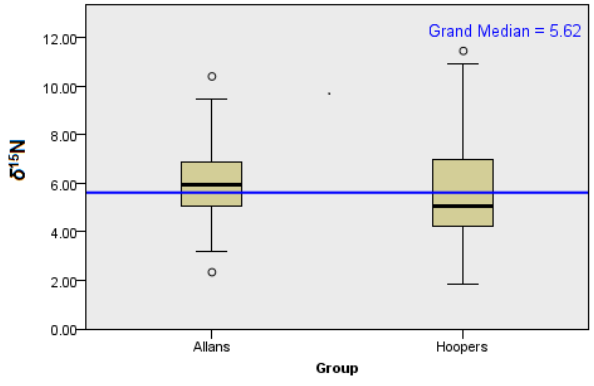
Variable	Site contrast	Interface	n	S/obs.	S/trim.	p (obs.)	P (trim.)
δ^{15}**N**	HPI-ANB	estuary-ocean	84	81	74	0.507	0.330
δ^{13}**C**	HPI-ANB	estuary-ocean	82	80	71	0.664	0.227
δ^{15}**N**	TMC-SMB	river-ocean	79	46	40	0.000	0.000
δ^{13}**C**	TMC-SMB	river-ocean	79	36	31	0.000	0.000

There was an insignificant deviation from the grand medians δ^{13}C-SPOM (i.e. the independent-samples median Chi square test) for the estuary-ocean interface (Table 31). A reflection of the proper mixing of carbon supply in the suspended particulate organic matter at the interface and lessening influence of freshwater. The δ^{15}N-SPOM median was found to be significant (Table 31) and dissimilar to the grand median suggestive of terrigenous

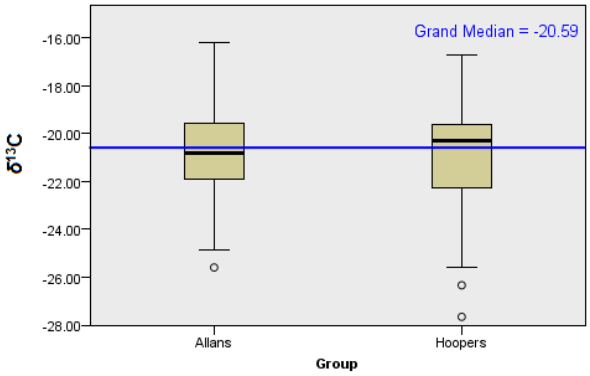
influence (i.e. slight deviation from the grand median) on the estuary (Hoopers Inlet) (Figure 26A-B).

The δ^{15}N- and δ^{13}C-SPOM had insignificant means for the estuary-ocean interface in contrast to the independent-samples median Chi square test findings (Table 32 and Figure 27A-B).

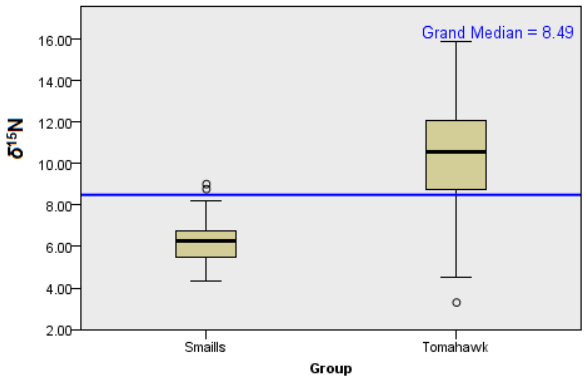
For the river-ocean interface, there existed a significant deviation (Table 31) from the grand medians δ^{15}N- and δ^{13}C-SPOM values in the suspended particulate organic matter was observed showing irregular mixing of freshwater and oceanic matter. However, the δ^{15}N seemed to show some sort of mixing agreement but not so strong to influence the continuous distribution and supply of δ^{15}N-SPOM riverine organic matter at the oceanic waters at Smaills Beach (Figure 26C-D). At the river-ocean interface, there was a similar occurrence for the comparison of the means (i.e. Independent-samples Moses test of extreme reaction) (Figure 27C-D). The δ^{15}N- and δ^{13}C-SPOM significant means were recorded for the river-ocean interface. However, the model assumed some of the δ^{13}C-SPOM measured (Figure 27D) as 'outliers' based on the fact that some have marine organic matter bearing while others were terrigenous (Table 32 and Figure27C-D). The same bearing was observed for the δ^{13}C-SPOM grand median assessment at the river-ocean interface. The only explanation the oceanic waters witnessed intermittent influence of flood plumes during high rainfall events.



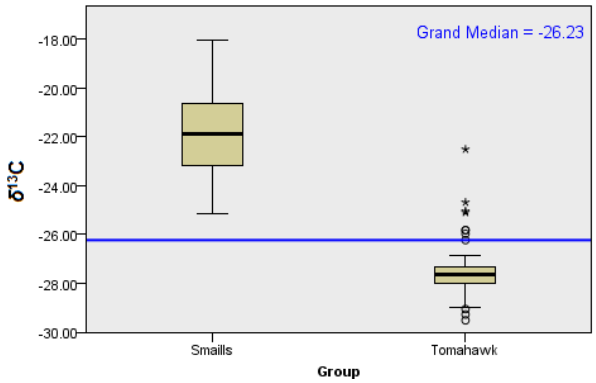
(A)



(B)

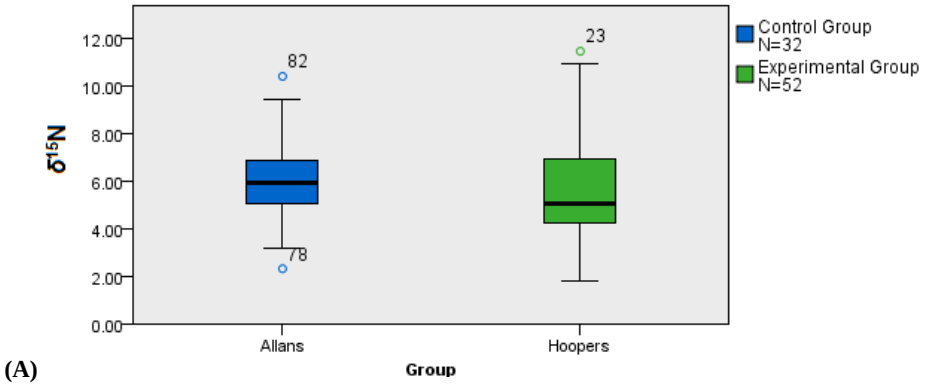


(C)

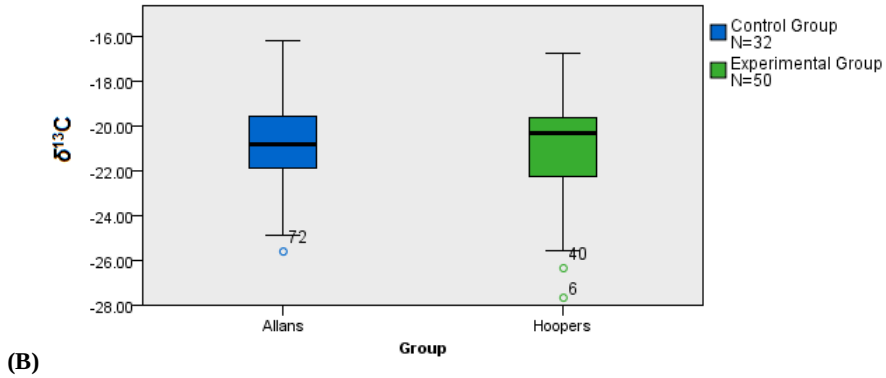


(D)

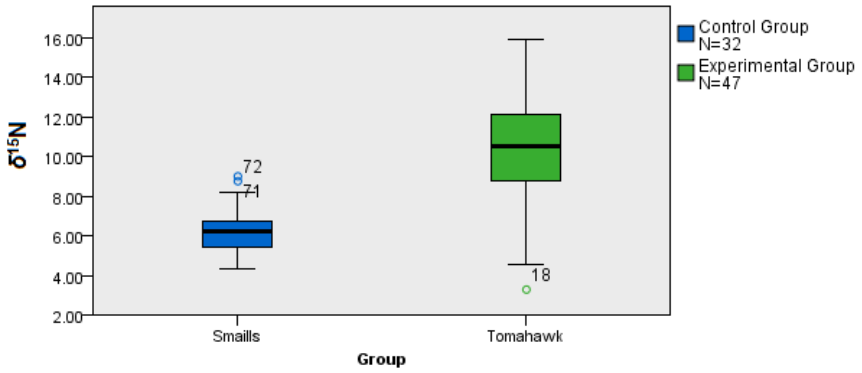
Figure 26A-D: Independent-samples median Chi square test of δ^{15}N- and δ^{13}C-SPOM comparison between the coastal marine waters and their respective adjacent water body interface.



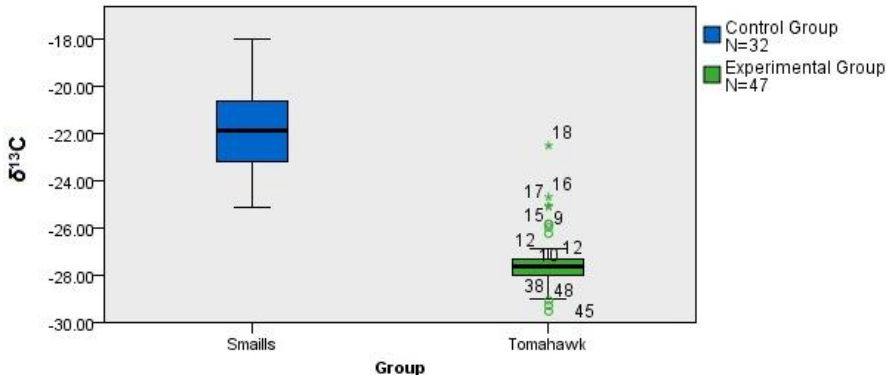
(A)



(B)



(C)



(D)

Figure 27A-D: Independent-samples Moses test of extreme reaction of the δ^{15}N- and δ^{13}C-SPOM between the coastal marine waters and their respective adjacent water body interfaces.

5.6 MixSIAR Model Framework and Hypotheses

The estimation of assimilated suspended organic matter and possible nutrient source land-based organic materials in *Mytilus galloprovincialis* at each of the adjacent water bodies to coastal marine water gradients was determined using the MixSIAR model framework. The stable isotope ratios (δ^{13}C and δ^{15}N) of suspended organic matter and possible nutrient source sewage-based organic materials of interest were incorporated into MixSIAR framework to compute their contributions in the digestive tissue of *Mytilus galloprovincialis*. The model is built upon a Gaussian likelihood with Dirichlet-distributed prior to the mean distribution (Parnell et al. 2013). A matrix correlation was also conducted by MixSIAR model to measure the degree of the relationship between each of the proportional contributions of the nutrient source of organic materials in the digestive tissues of *Mytilus galloprovincialis*. The Mix-SAIR model framework assessed the density indicate the intensity of the distribution of possible organic materials in *Mytilus galloprovincialis*.

A robust isotopic fractionation factor (i.e. trophic enrichment factor) of +0.4 ‰ for δ^{13}C and +3.4 ‰ for δ^{15}N with tolerance values (standard deviation) of ±1.3 and ±1 ‰ for δ^{13}C and δ^{15}N respectively as described in Post (2002) was adopted. This also agrees with the observed +3 (N) and +1 (C) enrichment factor described in Babaranti et al. 2019.

The evaluation of the proportional contributions for possible terrestrial-based organic materials in the digestive tissues of *Mytilus galloprovincialis* was examined using the following set-up assumptions:

- Hypothesis #1: Presence of sewage effluent
- Hypothesis #2: Absence of sewage effluent

Small ranges between the minimum and maximum estimates were well constrained and fixed in the framework model for each of the different nutrient source contributions in *Mytilus galloprovincialis.* For the purpose of consistency, the maximum proportional values were accepted as the estimated nutrient source contributions in *Mytilus galloprovincialis* (Table 33). The MixSIAR bi-plots, source proportional boxplots, proportional density histograms and matrix plots of SPOM and the land-based organic materials in the tissues of *Mytilus galloprovincialis* collected at the coastal marine waters along Otago Peninsula are represented in Table 33.

Figure 29A-G (presence of sewage effluent) and Figure 30A-G (absence of sewage effluent) and Figure 28A-F illustrated the analysis of the percentage proportions of suspended organic matter and other land-based nutrient source organic materials.

Table 33: δ^{13}C and δ^{15}N values (with standard deviations) of suspended organic matter and land-based nutrient source organic materials and their percentage proportions in *Mytilus galloprovincialis*

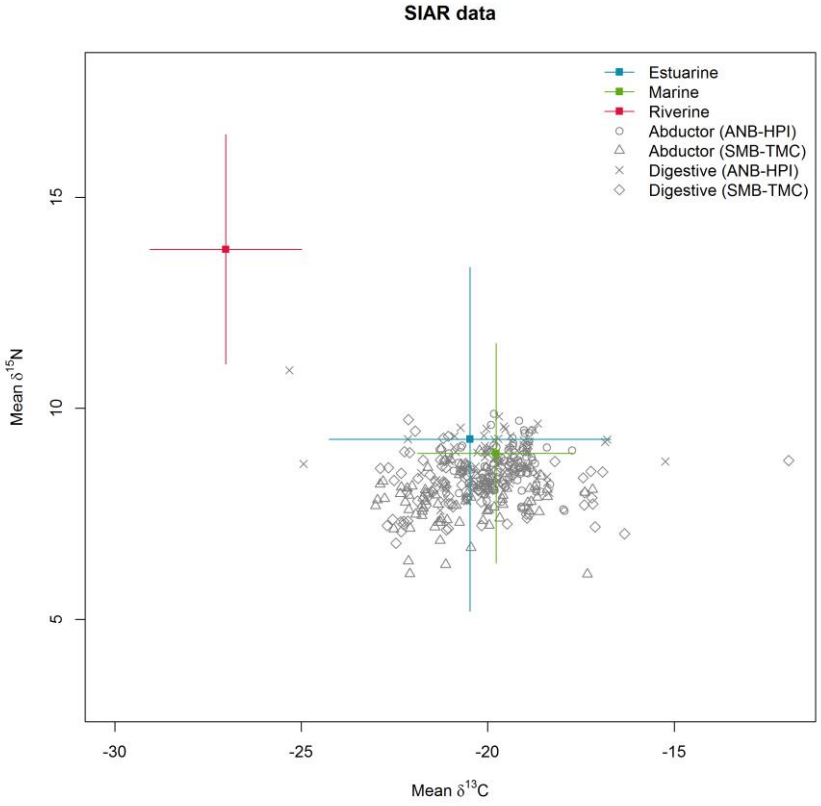
Organic material	δ^{15}N ± SD (‰)	δ^{13}C ± SD (‰)	% Proportion 1		% Proportion 2	
			ANB	SMB	ANB	SMB
		SPOM*				
Estuarine SPOM	5.87 ± 1.57	-20.88 ± 1.60	58	44	-	-
Marine SPOM	5.53 ± 0.07	-20.18 ± 0.32	76	98	-	-
Riverine SPOM	10.37 ± 0.40	-27.43 ± 0.18	1	1	-	-
		Land-based organic materials				
Compost	4.09 ± 0.20	-28.04 ± 0.11	20	22	22	30
Cow faeces	2.57 ± 0.84	-30.62 ± 0.23	30	30	40	38
Detrital matter	5.41 ± 0.21	-12.83 ± 0.03	50	50	54	56
Farm manure	5.93 ± 0.15	-21.47 ± 0.63	18	20	18	20
Sheep faeces	4.86 ± 0.31	-30.41. ± 0.10	4	10	4	10
Sea lion faeces	14.20 ± 0.60	-21.90 ± 0.30	1	2	1	1
Sewage effluent	3.07 ± 0.79	-25.80 ± 0.12	40	39	-	-
Ulva lactuca	3.07 ± 0.79	-15.95 ± 2.28	2	3	2	2

***Estimation percentage proportional contributions without land-based organic materials**

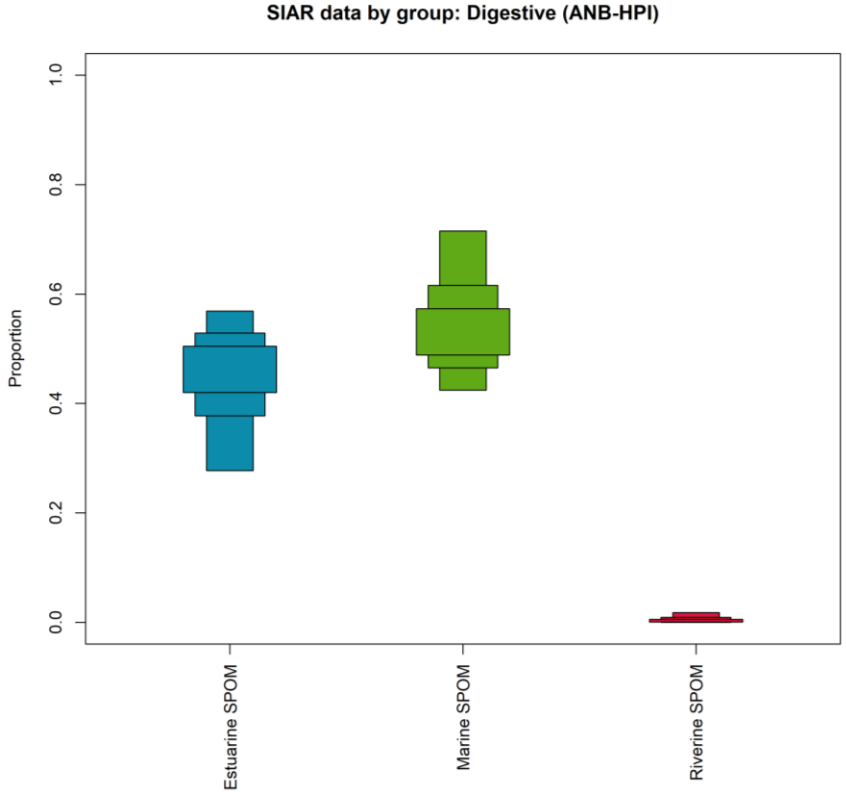
5.6.1 Proportional Contributions of Suspended Particulate Organic Matter in *Mytilus galloprovincialis*

This is apparent in their overlap of estuarine and marine organic matter in the isotope space (iso-space) with the digestive tissues of *Mytilus galloprovincialis* (Figure 28A), indicating their source contribution to the diet of the bivalve. The estimated proportional contributions

of suspended particulate organic matter in the digestive tissues of *Mytilus galloprovincialis* include; the estuarine SPOM (58 %), marine SPOM (76 %) at the estuarine-oceanic interface (HPI-ANB) (Table 33 & Figure 28B) and estuarine SPOM (44 %), marine SPOM (98 %) at the river-oceanic interface (TMC-SMB) (Figure 28C). At both interfaces, the contributions from riverine SPOM were observed to be insignificant. A matrix correlation was conducted via the MixSIAR model to measure the degree of the relationship between each of the proportional contributions of SPOM in the digestive tissues of *Mytilus galloprovincialis*. At both contrasting interfaces, the matrix correlation revealed a perfect linear association of -1.00 was recorded for marine-estuarine SPOM association (Figure 28D and 28E).

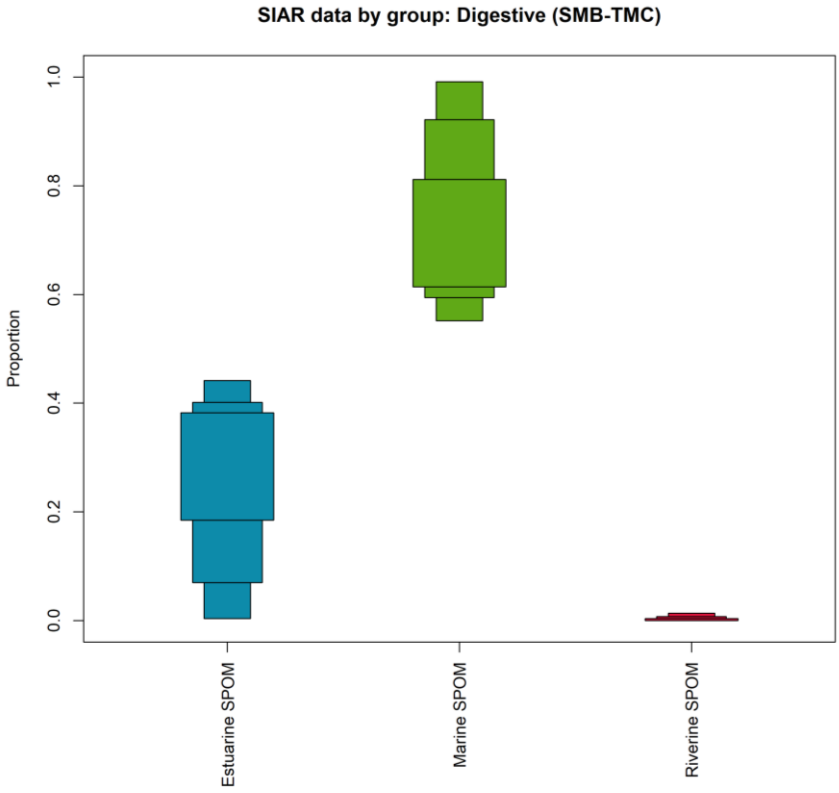

SIAR data
Estuarine
Marine
Riverine
Abductor (ANB-HPI)
Abductor (SMB-TMC)
Digestive (ANB-HPI)
Digestive (SMB-TMC)
Mean δ15N
Mean δ13C
5
10
15
-30
-25
-20
-15

(A)

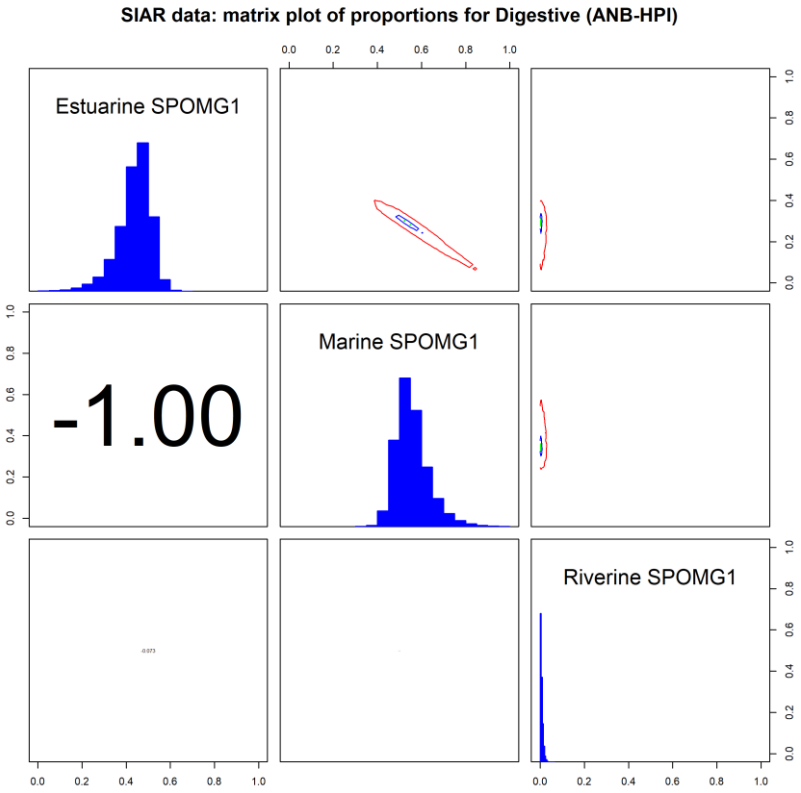

SIAR data by group: Digestive (ANB-HPI)
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
Estuarine SPOM
Marine SPOM
Riverine SPOM

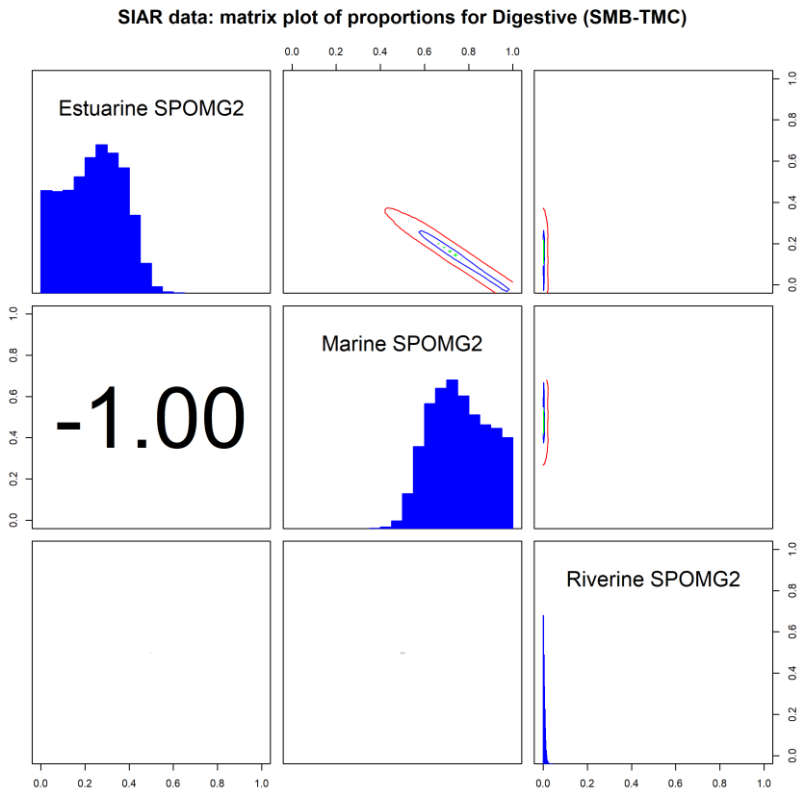
(B)

(C)

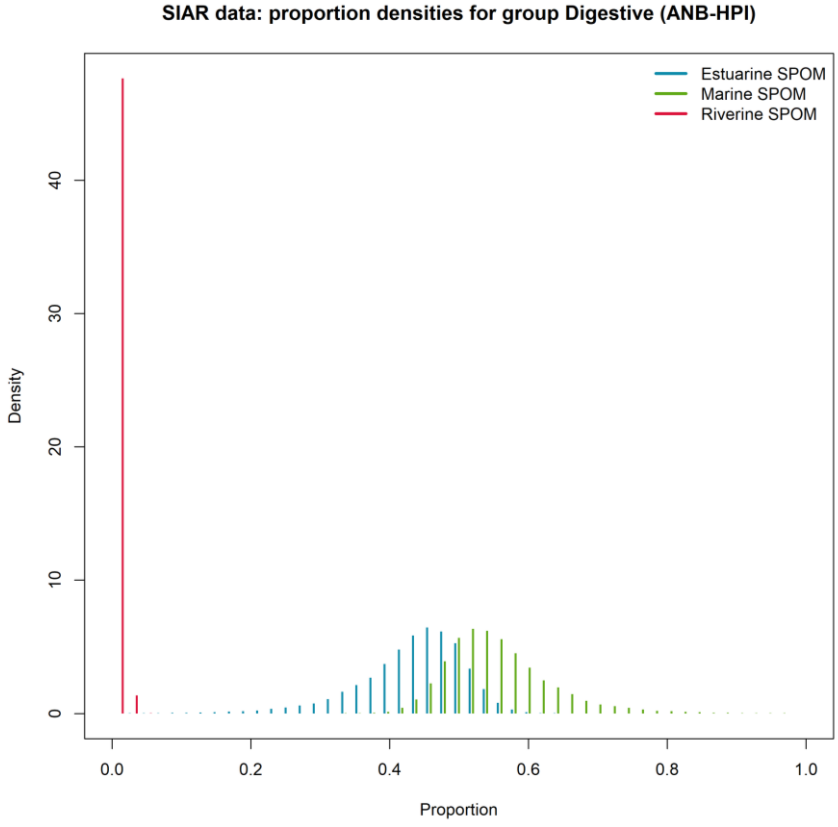

SIAR data by group: Digestive (SMB-TMC)
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
Estuarine SPOM
Marine SPOM
Riverine SPOM

(D)

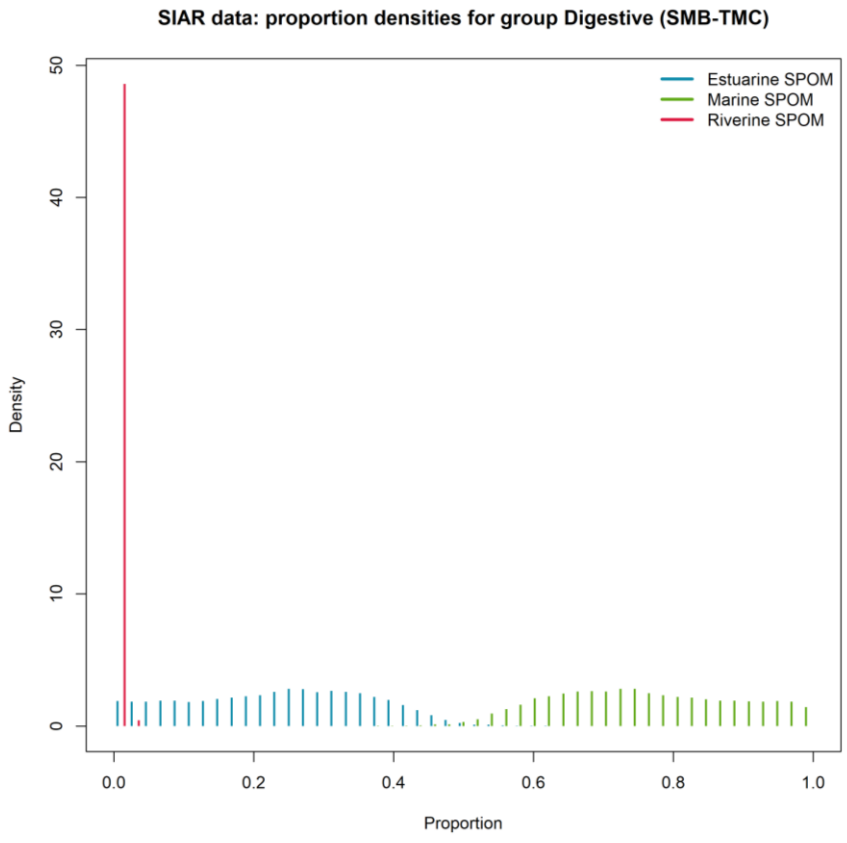

SIAR data: matrix plot of proportions for Digestive (ANB-HPI)
Estuarine SPOMG1
Marine SPOMG1
Riverine SPOMG1
-1.00
0.0
0.2
0.4
0.6
0.8
1.0


SIAR data: matrix plot of proportions for Digestive (SMB-TMC)
Estuarine SPOMG2
Marine SPOMG2
Riverine SPOMG2
-1.00

(E)


SIAR data: proportion densities for group Digestive (ANB-HPI)
Estuarine SPOM
Marine SPOM
Riverine SPOM
Density
Proportion

(F)



(G)

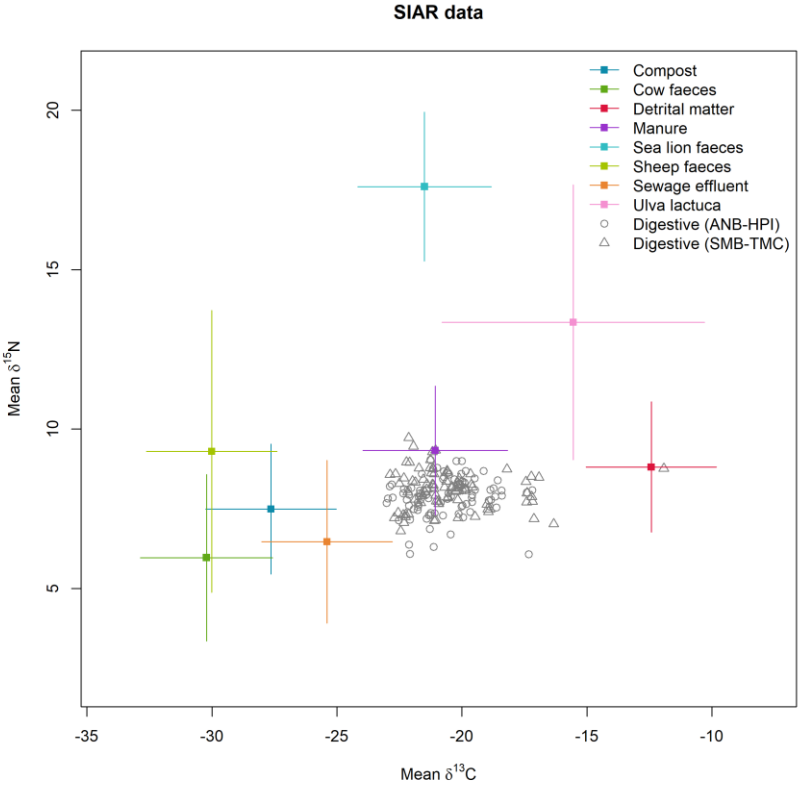
Figure 28A-G: Biplot, boxplots, histograms and matrix plots of carbon and nitrogen isotope ratios of suspended organic matter (excluding sewage effluent and animal sewage) in the digestive tissues of *Mytilus galloprovincialis* collected at the riverine-oceanic (ANB-TMC) and estuary-oceanic (HPI-SMB) waters along Otago Peninsula, New Zealand.

The iterated Mix-SAIR proportional histograms for both interfaces showed that the marine and estuarine SPOM at the estuary-ocean interface were uniformly distributed between 40 and 60 % proportion from 1 to 5 density intensity (Figure 28F) but were not uniformly distributed at the freshwater-ocean interface (Figure 28G). The marine and estuarine SPOM showed the possibility of proper mixing at the estuary-ocean interface. The intensity of the density for source proportion of the C- and N-SPOM riverine at both interfaces was not sensitive enough to estimate its correlation matrix with other organic matter sources (Figure 28F & 28G).

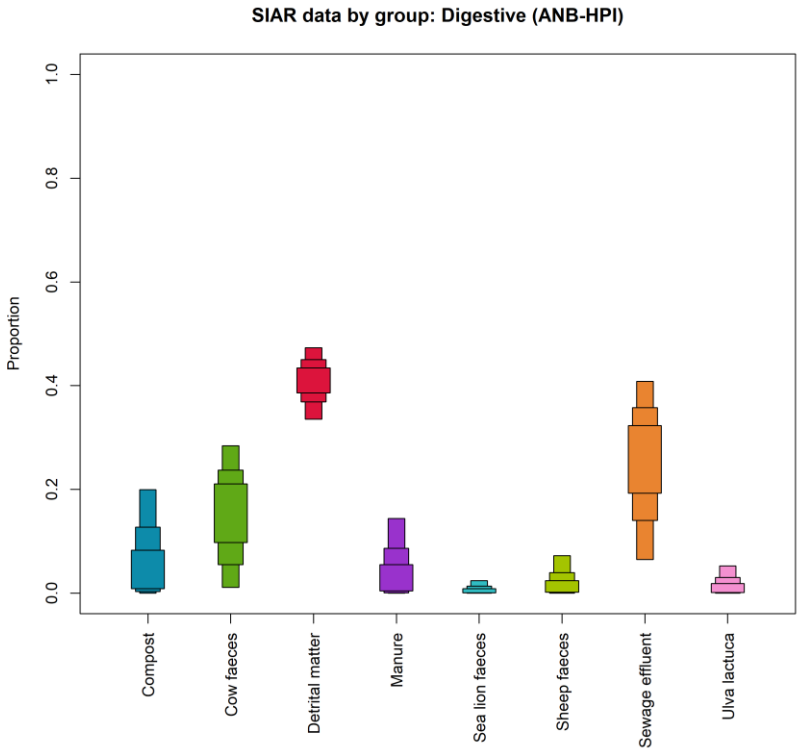
5.6.2 Proportional Contributions of Terrestrial-based Organic Materials in *Mytilus galloprovincialis*

Hypothesis #1: Presence of sewage effluent

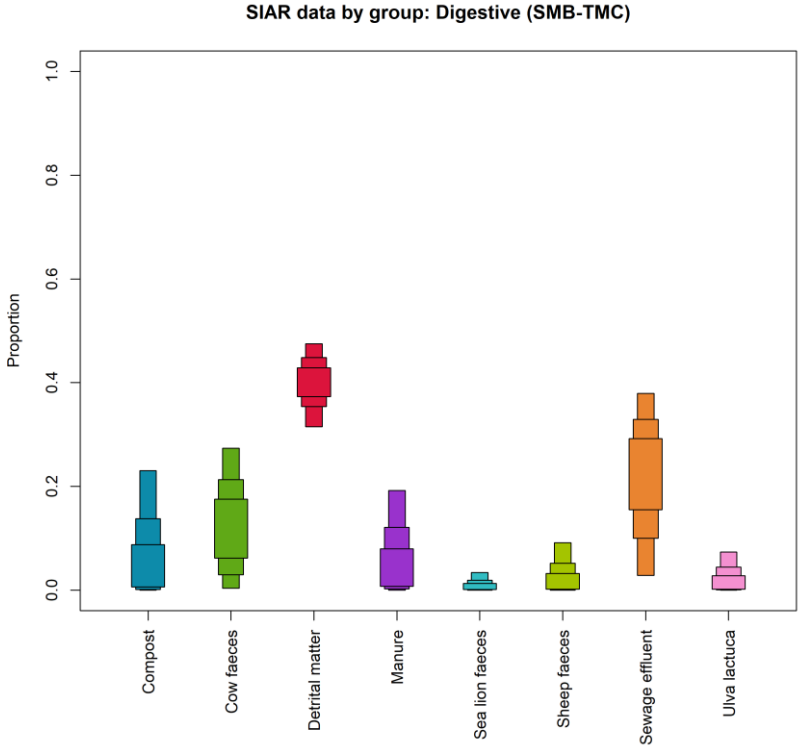
In general, sewage-derived organic materials (cow, sea lion faeces, sheep and sewage effluent) seemed to overlap one another and aggregate on the same side on the iso-space (Figure 29A). The estimated proportional contributions for each of the possible nutrient source organic materials in the digestive tissues of *Mytilus galloprovincialis* include; compost (20 %), cow faeces (30 %), detrital matter (50 %), sewage effluent (40 %), farm manure (18 %), sheep faeces (4 %), *Ulva* (2 %) and sea lion faeces (1 %) at the estuary-ocean interface (Figure 29B). The contributions from possible nutrient source organic materials in the digestive tissues of *Mytilus galloprovincialis* at the freshwater-ocean interface were: compost (22 %), cow faeces (30 %), detrital matter (50 %), sheep faeces (10 %), sewage effluent (39 %), farm manure (20 %), *Ulva lactuca* (0 – 8 %) and sea lion faeces (2 %) (Figure 29C). A matrix correlation value of 0.63 was recorded for the cow faeces-detrital matter association, -0.51 for the detrital matter-manure association, -0.78 for sewage effluent-detrital matter association and -0.80 for cow faeces-sewage effluent association in the digestive tissues of *Mytilus galloprovincialis* collected at the estuary-ocean interface (ANB-HPI) (Figure 2F). A matrix correlation value of 0.52 was recorded for cow faeces-detrital matter association, -0.56 between detrital matter and manure association, -0.70 for sewage effluent-detrital matter association and -0.72 between cow faeces and sewage effluent association in the digestive tissues of *Mytilus galloprovincialis* collected at the freshwater-oceanic interface (SMB-TMC) (Figure 2G).


SIAR data
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Sewage effluent
Ulva lactuca
Digestive (ANB-HPI)
Digestive (SMB-TMC)
Mean δ15N
Mean δ13C
20
15
10
5
-35
-30
-25
-20
-15
-10

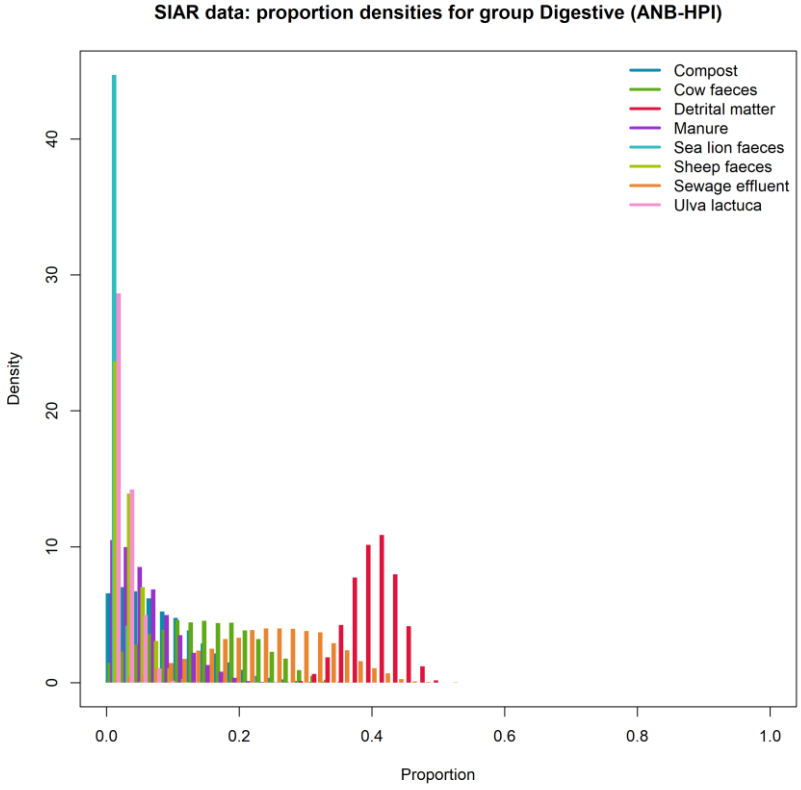
(A)


SIAR data by group: Digestive (ANB-HPI)
Proportion
1.0
0.8
0.6
0.4
0.2
0.0
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Sewage effluent
Ulva lactuca

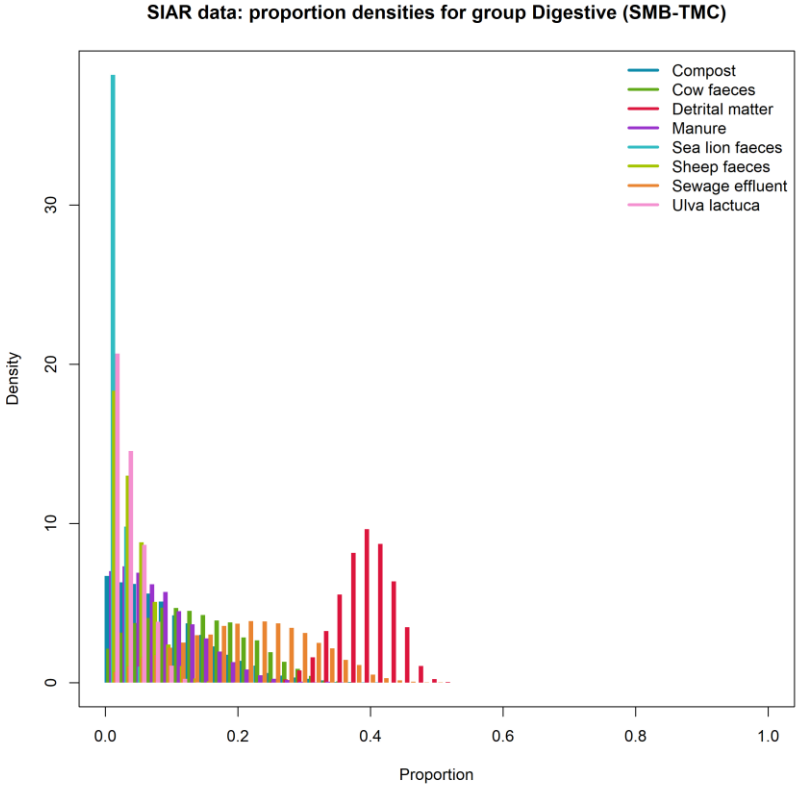
(B)


SIAR data by group: Digestive (SMB-TMC)
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Sewage effluent
Ulva lactuca

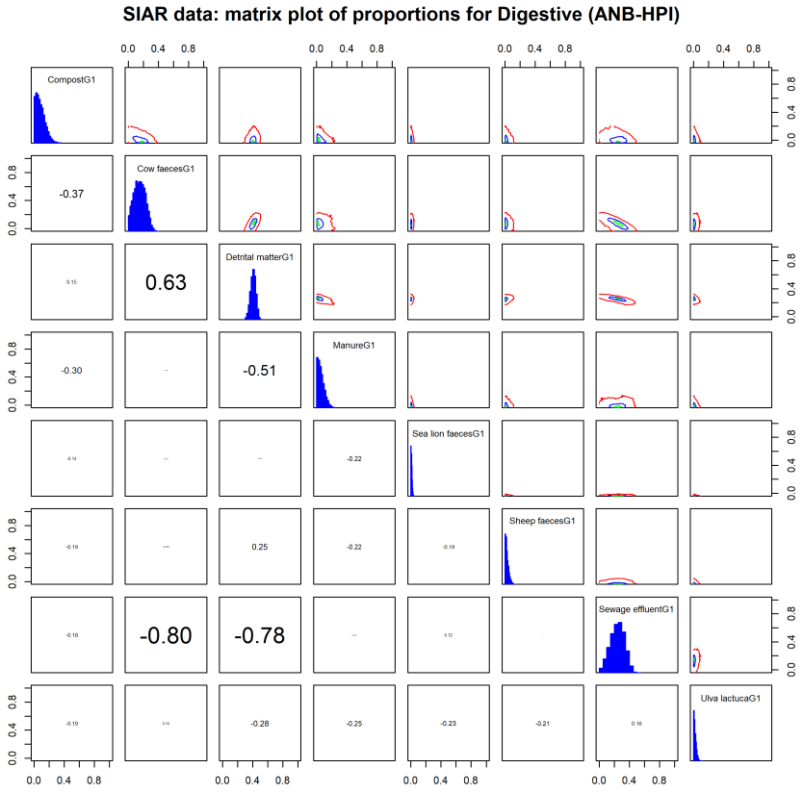
(C)


SIAR data: proportion densities for group Digestive (ANB-HPI)
Density
0
10
20
30
40
0.0
0.2
0.4
0.6
0.8
1.0
Proportion
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Sewage effluent
Ulva lactuca

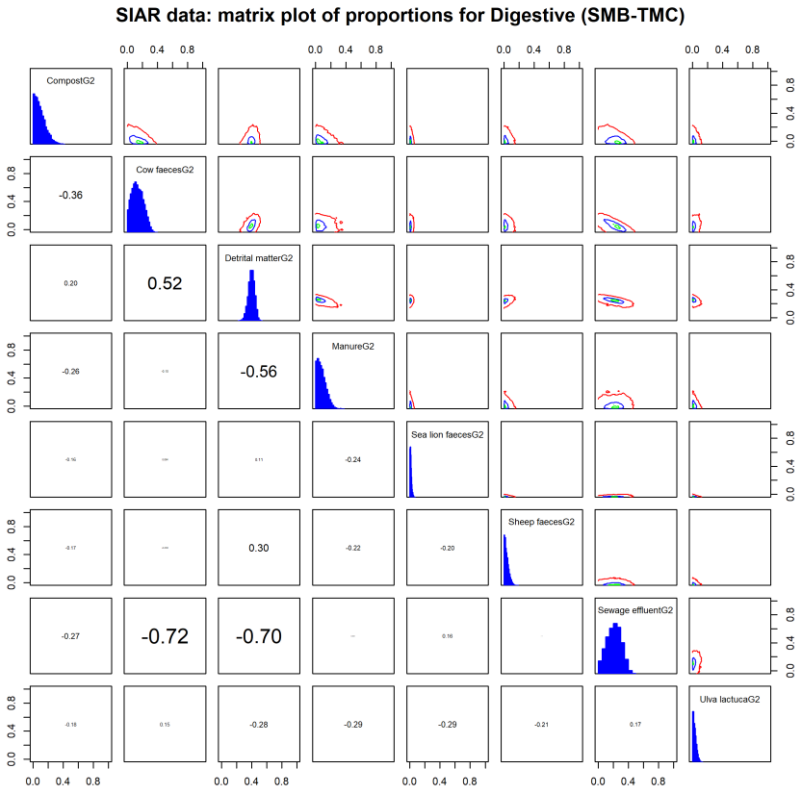
(D)


SIAR data: proportion densities for group Digestive (SMB-TMC)
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Sewage effluent
Ulva lactuca
Density
0
10
20
30
0.0
0.2
0.4
0.6
0.8
1.0
Proportion

(E)


SIAR data: matrix plot of proportions for Digestive (ANB-HPI)
CompostG1
Cow faecesG1
Detrital matterG1
ManureG1
Sea lion faecesG1
Sheep faecesG1
Sewage effluentG1
Ulva lactucaG1
-0.37
0.63
-0.51
-0.80
-0.78

(F)

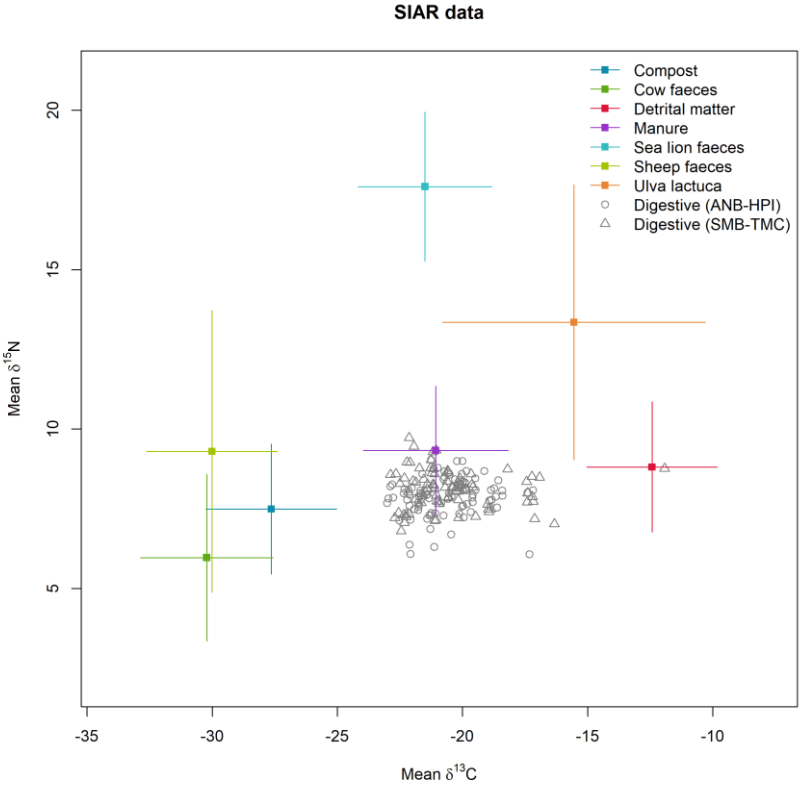


(G)

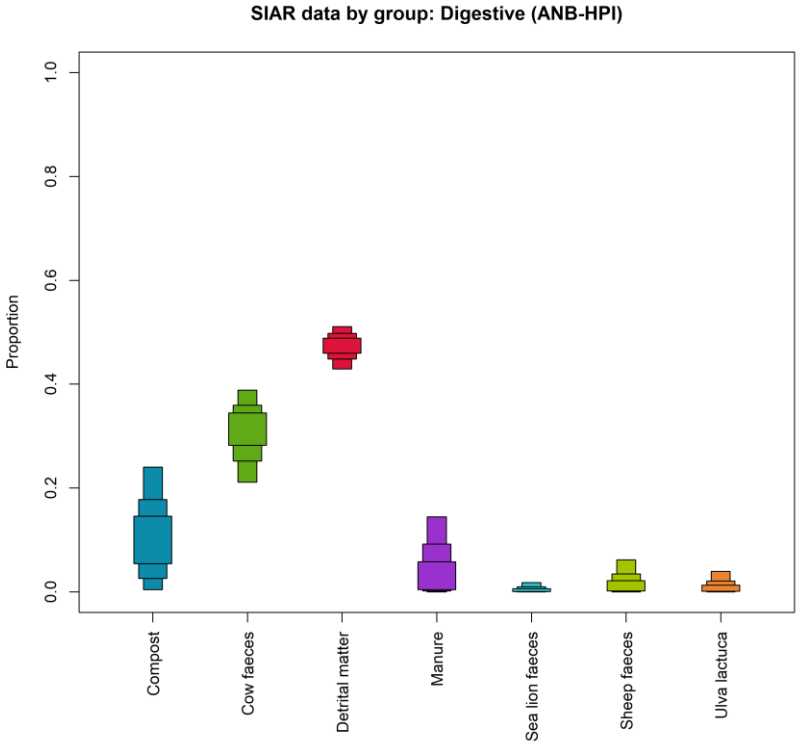
Figure 29A-G: Biplot, boxplots, histograms and matrix plots of carbon and nitrogen isotope ratios of possible nutrient source organic materials (including sewage effluent and animal sewage) in the digestive tissues of *Mytilus galloprovincialis* collected at the riverine-oceanic (ANB-TMC) and estuary-oceanic (HPI-SMB) waters along Otago Peninsula, New Zealand.

Hypothesis #2: Absence of sewage effluent

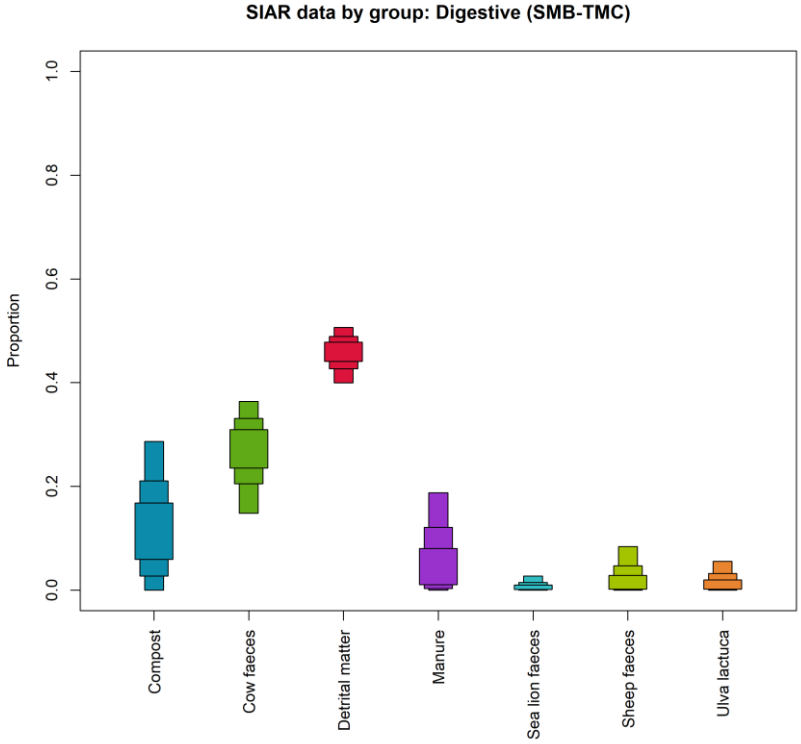
In the absence of effluent, other sewage-derived organic matter from pastoral farming (i.e. cow, sea lion faeces and sheep) overlapped one another and aggregated on the same side on the iso-space (Figure 30A). The estimated proportional contributions for each of the possible nutrient source organic materials in the digestive tissues of *Mytilus galloprovincialis* include; compost (22 %), cow faeces (40 %), detrital matter (54 %), farm manure (18 %), sheep faeces (4 %), *Ulva* (2 %) and sea lion faeces (1 %) at the estuarine-oceanic interface (Figure 30B). The source contributions from possible nutrient source organic materials in the digestive tissues of *Mytilus galloprovincialis* at the riverine-oceanic interface are compost (30 %), cow faeces (38 %), detrital matter (56 %), sheep faeces (10 %), farm manure (20 %), *Ulva lactuca* (2 %) and sea lion faeces (1 %) (Figure 30C).


SIAR data
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Ulva lactuca
Digestive (ANB-HPI)
Digestive (SMB-TMC)
Mean δ15N
Mean δ13C
5
10
15
20
-35
-30
-25
-20
-15
-10

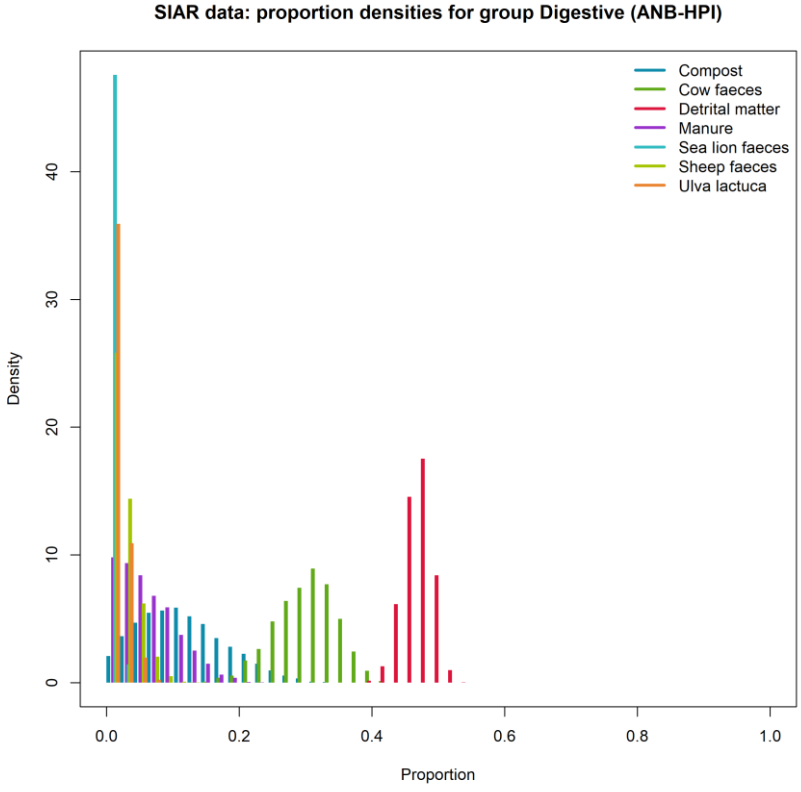
(A)


SIAR data by group: Digestive (ANB-HPI)
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Ulva lactuca

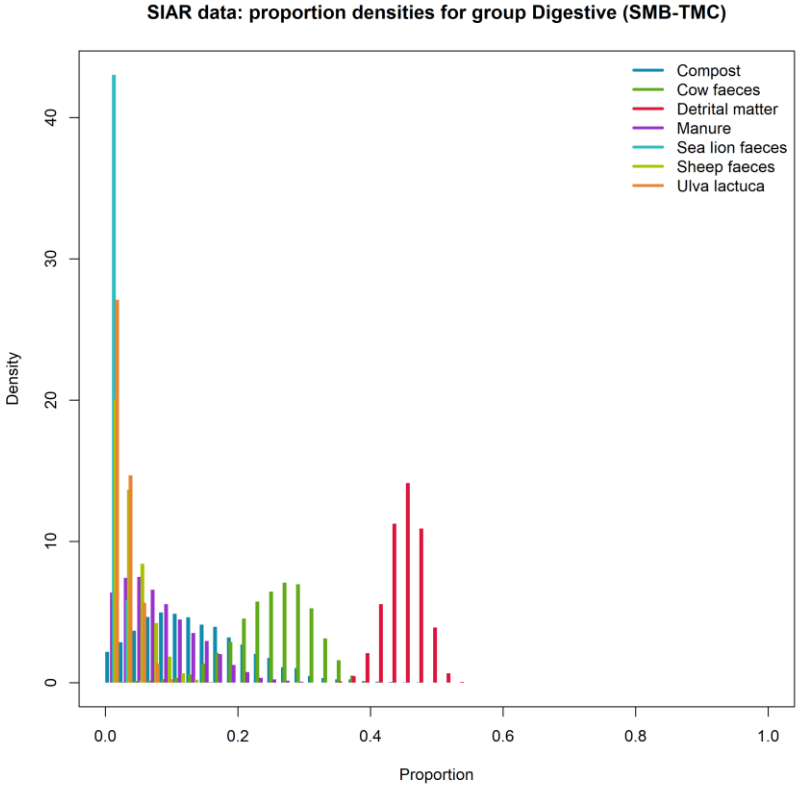
(B)


SIAR data by group: Digestive (SMB-TMC)
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Ulva lactuca

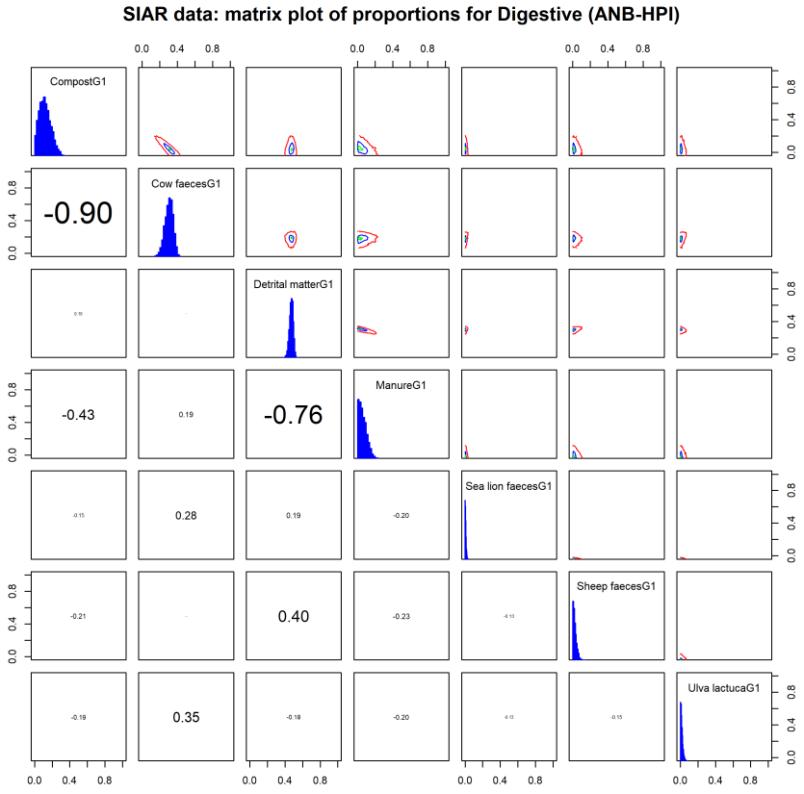
(C)


SIAR data: proportion densities for group Digestive (ANB-HPI)
Density
0
10
20
30
40
0.0
0.2
0.4
0.6
0.8
1.0
Proportion
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Ulva lactuca

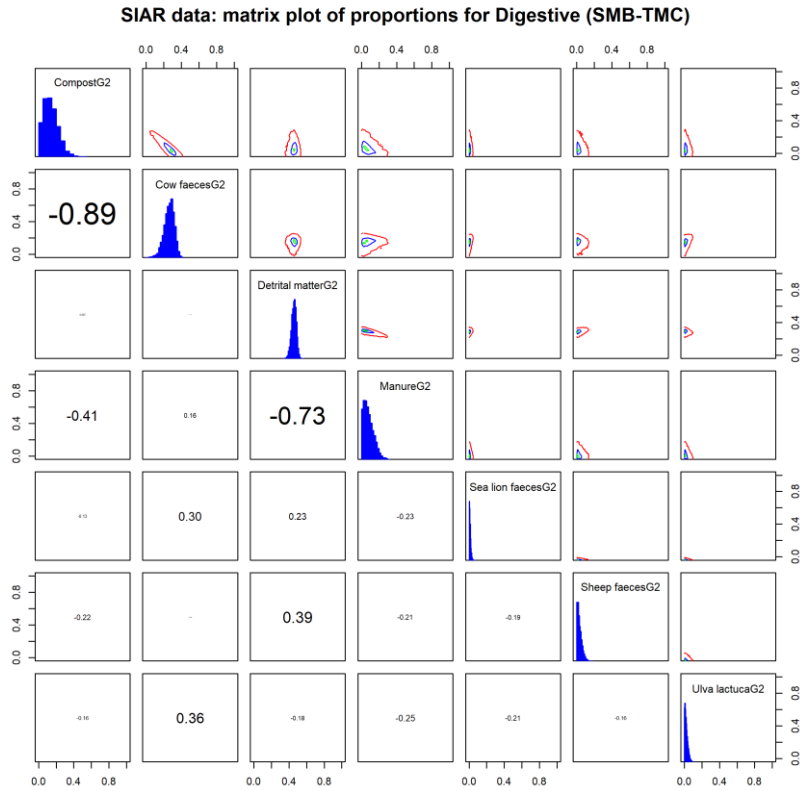
(D)


SIAR data: proportion densities for group Digestive (SMB-TMC)
Compost
Cow faeces
Detrital matter
Manure
Sea lion faeces
Sheep faeces
Ulva lactuca
Density
0
10
20
30
40
0.0
0.2
0.4
0.6
0.8
1.0
Proportion

(E)


SIAR data: matrix plot of proportions for Digestive (ANB-HPI)
CompostG1
Cow faecesG1
Detrital matterG1
ManureG1
Sea lion faecesG1
Sheep faecesG1
Ulva lactucaG1
-0.90
-0.43
0.19
-0.76
0.28
0.19
-0.20
-0.21
0.40
-0.23
-0.19
0.35
-0.18
-0.20
-0.15
0.0
0.4
0.8

(F)



(G)

Figure 30A-G: Biplot, boxplots, histograms and matrix plots of carbon and nitrogen isotope ratios of possible nutrient source organic materials (excluding sewage effluent) in the digestive tissues of Mytilus galloprovincialis collected at the riverine-oceanic (ANB-TMC) and estuary-oceanic (HPI-SMB) waters along Otago Peninsula, New Zealand.

A matrix correlation value of 0.90 was recorded for cow faeces-compost association, -0.76 between detrital matter and manure association, 0.40 for detrital matter-sheep faeces association in *Mytilus galloprovincialis* collected at the estuarine-oceanic interface (ANB-HPI) (Figure 30F). A matrix correlation value of 0.89 was recorded for cow faeces-compost association, -0.73 between detrital matter and manure association, 0.39 for detrital matter-sheep faeces association in *Mytilus galloprovincialis* collected at the riverine-oceanic interface (SMB-TMC) (Figure 30 G).

The exclusion and inclusion of the sewage effluent from the model simulation gave a different outlook for other terrestrial-based sources. There existed an overbearing effect existed between the detrital matter and sewage effluent in the model. This could lead to over estimation of the contribution of sewage matter at both interfaces. Since they can be affected by microbial breakdown processes (i.e. decomposition), such an effect is expected.

5.6.3 Marine, Estuarine Riverine and Sewage Organic Matter

To resolve the overbearing effect and uncertainty among the contributions of sewage organic matter and other nutrient sources of ecological relevance, a four-source member approach was fitted to address the ecological question of interest. The relevant four sources considered are marine, estuarine, riverine and sewage matter. A combined sewage matter for C and N isotope ratio values via the grand averages of sewage effluent, sheep faeces and cow faeces (see Table 34) was used. Since the sewage matter overlap, combining the sewage source groups of ecological importance and reduction of the sources seemed a better option to improve the mixing model tractability and inference of the sewage source contributions in *Mytilus galloprovincialis* collected at both interfaces.

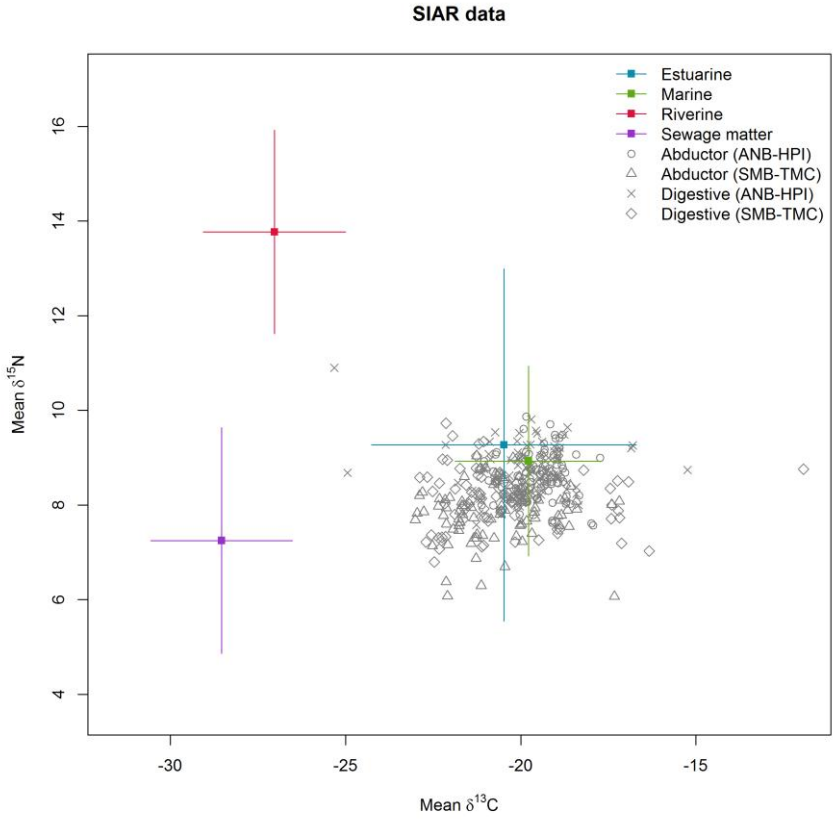
Using the four-source member approach, it is assumed that there are similar proportions of non-sewage materials at the sewage-unexposed (ANB-HPI) and previously sewage-exposed (SMB-TMC) sites, thereby avoiding the uncertainty and complex inferences of estimating the proportions of non-sewage materials in *Mytilus galloprovincialis* diet, and the measuring isotope ratios of all diet components by the Mix-SIAR. Since sewage-derived organic matter (SDOM) can be a mixture of organic detritus, other organic materials and microorganisms such as bacteria and algae, the uptake of SDOM by filter feeders could signify a major pathway of contaminants transfer to nearshore marine food web. The MixSIAR outputs are depicted in Figure 31A-G

Table 34: Isotope ratios of four-source member and percentage proportion in the digestive tissues of *Mytilus galloprovincialis*

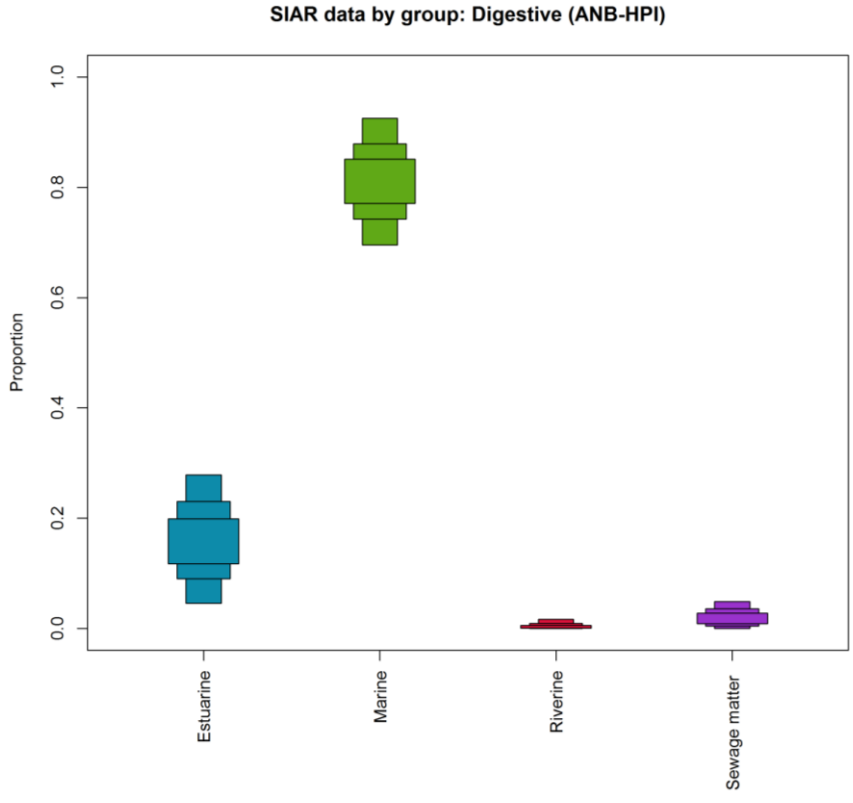
Source	$\delta^{15}N$	SD	$\delta^{13}C$	SD	% Proportion ANB-HPI	% Proportion SMB-TMC
Estuarine	5.87	1.57	-20.88	1.60	2-30	0-10
Marine	5.53	0.07	-20.18	0.32	70-95	70-90
Riverine	10.37	0.40	-27.43	0.18	negligible	negligible
Sewage matter	3.85	0.65	-28.94	0.15	negligible	10-18

The estimated proportional contributions for each of the four sources in the digestive tissues of *Mytilus galloprovincialis* include; estuarine (2-30 %), marine (70-95 %), riverine and sewage matter was negligible. The source contributions from possible nutrient source organic materials in the digestive tissues of *Mytilus galloprovincialis* at the riverine-oceanic interface

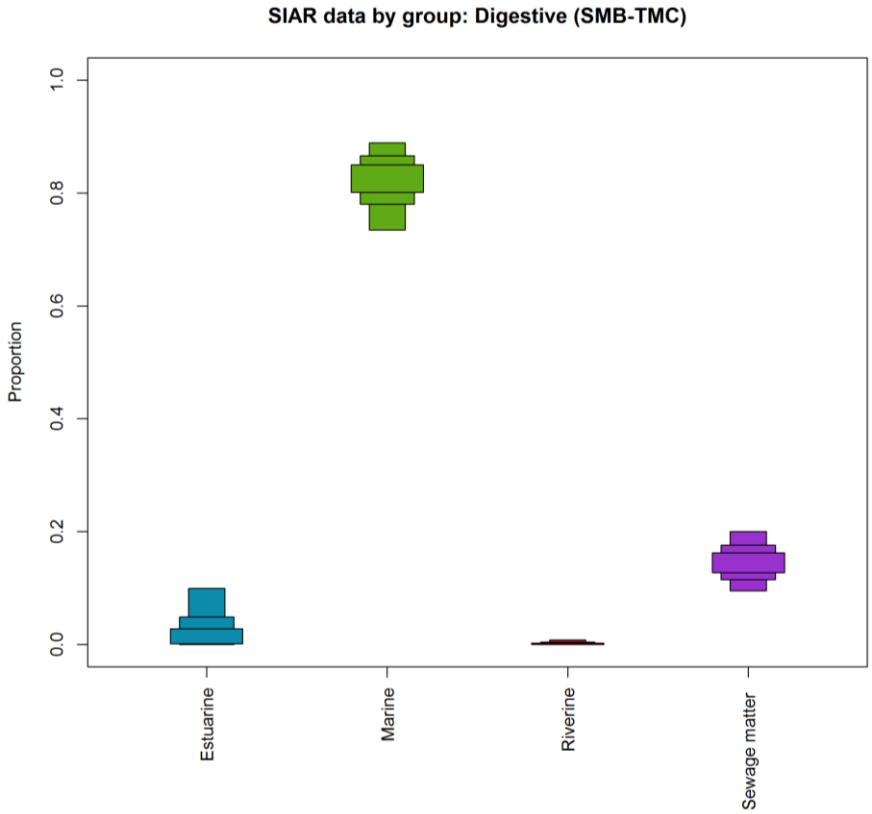
are estuarine (0-10 %), marine (70-90 %), and sewage matter (10-18 %). The riverine matter was insignificant (Table 34).


SIAR data
Estuarine
Marine
Riverine
Sewage matter
Abductor (ANB-HPI)
Abductor (SMB-TMC)
Digestive (ANB-HPI)
Digestive (SMB-TMC)
Mean δ15N
Mean δ13C
4
6
8
10
12
14
16
-30
-25
-20
-15

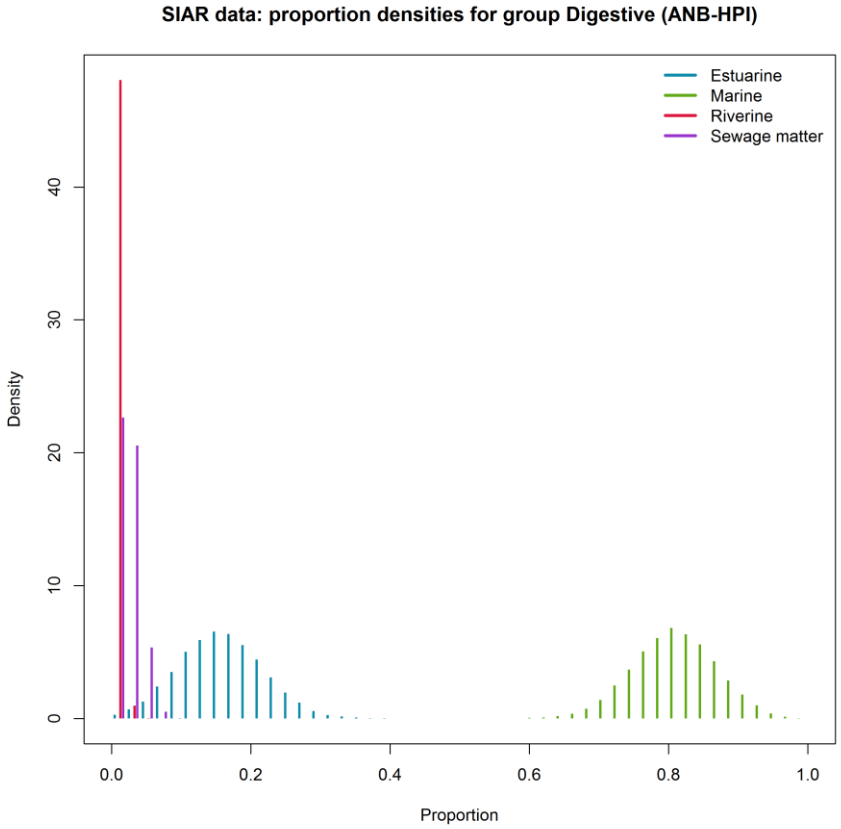
(A)


SIAR data by group: Digestive (ANB-HPI)
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
Estuarine
Marine
Riverine
Sewage matter

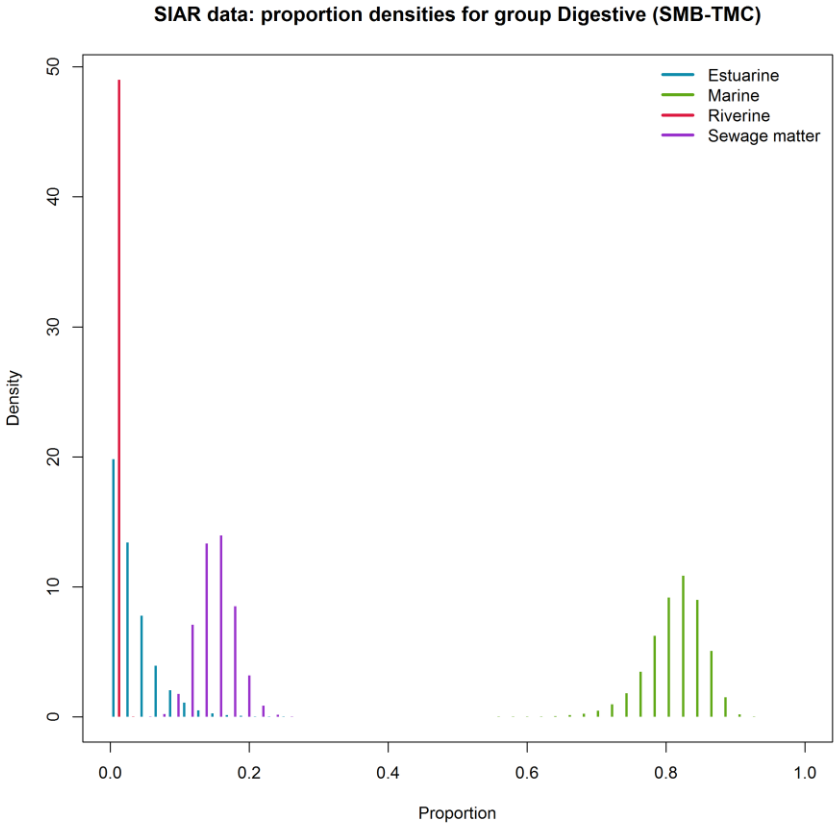
(B)


SIAR data by group: Digestive (SMB-TMC)
Proportion
0.0
0.2
0.4
0.6
0.8
1.0
Estuarine
Marine
Riverine
Sewage matter

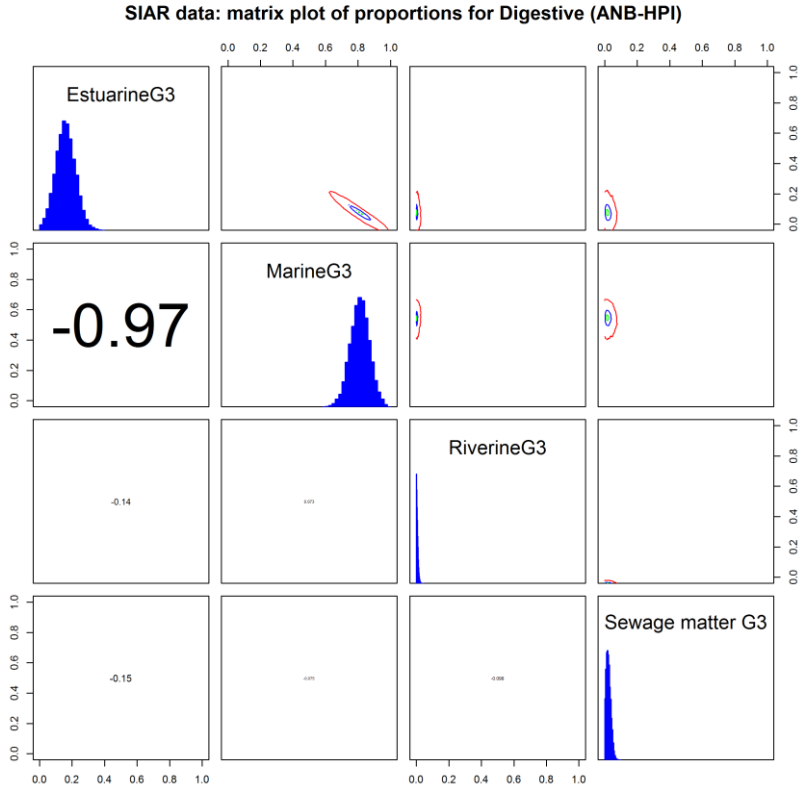
(C)


SIAR data: proportion densities for group Digestive (ANB-HPI)
Estuarine
Marine
Riverine
Sewage matter
Density
0
10
20
30
40
0.0
0.2
0.4
0.6
0.8
1.0
Proportion

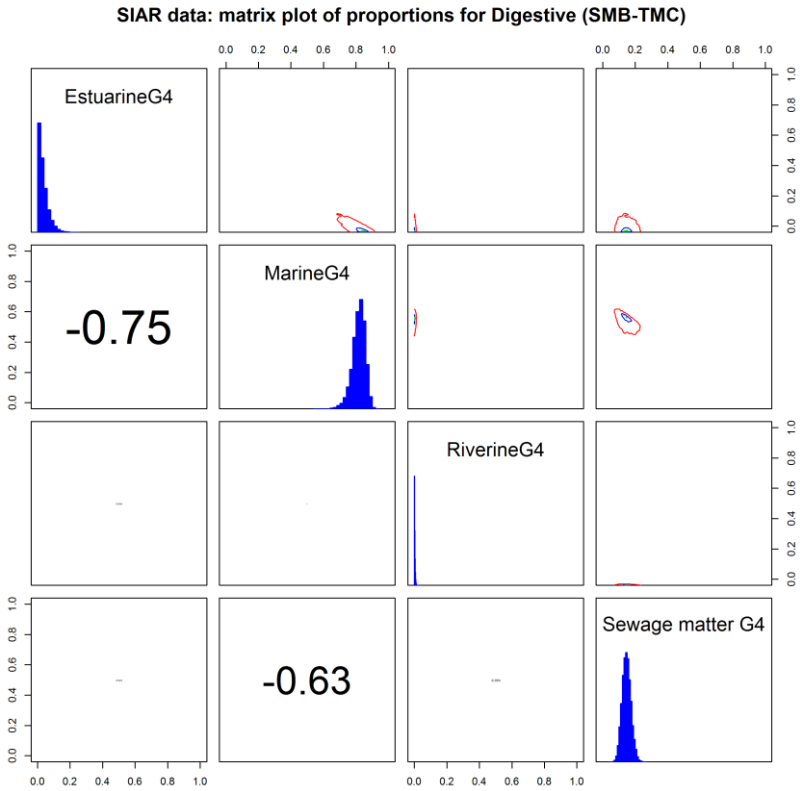
(D)


SIAR data: proportion densities for group Digestive (SMB-TMC)
Estuarine
Marine
Riverine
Sewage matter
Density
Proportion
0
10
20
30
40
50
0.0
0.2
0.4
0.6
0.8
1.0

(E)


SIAR data: matrix plot of proportions for Digestive (ANB-HPI)
EstuarineG3
MarineG3
RiverineG3
Sewage matter G3
-0.97
-0.14
-0.15

(F)



(G)

Figure 31A-G: Bi-plots, box plots, histograms and matrix plots of carbon and nitrogen isotope ratios of possible nutrient source organic materials (including sewage effluent and animal sewage) in the digestive tissues of *Mytilus galloprovincialis* collected at the riverine-oceanic (SMB-TMC) and estuary-oceanic (ANB-HPI) waters along Otago Peninsula, New Zealand.

A matrix correlation value of -0.97 was recorded for estuary-oceanic association, no robust matrix correlation values among marine, riverine, and sewage sources at the estuarine-oceanic interface (ANB-HPI) (Figure 31F). A matrix correlation value of -0.75 was recorded for estuarine-oceanic association and -0.63 between marine and sewage matter association in *Mytilus galloprovincialis* collected at the riverine-oceanic interface (SMB-TMC). No established matrix correlation value for riverine, estuarine and sewage matter sources (Figure 31G).

5.7 Discussion

In this study, the tidal channels influenced the seasonal patterns of physical, chemical and biological features of the proximate oceanic waters and played a vital role in the delivery of nutrients and organic materials to the intertidal mixing zone of the coastal marine waters. Studies have shown that the chemistry of water column in intertidal environments is complex and highly dynamic over slight spatio-temporal scales (Bouillon et al. 2007; Sakamaki et al.

2006; Taniguchi et al. 2008) and that the exchange of organic materials across the sediment–water interface play a significant role in intertidal water column chemistry. The composition of exchanged materials is influenced by the biogeochemistry of underlying intertidal sediments (Beck et al. 2008; Charette et al. 2005; Taillefert et al. 2007). The exchange processes themselves is often strongly influenced by tidally modulated hydrological processes (Bouillon et al. 2007; Cabrita et al. 1999; Povinec et al. 2008b; Rocha et al. 2009; Santos-Echeandía et al. 2010).

5.7.1 Hydro-geochemical Features of the Coastal Waters

The tidal estuary was hypersaline, mixed moderately throughout this study period. During early winter and autumn, the estuarine waters tend to be more saline than oceanic waters. An almost similar salinity of the downstream estuarine waters with the oceanic waters was observed in late winter and mid-spring suggestive of an intermittent euhaline and stratified prevailing conditions characteristic of strong tidal hypersaline stratified mudflat estuaries described in Uncles and Stephens (2000), Ralston and Stacey (2005).

Even though freshwater incursions seemed to influence the salinity gradients in Hoopers Inlet, the tidally mediated influx of saline water from the ocean was highly significant as it assisted in the mixing and flushing of organic materials in the estuarine system. In addition, the estuarine basin that is irregularly partially drained at low tide owing to residual tidal flow, wind-drift influence, and the estuarine storage and draining capability (triggered by the dynamic coupling to the coastal ocean) ensure a substantial volume of water remains in the estuarine during low tide. The shallowness of the downstream of the estuary, quantity of freshwater incursions, magnitude and duration of tidal flushing at the trade-off are influential factors that seem to control the distribution of nutrients and organic materials in the estuary.

Studies have shown that the movement of salt along an estuarine network to the physical forcing such as tides, freshwater flow, wind and evaporation is fundamental in predicting the extent of saline intrusion, which can provide insight into the dispersal of substances/materials (Becker et al. 2010; Monismith et al. 1996; Wells and Young 1992). Hydro-geomorphologic features such as water velocity, the channel width and type of riparian vegetation play a vital role in the balance between inputs of nutrients and organic materials from the terrestrial environment to the water column (Abrantes and Sheaves 2008; Bouillon et al. 2004).

During late summer and autumn, low level of dissolved oxygen levels was observed due to the build-up of macro-algal die-off, decomposed seagrasses, and marine organic materials.

The nitrate concentrations in the freshwater (Tomahawk Creek) were greater than 10 mg/l may indicate contamination by feedlot runoff, animal sewage, or fertilizer use. The level of nitrate in the freshwater affected the coastal marine waters at Smaills Beach.

5.7.2 Biogeochemical Cycling of Nutrients and Organic Materials in the Coastal Waters

The riverine system was highly eutrophic due to land-use influence, export of nutrients, fine sediment and other organic materials such as leaf litter from the watershed catchment and other drainage basin areas. The freshwater stream, Tomahawk Creek exerted a considerable influence on Smaills Beach. This is obvious from the slightly turbid coastal marine waters at Smaills Beach due to the incessant infiltration of large amounts of organic matter from the freshwater to the coastal marine waters. Some of the easily noticeable organic materials of interest that accountable for the turbid coastal marine waters were; fine sediment, excreta from grazing seabirds and sea lions, leaf litter and plant debris that could also supply additional nutrients to the nutrient-rich nearshore waters. However, a significant amount of nutrient contribution was recorded in the coastal waters during a period of high precipitation and runoff from the catchment area. In contrast, the coastal water at Allans Beach (estuary-ocean interface) was clear and moderately nutrient-rich despite the high presence of organic materials in the estuary (Hoopers Inlet).

A previous study on the estuary revealed that the estuary's sediment was variable with fine sand and had high organic matter content (Goerlitz et al. 2013). They attributed the low abundance of seagrasses (*Zostera muelleri*) and other marine vegetation to the burrowing activities via bioturbation of the sediment by polychaete lugworms (*Arenicola marina*) which are noticeable by the numerous faecal casts they produced in the estuary. The bioturbation sediment mixing of *Arenicola marina* had been reported to increase nutrient fluxes from the sediment to the surface water and improve decomposition of organic matter (Andersen and Kristensen 1992; Asmus and Asmus 1998; Kostka et al. 2002; Nielsen et al. 2003).

Fluxes of nutrient and organic material supply of interest to the coastal waters are; wood debris, decomposed grasses such as cutty grasses (*Carex spp*), knobby club rush (*Isolepis nodosa*) and faecal matter from grazing seabirds and sea lions. Human-induced activities such as pastoral farming and other agricultural practices undoubtedly accounted for the observed elevated nutrients at the adjacent and coastal marine waters. The application of inorganic phosphate fertilizer (particularly superphosphate to raise soil P levels) for top-dressing purposes by dairy farmers to encourage pasture growth resulting in high reactive

phosphate runoffs from catchment drainage basin (Mitchell 1989; Wheeler et al. 2004). Nitrate increases in the proximate water bodies and coastal marine waters were from the use of N-fertilizer, compost and farm manure, grazing farm animal faecal matter, defecation from grazing marine animals (i.e. sea lions and birds).

The elevated nutrient runoff and increase in temperature are responsible for high chlorophyll a concentration and microalgal growth (i.e. episodic eutrophication) recorded in mid-spring and summer in the estuary and along coastal marine waters studied. Innes et al. (2010) in their study of the Otago Harbour, considered phosphorus as a limiting nutrient initiating intermittent eutrophication in estuaries, responsible for the deterioration of water quality in estuaries. They observed that relatively small phosphate inputs could lead to algal blooms that, upon dying and decomposing, deplete oxygen to levels that can threaten aquatic life.

5.7.3 δ^{15}N and δ^{13}C-SPOM Distribution in Water and N-source Contributions in the Resident Organism

Organic materials in river waters can occur as organic colloids macromolecules infiltrated from soils, organic material in soil aggregates and organic material produced in the river system. Residual particulate organic materials (POMs) in rivers are typically associated with soil aggregates, which flocculate in seawater (Helland et al. 2003). Therefore flocculation is a key process which determines the distribution of colloidal materials, organic materials and elements at the terrestrial-aquatic interfaces of salinity gradients (i.e. estuary and coastal marine waters) (Furukawa et al. 2014; Gratiot et al. 2017; Hassani et al. 2017; Verney et al. 2009).

The continuous distribution in the δ^{15}N and δ^{13}C of the suspended organic matter across the estuarine-sea gradients (Allans and Hooper's Inlet) indicated that the marine bivalve, *Mytilus galloprovincialis* possibly derived nourishment from allochthonous suspended organic matter and nutrients from the estuaries. These exported resource subsidies from the estuaries are critical in the enhancement of the coastal biodiversity and intertidal ecosystem functioning.

The nonconformity of the mean distribution of δ^{15}N and δ^{13}C in the suspended particulate organic matter between coastal marine waters and the adjacent water bodies established the varied mixed compositions of the organic pool (suspended organic materials) assessed across the estuarine-ocean interface. Similar means would have revealed the relationship among the associated source of suspended organic materials. The outlook of the C/N-SPOM mole ratios of the coastal marine waters was expected to fall within the range 5 and 8 to suggest nutrient-

replete phytoplankton such as diatoms and algae (marine particulate organic matter) (Geider and La Roche 2002; Rullkötter 2006; Vuorio et al. 2006) but the marine particulate organic matter recorded fell within 6.98 and 9.84. This is suggestive of influence nutrients from agricultural watershed catchments, via freshwater and estuary waters of SPOM C/N mole ratio ranged between 9 and 12 (Lambert et al. 2017; Ngugi et al. 2017; Thornton and McManus 1994).

Inorganic nitrogen and phosphate inputs mainly derive from agricultural runoff carrying fertilizer and minor organic inputs consisting of dissolved and particulate forms of nitrogen associated with decomposing organisms and human and animal wastes seemed to influence the spatial and temporal N isotope signature of *Ulva lactuca*. The increase in biomass of *Ulva lactuca* in spring and summer, and reduction in autumn (and eventual decomposition in late autumn) attests to the fact that the sea lettuce's growth is nurtured by agricultural activities along the shoreline and further upstream of the estuary (i.e. Hoopers Inlet). Contribution of nutrient inputs from agricultural watershed catchment in the form of non-point nutrient additions and point sources from sewage matter from human and animal contribute nutrients that enter the coastal marine waters, and increase the amount of available nitrogen and phosphorus at Smaills Beach.

Ulva sp. typically grow throughout the summer due to solar radiation and decline in the autumn as light conditions. However, in summer, high water temperatures can cause decline in sea lettuce. The growth of *Ulva sp*. throughout the spring and summer serves as large sink of nutrients in the water column. Decomposed *Ulva sp*. contributes nutrients back into the sediment and the water column to aid productivity in summer (Martinez et.al. 2012 and Nelson et.al. 2003).

5.7.4 Mix-SIAR and Source Contributions

Terrestrial-based nutrient and sewage-derived organic matter arising from facility a land-use for pastoral farming and municipal wastewater treatment facilities are of great environmental concern to the health status of the proximate water bodies and coastal marine waters.

The MixSIAR model results revealed that the marine particulate organic matter is the major food source contributor to the marine bivalve at both interfaces. The estuarine particulate organic matter contributed a significant portion to its diet with a negligible subsidy from riverine particulate organic matter. The four-source member approach revealed moderate

contributions of sewage matter at Smaills Beach to be between 10-18 % and insignificant amount at Hoopers Inlet.

The moderate correlation value of -0.63 for marine-sewage matter association at the riverine-oceanic (previously sewage contaminated site) interface was indicative of possible integration of sewage matter in marine matter at Smaills Beach. However, there was no correlation of riverine-sewage matter association suggestive that sewage input from animal is insignificant or intermittent. Negligible correlation values obtained for marine-sewage matter, estuarine-sewage matter, and riverine-sewage matter associations at the estuarine-oceanic interface signify no sewage influence. This agrees with the notion that Allans Beach and Hoopers Inlet waters are probably pristine and are not sewage impacted.

However, the isotope ratios of the animal sewage organic materials used to mock-up the model as estimate of the sewage matter in *Mytilus galloprovincialis was* untainted. Hence the estimated sewage matter contribution maybe accepted provided the bulk of the animal sewage matter was ingested directly. The assumption is that the sewage matter from watershed basin as run-off was not transformed via microbiological processes in the water column.

5.8 Conclusion

The outcomes of this study show that the hydro-biogeochemical dynamics of the coastal marine waters and proximate water bodies were influenced by elevated nutrients from moderate agricultural activities that promote seasonal eutrophication in the course of spring and summer. However, natural processes in the coastal marine waters coped well the modest anthropogenic influences. The seasonal excessive growth of *Ulva lactuca* in the estuaries was driven by the run-off nutrients from organic manure and inorganic fertilisers usage to grow pastures for farm animals.

The Mix-SIAR survey on the impact of sewage nutrients and organic materials in the resident organism of coastal marine systems revealed that the riverine-oceanic interface had sewage matter indication alongside the major nourishment (i.e. marine matter). The estuarine-oceanic system dominated by marine and estuarine matter deprived of sewage matter. Both interfaces had negligible influence of riverine matter and significant contributions of estuarine matter at both interfaces. This emphasises the significance of estuarine processes in the transfer of organic materials as nutrients and contaminants, resource subsidy across ecosystem

boundaries and eventual assimilation / sequestration in the coastal marine biota and receiving adjacent coastal marine waters.

Generally, the major contributions of marine matter, use of terrestrial-derived particulate organic matter from the estuary by the bivalve and negligible subsidy riverine matter contributed to the sustenance of the nearshore marine bivalve. This asserts that land-use activities in river catchments from any or all of such as the use of inorganic fertilisers, compost and organic manure by pastoral farmers as well as sewage effluent from wastewater treatment facilities can contribute nutrients and organic materials capable of promoting periodic eutrophication in the estuary and coastal marine waters.

5.9 Future Direction

The pool of organic matter in the waters of Hoopers Inlet is characteristically large consists mainly of recalcitrant organic materials which are difficult to decompose within the time required for them to be tidally flushed out of the system. Therefore, there is the tendency for the integration of these materials in resident intertidal biota of the coastal oceanic waters. However, this study may not precisely identify the sources and nature of organic materials but provided hints on their respective influence as potential sources of nutrient and contaminant supply to the coastal marine waters.

This study reported the periodic variations in the export of riverine and estuarine matter that combine with the major marine matter with adequate attention given to sewage-derived organic materials and marine end-members in a resident organism (*Mytilus galloprovincialis*) via the developed MixSIAR isotope mixing models. However, these mixing models (either linear or Bayesian isotope mass balance models) did not take into consideration the decomposition processes in the water column, which can possibly cause a shift the isotopic signature (i.e. fractionation) of organic materials indicated in end-member source inputs. These models seem not to be quick solutions to answer questions about the food-web structure of the coastal marine waters. Consequently, there is a need to identify the nutrients and contaminants in the resident organism. The use of chemical tracers may indicate the nature and sources of nutrients and contaminants in the resident organism and perhaps in the coastal marine waters.

Additionally, the nature of the organic pool of nutrients in the riverine and estuarine systems are of ecological importance and can be resolved categorically via the development of a denitrified analytical system to differentiate between the water column and sedimentary

denitrification. The system will afford the opportunity of measuring the isotopes of oxygen and nitrogen of nitrate in the water samples to acquire further insight and give and understanding of the major N transformation that may occur in the coastal marine waters. Thereby the various sources of N and the role of microbes involved in the N transformation water column can be categorised.

CHAPTER 6

Biochemical compositions and trace metal levels in *Mytilus galloprovincialis* as tracers of nutrients and contaminants of coastal waters along Otago Coastal Waters, New Zealand

Overview

In the previous chapters, the stable isotope values were used to gain an understanding of the status of the coastal environment near Dunedin in terms of possible contamination by sewage. The model derived from that work indicate there is now insignificant impact of sewage on the *Mytilus galloprovincialis* sampled from the region. However, stable isotopes are a useful course tool and subject to numerous influences, including additional sources and /or transformations that may affect the conclusions. This chapter examines the use of independent chemical tracers to confirm results from the application of stable isotope study and mixing models. Therefore, the objectives are to:

- Use independent tracers to demonstrate that sewage-derived organic matter was no longer a significant influence in the nutrition of bivalves from the previously contaminated site (Smaills Beach).
- Develop the technique to assess the relative abundance of biochemical and elemental compositions in *Mytilus galloprovincialis* to indicate the source of nourishment in the bivalves and infer changes in nutrient supply and contaminant fluxes in the coastal marine waters.

The chapter focuses on the identification of biochemical tracers (i.e. faecal sterol and elemental compositions) in *Mytilus galloprovincialis* as an independent means of testing the conclusions from the stable isotope studies. The stable isotope-mixing model assumptions in chapter 5 gave an impress of the influence of sewage-derived organic materials in *Mytilus galloprovincialis* in the absence of decomposition processes in the water column, which can effect a shift in the isotope signature (i.e. fractionation) of organic materials signal in end-member source inputs. Therefore, the use of chemical tracers to indicate the source of nourishment to the resident organism to gain insight on the nutrient supply and contaminant dynamics of the coastal marine waters was required. Independent assessors such as sterol, fatty acid and elemental analyses can indicate the sources of nutrients and contaminants in *Mytilus galloprovincialis*. They serve as complementary indicative tools for inspecting the stable isotope-mixing model inferences, which was a key environmental tool for investigating the impact of discharged of sewage-derived organic matter on the nearshore marine waters

along Otago Peninsula, Dunedin, New Zealand. Sterol and fatty acid analyses on the bivalve provided insight into the nutrition of the bivalve. The elemental analysis on the bivalves was used to examine the health of the bivalves and any significant threat to human health associated to consuming the bivalves from the coastal marine waters.

6.1 Introduction

Organic matter, a large pool of carbon-based compounds exists naturally in terrestrial and aquatic environments. It can also come from decomposed plants and animals and their waste materials. Due to the heterogeneous nature of organic matter, it serves as a medium for fluxes of anthropogenic nutrients and contaminants in natural waters (Doney 2010; Schwarzenbach et al. 2006; Seitzinger et al. 2002; Zhang et al. 2003). In aquatic systems such as rivers, estuaries and coastal oceans, organic matter is susceptible to transformation or decomposition from processes such as microbial or metazoan grazing, viral infection and cell lysis, and photochemical breakdown (Bertrand et al. 2015; Bianchi and Canuel 2011; Kirchman 2018; Sinsabaugh and Foreman 2003; Stahl et al. 2013; Zonneveld et al. 2010).

The coastal ocean ecosystem receives organic matter from distinct but connected ecosystems such as rivers, estuaries, wetlands, and the continental shelf. The difficulty of distinguishing organic matter within this complex range of sub-habitats is due to the range of sources from which the organics originate (Bianchi 2011; Canuel 2001; Hopmans et al. 2004; Mannino and Harvey 2000; Stedmon et al. 2007). The intricacy of characterising organic matter sources in aquatic ecosystems and the complications accompanying the use of bulk measurements of suspended particulate organic materials from the water column to constrain sources has made the application of biochemical tracers desirable in aquatic studies (Bianchi 2007; Canuel and Hardison 2016). Biochemical tracers (i.e. chemical tracers) are organic compounds that convey information on the sources of organic matter or contamination in the environment (Eganhouse 2004). The chemical structures of biochemical tracers are associated with their specific origins; hence, the occurrence of associated compounds in organisms or the environment can be a pointer to the possible ‘source material’.

Biochemical tracers such as stable isotopes, trace elements, fatty acids and steroids are extensively used in environmental studies as markers for distinguishing and identifying sources of nutrients and contaminants in aquatic systems (Biache and Philp 2013; Chou and Liu 2004; Evershed et al. 2007; Figueira et al. 2002; Hu et al. 2009; McCorquodale et al. 1996; Miller et al. 2008; Peng et al. 2002; Roussiez et al. 2006; Standley et al. 2000). They

are also useful as tracers for numerous other geological and environmental processes (Simoneit 2005).

6.1.1 Sterols

The distribution of faecal sterols such as cholesterol, coprostanol, and 24-ethylcoprostanol in man and shellfish has been studied by many investigators to indicate and distinguish faecal contamination originating from human and higher animals (Cathum and Sabik 2001; Gagné et al. 2001; Hellou et al. 2003; Yeats et al. 2008). Sterols have been found to be persistent in the environment and transferred from the watershed or catchment basin areas to coastal waters where shellfish are situated (Jeanneau et al. 2012; Solecki et al. 2011). During excretion of sterols in humans, it has been shown that cholesterol is reduced in the gut by microorganisms (i.e. Human intestinal microflora) to 5β −coprostanol via 5β −Cholest-4-en-3-one and 5β −Cholestan-3-one (Drasar and Hill 1974; Grimalt et al. 1990; Walker et al. 1982; Yasuda 1978) (Figure 32).

Grimalt et al. (1990) affirmed that occurrence of coprostanol in aquatic samples (from coastal areas) such water particulates and sediments collected in urban polluted and pristine areas could not by itself be unambiguously attributed to human faecal matter inputs unless compared with other faecal-linked sterols. Coprostanol with other faecal steroids, though considered an indicator of human faecal contamination by a large number of researchers since late 1960s; it is still rarely embraced as a sanitary indicator for sewage contamination because it has no known health risk (Leeming et al. 1996).

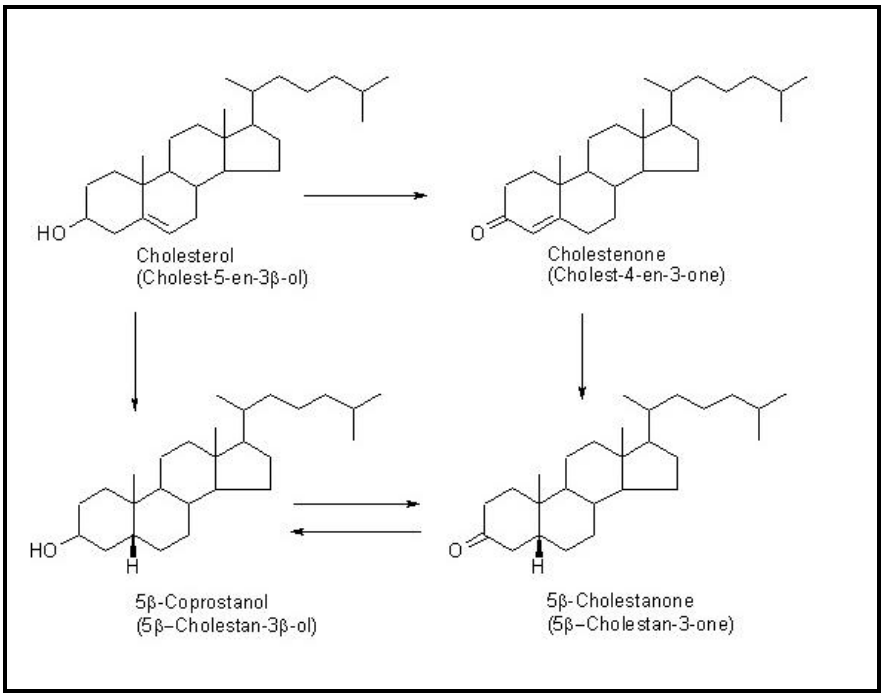


Figure 32: Pathway for the reduction of cholesterol to coprostanol [modified from Grimalt et al. (1990)]

Faecal-associated sterol, 24-ethylcoprostanol is an unsaturated phytostanol (i.e. plant sterol) found only in the plant fats or oils, in the gut and defecation of herbivores. It is an applicable biomarker for the presence of animal (non-human) faecal matter in the environment. Leeming et al. (1996) in an attempt to elucidate the sources of faecal pollution in water particulates, sediment samples and faecal samples from other possible sources (i.e., humans, dogs, sheep, birds) from Australian inland and coastal regions found that herbivores have a different dominant faecal sterol form (i.e. 24-ethylcoprostanol). He observed that human faecal pollution typically has a ratio of > 0.73 based on a fraction index in Equation 20 below:

[(coprostanol / (coprostanol + 24-ethylcoprostanol)]…....Equation 20

This fraction index evaluation is widely used by researchers in the determining the degree contribution of faecal matter from herbivore and human sources relative to each other. However, the distribution of stanols (i.e. coprostanol and 24-ethyl coprostanol) in animal faecal matter depends on the animal's diet, the ability of the animal to biosynthesize endogenous sterols and existence of intestinal flora, gut microorganisms responsible for cholesterol biohydrogenation to coprostanol. In practice, these two stanols are appropriate indicators of faecal matter sources and contamination.

6.1.2 Trace Metals

Sewage derived organic matter (SDOM) discharged within a few meters from coastal waters via ocean outfalls and other agricultural activities can increase the concentrations of trace metals in resident nearshore biota to hazardous levels (Al-Ghadban et al. 2002; de Mora et al. 2004; Lawal-Are and Babaranti 2014). Terrestrial runoff is one of the most common conduits for trace metals entering the coastal marine waters via estuaries and rivers (Förstner and Wittmann 2012; Rose and Shea 2007). Agricultural fertilizers, mining tailings, municipal wastewater, industrial and landfill discharges are other examples of point sources of toxic soluble trace metals in rivers estuaries and coastal marine areas (Daby 2006; Förstner and Wittmann 2012; Prasad 2003; Rieuwerts 2017; Schwarzenbach et al. 2010).

From an ecological and health perspective, hyper-accumulation of As, Cd, Cr, Cu, Ni, Pb and Zn (Perez et al. 2005; Soto-Jiménez et al. 2001 and Zhang et al. 2007) in marine aquatic organisms and higher plants grown in the soil is deleterious (Kabata-Pendias and Mukherjee 2007; Peralta-Videa et al. 2009; Prasad 2003; Sposito 1981; Wuana and Okieimen 2011; Zhou et al. 2008).

The pathways for toxic soluble metals in marine organisms such as finfish and shellfish may include; the ingestion of particulate suspended materials from the water column and pre-concentrated metals in the diet. Other possible forms are; organometallic materials derived from linkages of metals and organic compounds through chemical complexation and incorporation of metal ions into systems of physiologically important. In addition, uptake by diffusive exchange onto the mucous sheets located in the gills (Apeti et al. 2005; Armstrong and Atkins 1950; Bowen and Sutton 1951; Brooks and Rumsby 1965; Lehninger 1950; Rüdel et al. 2003; Schubert 1954). These toxic metals are concentrated many times higher than present in the water column in finfish, and shellfish tissues then passed higher up the food chain (Goerke et al. 2004; Lawal-Are and Babaranti 2014).

6.1.3 Dietary Contributions

Mytilus galloprovincialis, a marine shellfish, long-lived filter-feeders with high clearance rate and capable of directly ingesting (via the gills) and assimilating sewage particulate organic matter (POM) (containing carbon and nitrogen) was used for an isotopic study designed to investigate the impact of sewage-derived organic matter (SDOM) on the nearshore marine ecosystem of the Otago Coast. The marine bivalve exhibited a trophic enrichment factor of +3 ‰ (δ^{15}N) and +1 ‰ (δ^{13}C) in the digestive tissues when compared to the marine

particulate organic matter (POM), suggestive of a dietary change away from the SDOM (Babaranti et al 2019). The suspended particulate matter collected from freshwater and estuarine sources was suggestive of other possible carbon and nitrogen sources from human-driven activities such as pastoral farming, application of organic manure and inorganic fertilisers, nitrification of ammonium from semi-urban septic tanks, and animal organic waste residues (Babaranti et al. 2019).

In chapter 5, the Bayesian stable isotopic mixing model in R (MixSIAR) simulation was used to determine the possible contributions of combined sewage matter from human and animal sources in the digestive tissues of *Mytilus galloprovincialis*. The estimated proportional contributions from sewage-derived organic materials in the marine bivalve was between 10 - 18 % at the riverine-oceanic interface and an insignificant contribution at the estuarine-oceanic interface. At both interfaces, marine matter was observed to be the major component of *Mytilus galloprovincialis* with significant subsidy from estuarine matter. The application of the stoichiometric and stable isotope analyses with the mixing models was found to aid the pseudo-quantification of the flow of C and N land-based organic materials in bivalve via the coastal marine food chain (producer-consumer interactions), identified key members and linkages in the recipient food chain. The findings provided the basis for further assessment of the community- and ecosystem-based implications of sewage matter cycling and dynamics in the coastal marine waters.

In addition, the movement of sewage matter and other elements through food chain and their consequences for nutrient cycling will be of assistance in the prediction of organic matter sources and management of the human-altered surface waters of the coastal marine waters. However, the shortfall of the applied stable isotope-mixing model is its incapability to account for the decomposition processes in the water column that can change the isotopic signature of organic materials reflected in the end-member source inputs. This created an uncertainty in the inference of the source of nutrients and contaminants in the Otago's Coastal waters.

Consequently, there is the need for further assess the biochemical compositions of the resident organism using chemical tracers to ascertain the nutrient supply and contaminant dynamics of the coastal waters. Therefore, the specific objectives of this study are to:

1. Assess faecal-linked sterol abundance in *Mytilus galloprovincialis* as independent assessor to determine if there is any faecal contamination in the coastal marine waters.

2. Determine the levels of toxic soluble trace metals such as As, Cd, Cr, Cu, Ni, Pb and Zn in *Mytilus galloprovincialis* to indicate the magnitude of faecal contamination and healthiness of coastal marine fisheries and waters
3. Assess the potential health risks to human consumers of *Mytilus galloprovincialis* collected from the coastal marine waters

The working hypothesis is that the negative impacts from intensive pastoral farming activities, storm water drains, ground water from septic tanks of semi-rural communities, animal faecal matter from grazing animals, farm effluent, landfill leachates and human wastewater discharges will increase the level of sterols and toxic metal concentrations in the resident marine bivalves of the coastal marine waters. In addition, since there are sterols that are specific to particular sewage sources, they could be used to indicate the source of the contaminant. Coprostanol and 24-ethylcoprostanol are considered indicative of human and animal sewage matter in natural aquatic waters accordingly (Furtula et al. 2012; Gilpin et al. 2002)

Since, there are no information to suggest that shellfish and finfish metabolise coprostanol and 24-ethylcoprostanol, the concentrations of these steroids can be measured from their digestive matter. These two sterols are emblematic to mammals only. Marine bivalves do not possess any anaerobic bacteria in their digestive tract to biohydrogenate sterols to various stanol isometric structures, unlike many animals which irrespective their diet can biosynthesise sterols. For instance; Kanazawa and Teshima (1978) could not detect coprostanol in the faeces of *Tilapia* fed on a diet containing cholesterol as a sterol source.

Hence, information on levels of these two faecal-linked sterols and toxic metal concentrations in *Mytilus galloprovincialis* could shed light to the uncertainty of the potential sources of faecal contamination in the coastal waters, ecosystem health of the coastal waters and well-being of the resident coastal biota. This will further give a reliable fingerprint of the nutrient supply and contaminant dynamics of the coastal waters and lay to rest the rising public concerns on the standard of the coastal waters for recreational activities, safe collection and consumption of the resident coastal marine fisheries. Thus, such information will assist environmental bodies and monitoring agencies in taking key decisions on protecting public health, monitoring coastal fishery resources and recreational coastal waters to ensure faecal pathogens and other metal-linked toxins are not present.

6.2 Materials and Methods

6.2.1 Study Area

The study sites for this study is the same as that of Chapter 5 as described in section 5.2.1. The two shoreline waters chosen had been studied by (Babaranti et al. 2019) and categorised based on discharged sewage contamination. Allans Beach (uncontaminated) as a reference site and Smaills Beach as previously contaminated).

6.2.2 Sample Collection and Preparation

A monthly collection of *Mytilus galloprovincialis* from 14/7/16 to 29/06/17 from the intertidal mixing zones of two nearshore marine waters with contrasting proximate tidal channels along the Otago's Coastline was carried out. The two coastal waters were portions of the Otago Marine Area and 10 km apart. *Mytilus galloprovincialis* individually placed into clean ziplock plastic bags labelled with date and sample site location. Sealed off in a plastic cooler for onward transport to the laboratory, later rinsed in deionised water, and frozen samples were freeze dried at a temperature of -80 °C for 24 h at the PC 2 microbiology laboratory after collection prior to analysis. The gut (digestive tissue) was removed for faecal analysis and bulk tissues of the mussels removed with an acid-washed ceramic knife (soaked in 10 % HNO_3 solution for at least 24 h). The gut and the bulk tissue were freeze-dried in a 4.5-litre Labconco FreeZone® Freeze Dry System, for at least 12 h separately in plastic vials hitherto acid-washed. Once dry the gut was homogenised separately with the aid of an MM400 bench-top Retsch ball mill using agate cups and balls. The residual bulk tissues were homogenised with the aid of a plastic mortar and pestle hitherto washed in acid. Ground samples stored in acid-washed plastic vials individually, which were placed under vacuum overnight for further drying.

6.2.3 Biochemical Tracer Analysis

As described in section 2.5 to 2.5.7 of Chapter 2

6.2.4 Trace Elemental Analysis

As explained in section 2.6 to 2.6.4 of Chapter 2

6.2.5 Statistical Analysis

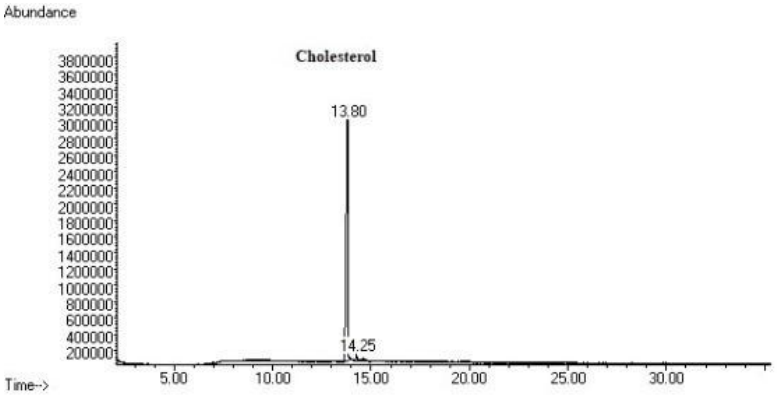
Parameters such as the minimum value, maximum value, mean value, relative standard deviation and error were determined for the trace metal concentrations in *Mytilus galloprovincialis* using Excel 2013 (Microsoft Corporation, USA). The SPSS 20.0 software

(IBM Corporation, USA) was used to conduct a one-tail F-Test two-Sample variances analysis using a significant level of 95 % with $\alpha \leq 0.05$ benchmark for the validation of the acceptance of the set hypothesis (see Table 36). The variance analysis was to test the normal distribution of each metal concentrations in the marine bivalve between the two sites studied. The intention for the mean-variance analysis was to examine whether if site influences metal concentrations in the bivalve since the marine bivalves analysed were randomly selected and independent of one another.

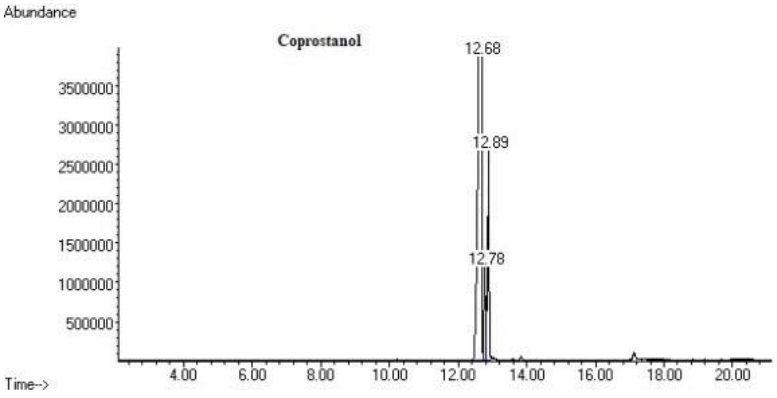
6.3 Results

6.3.1 Sterol Standard Chromatograms

In the GC, the hydroxyl groups of sterols standards transformed into alkyl silyl ether derivatives to improve the peak outline, determination and sensitivity of sterol standards. The GC chromatogram of the sterol standards and retention time represented in Figure 33a-c and Table 35.



a



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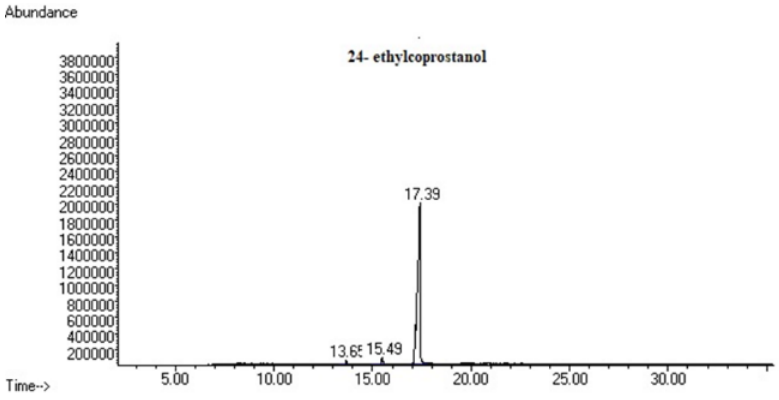


c

Figure 33a-c: Distinct GC chromatograms of each of the sterol derivatives with their respective retention time.

Table 35: Chemical formula, derivative nomenclature, mass (MW), retention time (RT), peak area (PA) (indicated by total ion chromatogram) of sterol standards with standard error and number of samples measured

Sterol	Chemical formula	Silylated derivative chemical name	MW	RT (min)	TIC ± SE (%)
cholesterol	$C_{27}H_{46}O$	Cholesterol trimethylsilyl ether ($C_{30}H_{54}OSi$)	459	13.80	77.58 ± 0.58 (n = 3)
coprostanol	$C_{27}H_{48}O$	3-[(trimethylsilyl)oxy] cholestane ($C_{30}H_{56}OSi$)	461	12.68	71.84 ± 0.88 (n = 3)
24-ethyl coprostanol	$C_{29}H_{60}O_3Si$	(24R)-24-ethyl-5α-cholestan-3β-ol ($C_{32}H_{60}OSi$)	489	17.39	96.60 ± 1.04 (n = 3)

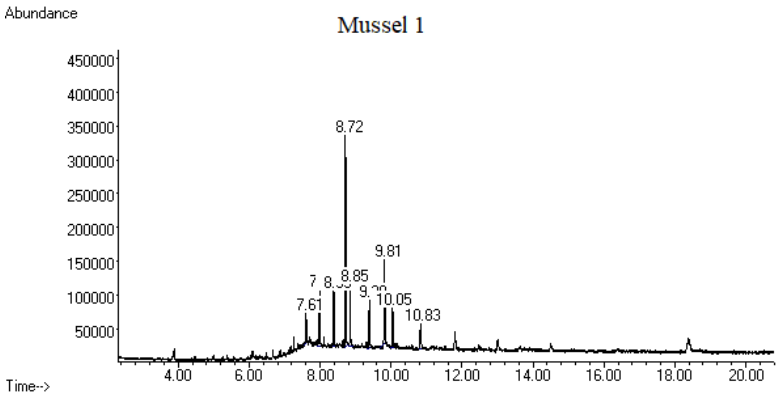
6.3.2 Distribution of Sterols, Fatty Acids and Hydrocarbons in *Mytilus galloprovincialis*

Practically no coprostanol or 24-ethyl coprostanol were found in gut tissues of *Mytilus galloprovincialis* (n =3 from Allans Beach and n = 4 from Smaills Beach) nevertheless GC-MS analysis revealed the presence of various other biochemical compounds with different chemical structures. Derivatives of cholesterol, fatty acids and hydrocarbons were identified. These noticeable biochemical compounds with their unique parameters are listed in Table 36 and depicted in Figure 34a-g.

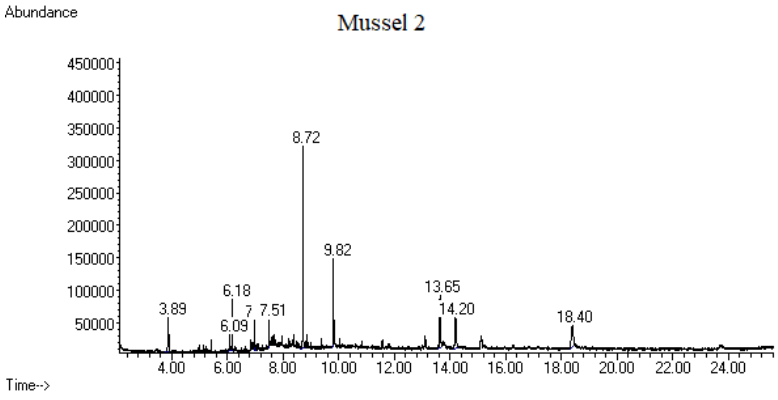
Table 36: Compounds identified from GC-MS analysis of the gut tissues of *Mytilus galloprovincialis* and their respective derivative compounds with molecular formula, weight (MW), retention time (RT) and total ion count (TIC) with standarad error

Compound	Derivative chemical name	MW g/mol	RT (min)	Mean TIC ± SE (%)
Cholesterol ($C_{27}H_{46}O$)	Cholesterol trimethylsilyl ether ($C_{30}H_{54}OSi$)	458	13.65 **(B,E,G)**	12.66 ± 1.71 (n = 3)
Hexadecanoic acid ($C_{16}H_{32}O_2$)	Hexadecanoic acid 2,3-bis (trimethylsilyl) oxy propyl ester ($C_{25}H_{54}O_4Si_2$)	475	8.72 – 8.86 **(A-G)**	35.46 ± 5.60 (n = 7)
2,6-Di-tert-butylphenol ($C_{14}H_{22}O$)	Trimethyl (2 6 di tert.-butylphenoxy) silane ($C_{17}H_{30}OSi$)	279	3.89 **(B,D-G)**	6.30 ± 0.79 (n = 5)
Octadecenoic acid (Oleic acid) ($C_{18}H_{34}O_2$)	9-Octadecenoic acid, 2,3bis [(trimethylsilyl)oxy] propyl ester ($C_{27}H_{56}O_4Si_2$)	501	9.81 – 9.82 **(A-G)**	17.30 ± 3.58 (n = 7)
Phenol (C_6H_6O)	Phenol, 3,5-bis(1,1-dimethylethyl)- ($C_{14}H_{22}O$)	206	18.40 – 18.42**(B,E-G)**	11.39 ± 2.02 (n = 4)
Nonadecane ($C_{19}H_{40}$)	Nonadecane, 9-methyl- ($C_{20}H_{42}$)	283	7.70 – 8.40 **(A,D,F,G)**	13.99 ± 4.18 (n = 4)
Heptacosane ($C_{27}H_{56}$)	-	381	7.61**(A,D,F)**	4.21 ± 0.13 (n = 3)
Ergosta-5,22-dien-3-ol, acetate (Brassicasterol acetate) ($C_{30}H_{48}O_2$)	-	441	14.20 – 14.21**(B,E,G)**	7.30 ± 2.78 (n = 2)
Methoxyacetic acid ($C_{16}H_{32}O_3$)	-	272	7.00 – 7.26 **(B,D-G)**	2.54 ± 0.43 (n = 5)
Tetracontane ($C_{40}H_{82}$)	Tetracontane 3, 5, 24-trimethyl- ($C_{43}H_{88}$)	605	10.05 – 13.03 **(A,D,F,G)**	13.24 ± 3.78 (n = 4)
Monostearin ($C_{21}H_{42}O_4$)	Bis(trimethylsilyl)monostearin ($C_{27}H_{58}O_4Si_2$)	503	9.00 – 9.40 **(A,D)**	8.14 ± 0.79 (n = 2)
Eicosapentaenoic acid ($C_{20}H_{30}O_2$)	5,8,11,14,17-Eicosapentaenoic acid, methyl ester ($C_{22}H_{34}O_2$)	331	7.51**(B,E)**	2.44 ± 0.10 (n = 2)
9-Hexadecenoic acid ($C_{16}H_{30}O_2$)	9-Hexadecenoic acid, methyl ester ($C_{17}H_{32}O_2$)	268	6.09 – 6.18 **(B,E,G)**	6.03 ± 0.71 (n = 3)

NB- Bold alphabets in brackets expressed as indexes alongside the retention times represent the Figure 3a-g


Abundance
Mussel 1
450000
400000
350000
300000
250000
200000
150000
100000
50000
8.72
9.81
8.85
7.61
10.05
10.83
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Time-->

a


Abundance
Mussel 2
450000
400000
350000
300000
250000
200000
150000
100000
50000
8.72
9.82
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13.65
14.20
3.89
6.09
7.51
18.40
4.00
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8.00
10.00
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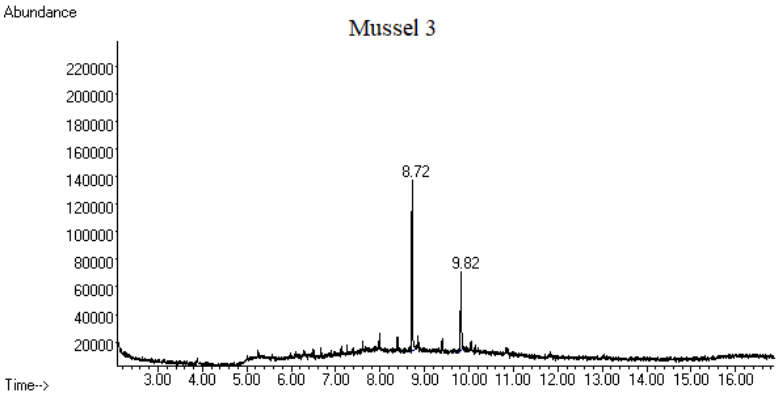
14.00
16.00
18.00
20.00
22.00
24.00
Time-->

b


Abundance
Mussel 3
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
8.72
9.82
3.00
4.00
5.00
6.00
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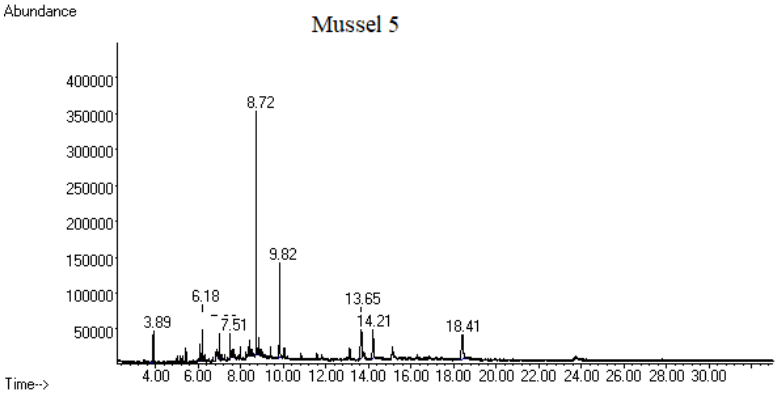
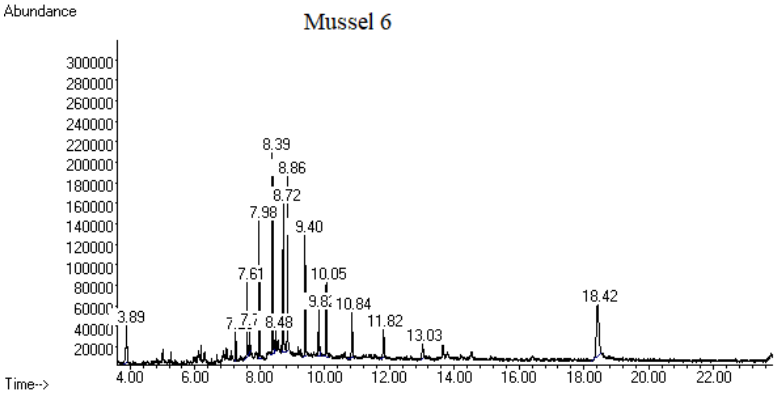
Time-->

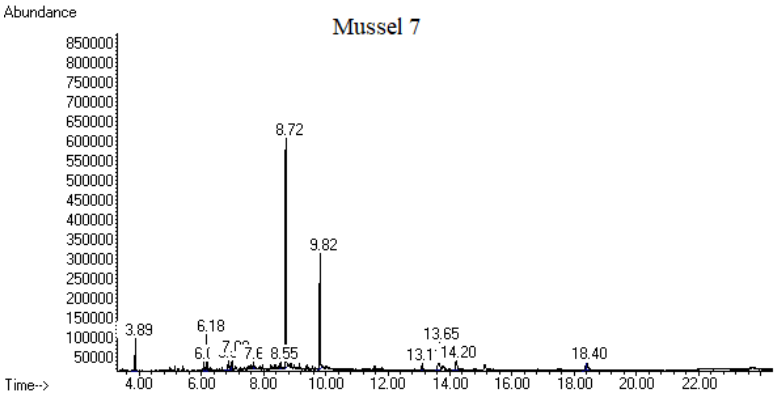
c



e



f



g

Figure 34a-g : GC-MS chromatograms of biochemical compounds extracted in the gut tissues of *Mytilus galloprovincialis* (a-c mussels from Allans Beach and d-g from Smaills Beach).

The average relative abundances of the biochemical compounds in *Mytilus galloprovincialis* (n = 7) are as follows:

- Sterols – cholesterol (12.66 ± 1.71 %) and Ergosta-5, 22-dien-3-ol, acetate (7.30 ± 2.78 %)
- Fatty acids – Hexadecanoic acid (35.46 ± 5.60 %,), Octadecenoic acid (17.30 ± 3.58 %), Monostearin (8.14 ± 0.79 %), 9-Hexadecenoic acid (2.54 ± 0.43 %), Methoxyacetic acid (2.54 ± 0.43 %) and Eicosapentaenoic acid (2.44 ± 0.10 %)
- Hydrocarbons – 2, 6-Di-tert-butylphenol (6.30 ± 0.79 %), phenol (11.39 ± 2.02 %), Tetracontane (13.24 ± 3.78 %), Nonadecane (13.99 ± 4.18 %) and Heptacosane (4.21 ± 0.13 %).

The biochemical compounds categories and their respective percentage compositions in *Mytilus galloprovincialis* depicted in Figure 35. The estimated mean concentration of cholesterol in *Mytilus galloprovincialis* was 0.03 ± 0.07 mg/kg.

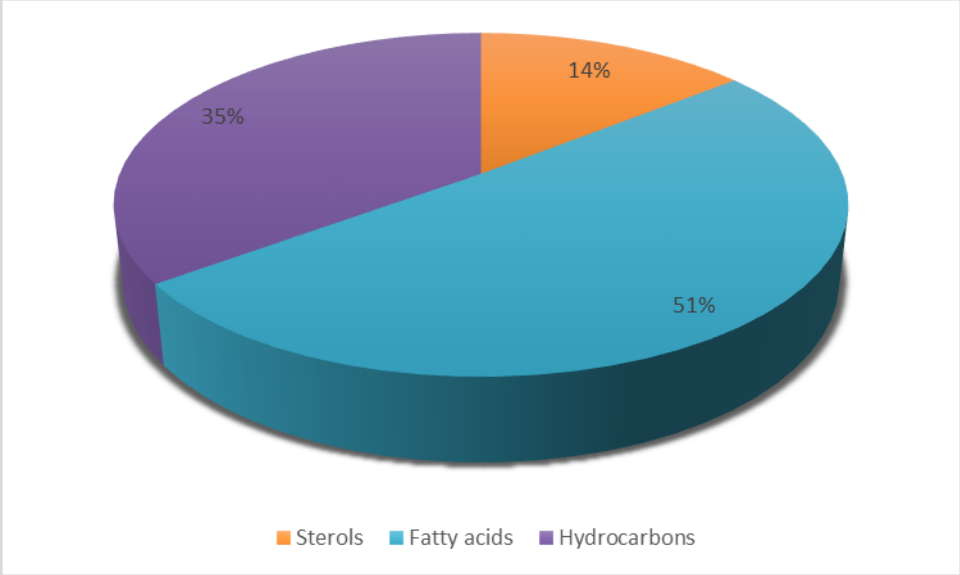


Figure 35: Pie chart of biochemical compounds identified in *Mytilus galloprovincialis*

6.3.3 Trace Metal Quantification

6.3.4 Quality Assurance

Five blanks analysed for background values of arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), and lead (Pb). Arsenic and chromium had higher mean values of 0.12 mg/kg and 0.02 mg/kg, respectively (Table 7).

6.3.5 Accuracy and Precision Check

The accuracy and precision checks of the method validated by comparing the percent recoveries for each of the metal concentration from the analysis of certified reference materials (in triplicate) (DORM 4). There was a good agreement between the certified values and obtained analytical results (see Table 8).

6.3.6 Trace Metal Levels in *Mytilus galloprovincialis*

Trace metal concentrations measured in whole tissues of *Mytilus galloprovincialis* collected from Allans and Smaills Beaches, Otago Peninsula, expressed on wet weight basis expressed in Table 37 and represented graphically in Figure 36. The number of samples analysed was 11 and 10 bivalves for Allans and Smaills Beaches respectively.

Table 37: Metal concentrations in *Mytilus galloprovincialis* collected along the Otago Peninsula

	Metal					
	As	Cd	Cr	Cu	Ni	Pb
Site	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
			Allans Beach (n = 10)			
Mean ± SE	9.40 ± 0.59	2.16 ± 0.62	1.55 ± 0.31	5.09 ± 0.16	1.87 ± 0.20	0.71 ± 0.07
Minimum	6.56	0.81	0.71	3.76	1.27	0.38
Maximum	13.20	7.16	3.76	5.75	3.25	1.05
SD	1.87	1.95	0.97	0.51	0.64	0.22
			Smaills Beach (n = 10)			
Mean ± SE	8.66 ± 0.40	1.36 ± 0.42	1.34 ± 0.19	4.75 ± 0.29	2.32 ± 0.36	0.71 ± 0.08
Minimum	7.14	0.50	0.63	3.57	3.57	0.20
Maximum	11.1	5.28	2.43	6.61	6.61	1.08
SD	1.12	1.47	0.57	1.02	1.21	0.27

The mean trace metal concentrations detected in *M. galloprovincialis* at Allans Beach decreased in the order As > Cu >Ni >Cd > Cr > Pb. The same order noted at Smaills Beach.

Similar Pb concentrations (0.71 mg/kg) observed at two beaches studied. A slightly differential value of 0.02 mg/kg was detected between Cd and Cr concentrations in the tissue of *M. galloprovincialis* at Smaills Beach.

Inter-site comparisons revealed that mean concentrations of all metals studied with the exception of nickel were higher at Allans Beach than Smaills Beach. The levels of arsenic and cadmium in the bivalve found to be 0.74 mg/kg and 0.80 mg/kg higher at Allans Beach than Smaills Beach. Nickel concentration found to be 0.45 mg/kg higher in Smaills Beach than Allans Beach.

However, the standard errors, a measure to test the level of confidence of the measured trace metal mean concentrations overlap as depicted in Figure 36, indicated the measured mean metal concentrations do not differ across the two studied sites.

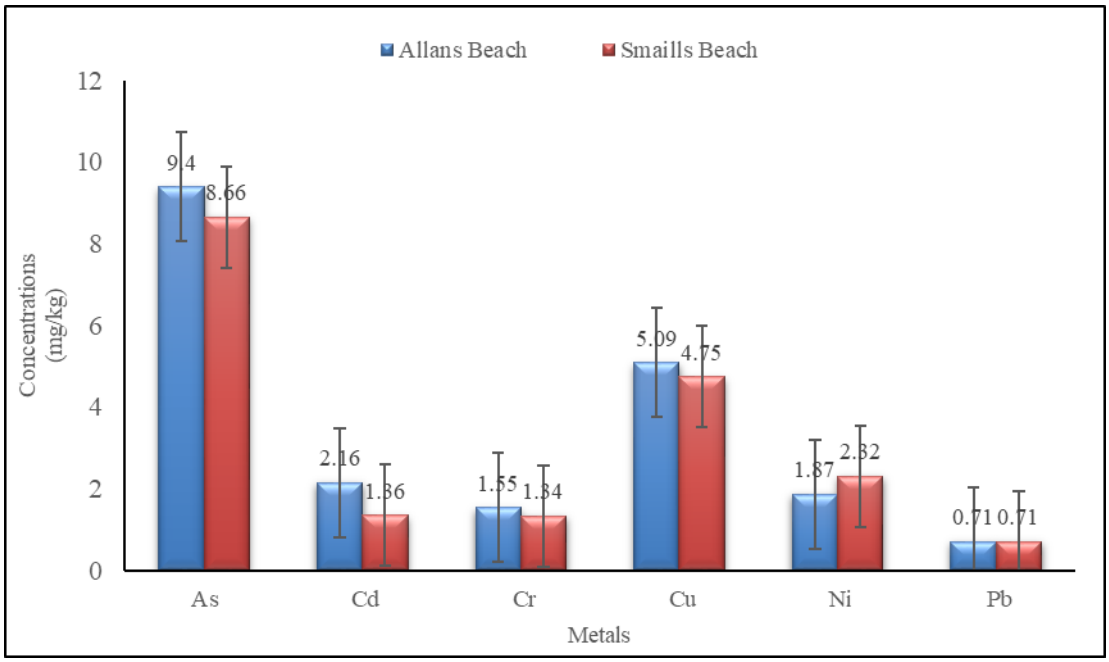


Figure 36: Mean comparison of the concentrations of As, Cd, Cr, Cu, Ni and Pb with their respective standard errors in *Mytilus galloprovincialis* collected from Allans and Smaills Beaches

6.3.7 Metal Concentration Variance Analysis

The variances of the mean metal concentrations in the bivalves from the two studied sites were compared to ascertain if they are similar (Table 38). This is to determine site influence on the metal distribution in the bivalve.

Hypothesis

Rejection of the null hypothesis: F-value greater than the F-critical (unequal variances)

Accept the null hypothesis: F-value less than the F-critical (equal variances)

Table 38: F-Test two-Sample variances analysis of As, Cd, Cr, Cu, Ni and Pb mean concentrations in *Mytilus galloprovincialis*

Variable	As	Cd	Cr	Cu	Ni	Pb
F-value	1.96	1.95	2.29	0.28	0.29	0.78
p*-value	0.16	0.16	0.11	0.03	0.04	0.36
F-critical	3.02	3.02	3.02	0.32	0.32	0.32

*** Significant level at 5% ($\alpha = 0.05$).**

The variances of As, Cd, Cr, and Cu mean concentrations not differ between the two sites. The variance of Pb mean concentrations differ between the two sites. The F-critical value of 3.02 and *p*-value of 0.16 was recorded for As and Cd concentrations. Pb, Cu and Ni had an F-critical value of 0.32. The site was an influential factor for the variance in mean Pb concentrations in the bivalve.

6.3.8 Human Health Risk Assessment

A comparison of the trace metal concentrations obtained in the bivalve tissue samples to the limit values and guidelines of shellfish depicted in Table 37 revealed that the cadmium levels in *Mytilus galloprovincialis* collected from the two beaches were well above the maximum set guideline values while other metal concentrations were lower than the maximum safe levels and guideline values.

Table 39: Maximum set safe limit standard levels of metal concentrations in *Mytilus galloprovincialis*

Organisation/ Country	Metal					
	As (mg/kg)	Cd (mg/kg)	Cr (mg/kg)	Cu (mg/kg)	Ni (mg/kg)	Pb (mg/kg)
FAO/WHO[1]	-	1.0	2.0	30.0	-	1.0
EU[2]	-	0.1	-	30.0	-	1.5
New Zealand[3]	1	1.0	2.0	-	-	1.0

[1](FAO/WHO 2007), [2](Commission 2001), [3] (FSANZ 2007)

There were no available safe limit values and guidelines of nickel concentrations in shellfish. Nevertheless, arsenic and cadmium concentrations recorded for both sites were higher than the maximum permissible levels in shellfish (Table 37). Therefore, a human health risk assessment for the acute intake of Cd levels in *Mytilus galloprovincialis* from the two beaches were required. The provisional tolerable weekly intake (PTWI) for As is 0.0008 mg/kg/day (FSANZ 2007; USEPA 2013) while that of Cd is 0.0001 mg/kg/day (FAO/WHO 2007) both calculated using average human body weight of approximately 70 kg. The estimated daily consumption (EDC) for the intake of chronic As and Cd levels measured in *Mytilus galloprovincialis* from Allans and Smaills Beaches were calculated using Equation 20. To determine the potential consumer carcinogenic risk effect (CCRE) for consuming chronic As and Cd levels in *Mytilus galloprovincialis*, a modified equation 4 described in (USEPA 2013) applied as in Equation 21:

$$EDC = PTWI \times C \ldots \text{Equation 21}$$

Where;

EDC is the estimated consumer daily metal intake (mg/kg/day)

C is the metal concentration measured in mussel

$$CCRE = EDC \times S \ldots \text{Equation 22}$$

Where;

CCRE is the consumer carcinogenic risk effect

SF is the metal cancer slope factor that is a plausible upper-bound estimate of the probability of a response per unit intake of the metal over a lifetime (mg/kg/day)

Note: Cancer slope factor for arsenic and cadmium are 1.50 and 6.1 mg/kg/day respectively

The estimated CCRE for arsenic in Allans and Smaills Beaches are 1.13×10^{-2} and 1.04×10^{-2} accordingly. The estimated CCRE for cadmium in Allans and Smaills Beaches are 1.3×10^{-3} and 8.3×10^{-4} respectively. Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1000000).

6.3.9 Target Hazard Quotient (THQ) and Hazard Index (HI)

A non-carcinogenic risk assessment, target hazard quotient (THQ) was conducted to estimate the potential health risks of long-term consumption of chronic As and Cd levels in *Mytilus galloprovincialis* from Allans and Smaills Beaches by local residents. The mathematical calculations for THQ with the standard assumptions for an integrated risk analysis described in USEPA (2017) are expressed in Equation 22 below:

$$THQ = [(MCM\times MCR \times EXD\times EXF) / (BW \times EXT \times RFD)] \times 10^{-3} \quad \ldots\text{Equation 23}$$

Where,

MCM is metal concentration in mussel (mg/kg wet weight),

MCR is the mussel consumption rate set to 6g/day/person (MPI 2016)

EXF is the exposure frequency (365 days/year) based on USEPA (2013),

EXD is the exposure duration (30years or 10950 days) for non-cancer risk as in USEPA (2013)

BW is the average adult body weight (70kg)

RFD is the oral reference dose of metal using an average adult body weight (70 kg) as in USEPA (2000) (As: 3×10^{-4} ; Cd: 1×10^{-3} ; Cu: 4×10^{-3} ; Cr: 3×10^{-3} ; Ni: 2×10^{-2} ; Pb: 5×10^{-5})

EXT is the average exposure time for non-carcinogens (10,950 days).

The THQ assessment outcome is depicted in Table 39. The acceptable benchmark for the THQ outcome is that a THQ less than 1 suggests that the level of exposure is smaller than the

reference dose and that exposed population is unlikely to experience any adverse health hazard. Conversely, if the THQ is equal to or higher than 1, there is a potential health risk of a daily exposure at this level and may cause any adverse effects during the consumer's lifetime hence relative interventions, and protective measures should be taken. Both THQ and HI have no units.

Table 40: Target hazard quotient (THQ) for As, Cd, Cu, Cr, Ni and Pb in *Mytilus galloprovincialis*

	Metal					
Site	**As**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**
Allans	2.69	0.19	0.44	0.01	0.01	1.22
Smaills	2.47	0.12	0.38	0.01	0.01	1.22

Arsenic and Lead had higher THQ values than 1 and could pose any potential health threat during the consumers' lifetime while other metals were found to have less than 1 in their THQ values hence exposed consumers are unlikely to experience any adverse health hazard.

The cumulative health risk is a measure of the Hazard index (HI) was evaluated for the marine bivalves collected from the two study sites. It was calculated by adding up the THQ values of individual metals (Table 38). The HI of the marine bivalves collected from Allans and Smaills Beaches were observed to be 4.55 and 4.21 accordingly.

6.4 Discussion

6.4.1 Tracers in *Mytilus galloprovincialis*

The main chemical components detected in the gut of the mussels from the coastal waters of the Otago Peninsula are essential fatty acids needed for metabolic processes. Some of the fatty acids detected in this study have a characteristic feature of a carbonyl group at one end of their aliphatic chain, and a methyl group at the opposite end for instance, polyunsaturated fatty acids (PUFAs) and Eicosapentaenoic acid (EPA) of more than one double bond structure. The EPA (omega-3 fatty acid) found in the bivalve is known to be produced by fin and shellfish from fatty acids precursors in their nourishment, or from the phytoplankton they consume (Brown 2002; Council 2011; Hendriks et al. 2003; Patil et al. 2007). Other fatty acid esters such as hexadecanoic acid, octadecenoic acid (oleic acid) and 9-Hexadecenoic acid that are mainly phospholipid ester-linked fatty acids (PLFAs) were also part of the fatty profile detected in the marine bivalve studied.

This array of fatty acids provided chemical tracers for assessing bacterial activities in an aquatic ecosystem, as they are essential structural, chemical components of all microbial cellular membrane structures. Such structures regulate cell flexibility and permeability (Denich et al. 2003; Dowhan and Bogdanov 2002; Li et al. 2007). The presence offers the opportunity for fingerprinting the microbial community of the coastal waters because the relative abundance of (Phospholipid-linked fatty acids) PLFAs are known to differ considerably among a particular group of microorganisms (Drenovsky et al. 2004; Fierer et al. 2003; Green and Scow 2000; Kaur et al. 2005; Schutter and Dick 2000).

Elevated hexadecanoic acid in the marine bivalve can indicate the presence of alkane-utilizing anaerobic microorganisms. The high alkane hydrocarbon compounds (35.09 ± 1.94 %) in the bivalve affirmed this, as described in Dashti et al. (2008). These microorganisms possess enzymes (Acetyl CoA synthetase) that can oxidise hydrocarbons in media sources of carbon and energy and are likely to originate from the sediment organic matter (SOM) which are also dietary components of the marine bivalve. *Mytilus spp*. receive an ample supply of essential dietary algal fatty acids, store a constant amount as energy resources and catabolised higher amount in their tissues (5-15 % of the dry weight) (Brett et al. 2006; Caramujo et al. 2008; Chen et al. 2007; Gladyshev et al. 2009; Kluytmans 1985).

Fatty acids such as octadecenoic acid, eicosapentaenoic acid, hexadecanoic acid and 9-Hexadecenoic acid are markers for different eukaryotic photosynthetic micro- and macro-algal groups (Basova 2005; Caramujo et al. 2008; Cardoso et al. 2017; Dawczynski et al. 2007; Kumari et al. 2011; Kumari et al. 2010; Kumari et al. 2013; Lang et al. 2011; Leblond et al. 2005; Siegenthaler and Trémolières 1998; Siegenthaler and Murata 2006). Monostearin, glycerol ester of stearic acid found in the marine bivalve is a by-product from the breakdown of fats. It is a food emulsifier and essential in the digestion of fats.

In this study, sterols measured in *M. galloprovincialis* accounted for 14 % of the total biochemical compounds measured in the gut of the marine bivalve. Cholesterol (a dominant sterol) and brassicasterol (phytosterol) are markers for Rhodophyta (red algae) Phaeophyta (brown algae) and found to vary among other micro- and macro-algal groups (Al Easa et al. 1995; Kamenarska et al. 2006; Lopes et al. 2011; Nasir et al. 2011; Sánchez- Machado et al. 2004; Shevchenko et al. 2009). For instance; *Sargassum spp*. contain cholesterol (21.3 – 43.2 %) and brassicasterol (9.6 %), *Laurencia papillosa* had cholesterol (68.7 %); *Polysiphonia brodiei* had cholesterol (32 %); *Chondria collinsiana* composed cholesterol (72.9 %) and

Caulerpa spp. had cholesterol (13.1 %) (Al Easa et al. 1995; Kamenarska et al. 2006; Lopes et al. 2011). In another study reported Kendel et al. (2015) that *Ulva sp.* (Chlorophyta) contain cholesterol (35 %) and brassicasterol (3.0%).

Murphy et al. (2002) compared the lipid, fatty acid and sterol compositions in *Perna canaliculus* from South Island, New Zealand and *Mytilus edulis* from Tasmania, Australia. A higher proportion of phospholipids (57 – 79 %), triacylglycerols (10 – 25 %), fatty acids (7 – 12 %), and sterols (12 – 18 %) characterized both mussel species. Cholesterol accounted for 30 % of lipid, fatty acid and sterol composition in *Mytilus edulis* and 29 % in *Perna canaliculus*. *Perna canaliculus* (14.6 ± 1.1 %) had a significantly higher amount of brassicasterol than *Mytilus edulis* (9.6 ± 1.1 %). Some of the predominant fatty acids observed in both mussel species are palmitic acid/hexadecanoic acid (16:0), Eicosapentaenoic acid (EPA) 20:5n-3 and docosahexaenoic acid/omega-3 fatty acid (DHA) 22:6n-3 in both mussel while palmitic acid16:0 was significantly in higher proportions in *Mytilus edulis*. No faecal sterols were present in either mussel species studied from South Island, New Zealand and Tasmania, Australia by (Murphy et al. 2002). In the same vein, no faecal sterols were found in *Mytilus galloprovincialis* analysed in this current study. However, there is faecal matter contribution from the sea lion seal colonies foraging at the intertidal mixing zone. Martins et al. (2002) reported that cholesterol typically accounted for more than 90 % of the sterols in the fresh faeces of *Leptonychotes weddellii*, *Mirounga leonine* (elephant seal), *Hudrurga leptonyx*, and *Arctocephalus gazelle*. According to Venkatesan and Santiago (1989), seals and sea lions excrements contained very a low level of coprostanol and no epicoprostanol. The New Zealand sea lion (*Phocarctos hookeri*) which is endemic to New Zealand and regionally localized (Gales 2009) was noted frequently at the study sites and could be the culprit.

Hydrocarbons in shellfish may be due to environmental contamination from hydrocarbons from fuel oil combustion, crude oil, or marine oil spill from vessels, wastewater and other petroleum products (Baumard et al. 1999; Blumer et al. 1970; Phillips 1999; Stołyhwo and Sikorski 2005; Tolosa et al. 2005). Since bivalves are filter feeders and have a low metabolic capacity for hydrocarbons, they can accumulate these 'odd' long-chain aliphatic hydrocarbons along with their diet. In this case, the estimated hydrocarbons are from algae/phytoplankton. For instance, Cranwell et al. (1990) found *n*-C_{17} alkane dominant in *Dtctyosphaerwn sp* (55 %), a minor constituent (18 %) in *Scenedesmus sp*. Cranwell et al. (1990) reported *n*-C_{17} and *n*-C_{19} in two marine chlorophytes. Eltgroth et al. (2005) found long

chain (*n*-C_{35} – *n*-C_{40}) alkenes, alkenones and alkenoates in *Emiliania huxleyi* and *Isochrysis galbana*. Achitouv et al. (2004) and Metzger and Largeau (2005) reported hydrocarbons (*n*-C_{23} – *n*-C_{40}) and ether lipids accounting to 80 % of cell dry weight in *Botryococcus braunii*.

The reported hydrocarbons in the marine bivalve in this study are from autochthonous sources such as algae and bacteria as well as allochthonous inputs from terrigenous plants. Marine algae (phytoplankton) can synthesize n-alkanes with chain lengths ranging from C_{14} to C_{32}. Hydrocarbons in algae are in low concentrations and may account for 3 – 5 % of the lipid fraction. (Bianchi and Canuel 2011). Hydrocarbon fractions present in phytoplankton are dominated by saturated and unsaturated hydrocarbon having both straight and branched chains. For this study, we hypothesized that nonadecane observed is from algae and diatoms, heptacosane for terrigenous plants and aquatic macrophytes (Cranwell 1982; Ficken et al. 2000) and Tetracontane (a waxy substance commonly found in the leaf cuticle of angiosperms) for seagrasses (Eglinton and Hamilton 1967; Lavergne et al. 2018).

Seagrasses are rich sources of phenolic substances including phenolic acids, sulphated phenolic acids, flavones, condensed tannins and lignins (Papenbrock 2012; Subhashini et al. 2013). Phenol compounds found in the marine bivalve could be attributed to the ingestion of detrital seagrasses and other vascular plants from both autochthonous and allochthon-nous sources (Arnold and Targett 2002; Hernes and Benner 2006). Lignin-phenol association is considered biomarker for sources and transport of land-derived particulate and dissolved organic matter in aquatic systems (Bianchi et al. 1997; Hatten et al. 2012; Hernes et al. 2009; Pautler et al. 2010). 2,6-di-tert-butylphenol, a widely derivative of industrial use as UV stabilizers and antioxidants for hydrocarbon-based products ranging from petrochemicals to plastics. It is a marker of municipal wastewater effluent and landfill leachates (Barnes et al. 2004; Björklund 2011; Rochman 2015).

The discovery of methoxyacetic acid in the marine bivalve (5 out of 7 marine bivalves sampled) could be of environmental and human health concern. Methoxyacetic acid is a primary active metabolite of ester phthalates widely used in industry as gelling, viscosity and stabilizer reagents for plastics (Priyandoko et al. 2011). It is also used in agrochemical for pesticide manufacturing (Blei et al. 2012; Burke 2013; Griveau and Barthaburu 2018). It is an endocrine-disrupting chemical concomitant with various developmental and reproductive health risk in mammals such as neural toxicity, blood and immune disorders, limb degeneration and testicular toxicity (Li et al. 1996; Pelch et al. 2011). It induces the

production of reactive oxygen species, causing DNA damage and loss of mitochondrial membrane potential in normal human fibroblasts (Bagchi et al. 2009; Bagchi et al. 2010) and harmful to aquatic life with long lasting effects. The biodegradation of phthalate esters is of ecological significance in contaminated aquatic systems (Staples et al. 1997). Metabolic breakdown of phthalic acid esters by microorganisms is considered to be one of the major routes of environmental degradation for these widespread pollutants (Chang et al. 2004; Gao and Wen 2016). Many studies have demonstrated the degradation of several phthalate esters under aerobic conditions in soil, natural water and wastewater. Also, numerous experiments have shown that the bioaccumulation of phthalate esters in the aquatic and terrestrial food chain is limited by biotransformation, which increases with increasing trophic level (Chang et al. 2004; Staples et al. 1997). Phthalate esters biodegradation or biotransformation can occur aerobically or anaerobically.

To the best of my knowledge, there is no previous study to validate the discovery of this carboxylic acid compound (i.e. methoxyacetic acid) in the marine bivalves. A similar synthetic form of the compound with perfluoroalkyl ether acid, perfluoro-2-methoxyacetic acid was detected in an important drinking water source in the Cape Fear River, North Carolina (Hopkins et al. 2018; Sun et al. 2016). An oral dose of methoxyacetic acid 2 g/kg was found to be lethal in rats (Miller et al. 1982). However, methoxyacetic acid was reported in microalgae as part of bioactive metabolites acting as antioxidant and antibacterial in response to ecological pressures to prevent antifouling in water (Shobier et al. 2016; Tanna et al. 2018). Blessy and Iyer (2016) identified the compound in *Chlorella sp.* (in water samples collected from Thoothukudi, India) in a study involving the extraction and production of biodiesel from algal culture.

6.4.2 Bioaccumulation and Human Health Implications of Trace Metals

Metal concentrations vary among bivalve species due to differences in metal biokinetics and bioaccumulation strategies (Wang and Lu 2017). For instance, metals such as copper and zinc accumulate in oysters at very high concentrations as compared with other metals. Marine invertebrates (i.e. bivalves) are exposed to metals from both dissolved and particulate phase forms. The dissolved metals were accumulated by direct adsorption to body surfaces while the particulate metals by ingestion and digestion of food materials (Wang and Fisher 1999; Wang et al. 1995).

Adsorption of metals by organisms is a function of physical and chemical properties while the rate and degree of metal bioaccumulation in organisms depend on metal concentration, time of exposure, bioavailability and speciation (John and Leventhal 1995). Marine invertebrates such as filter feeders are able to accumulate bioavailable forms of metals from their nourishment. They consume a lower trophic organism; any metals accumulated in the tissues of the consumed organism are transferred (biomagnified) to them (via trophic transfer). This process occurs primarily or exclusively in the unique environment of their gut. Adsorbed metals bound to the tissues of the nourished item are introduced into the gut of the consumer. They are desorbed from the food, dissolved in the gut fluids during digestion (aided by digestive enzymes), then partitioned from the gut fluids across the gut lining into the consumers' tissues.

The factors influencing the bioavailability of metals in the water column are metal speciation and biotransformation, availability of complexing ligands (e.g., organic carbon, chloride, carbonate, sulphide, manganese and ferrous oxides). Other factors are; competition by other cations for membrane adsorption sites (e.g. calcium, magnesium), pH, redox, particle sorption, suspended and bed sediments physicochemical properties and hydrology (Bridges et al. 2010; Cantwell et al. 2008; Eggleton and Thomas 2004; Hong et al. 2011; Idris et al. 2007).

Among the toxic metals in this study, Cd probably is of human health concern. Cadmium causes pulmonary disease, reduced glucose tolerance, severe kidney damage and death in humans (Bernard and Lauwerys 1986; Friberg 2017; Waalkes 2000). Cadmium exists in water as dissolved Cd, in particulate matter, and sediment phases. All three phases may be in equilibrium with each other. The relocation rates between these phases vary depending upon environmental conditions. The bioavailability of Cd in these phases for marine organisms is associated with the chemical species in the particulate matter and sediment and depends upon particle size, organic matter content, and ion-exchange capacity of the sediment.

The level of Cd reported in the bivalve this current study was above WHO maximum permissible levels in shellfish (Table 37). It was also found to be higher than reported by Birch and Apostolatos (2013) in *Mytilus galloprovincialis* (1.30 mg/kg) sampled in Sydney Estuary, Australia and Chandurvelan et al. (2015) in *Perna spp.* (0.77 ± 0.14 mg/kg) collected from West Coast and Nelson, New Zealand. Cd concentrations in the subtropical mussel generally low (< 2 mg/kg, dry weight) from different regions. The Cd concentrations reported

in this study was lower than reported by Bartolomé et al. (2010) in *Mytilus galloprovincialis* (3.10 – 11.20 mg/kg) from Bilbao Estuary, Spain and Vázquez-Luis et al. (2016) in *Pninna nobilis* (8.92 ± 3.01 mg/kg) collected from Andratx, Spain. Cd was reported to be lower than 9 mg/kg of wet weight in the dredge oyster, *Ostrea lutaria* (Hutton) from Foveaux Strait, New Zealand in Nielsen (1975) (Table 40). Nielsen (1975) attributed the source of Cd to natural occurrence due to the prevailing currents to the west of Foveaux Strait, possibly in Fiordland since there was no industrial pollution or any other obvious anthropogenic sources in the area of study. Frew et al. (1989) reported a mean Cd concentration of 30.6 mg/kg (dry weight) and a standard deviation of 14.6 mg/kg (n = 40) at a single site in Foveaux Strait, New Zealand.

Table 41: Comparison of the measured levels Cd in tissues of bivalves (mg/kg dry weight) with the levels previously reported in other studies.

		Cadmium concentration (mg/kg dry weight)		
Location	**Organism**	**This study**	**Reference**	
Allans Beach, New Zealand	*Mytilus galloprovincialis*	2.16 ± 0.62	-	
Smaills Beach, New Zealand	*Mytilus galloprovincialis*	1.36 ± 0.42	-	
Boulder Beach, New Zealand	*Mytilus galloprovincialis*	-	2.31	(1)
Second Beach, New Zealand	*Mytilus galloprovincialis*	-	1.66	(1)
Lawyers Head, New Zealand	*Mytilus galloprovincialis*	-	1.38	(1)
Victory Beach, New Zealand	*Mytilus galloprovincialis*	-	1.65	(1)
Sydney Estuary, Australia	*Mytilus galloprovincialis*	-	1.30	(2)
Nelson, New Zealand	*Perna spp.*	-	0.77 ± 0.14	(3)
Bilbao Estuary, Spain	*Mytilus galloprovincialis*	-	3.10 – 11.20	(4)
Andratx, Spain	*Pninna nobilis*	-	8.92 ± 3.01	(5)
Foveaux Strait, New Zealand	*Tiostrea chilensis*	-	30.60 ± 14.60	(6)

1 = (Citilab 2016), 2 = (Birch and Apostolatos 2013), 3 = (Chandurvelan et al. 2015), 4 = (Bartolomé et al. 2010) 5 = (Vázquez-Luis et al. 2016), 6 = (Frew et al. 1989)

In another related study by Frew et al. (1989) implied that most of the Cd was accumulated by the ingestion of food particles rather than by direct absorption from the water. They reported Cd (69 %) was found in the visceral mass, with small amounts (15 – 16 %) in each of the gills and muscle tissue. Frew et al. (2001) found the variations in Cd/PO_4^{3-} was related to direct productivity in the Southern Ocean. This was a departure from the notion that that Cd/PO_4^{3-} remains constant both spatially and temporally in the global ocean. Gault-Ringold et al. (2012) and Baars et al. (2014) reported that the decrease in dissolved Cd concentrations in subantarctic waters is due to biological uptake by phytoplankton (i.e. diatoms). This confirms that the high Cd recorded in the bivalve is from the uptake of phytoplankton and eventual accumulation in the tissues.

Cadmium in mussels is of less concern than the oysters in terms of seafood safety. The marine bivalve can accumulate Cd directly from water or indirectly from food and detritus particles, and subsequently, transport it throughout the body by active and passive transport mechanisms. The lower bioaccumulation potentials of Cd in mussels, when compared with oysters, were mainly because of its lower dissolved uptake and dietary assimilation efficiency, and a higher efflux (active transport), which effectively removed Cd from the mussel body.

The efflux rates of Cd in mussels are lower than in oysters (Wang and Lu 2017). In this study, the level of arsenic was higher than the acceptable FSANZ limit. However, the predominant species of arsenic in shellfish, molluscs and seaweeds are the arsenosugars (dimethylarsinyl riboside derivatives), while in fish and crustaceans the predominant arsenic compound is arsenobetaine; a form of arsenic considered virtually non-toxic (Cava-Montesinos et al. 2005; Kaise and Fukui 1992; Le et al. 2004).

Most of the consumed As in fish is in the less toxic organic forms. The major anthropogenic source of As and Cd pollution in aquatic environments is the application of agricultural chemicals. The THQ value for Pb was above 1 (see Table 38), suggesting that the consumption of the marine bivalve could pose a potential health threat during the consumers' lifetime.

6.4.3 Tracers and Sewage

Faecal sterols were not present in *Mytilus galloprovincialis* indicative of the absence of sewage-derived organic matter from human and animal faecal matter in the nearshore marine waters. The Cd concentrations measured here are consistent with natural occurrence, and there is no evidence of sewage or any anthropogenic influence. Convincingly, the Cd levels were higher at the reference site and other clean sites than the previously sewage contaminated site, an indication of the remarkable recovery from sewage contamination. There is an agreement among the tracer approaches used to indicate the source of nourishment in *Mytilus galloprovincialis* and the absence of municipal sewage effluent influence (Table 42). The tracers confirm the mussels' diet constituent are marine particulate organic matter, *Ulva lactuca* and sea lion faeces.

Table 42 : Comparison of the tracer approaches

	Tracer		
N-Source	**Stable Isotope**	**Biochemical**	**Trace metal**
Marine (Algae)	✓	✓	✓
Municipal sewage effluent	✓	•	•
Farm organic manure	✓	•	•
Sheep faeces	✓	•	•
Cow faeces	✓	•	•
Ulva lactuca	✓	✓	•
Sea lion faeces	✓	✓	✓

6.5 Conclusion

The independent assessment of biochemical markers in the gut of the marine bivalve confirmed that the marine bivalves receive nourishment from marine particulate organic matter without significant subsidy from municipal sewage. The variances of the mean concentrations of metals studied do not differ across the two locations except for lead concentrations. The only suggestion for the observed difference in the variance in Pb may be due to the varying degree and route of exposure in the marine bivalve. Trace metal accumulated in the tissue of the marine bivalve were at levels expected from ingestion and assimilation from marine particulate organic matter. Algae, the main part of their diet, contain substantial amounts of wide-ranging fatty acids and sterols (phtytosterol) and metal contents relative to its ambient waters.

Anthropogenic contaminants found in the marine bivalves are chemicals from plastics and pesticides. No evidence of sewage contamination from either of the tracers applied here (biomolecules and trace metals) providing support for the conclusions derived from the stable isotope studies. However, the Bayesian mixing model (MixSIAR) inferred sewage matter contributions of 10 - 18 % of sewage matter, the absence of sewage steroids and chemical tracers indicated marine particulate organic matter as the main diet of *Mytilus galloprovincialis*.

Lastly, the mussels from the study regions have levels of cholesterol (absence of faecal sterols) and trace metal contents that do not present a danger to human health from moderate consumption. The improvement in the treatment and disposal of the municipal wastewater plant had a positive influence on the coastal marine waters and resident biota.

CHAPTER 7

7.1 Summary, Conclusions and Direction for Future Studies

The main purpose of this thesis is to assess the impact of the improvement in Tahuna municipal wastewater plant on the nearshore marine waters along Otago Peninsula, Dunedin, New Zealand. Before the advancement of the wastewater treatment plant, the nearshore marine water was affected by long-term discharged of raw sewage effluent from the plant. The negative responses created by the dumping of raw sewage include poor water quality, disruption of the nearshore marine food web and sporadic eutrophication based on several previous studies. This led to an advancement in the treatment and disposal processes (i.e. upgrade of wastewater facilities and extension of the ocean outfall pipes). After the upgrade, there was the need for a revaluation of the nearshore marine waters. This thesis reassessed the nearshore marine waters using various approaches such as stable isotope (i.e. using stable isotope mass balance models) as the main environmental tool. Other independent tracer techniques (i.e. complementary assessors) used are faecal sterol, fatty acid and trace metal contents to indicate the source of nourishment to the resident nearshore marine bivalves (*Mytilus galloprovincialis*) as a pointer to the nutrient and contaminants sources in the nearshore marine waters. In order to achieve the main set goal of the study, the aims and objectives of the thesis are to:

(1) Evaluate the possibility of using the differences in stable isotope compositions of carbon and nitrogen in the tissues of *Mytilus galloprovincialis* and *Ulva lactuca* as indicators of organic matter sources as nutrient and contaminants in the coastal marine waters.

(2) Examine the impact of discharged of sewage-derived organic materials on the nearshore marine waters by way of using the resident marine flora (*Ulva lactuca*) and fauna (*Mytilus galloprovincialis*) as indicator organisms for exploring the nutrient supply and contaminant fluxes in the coastal marine waters.

(3) Appraise the outcome of the advancement in the municipal waste treatment plant and disposal process (extension of sewage outfall) on the sewage-derived organic matter deposited in the nearshore waters and preserved in the coastal marine flora (*Ulva lactuca*) and fauna (*Mytilus galloprovincialis*).

(4) Set up isotope mixing mass balance models on measured bulk stable isotope compositions of carbon and nitrogen suspended particulate organic matter in water, and land-based materials (i.e. sewage matter) to quantify the contributions of organic materials and infer changes in organic matter sources in the resident fauna (*Mytilus galloprovincialis*) and probably in the nearshore marine waters.

(5) Quantify the biochemical and elemental compositions (as chemical tracers) in resident organisms as independent assessors to infer and ascertain the changes in the organic matter sources (nutrient supply) and contaminant dynamics in the nearshore marine waters.

(6) Assess the potential human health risks associated with the consumption of the coastal marine fisheries (i.e. *Mytilus galloprovincialis*) collected from the coastal.

The accomplishment of the stated aims and objectives above are as follows:

The Chapter 3 of this thesis examined the differences in the dual-isotope ratios of *Mytilus galloprovincialis* as a preliminary survey to determine the tissues to use as an indicative tool for the long-term ecological-based study aimed at marking out the sources and fate of organic materials in the nearshore marine waters along Otago Peninsula, New Zealand. The survey aided the experimental design of the study. The variance in the isotope signatures of the bivalves was influenced by land-use activities that transfer organic materials (in the form of nutrients and contaminants) into the coastal marine waters and their proximate water bodies. The surveyed variance of isotope ratios of six diverse tissues of *Mytilus galloprovincialis* exhibited an almost similar trend in δ^{15}N and δ^{13}C enrichment. This indicated the aptness of the tissues as indicators for elucidating the consequence of land-use activities in nutrient loading and contamination of the coastal waters. Variance analyses (ANOVA) on δ^{15}N and δ^{13}C isotopic ratios in *Mytilus galloprovincialis* were performed to ascertain the responses in tissue and site interactions. The abductor and digestive tissues showed consistent responses in their δ^{15}N and δ^{13}C isotopic ratios indicative of better choice as indicator tissues of choice for indicating nutrient supply and contamination in the coastal marine waters. The byssus tissue was detected as a possible indicative tool of choice for indicating contaminants in the coastal marine waters. The digestive tissue as an indicator for marking out nutrients and contaminants in the nearshore marine waters could provide dietary information based on long-term dietary assimilation and ingested materials.

Following attainment of the objective two and three of the thesis, Chapter 4 used stable isotope measurements from the digestive tissue of the marine bivalves and seaweeds to assess the effect of sewage-derived organic matter (SDOM) on the nearshore marine ecosystem of the Otago Coast before, and 15 years after upgrade of the Tahuna Wastewater Treatment Plant, Dunedin. Carbon and nitrogen isotopic ratios in the tissues of sentinel organisms (*Mytilus galloprovincialis* and *Ulva lactuca*) were utilised as indicators to mark out the primary sources of nutrition in the coastal marine waters. *Mytilus galloprovincialis* exhibited a strong influence of SDOM from two sites in 2001. In 2015, *M. galloprovincialis* had a trophic enrichment factor of +3 ‰ (δ^{15}N) and +1 ‰ (δ^{13}C) when compared to the marine particulate organic matter (POM), suggestive of a dietary change (diet switching) away from the sewage-derived organic matter (SDOM). The two-source isotopic mixing model used revealed that the contribution of terrigenous organic materials in the marine bivalve varied from 1 % to 5 % for δ^{13}C and 13 % to 51 % for δ^{15}N. The suspended particulate organic matter collected from freshwater and estuarine sources revealed possible nitrogen sources from human-driven activities such as pastoral farming, application of organic manure and inorganic fertilisers, nitrification of ammonium from semi-urban septic tanks, and animal organic waste residues.

In Chapter 5, two of the coastal marine waters and their proximate water bodies (i.e. tidal channels) of divergent hydrographical features were examined for a full seasonal cycle on the influence of sewage–derived organic material incursions and other anthropogenic influences. The stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope ratios in *Ulva lactuca* (primary producer), *Mytilus galloprovincialis* (primary consumer) and other N-source land input organic materials were fitted into a Bayesian stable isotopic mixing model, R (MixSIAR). The model was built upon a Gaussian likelihood with a mixture Dirichlet-distributed prior to the mean distribution of stable isotope ratios of one consumer and four souces with the adoption of enrichment factor. The model was used to quantify the contributions from sewage-derived organic matter as dietary components in digestive tissues of *Mytilus galloprovincialis*. Sewage matter was found to contribute 10 to 18 % as apart of the dietary component of the marine bivalve at Smaills. No sewage matter indication at Allans Beach. The marine matter was found to be the major dietary component of the bivalves at both beaches with significant contribution from estuarine matter. The riverine matter was insignificant at both beaches Run-off from agrarian watershed areas were found to be a major negative influence for the episodic growth of *Ulva lactuca* and the elevated nutrients in the

coastal marine waters and adjacent water bodies. The estuary was found to act as a filter of the fluxes of terrestrial-based source materials that reach the oceans and contribute a significant portion of allochthonous nutrients to the dietary configuration of the nearshore marine bivalve. Overall, the estuarine processes played a significant role in the transfer of organic materials and nutrients, allocation of resource subsidy across land and sea frontiers. The estuarine subsidies were found to be eventually assimilated/sequestered in resident organisms of the receiving adjacent coastal marine waters..

Chapter 6 focused on using independent analytical techniques (i.e. molecular tracers) to test the inference of the mixing model model developed in the previous chapter to see if there was any indication of sewage matter in diet of the mussels . The analyses of chemical tracers involving biochemical composition and elemental analyses on *Mytilus galloprovincialis* were conducted to substantiate the levels of faecal contamination and toxic trace metals as a pointer to organic matter sources and the ecosystem health status of the coastal marine waters and well-being of the resident coastal biota. The independent assessment of biochemical compositions in the gut of the marine bivalve confirmed that the marine bivalves receive nourishment from marine particulate organic matter. Trace metal levels accumulated in the tissue of the marine bivalve were found to be due to ingestion and assimilation of marine particulate organic matter. Algae, the main diet, contain substantial amounts of wide-ranging fatty acids and sterols (phytosterol). Susceptible contaminants found in the marine bivalves were industrial chemicals used in the production of plastics and pesticides.

The upgrade of the municipal wastewater facilities aided the processing of wastewaters from homes and commercial establishments by employing primary (grit removal, screening, grinding, flocculation, sedimentation), secondary (oxidation of dissolved organic matter), and tertiary (nutrient removal) methods of treatment. This had helped in the improvement of water quality of the coastal marine waters. The ultimate goal of the upgrade was to the reduce or eliminate suspended solids, oxygen-demanding substances, dissolved inorganic (nitrogen and phosphorus) compounds, and bacteria that may affect receiving ocean waters. However, there is still a concern of nonpoint contaminant sources from agricultural runoff, urban stormwater, dredged material, landfill leachates and industrial waste that can transfer organic materials to water bodies within a catchment and ending up at the nearshore marine waters. These nonpoint sources can intermittently cause coastal enrichment that may produce acute oxygen deficiency in the coastal marine waters and their proximate bodies.

Oxygen depletion witnessed during late summer and winter period of the study may have serious effects on the health of the nearshore marine ecosystem, as shellfish finfish and phytoplankton need favourable oxygen levels to conserve in order to survive. The application of inorganic fertilisers, compost and organic manure by pastoral farmers to enhance pasture growth for livestock (i.e. land-use influence) contributed nutrients and organic materials via river catchments, which fuel intermittent eutrophication in the estuary and coastal waters during summer that might result in oxygen depletion due to increase consumption of oxygen by microorganisms.

Since the export of land-derived nutrients and organic materials affect the coastal marine systems, it is a regional rather than local concern for the effective management, conservation and preservation of the coastal marine ecosystem and resources. Normal processes in natural waters (i.e. self-purification) may not always be well equipped for the reduction of elevated anthropogenic nutrients and organic material masses in the proximate water bodies. The creation of proper policy to improve nutrient management by various environmental protection agencies and other stakeholders will probably be a viable option, as this will assist in reducing episodic human-enhanced coastal marine productivity. The relevant environmental agencies should institute quarterly monitoring programmes to assess the level and impact of nutrients, contaminants and other discharged anthropogenic inputs into the proximate water bodies via catchments as remedial measures to ensure the health of the invaluable marine shoreline. Regular proper public education and engagement on how to reduce the level of nutrients and contaminants in water bodies and the environment are also required.

7.1.2 Future Studies

One of the intriguing findings from this study was the elevated Cd levels in shellfish common for the Southern region of New Zeland. The observed elevated Cd in the marine bivalve indicated natural source via ingestion of high Cd-rich phytoplankton rather than anthropogenic sources. Therefore, further research questions that require answers are:

- What is the structure of the natural community of phytoplankton biomass at the nearshore marine waters?
- What is the relationship between Cd levels and the primary production in the nearshore marine waters?

- Why do the natural phytoplankton biomass assemblages at the nearshore marine waters have an affinity for high Cd uptake?
- What kinetics and biochemical mechanisms are responsible for the high uptake and accumulation of Cd in the natural nearshore marine phytoplankton biomass community?

The questions raised here have wider implications for understanding the functioning of the marine system. Phytoplankton are responsible for nearly half of global primary productivity and are a major regulator of the exchange of CO_2 between ocean and atmosphere (Litchman et al. 2015; Maranon et al. 2014). These unicellular organisms form the base of the marine food web. They provide food for higher organisms and process much of the global carbon budget (Raven and Falkowski 1999), thus regulating global climate (Falkowski 1994) and oxygen levels. The Southern Ocean plays a particularly important role in the global carbon budget (Sigman et al. 2010), but we know little of the factors that govern plankton growth and activity there. Cycles of micronutrients such as iron (Fe) significantly affect primary productivity, but our understanding of these interactions remains somewhat limited, possibly because other metals also play a role (Middag et al. 2013; Moore et al. 2013; Sunda 2012).

The metal Cd has intrigued oceanographers for decades (Boyle et al. 1976; Frew and Hunter 1992; Xie et al. 2015; Xu et al. 2012). Despite its toxicity, in the ocean its behaviour mimics that of an essential nutrient, implying a biological role. Cadmium may be a key micronutrient in primary productivity in the Southern Ocean as phytoplankton can substitute Cd for other metals in some enzymes when elements such as Zn are scarce (Morel et al. 1994; Park et al. 2007; Sunda 2012).

Recent work is contradictory. A model system based on the bacterium *E. coli* suggests that Cd uptake is simply accidental in metal-replete conditions (Horner et al. 2013), but there are doubts about the biological significance of this work (Morel 2013). Our recent findings imply a significant role for Cd and provide evidence for active uptake in marine diatoms. Remarkably, the only known biological role for Cd, as a cofactor in the enzyme carbonic anhydrase, does not seem to be important (Bulbul 2013). Understanding the role of Cd is essential in understanding the control of primary productivity and its interaction with climate change. Moreover, as Cd levels in biogenic sediment particles are widely used as an indicator for surface ocean nutrient utilisation (Elderfield and Rickerby 2000) we need to have a much

clearer understanding of the drivers and stressors of phytoplankton if we are to interpret past climate from ocean sediments properly.

The sub-Antarctic water has anomalously low Cd concentrations (Frew and Hunter 1992; Frew and Hunter 1995), unusual isotopic composition (Gault-Ringold et al. 2012), and is characterised by intense seasonality in the concentrations of Cd and other nutrients (Adu 2012). Primary productivity in this region is known to be limited by Fe (Boyd et al. 1999), and so seasonality in Fe concentrations is expected. The surprise is the much larger seasonal variation in Cd (11-fold) when compared with Fe (4-fold) (Adu 2016). Moreover, close examination reveals Cd concentrations decrease earlier in the season than Fe.

Two important questions arise; 1) what stimulates the Cd uptake? - E.g. amelioration of a nutrient limitation or change in community structure. 2) Why is Cd the seasonally most affected element - is it an essential nutrient, does it reflect a detoxification mechanism, or is it accidental?

We can address the questions through a long–term field and laboratory-based studies that will:

A. Conduct a detailed time-resolved study of the Sub-antarctic Ocean chemistry, phytoplankton community structure, and cell function and composition. Specifically;

1) Field measurements of nutrients, trace metals, algal community (Vongsvivut et al. 2015) and cell composition and function(Ellwood et al. 2013) in sub-Antarctic waters

2) Culture and manipulate natural samples to determine the effects of adding Cd (and other metals) on the growth, composition and metal content of the algal cells (Lane et al. 2009; Strzepek et al. 2011).

3) Using the culture conditions identified in 2, where Cd has the greatest positive effect on cell growth, we will grow axenic cultures (Strzepek et al. 2011) of suitable phytoplankton and use vibrational spectroscopy (Vongsvivut et al. 2015), untargeted high–resolution mass spectrometry and molecular approaches to determine where the Cd is localised within the cells and what molecular forms it takes

4) Use stable isotopes (C, N, Cd) in culture experiments to help identify the mechanisms controlling uptake and distinguish uptake from adsorption to particle surfaces (Gault-Ringold et al. 2012).

This work will unravel the role of Cd in primary productivity, identify the role of Cd in shaping ecosystem function (including carbon uptake) and provide key information underpinning the use of Cd as a proxy for past oceanic nutrient utilisation. Resolving this important gap in our knowledge is imperative for understanding the consequences of changes in ocean chemistry on primary productivity, global climate and ocean geochemical cycles.

A comprehensive time-series study of the natural phytoplankton biomass community that will investigate the interaction of nutrient and plankton dynamics of the nearshore marine waters is required to understand the controlling factors of primary production and the role of Cd in the biogeochemical trajectories of the nearshore marine ecosystem. The use of the stable Cd isotope ($^{110}Cd/^{112}Cd$) fractionation and the correlation between Cd and PO_4 will assist in detecting the differences in biological productivity in the nearshore marine waters. Laboratory-based studies involving selected species collected from the natural community of phytoplankton biomass at the nearshore marine waters can be grown in vitro via phytoplankton inoculation in water culture media with varying Cd concentrations in the culture media to get a better insight on the removal of Cd by phytoplankton uptake during phytoplankton growth. The plankton cells can then be extracted for the measurement of Cd isotopic composition. The information obtained from the study will be developed into an isotope mass balance model to predict the interrelationship of Cd isotopic fractionation associated with the various aspects of the biological uptake, adsorptive scavenging and re-mineralization of organic matter in the nearshore marine waters.

Another result to follow up from this thesis is the presence of methoxyacetic acid in some *Mytilus galloprovincialis* samples. The toxicity and ecological effect of methoxyacetic acid on marine organisms and other biotic communities are of human health and environmental concerns. There seems to be a research gap here, which needs to be studied. Therefore, a laboratory study involving toxicological assessment of sources and the effects of methoxyacetic acid on *Mytilus galloprovincialis* could be conducted alongside the culture experiments for the Cd study.

Finally, the development of a laboratory protocol to quantify both the nitrogen and oxygen isotopes of nitrate in the water samples collected during the course of this study would have

assisted in distinguishing the source of nitrate contamination in the freshwater stream, estuary and coastal marine waters studied. To achieve this, the measurement of the isotopic fractionation of the isotopes of nitrogen and oxygen in water samples during the bacterial reduction of nitrate to nitrous oxide seemed to be more expedient. The use of denitrifying strains of microbes will be crucial for the success of the protocol. However, other methods of the nitrogen and oxygen isotopes of nitrate in the water samples are existing and been developed.

However, owing to the complexity of the nitrate pollution source and the influence of isotopic fractionation, the application of the bacterial reduction method has some limitations. Hence, the need for further testing proper and inter-calibration to demonstrate the accuracy of the method is required. A successful protocol to resolve nitrogen (δ^{15}N) and oxygen (δ^{18}O) isotopes of nitrate in the water samples will provide additional information that this study failed to provide on the source, nature and fate of nitrate contamination in the proximate water bodies and coastal marine waters. The measurement of the nitrogen isotope of the nitrate in the water samples will assist in identifying the nitrate from ammonium nitrogen fertilizer, soil nitrogen, manure, and sewage because the nitrogen isotope values of nitrate (in solid samples) from precipitation, soil, chemical fertilizer, manure, and sewage relatively overlap. The oxygen isotope, an isotope of precipitation, will assist in identifying nitrate source as well and reducing the uncertainties that may arise from nitrogen isotope values. This will provide further information, and a better understanding of the sources and transformation pathways of organic matter in the coastal marine system studied.

REFERENCES

Abbasi, T., and Abbasi, S. 2011. Water quality indices based on bioassessment: The biotic indices. Journal of Water and Health **9**(2): 330-348.

Abrantes, K., and Sheaves, M. 2008. Incorporation of terrestrial wetland material into aquatic food webs in a tropical estuarine wetland. Estuarine, Coastal and Shelf Science **80**(3): 401-412.

Achitouv, E., Metzger, P., Rager, M.-N., and Largeau, C. 2004. C31–C34 methylated squalenes from a Bolivian strain of *Botryococcus braunii*. Phytochemistry **65**(23): 3159-3165.

Adu, T. 2012. Assessing the Cadmium Paleo-nutrient Proxy. Doctoral Dissertation, University of Otago.

Adu, T., Hunter K., and Frew R. 2016. Strong seasonality in the cadmium and phosphate cycling at the subtropical convergence, Southeast New Zealand. Global Biogeochemical Cycles **submitted**.

Ahad, J.M., Barth, J.A., Ganeshram, R.S., Spencer, R.G., and Uher, G. 2008. Controls on carbon cycling in two contrasting temperate zone estuaries: The Tyne and Tweed, UK. Estuarine, Coastal and Shelf Science **78**(4): 685-693.

Al-Ghadban, A., Al-Majed, N., and Al-Muzaini, S. 2002. The state of marine pollution in Kuwait: Northern Arabian Gulf. Technology **8**(1-2): 7-26.

Al Easa, H.S., Kornprobst, J.-M., and Rizk, A. 1995. Major sterol composition of some algae from Qatar. Phytochemistry **39**(2): 373-374.

Albrecht, N., and Vennell, R. 2007. Tides in two constricted New Zealand lagoons. New Zealand Journal of Marine and Freshwater Research **41**(1): 103-118.

Alfaro, A.C., Jeffs, A.G., and Hooker, S.H. 2001. Reproductive behavior of the green-lipped mussel, *Perna canaliculus*, in northern New Zealand. Bulletin of Marine Science **69**(3): 1095-1108.

Allan, J.D., and Castillo, M.M. 2007. Stream ecology: Structure and function of running waters. Springer Science & Business Media.

Ameztegui, A., Coll, L., Brotons, L., and Ninot, J.M. 2016. Land-use legacies rather than climate change are driving the recent upward shift of the mountain tree line in the Pyrenees. Global ecology and Biogeography **25**(3): 263-273.

Andersen, F.O., and Kristensen, E. 1992. The importance of benthic macrofauna in decomposition of microalgae in a coastal marine sediment. Limnology and Oceanography **37**(7): 1392-1403.

Anderson, D.M., Glibert, P.M., and Burkholder, J.M. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries **25**(4): 704-726.

Anderson, I.C., Stanhope, J.W., Hardison, A.K., and McGlathery, K.J. 2010. Sources and fates of nitrogen in Virginia coastal bays. Coastal lagoons: Critical habitats of Environmental Change: 43-72.

Apeti, D.A., Johnson, E., and Robinson, L. 2005. A model for bioaccumulation of metals in *Crassostrea virginica* from Apalachicola Bay, Florida. American Journal of Environmental Sciences **1**(3): 239-248.

Aravena, R., Evans, M., and Cherry, J.A. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. Groundwater **31**(2): 180-186.

Archana, A., Li, L., Shuh-Ji, K., Thibodeau, B., and Baker, D.M. 2016. Variations in nitrate isotope composition of wastewater effluents by treatment type in Hong Kong. Marine Pollution Bulletin **111**(1-2): 143-152.

Armstrong, F., and Atkins, W. 1950. The suspended matter of sea water. Journal of the Marine Biological Association of the United Kingdom **29**(1): 139-143.

Arnold, T.M., and Targett, N.M. 2002. Marine tannins: The importance of a mechanistic framework for predicting ecological roles. Journal of Chemical Ecology **28**(10): 1919-1934.

Ashoka, S., Peake, B.M., Bremner, G., Hageman, K.J., and Reid, M.R. 2009. Comparison of digestion methods for ICP-MS determination of trace elements in fish tissues. Analytica Chimica Acta **653**(2): 191-199.

Asmus, H., and Asmus, R. 1998. The role of macrobenthic communities for sediment-water material exchange in the Sylt-Rømø tidal basin Die Rolle der Makrobenthos-Gemeinschaften für den Stoffaustausch des Sylt-Rømø Tidebeckens. Senckenbergiana Maritima **29**(1-6): 111-119.

Baars, O., Abouchami, W., Galer, S.J., Boye, M., and Croot, P.L. 2014. Dissolved cadmium in the Southern Ocean: Distribution, speciation, and relation to phosphate. Limnology and Oceanography **59**(2): 385-399.

Babaranti, O., Horn, S., Jowett, T., and Frew, R. 2019. Isotopic signatures in *Mytilus galloprovincialis* and *Ulva lactuca* as bioindicators for assessing discharged sewage

effluent in coastal waters along Otago Peninsula, New Zealand. Geology, Ecology, and Landscapes **3**(1): 53-64.

Baeta, A., Valiela, I., Rossi, F., Pinto, R., Richard, P., Niquil, N., and Marques, J.C. 2009. Eutrophication and trophic structure in response to the presence of the eelgrass *Zostera noltii*. Marine Biology **156**(10): 2107-2120.

Bagchi, G., Hurst, C.H., and Waxman, D.J. 2009. Interactions of methoxyacetic acid with androgen receptor. Toxicology and Applied Pharmacology **238**(2): 101-110.

Bagchi, G., Zhang, Y., and Waxman, D.J. 2010. Impact of methoxyacetic acid on mouse Leydig cell gene expression. Reproductive Biology and Endocrinology **8**(1): 65.

Baker, D.M., MacAvoy, S.E., and Kim, K. 2007. Relationship between water quality, δ^{15}N, and aspergillosis of Caribbean sea fan corals. Marine Ecology Progress Series **343**: 123-130.

Barnes, K.K., Christenson, S.C., Kolpin, D.W., Focazio, M.J., Furlong, E.T., Zaugg, S.D., Meyer, M.T., and Barber, L.B. 2004. Pharmaceuticals and other organic waste water contaminants within a leachate plume downgradient of a municipal landfill. Groundwater Monitoring & Remediation **24**(2): 119-126.

Barr, N.G., Dudley, B.D., Rogers, K.M., and Cornelisen, C.D. 2013. Broad-scale patterns of tissue-δ^{15}N and tissue-N indices in frondose *Ulva spp*.; Developing a national baseline indicator of nitrogen-loading for coastal New Zealand. Marine Pollution Bulletin **67**(1-2): 203-216.

Bartolomé, L., Etxebarria, N., Martínez-Arkarazo, I., Raposo, J.C., Usobiaga, A., Zuloaga, O., Raingeard, D., and Cajaraville, M.P. 2010. Distribution of organic microcontaminants, butyltins, and metals in mussels from the estuary of Bilbao. Archives of Environmental Contamination and Toxicology **59**(2): 244-254.

Basova, M. 2005. Fatty acid composition of lipids in microalgae. International Journal on Algae **7**(1).

Bates, D.M. 2010. lme4: Mixed-effects modeling with R. URL http://lme4. r-forge. r-project. org/book.

Baumard, P., Budzinski, H., Garrigues, P., Dizer, H., and Hansen, P. 1999. Polycyclic aromatic hydrocarbons in recent sediments and mussels (*Mytilus edulis*) from the Western Baltic Sea: occurrence, bioavailability and seasonal variations. Marine Environmental Research **47**(1): 17-47.

Bayne, B., Hawkins, A., and Navarro, E. 1988. Feeding and digestion in suspension-feeding bivalve molluscs: The relevance of physiological compensations. American Zoologist **28**(1): 147-159.

Bayne, B.L. 1976. Marine mussels: Their ecology and physiology. Cambridge University Press.

Bayne, B.L. 1993. Feeding physiology of bivalves: Time-dependence and compensation for changes in food availability. *In* Bivalve filter feeders. Springer. pp. 1-24.

Beck, M., Dellwig, O., Schnetger, B., and Brumsack, H.-J. 2008. Cycling of trace metals (Mn, Fe, Mo, U, V, Cr) in deep pore waters of intertidal flat sediments. Geochimica et Cosmochimica Acta **72**(12): 2822-2840.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B.S., Hayes, C.G., Hoshino, K., and Minello, T.J. 2003. The role of nearshore ecosystems as fish and shellfish nurseries.

Becker, M.L., Luettich Jr, R.A., and Mallin, M.A. 2010. Hydrodynamic behavior of the Cape Fear River and estuarine system: a synthesis and observational investigation of discharge–salinity intrusion relationships. Estuarine, Coastal and Shelf Science **88**(3): 407-418.

Bedard-Haughn, A., Van Groenigen, J., and Van Kessel, C. 2003. Tracing ^{15}N through landscapes: potential uses and precautions. Journal of Hydrology **272**(1-4): 175-190.

Berg, B., and Staaf, H. 1981. Leaching, accumulation and release of nitrogen in decomposing forest litter. Ecol. Bull **33**: 163-178.

Bergman, Å., Heindel, J.J., Jobling, S., Kidd, K.A., and Zoeller, R.T. 2013. Endocrine Disrupting Chemicals-2012. United Nations Environment Programme and the World Health Organization: Geneva, Switzerland.

Bernard, A., and Lauwerys, R. 1986. Effects of cadmium exposure in humans. *In* Cadmium. Springer. pp. 135-177.

Bertrand, J.-C., Bonin, P., Caumette, P., Gattuso, J.-P., Grégori, G., Guyoneaud, R., Le Roux, X., Matheron, R., and Poly, F. 2015. Biogeochemical cycles. *In* Environmental microbiology: Fundamentals and applications. Springer. pp. 511-617.

Biache, C., and Philp, R.P. 2013. The use of sterol distributions combined with compound specific isotope analyses as a tool to identify the origin of fecal contamination in rivers. Water Research **47**(3): 1201-1208.

Bianchi, T.S. 2007. Biogeochemistry of estuaries. Oxford University Press on Demand.

Bianchi, T.S. 2011. The role of terrestrially derived organic carbon in the coastal ocean: A changing paradigm and the priming effect. Proceedings of the National Academy of Sciences **108**(49): 19473-19481.

Bianchi, T.S., and Canuel, E.A. 2011. Chemical biomarkers in aquatic ecosystems. Princeton University Press.

Bianchi, T.S., Lambert, C.D., Santschi, P.H., and Guo, L. 1997. Sources and transport of land-derived particulate and dissolved organic matter in the Gulf of Mexico (Texas shelf/slope): The use of ligninphenols and loliolides as biomarkers. Organic Geochemistry **27**(1): 65-78.

Biggs, R.B., and Cronin, L.E. 1981. Special characteristics of estuaries. *In* Estuaries and nutrients. Springer. pp. 3-23.

Birch, G., and Apostolatos, C. 2013. Use of sedimentary metals to predict metal concentrations in black mussel (*Mytilus galloprovincialis*) tissue and risk to human health (Sydney estuary, Australia). Environmental Science and Pollution Research **20**(8): 5481-5491.

Björk, M. Bioavailability and uptake of hydrophobic organic contaminants in bivalve filter-feeders. *In* Annales Zoologici Fennici. 1995. JSTOR. pp. 237-245.

Björklund, K. 2011. Sources and fluxes of organic contaminants in urban runoff. Doctoral Thesis, Chalmers University of Technology.

Blei, S., Schneider, K.-H., Fricke, H.-M., and Werner, A. 2012. Production of pesticide granulates in a spouted bed apparatus. Google Patents.

Blessy, S., and Iyer, P.R. 2017. Extraction and production of biodiesel from *Chlorella Spp*. World Journal of Pharmacy and Pharmaceutical Sciences **6**(1): 424-432.

Blumer, M., Souza, G.t., and Sass, J. 1970. Hydrocarbon pollution of edible shellfish by an oil spill. Marine Biology **5**(3): 195-202.

Bocchetti, R., and Regoli, F. 2006. Seasonal variability of oxidative biomarkers, lysosomal parameters, metallothioneins and peroxisomal enzymes in the Mediterranean mussel *Mytilus galloprovincialis* from Adriatic Sea. Chemosphere **65**(6): 913-921.

Bocchetti, R., Fattorini, D., Pisanelli, B., Macchia, S., Oliviero, L., Pilato, F., Pellegrini, D., and Regoli, F. 2008. Contaminant accumulation and biomarker responses in caged mussels, *Mytilus galloprovincialis*, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbour areas. Aquatic Toxicology **89**(4): 257-266.

Bódis, E., Nosek, J., Oertel, N., Tóth, B., Hornung, E., and Sousa, R. 2011. Spatial distribution of bivalves in relation to environmental conditions (middle Danube catchment, Hungary). Community Ecology **12**(2): 210-219.

Bolin, B. and Cook, R.B., 1983. The major biogeochemical cycles and their interactions (Scope Report 21).

Bouillon, S., Moens, T., Overmeer, I., Koedam, N., and Dehairs, F. 2004. Resource utilization patterns of epifauna from mangrove forests with contrasting inputs of local versus imported organic matter. Marine Ecology Progress Series **278**: 77-88.

Bouillon, S., Middelburg, J.J., Dehairs, F., Borges, A.V., Abril, G., Flindt, M.R., Ulomi, S., and Kristensen, E. 2007. Importance of intertidal sediment processes and porewater exchange on the water column biogeochemistry in a pristine mangrove creek (Ras Dege, Tanzania). Biogeosciences Discussions **4**(1): 317-348.

Bouman, R., and Archer, H. 2014. Tahuna WWTP, Dunedin, New Zealand: An Innovative Upgrade. Proceedings of the Water Environment Federation **2014**(16): 4096-4116.

Bowen, V.T., and Sutton, D. 1951. Comparative studies of mineral constituents of marine sponges. I. The genera *Dysidea, Chondrilla, Terpios*. Brookhaven National Lab.; Yale Univ.

Boyd, P., LaRoche, J., Gall, M., Frew, R.D., and McKay, R. 1999. Role of iron, light and silicate in controlling algal biomass in subantarctic waters SE of New Zealand. Journal of Geophysical Research **104**(C6): 13,395-313,408.

Boyle, E.A., Sclater, F., and Edmond, J.M. 1976. On the marine geochemistry of cadmium. Nature **263**(September 2): 42-44.

Bradley, J. 2016. Wastewater Outfalls - International Perspectives Relative to New Zealand. IWA International Symposium on Outfall Systems (ISOS) in Ottawa: 19.

Brazee, S.L., and Carrington, E. 2006. Interspecific comparison of the mechanical properties of mussel byssus. The Biological Bulletin **211**(3): 263-274.

Brett, M.T., Müller-Navarra, D.C., Ballantyne, A.P., Ravet, J.L., and Goldman, C.R. 2006. Daphnia fatty acid composition reflects that of their diet. Limnology and Oceanography **51**(5): 2428-2437.

Bridges, T.S., Gustavson, K.E., Schroeder, P., Ells, S.J., Hayes, D., Nadeau, S.C., Palermo, M.R., and Patmont, C. 2010. Dredging processes and remedy effectiveness: Relationship to the 4 Rs of environmental dredging. Integrated Environmental Assessment and Management **6**(4): 619-630.

Brooks, R.R., and Rumsby, M.G. 1965. Trace Element Uptake by some New Zealand Bivalves1. Limnology and Oceanography **10**(4): 521-527.

Brown, M.R. 2002. Nutritional value and use of microalgae in aquaculture. Avances en Nutrición Acuícola VI. Memorias del VI Simposium Internacional de Nutrición Acuícola **3**: 281-292.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., and Thompson, R. 2011. Accumulation of microplastic on shorelines woldwide: Sources and sinks. Environmental Science & Technology **45**(21): 9175-9179.

Bulbul, A. 2013. The biogeochemical cycle of cadmium in subantarctic water : Studies of carbonic anhydrase enzyme activity. Chemistry, University of Otago, Dunedin.

Bump, J.K., Fox-Dobbs, K., Bada, J.L., Koch, P.L., Peterson, R.O., and Vucetich, J.A. 2007. Stable isotopes, ecological integration and environmental change: Wolves record atmospheric carbon isotope trend better than tree rings. Proceedings of the Royal Society of London B: Biological Sciences **274**(1624): 2471-2480.

Burke, T.R. 2013. Foamable pesticide compositions. Google Patents.

Buswell, A.M., Shive, R., and Neave, S. 1928. Removal of colloids from sewage. Circular no. 003.

Cabana, G., and Rasmussen, J.B. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences **93**(20): 10844-10847.

Cabrita, M., Catarino, F., and Vale, C. 1999. The effect of tidal range on the flushing of ammonium from intertidal sediments of the Tagus estuary, Portugal. Oceanologica Acta **22**(3): 291-302.

Cantwell, M.G., Burgess, R.M., and King, J.W. 2008. Resuspension of contaminated field and formulated reference sediments Part I: Evaluation of metal release under controlled laboratory conditions. Chemosphere **73**(11): 1824-1831.

Canuel, E.A. 2001. Relations between river flow, primary production and fatty acid composition of particulate organic matter in San Francisco and Chesapeake Bays: A multivariate approach. Organic Geochemistry **32**(4): 563-583.

Canuel, E.A., and Hardison, A.K. 2016. Sources, ages, and alteration of organic matter in estuaries. Annual Review of Marine Science **8**: 409-434.

Canuel, E.A., Cloern, J.E., Ringelberg, D.B., Guckert, J.B., and Rau, G.H. 1995. Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay. Limnology and Oceanography **40**(1): 67-81.

Caramujo, M.J., Boschker, H.T., and Admiraal, W. 2008. Fatty acid profiles of algae mark the development and composition of harpacticoid copepods. Freshwater Biology **53**(1): 77-90.

Cardoso, C., Ripol, A., Afonso, C., Freire, M., Varela, J., Quental-Ferreira, H., Pousão-Ferreira, P., and Bandarra, N. 2017. Fatty acid profiles of the main lipid classes of green seaweeds from fish pond aquaculture. Food Science & Nutrition **5**(6): 1186-1194.

Carmichael, R.H., Walton, W., and Clark, H. 2012. Bivalve-enhanced nitrogen removal from coastal estuaries. Canadian Journal of Fisheries and Aquatic Sciences **69**(7): 1131-1149.

Carrington, E. 2002. Seasonal variation in the attachment strength of blue mussels: Causes and consequences. Limnology and Oceanography **47**(6): 1723-1733.

Cathum, S., and Sabik, H. 2001. Determination of steroids and coprostanol in surface water, effluent and mussel using gas chromatography-mass spectrometry. Chromatographia **53**(1): 394-399.

Cava-Montesinos, P., Nilles, K., Cervera, M.L., and de la Guardia, M. 2005. Non-chromatographic speciation of toxic arsenic in fish. Talanta **66**(4): 895-901.

Cave, R., Andrews, J., Jickells, T., and Coombes, E. 2005. A review of sediment contamination by trace metals in the Humber catchment and estuary, and the implications for future estuary water quality. Estuarine, Coastal and Shelf Science **62**(3): 547-557.

Chandurvelan, R., Marsden, I.D., Glover, C.N., and Gaw, S. 2015. Assessment of a mussel as a metal bioindicator of coastal contamination: relationships between metal bioaccumulation and multiple biomarker responses. Science of the Total Environment **511**: 663-675.

Chang, B., Yang, C., Cheng, C., and Yuan, S. 2004. Biodegradation of phthalate esters by two bacteria strains. Chemosphere **55**(4): 533-538.

Chapman, P.M., and Wang, F. 2001. Assessing sediment contamination in estuaries. Environmental Toxicology and Chemistry **20**(1): 3-22.

Charette, M.A., Sholkovitz, E.R., and Hansel, C.M. 2005. Trace element cycling in a subterranean estuary: Part 1. Geochemistry of the permeable sediments. Geochimica et Cosmochimica Acta **69**(8): 2095-2109.

Chary, N.S., Kamala, C., and Raj, D.S.S. 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicology and Environmental Safety **69**(3): 513-524.

Cheevaporn, V., and Menasveta, P. 2003. Water pollution and habitat degradation in the Gulf of Thailand. Marine Pollution Bulletin **47**(1–6): 43-51.

Chen, G.-Q., Jiang, Y., and Chen, F. 2007. Fatty acid and lipid class composition of the eicosapentaenoic acid-producing microalga, *Nitzschia laevis*. Food chemistry **104**(4): 1580-1585.

Cheung, C., Zheng, G., Li, A., Richardson, B., and Lam, P. 2001. Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, *Perna viridis*. Aquatic Toxicology **52**(3-4): 189-203.

Chikaraishi, Y., Suzuki, Y., and Naraoka, H. 2004. Hydrogen isotopic fractionations during desaturation and elongation associated with polyunsaturated fatty acid biosynthesis in marine macroalgae. Phytochemistry **65**(15): 2293-2300.

Chou, C.-C., and Liu, Y.-P. 2004. Determination of fecal sterols in the sediments of different wastewater outputs by GC-MS. International Journal of Environmental Analytical Chemistry **84**(5): 379-388.

Ciborowski, J.J., Niemi, G.J., Brady, V.J., Doka, S.E., Johnson, L.B., Keough, J.R., Mackey, S.D. and Uzarski, D.G., 2008. Ecosystem responses to regulation-based water level changes in the upper Great Lakes. International Upper Great Lakes Study Technical Work Group, International Joint Commission, Washington DC and Ottawa Canada.

Citilab. 2016. Trace metal concentrations in *Mytilus galloprovincialis*. Monthly Laboratory Report for theTahuna Wastewater Treatment Plant.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series **210**: 223-253.

Cloern, J.E., Powell, T.M., and Huzzey, L.M. 1989. Spatial and temporal variability in South San Francisco Bay (USA). II. Temporal changes in salinity, suspended sediments, and phytoplankton biomass and productivity over tidal time scales. Estuarine, Coastal and Shelf Science **28**(6): 599-613.

Cloern, J.E., Canuel, E.A., and Harris, D. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnology and Oceanography **47**(3): 713-729.

Cloern, J.E., Foster, S., and Kleckner, A. 2014. Phytoplankton primary production in the world's estuarine-coastal ecosystems. Biogeosciences **11**(9): 2477-2501.

Cohen, I., and Neori, A. 1991. *Ulva lactuca* biofilters for marine fishpond effluents. I. Ammonia uptake kinetics and nitrogen content. Botanica Marina **34**(6): 475-482.

Cohen, J.E., Small, C., Mellinger, A., Gallup, J., Sachs, J., Vitousek, P.M., and Mooney, H.A. 1997. Estimates of coastal populations. Science **278**: 1209.

Commission, E. 2001. EU Commission Regulation as regards heavy metals. Directive 2001/22/EC.

Connolly, R.M., Gorman, D., Hindell, J.S., Kildea, T.N., and Schlacher, T.A. 2013. High congruence of isotope sewage signals in multiple marine taxa. Marine Pollution Bulletin **71**(1-2): 152-158.

Coplen, T.B. 2011. Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. Rapid Communications in Mass Spectrometry **25**(17): 2538-2560.

Correll, D.L. 1978. Estuarine productivity. BioScience **28**(10): 646-650.

Council, D.C. 2012. Stormwater Compliance Monitoring 2013.

Council, N.R. 2011. Nutrient requirements of fish and shrimp. National Academies Press.

Council, O.R. 2001. Regional Plan: Coast. Accessed online January **12**: 2008.

Council, O.R. 2004. Regional Plan: Water for Otago. Otago Regional Council, Dunedin.

Council, O.R. 2005. Environmental Status of the Near Shore Coastal Environment (1): 1-77.

Cranwell, A., Jaworski, G., and Bickley, H. 1990. Hydrocarbons, sterols, esters and fatty acids in six freshwater chlorophytes. Phytochemistry **29**(1): 145-151.

Cranwell, P. 1982. Lipids of aquatic sediments and sedimenting particulates. Progress in Lipid Research **21**(4): 271-308.

Crawford, C. 2006. Indicators for monitoring the condition of estuaries and coastal waters.

Criss, R.E. 1999. Principles of stable isotope distribution. Oxford University Press on Demand.

Davies-Colley, R.J., 2013. River water quality in New Zealand: An introduction and overview. Ecosystem services in New Zealand: Conditions and trends. Manaaki Whenua Press, Lincoln, pp.432-447.

Da Ros, L., Moschino, V., Guerzoni, S., and Halldórsson, H. 2007. Lysosomal responses and metallothionein induction in the blue mussel *Mytilus edulis* from the south-west coast of Iceland. Environment International **33**(3): 362-369.

Daby, D. 2006. Coastal pollution and potential biomonitors of metals in Mauritius. Water, Air, and Soil Pollution **174**(1-4): 63-91.

Dagg, M.J., Ammerman, J.W., Amon, R.M., Gardner, W.S., Green, R.E., and Lohrenz, S.E. 2007. A review of water column processes influencing hypoxia in the northern Gulf of Mexico. Estuaries and Coasts **30**(5): 735-752.

Dalerum, F., and Angerbjörn, A. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia **144**(4): 647-658.

Das, A., Justic, D., Swenson, E., Turner, R.E., Inoue, M., and Park, D. 2011. Coastal land loss and hypoxia: the 'outwelling'hypothesis revisited. Environmental Research Letters **6**(2): 025001.

Dashti, N., Al-Awadhi, H., Khanafer, M., Abdelghany, S., and Radwan, S. 2008. Potential of hexadecane-utilizing soil-microorganisms for growth on hexadecanol, hexadecanal and hexadecanoic acid as sole sources of carbon and energy. Chemosphere **70**(3): 475-479.

Dawczynski, C., Schubert, R., and Jahreis, G. 2007. Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chemistry **103**(3): 891-899.

de Almeida, E.A., Bainy, A.C.D., de Melo Loureiro, A.P., Martinez, G.R., Miyamoto, S., Onuki, J., Barbosa, L.F., Garcia, C.C.M., Prado, F.M., and Ronsein, G.E. 2007. Oxidative stress in *Perna perna* and other bivalves as indicators of environmental stress in the Brazilian marine environment: Antioxidants, lipid peroxidation and DNA damage. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology **146**(4): 588-600.

De Jonge, V., and Van Beusekom, J. 1995. Wind-and tide-induced resuspension of sediment and microphytobenthos from tidal flats in the Ems estuary. Limnology and Oceanography **40**(4): 766-778.

de Mora, S., Fowler, S.W., Wyse, E., and Azemard, S. 2004. Distribution of heavy metals in marine bivalves, fish and coastal sediments in the Gulf and Gulf of Oman. Marine Pollution Bulletin **49**(5-6): 410-424.

Dede, O.T., Telci, I.T., and Aral, M.M. 2013. The use of water quality index models for the evaluation of surface water quality: A case study for Kirmir Basin, Ankara, Turkey. Water Quality, Exposure and Health **5**(1): 41-56.

Deegan, L.A., and Buchsbaum, R. 2005. The effect of habitat loss and degradation on fisheries. The decline of disheries resources in New England: Evaluating the impact of overfishing, contanmination, and habitat degradation. Massachusetts: MIT Sea Grant College Program: 67-96.

Degger, N., Wepener, V., Richardson, B., and Wu, R. 2011. Brown mussels (*Perna perna*) and semi-permeable membrane devices (SPMDs) as indicators of organic pollutants in the South African marine environment. Marine Pollution Bulletin **63**(5): 91-97.

Denich, T., Beaudette, L., Lee, H., and Trevors, J. 2003. Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. Journal of Microbiological Methods **52**(2): 149-182.

Deudero, S., Cabanellas, M., Blanco, A., and Tejada, S. 2009a. Stable isotope fractionation in the digestive gland, muscle and gills tissues of the marine mussel Mytilus galloprovincialis. Journal of Experimental Marine Biology and Ecology **368**(2): 181-188.

Deudero, S., Box, A., Tejada, S., and Tintoré, J. 2009b. Stable isotopes and metal contamination in caged marine mussel Mytilus galloprovincialis. Marine Pollution Bulletin **58**(7): 1025-1031.

Diaz, R.J., Rhoads, D.C., Blake, J.A., Kropp, R.K., and Keay, K.E. 2008. Long-term trends of benthic habitats related to reduction in wastewater discharge to Boston Harbor. Estuaries and Coasts **31**(6): 1184-1197.

Directive, W.F. 2003. Common implementation strategy for the water framework directive (2000/60/EC).

Dolenec, M., Žvab, P., Mihelčić, G., Lambaša Belak, Ž., Lojen, S., Kniewald, G., Dolenec, T., and Rogan Šmuc, N. 2011. Use of Stable Nitrogen Isotope Signatures of Anthropogenic Organic Matter in the Coastal Environment: The Case Study of the Kosirina Bay (Murter Island, Croatia). Geologia Croatica **64**(2): 143-152.

Domouhtsidou, G., Dailianis, S., Kaloyianni, M., and Dimitriadis, V. 2004. Lysosomal membrane stability and metallothionein content in *Mytilus galloprovincialis* (L.), as biomarkers: combination with trace metal concentrations. Marine Pollution Bulletin **48**(5-6): 572-586.

Doney, S.C. 2010. The growing human footprint on coastal and open-ocean biogeochemistry. Science **328**(5985): 1512-1516.

Doré, W.J., Henshilwood, K., and Lees, D.N. 2000. Evaluation of F-specific RNA bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish. Applied and Environmental Microbiology **66**(4): 1280-1285.

Dowhan, W., and Bogdanov, M. 2002. Functional roles of lipids in membranes. *In* New comprehensive biochemistry. Elsevier. pp. 1-35.

Drake, D.C., Peterson, B.J., Galván, K.A., Deegan, L.A., Hopkinson, C., Johnson, J.M., Koop-Jakobsen, K., Lemay, L.E., and Picard, C. 2009. Salt marsh ecosystem biogeochemical responses to nutrient enrichment: A paired ^{15}N tracer study. Ecology **90**(9): 2535-2546.

Drasar, B.S., and Hill, M.J. 1974. Human intestinal flora. Academic Press (London) Ltd., 24/28 Oval Road, London, NWI.

Drenovsky, R.E., Elliott, G.N., Graham, K.J., and Scow, K.M. 2004. Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME) for characterizing soil microbial communities. Soil Biology and Biochemistry **36**(11): 1793-1800.

Dudley, B.D., and Shima, J.S. 2010. Algal and invertebrate bioindicators detect sewage effluent along the coast of Titahi Bay, Wellington, New Zealand. New Zealand Journal of Marine and Freshwater Research **44**(1): 39-51.

Dudley, B.D., Milne, J. R., Zeldis, J. 2018. Review of Estuarine Water Quality Monitoring for the Horizons Region [Environmental Report]. Horizons Report 2018/EXT/1608 Envirolink Report No. 1872-HZLC148: 1-41.

Duggins, D. 1988. The effects of kelp forests on nearshore environments: biomass, detritus, and altered flow. *In* The community ecology of sea otters. Springer. pp. 192-201.

Dutertre, M., Hamon, D., Chevalier, C., and Ehrhold, A. 2013. The use of the relationships between environmental factors and benthic macrofaunal distribution in the establishment of a baseline for coastal management. ICES Journal of Marine Science **70**(2): 294-308.

Ebner, B.C., McAllister, R.R., and Suter, P. 2009. Effects of sample size on numerical estimates of diel prey consumption in a fish population. New Zealand Journal of Marine and Freshwater Research **43**(2): 579-590.

Eganhouse, R.P. 2004. Molecular markers and their use in environmental organic geochemistry. *In* The Geochemical Society Special Publications. *Edited by* R.J. Hill and J. Leventhal and Z. Aizenshtat and M.J. Baedecker and G. Claypool and R. Eganhouse and M. Goldhaber and K. Peters. Elsevier. pp. 143-158.

Eggleton, J., and Thomas, K.V. 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environment International **30**(7): 973-980.

Eglinton, G., and Hamilton, R.J. 1967. Leaf epicuticular waxes. Science **156**(3780): 1322-1335.

Egozcue, J.J., Pawlowsky-Glahn, V., Mateu-Figueras, G., and Barcelo-Vidal, C. 2003. Isometric logratio transformations for compositional data analysis. Mathematical Geology **35**(3): 279-300.

Elderfield, H., and Rickerby, R.E.M. 2000. Oceanic Cd/P and nutrient utilisation in the glacial Southern Ocean. Nature **405**: 305-310.

Ellis, T.G. 2004. Chemistry of wastewater. Encyclopedia of Life Support System (EOLSS).

Ellwood, M.J., Law, C.S., Hall, J., Woodward, E.M.S., Strzepek, R., Kuparinen, J., Thompson, K., Pickmere, S., Sutton, P., and Boyd, P.W. 2013. Relationships between nutrient stocks and inventories and phytoplankton physiological status along an oligotrophic meridional transect in the Tasman Sea. Deep-Sea Research Part I-Oceanographic Research Papers **72**: 102-120.

Eltgroth, M.L., Watwood, R.L., and Wolfe, G.V. 2005. Production and cellular localization of neutral long-chain lipids in the haptophyte algae *Isochrysis galbana* and *Emiliania huxleyi* Journal of Phycology **41**(5): 1000-1009.

Evershed, R.P., Bull, I.D., Corr, L.T., Crossman, Z.M., van Dongen, B.E., Evans, C.J., Jim, S., Mottram, H.R., Mukherjee, A.J., and Pancost, R.D. 2007. Compound-specific stable isotope analysis in ecology and paleoecology. Stable Isotopes in Ecology and Environmental Science: 480.

Evgenidou, A., and Valiela, I. 2002. Response of growth and density of a population of Geukensia demissa to land-derived nitrogen loading, in Waquoit Bay, Massachusetts. Estuarine, Coastal and Shelf Science **55**(1): 125-138.

Fairchild, J., Boyle, T., Robinson-Wilson, E., and Jones, J. 1983. Microbial action in detrital leaf processing and the effects of chemical perturbation. Dynamics of Lotic Systems, Ann Arbor Science, Ann Arbor MI. 1983, 437-456.

Falkowski, P.G. 1994. The role of phytoplankton phtotsynthesis in global biogeochemical cycles. Photosynthesis Research **39**(3): 235-258.

FAO/WHO. 2007. Evaluation of certain food additives and contaminants: sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization.

Ficken, K.J., Li, B., Swain, D., and Eglinton, G. 2000. An n-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. Organic Geochemistry **31**(7-8): 745-749.

Fierer, N., Schimel, J.P., and Holden, P.A. 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry **35**(1): 167-176.

Figueira, R., Sérgio, C., and Sousa, A. 2002. Distribution of trace metals in moss biomonitors and assessment of contamination sources in Portugal. Environmental Pollution **118**(1): 153-163.

Findlay, S., Pace, M.L., Lints, D., and Howe, K. 1992. Bacterial metabolism of organic carbon in the tidal freshwater Hudson Estuary. Marine Ecology Progress Series. Oldendorf **89**(2): 147-153.

Finlay, J.C. 2001. Stable-carbon-isotope ratios of river biota: Implications for energy flow in lotic food webs. Ecology **82**(4): 1052-1064.

Finlay, J.C. 2004. Patterns and controls of lotic algal stable carbon isotope ratios. Limnology and Oceanography **49**(3): 850-861.

Finlay, J.C., Power, M.E., and Cabana, G. 1999. Effects of water velocity on algal carbon isotope ratios: implications for river food web studies. Limnology and Oceanography **44**(5): 1198-1203.

Frolander, H.F. 1964. Biological and chemical features of tidal estuaries. Journal (Water Pollution Control Federation): 1037-1048.

Förstner, U., and Wittmann, G.T. 2012. Metal pollution in the aquatic environment. Springer Science & Business Media.

Frew, R., Bowie, A., Croot, P., and Pickmere, S. 2001. Macronutrient and trace-metal geochemistry of an in situ iron-induced Southern Ocean bloom. Deep Sea Research Part II: Topical Studies in Oceanography **48**(11-12): 2467-2481.

Frew, R.D., and Hunter, K.A. 1992. Influence of Southern Ocean waters on the cadmium-phosphate properties of the global ocean. Nature **360**: 144-146.

Frew, R.D., and Hunter, K.A. 1995. Cadmium-phosphorus cycling at the subtropical convergence south of New Zealand. Marine Chemistry **51**(3): 223-237.

Frew, R.D., Hunter, K.A., and Beyer, R. 1989. Cadmium in the dredge oyster *Ostrea lutaria*—dependence on age, body weight and distribution in internal organs. Marine Pollution Bulletin **20**(9): 463-464.

Friberg, L. 2017. Cadmium in the Environment:CRC press. Cleveland, Ohio.

Fry, B. 1981. Natural stable carbon isotope tag traces Texas shrimp migrations. Fish Bull **79**(2): 337-345.

Fry, B. 2002. Conservative mixing of stable isotopes across estuarine salinity gradients: A conceptual framework for monitoring watershed influences on downstream fisheries production. Estuaries **25**(2): 264-271.

Fry, B. 2006. Stable isotope ecology. Springer.

Fry, B., and Arnold, C. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). Oecologia **54**(2): 200-204.

Fry, B., Gace, A., and McClelland, J.W. 2003. Chemical indicators of anthropogenic nitrogen-loading in four Pacific estuaries. Pacific Science **57**(1): 77-101.

FSANZ. 2007. Australia New Zealand Food Standards Code. Food Standards Australia New Zealand.

Fuhrman, J. 1992. Bacterioplankton roles in cycling of organic matter: the microbial food web. Primary productivity and biogeochemical cycles in the sea. Plenum Press, New York: 361-383.

Furtula, V., Liu, J., Chambers, P., Osachoff, H., Kennedy, C., & Harkness, J. (2012). Sewage treatment plants efficiencies in removal of sterols and sterol ratios as indicators of fecal contamination sources. Water, Air, & Soil Pollution, **223**(3), 1017-1031.

Furukawa, Y., Reed, A.H., and Zhang, G. 2014. Effect of organic matter on estuarine flocculation: A laboratory study using montmorillonite, humic acid, xanthan gum, guar gum and natural estuarine flocs. Geochemical Transactions **15**(1): 1.

Gagné, F., Blaise, C., Lachance, B., Sunahara, G., and Sabik, H. 2001. Evidence of coprostanol estrogenicity to the freshwater mussel *Elliptio complanata*. Environmental Pollution **115**(1): 97-106.

Gales, N.J. 2009. New Zealand Sea Lion: Phocarctos hookeri. *In* Encyclopedia of Marine Mammals (Second Edition). Elsevier. pp. 763-765.

Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B., and Cosby, B.J. 2003. The nitrogen cascade. Bioscience **53**(4): 341-356.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., and Sutton, M.A. 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science **320**(5878): 889-892.

Gao, D.-W., and Wen, Z.-D. 2016. Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. Science of the total Environment **541**: 986-1001.

García-Robledo, E., Corzo, A., and Papaspyrou, S. 2014. A fast and direct spectrophotometric method for the sequential determination of nitrate and nitrite at low concentrations in small volumes. Marine Chemistry **162**: 30-36.

Gartner, A., Lavery, P., and Smit, A. 2002. Use of δ^{15}N signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Marine Ecology Progress Series **235**: 63-73.

Gaston, T.F., and Suthers, I.M. 2004. Spatial variation in δ^{13}C and δ^{15} N of liver, muscle and bone in a rocky reef planktivorous fish: The relative contribution of sewage. Journal of Experimental Marine Biology and Ecology **304**(1): 17-33.

Gault-Ringold, M., Adu, T., Stirling, C.H., Frew, R.D., and Hunter, K.A. 2012. Anomalous biogeochemical behavior of cadmium in subantarctic surface waters: Mechanistic constraints from cadmium isotopes. Earth and Planetary Science Letters **341**: 94-103.

Geider, R.J., and La Roche, J. 2002. Redfield revisited: variability of C [ratio] N [ratio] P in marine microalgae and its biochemical basis. European Journal of Phycology **37**(1): 1-17.

Gladyshev, M., Sushchik, N., Makhutova, O., Kalacheva, G., Kolmakova, A., Kravchuk, E., and Dubovskaya, O. Efficiency of transfer of essential polyunsaturated fatty acids along trophic chains in aquatic ecosystems. *In* Doklady Biochemistry and Biophysics. 2009. Springer. pp. 158-160.

Glickman, L., Fairbrother, A., Guarino, A., Bergman, H., and Buck, W. 1991. Animals as sentinels of environmental health hazards. National Research Council, Washington, DC (United States). Committee on Animals as Monitors of Environmental Hazards.

Gilpin, B. J., Gregor, J. E., & Savill, M. G. (2002). Identification of the source of faecal pollution in contaminated rivers. Water Science and Technology, **46**(3), 9-15.

Goerke, H., Weber, K., Bornemann, H., Ramdohr, S., and Plötz, J. 2004. Increasing levels and biomagnification of persistent organic pollutants (POPs) in Antarctic biota. Marine Pollution Bulletin **48**(3-4): 295-302.

Goerlitz, S., Berkenbusch, K., and Probert, P. 2013. Distribution and abundance of Abarenicola affinis (Arenicolidae, Polychaeta) in tidal inlets of Otago, New Zealand. New Zealand Journal of Marine and Freshwater Research **47**(4): 548-560.

Gormley, A.M., Slooten, E., Dawson, S., Barker, R.J., Rayment, W., du Fresne, S., and Bräger, S. 2012. First evidence that marine protected areas can work for marine mammals. Journal of Applied Ecology **49**(2): 474-480.

Gosling, E. 2003. Reproduction, settlement and recruitment. Bivalve molluscs: biology, ecology and culture. Fishing news books, Blackwell, Oxford: 131-168.

Gratiot, N., Bildstein, A., Anh, T.T., Thoss, H., Denis, H., Michallet, H., and Apel, H. 2017. Sediment flocculation in the Mekong River estuary, Vietnam, an important driver of geomorphological changes. Comptes Rendus Geoscience **349**(6): 260-268.

Green, C.T., and Scow, K.M. 2000. Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeology Journal **8**(1): 126-141.

Green, S.B., and Salkind, N.J. 2008. Using SPSS for Windows and Macintosh: Analyzing and. Upper Saddle: Pearson Prentice Hall.

Greening, G.E., Lewis, G.D., and Dollimore, J. 2007. FRST Programme C03X0301 Safeguarding Environmental Health and Market Access for NZ Foods Objective 2: Virus Prevalence in Shellfish.

Grey, J. 2000. Trophic fractionation and the effects of diet switch on the carbon stable isotopic 'signatures' of pelagic consumers. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen **27**(5): 3187-3191.

Gribsholt, B., Boschker, S., Struyf, E., Andersson, M., Tramper, A., De Brabandere, L., Van Damme, S., Brion, N., Meire, P., and Dehairs, F. 2005. Nitrogen processing in a tidal freshwater marsh: A whole-ecosystem ^{15}N labeling study. Limnology and Oceanography **50**(6): 1945-1959.

Grimalt, J.O., Fernandez, P., Bayona, J.M., and Albaiges, J. 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environmental Science & Technology **24**(3): 357-363.

Griveau, Y., and Barthaburu, E. 2018. Method for managing flea beetles of the family chrysomelidae in brassica crops. Google Patents.

Grossart, H.-P., Brinkhoff, T., Martens, T., Duerselen, C., Liebezeit, G., and Simon, M. 2004. Tidal dynamics of dissolved and particulate matter and bacteria in a tidal flat ecosystem in spring and fall. Limnology and Oceanography **49**(6): 2212-2222.

Gruber, N., and Galloway, J.N. 2008. An Earth-system perspective of the global nitrogen cycle. Nature **451**(7176): 293-296.

Guelinckx, J., Maes, J., Van Den Driessche, P., Geysen, B., Dehairs, F., and Ollevier, F. 2007. Changes in $\delta^{13}C$ and $\delta^{15}N$ in different tissues of juvenile sand goby *Pomatoschistus minutus*: A laboratory diet-switch experiment. Marine Ecology Progress Series **341**: 205-215.

Guinder, V.A., Popovich, C.A., and Perillo, G.M. 2009. Short-term variability in the phytoplankton and physico-chemical variables in a high-tidal regime, Bahía Blanca Estuary, Argentina. Brazilian Journal of Oceanography **57**(3): 249-258.

Hallegraeff, G. 2003. Harmful algal blooms: a global overview. Manual on harmful marine Microalgae **33**: 1-22.

Hamilton, S.K. and Lewis Jr, W.M., 1992. Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. Geochimica et Cosmochimica Acta, **56**(12), pp.4237-4246.

Hammer, Ø., Harper, D., and Ryan, P. 2001. Paleontological statistics software: Package for education and data analysis. Palaeontologia Electronica **4**(1) 9.

Hamza-Chaffai, A. 2014. Usefulness of bioindicators and biomarkers in pollution biomonitoring. International Journal of Biotechnology for Wellness Industries **3**(1): 19-26.

Hanisak, M. 1983. The nitrogen relationships of marine macroalgae.

Hansen, N., Jensen, V.B., Appelquist, H., and Morch, E. 1978. Uptake and release of petroleum hydrocarbons by the marine mussel *Mytilus edulis*. Progress in Water Technology.

Hargrave, B., Holmer, M., and Newcombe, C. 2008. Towards a classification of organic enrichment in marine sediments based on biogeochemical indicators. Marine Pollution Bulletin **56**(5): 810-824.

Harriague, A.C., Gaozza, L., Montella, A., and Misic, C. 2006. Benthic communities on a sandy Ligurian beach (NW Mediterranean). Hydrobiologia **571**(1): 383-394.

Hassani, S., Karbassi, A., and Ardestani, M. 2017. Role of estuarine natural flocculation process in removal of Cu, Mn, Ni, Pb and Zn. Global Journal of Environmental Science and Management **3**(2): 187-196.

Hatten, J.A., Goñi, M.A., and Wheatcroft, R.A. 2012. Chemical characteristics of particulate organic matter from a small, mountainous river system in the Oregon Coast Range, USA [journal article]. Biogeochemistry **107**(1): 43-66.

Hauber, G. 1995. Wastewater treatment in NZ: Evaluation of 1992/93 performance data—ORGD. Water & Wastes in NZ **85**: 28-34.

Hawkins, A., and Bayne, B. 1985. Seasonal variation in the relative utilization of carbon and nitrogen by the mussel Mytilus edulis: Budgets, conversion efficiencies and maintenance requirements. Marine ecology progress series. Oldendorf **25**(2): 181-188.

Hayes, J.M. 2004. An introduction to isotopic calculations. Woods Hole Oceanographic Institution, Woods Hole, MA **2543**.

Heath, R. 1972. The southland current. New Zealand journal of marine and freshwater research **6**(4): 497-533.

Hedges, J., Keil, R., and Benner, R. 1997. What happens to terrestrial organic matter in the ocean? Organic Geochemistry **27**(5): 195-212.

Heip, C. 1995. Eutrophication and zoobenthos dynamics. Ophelia **41**(1): 113-136.

Helland, A., Holtan, G., and Jørgensen, P. 2003. Riverine inputs of organic carbon and nitrogen to Norwegian coastal areas. Ambio: 412-417.

Hellou, J., Yeats, P., Steller, S., and Gagné, F. 2003. Chemical contaminants and biological indicators of mussel health during gametogenesis. Environmental Toxicology and Chemistry: An International Journal **22**(9): 2080-2087.

Hendriks, I.E., van Duren, L.A., and Herman, P.M. 2003. Effect of dietary polyunsaturated fatty acids on reproductive output and larval growth of bivalves. Journal of Experimental Marine Biology and Ecology **296**(2): 199-213.

Hennebicq, R., Fabra, G., Pellerin, C., Marcotte, I., Myrand, B., and Tremblay, R. 2013. The effect of spawning of cultured mussels (*Mytilus edulis*) on mechanical properties, chemical and biochemical composition of byssal threads. Aquaculture **410**: 11-17.

Henze, M., and Comeau, Y. 2008. Wastewater characterization. Biological wastewater treatment: Principles Modelling and Design: 33-52.

Hepburn, C.D., Holborow, J.D., Wing, S.R., Frew, R.D., and Hurd, C.L. 2007. Exposure to waves enhances the growth rate and nitrogen status of the giant kelp *Macrocystis pyrifera*. Marine Ecology Progress Series **339**: 99-108.

Hernes, P.J., and Benner, R. 2006. Terrigenous organic matter sources and reactivity in the North Atlantic Ocean and a comparison to the Arctic and Pacific oceans. Marine Chemistry **100**(1-2): 66-79.

Hernes, P.J., Bergamaschi, B.A., Eckard, R.S., and Spencer, R.G. 2009. Fluorescence-based proxies for lignin in freshwater dissolved organic matter. Journal of Geophysical Research: Biogeosciences **114**(G4).

Hillebrand, H., and Sommer, U. 2000. Diversity of benthic microalgae in response to colonization time and eutrophication. Aquatic Botany **67**(3): 221-236.

Hobson, K.A., and Clark, R.G. 1992. Assessing avian diets using stable isotopes I: Turnover of ^{13}C in tissues. Condor: 181-188.

Hoefs, J. 1997. Stable isotope geochemistry. Springer.

Hoefs, J. 2009. Stable Isotope Geochemistry. 6th ed ed. Springer-Verlag Berlin Heidelberg. pp. 5-7.

Hoefs, J. 2018. Isotope fractionation processes of selected elements. *In* Stable Isotope Geochemistry. Springer. pp. 53-227.

Holmes, R.M., Peterson, B.J., Deegan, L.A., Hughes, J.E., and Fry, B. 2000. Nitrogen biogeochemistry in the oligohaline zone of a New England estuary. Ecology **81**(2): 416-432.

Hong, Y.S., Kinney, K.A., and Reible, D.D. 2011. Acid volatile sulfides oxidation and metals (Mn, Zn) release upon sediment resuspension: Laboratory experiment and model development. Environmental toxicology and chemistry **30**(3): 564-575.

Hopmans, E.C., Weijers, J.W., Schefuß, E., Herfort, L., Damsté, J.S.S., and Schouten, S. 2004. A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. Earth and Planetary Science Letters **224**(1-2): 107-116.

Horn, S. 2001. Tracking marine sewage outfall along the Otago Peninsula using stable isotope analysis of blue mussels *Mytilus galloprovincialis*, and sea lettuce *Ulva lactuca*, as biotracer species, pp33. Dissertation, Chemistry, Otago, Dunedin, New Zealand.

Horner, T.J., Lee, R.B.Y., Henderson, G.M., and Rickaby, R.E.M. 2013. Nonspecific uptake and homeostasis drive the oceanic cadmium cycle. Proceedings of the National Academy of Sciences of the United States of America **110**(7): 2500-2505.

Howarth, R. W., Marino, R., & Cole, J. J. (1988). Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls 1. Limnology and Oceanography, **33**(4part2), 688-701.

Howarth, R.W. 2008. Coastal nitrogen pollution: a review of sources and trends globally and regionally. Harmful Algae **8**(1): 14-20.

Hu, J., Peng, P.a., and Chivas, A.R. 2009. Molecular biomarker evidence of origins and transport of organic matter in sediments of the Pearl River estuary and adjacent South China Sea. Applied Geochemistry **24**(9): 1666-1676.

Hubbard Jr, K., Lautz, L., Mitchell, M., Mayer, B., and Hotchkiss, E. 2010. Evaluating nitrate uptake in a Rocky Mountain stream using labelled ^{15}N and ambient nitrate chemistry. Hydrological Processes **24**(23): 3322-3336.

IANZ. 2004. "Uncertainty of measurement, precision and limits of detection chemical and microbiological testing laboratories"- International Accrediation New Zealand, Auckland.

Idris, A.M., Eltayeb, M., Potgieter-Vermaak, S.S., Van Grieken, R., and Potgieter, J. 2007. Assessment of heavy metals pollution in Sudanese harbours along the Red Sea Coast. Microchemical Journal **87**(2): 104-112.

Innes, A., Rusak, S.A., Peakeb, B., and Warrenb, D. 2010. Healthy harbour watchers: community-based water quality monitoring and chemistry education in Dunedin. Chemistry in New Zealand **141145**.

Ip, C.C., Li, X.-D., Zhang, G., Wai, O.W., and Li, Y.-S. 2007. Trace metal distribution in sediments of the Pearl River Estuary and the surrounding coastal area, South China. Environmental Pollution **147**(2): 311-323.

James, W.F., Kennedy, R.H., Sham, W.E., and Myers, R.K. 1988. Leaf litter breakdown in a recently impounded reservoir. JAWRA Journal of the American Water Resources Association **24**(4): 831-837.

Jarvie, H.P., Neal, C., and Withers, P.J. 2006. Sewage-effluent phosphorus: a greater risk to river eutrophication than agricultural phosphorus? Science of the total environment **360**(1-3): 246-253.

Jeanneau, L., Solecki, O., Wery, N., Jardé, E., Gourmelon, M., Communal, P.-Y., Jadas-Hecart, A., Caprais, M.-P., Gruau, G., and Pourcher, A.-M. 2012. Relative decay of fecal indicator bacteria and human-associated markers: a microcosm study simulating wastewater input into seawater and freshwater. Environmental Science & Technology **46**(4): 2375-2382.

Jickells, T., An, Z., Andersen, K.K., Baker, A., Bergametti, G., Brooks, N., Cao, J., Boyd, P., Duce, R., and Hunter, K. 2005. Global iron connections between desert dust, ocean biogeochemistry, and climate. Science **308**(5718): 67-71.

John, D.A., and Leventhal, J.S. 1995. Bioavailability of metals. Descargado de http://www. unalmed. edu. co/rrodriguez/MODELOS/depositos-ambiente/BioaviabilityOf Metal. pdf/el **17**.

Johnson, A.C., Jürgens, M.D., Lawlor, A.J., Cisowska, I., and Williams, R.J. 2014. Particulate and colloidal silver in sewage effluent and sludge discharged from British wastewater treatment plants. Chemosphere **112**: 49-55.

Jørgensen, Bo. Barker, & Revsbech, N. P. (1989). Oxygen uptake, bacterial distribution, and carbon-nitrogen-sulfur cycling in sediments from the Baltic Sea-North Sea transition. Ophelia, **31**(1), 29-49.

Kabata-Pendias, A., and Mukherjee, A.B. 2007. Trace elements from soil to human. Springer Science & Business Media.

Kaise, T., and Fukui, S. 1992. The chemical form and acute toxicity of arsenic compounds in marine organisms. Applied organometallic chemistry **6**(2): 155-160.

Kamenarska, Z., Stefanov, K., Stancheva, R., Dimitrova-Konaklieva, S., and Popov, S. 2006. Comparative investigation on sterols from some Black Sea red algae. Natural product research **20**(2): 113-118.

Kanazawa, A., and Teshima, S.-l. 1978. Occurrence of coprostanol, an indicator of fecal pollution, in sea-water and sediments. Oceanologica Acta **1**(1): 39-44.

Kanduč, T., Medaković, D., and Hamer, B. 2011. *Mytilus galloprovincialis* as a bioindicator of environmental conditions: The case of the eastern coast of the Adriatic Sea. Isotopes in Environmental and Health Studies **47**(1): 42-61.

Kaur, A., Chaudhary, A., Kaur, A., Choudhary, R., and Kaushik, R. 2005. Phospholipid fatty acid–a bioindicator of environment monitoring and assessment in soil ecosystem. Current Science: 1103-1112.

Kelly, J.R., and Levin, S.A. 2012. A comparison of aquatic and terrestrial nutrient cycling and production processes in natural ecosystems, with reference. The Role of the oceans as a waste disposal option **172**: 165.

Kendall, C., and Caldwell, E.A. 1998. Fundamentals of isotope geochemistry. *In* Isotope tracers in catchment hydrology. Elsevier. pp. 51-86.

Kendall, C., Elliott, E.M., and Wankel, S.D. 2007. Tracing anthropogenic inputs of nitrogen to ecosystems. Stable Isotopes in Ecology and Environmental Science: 375-449.

Kendall, C., Young, M., Silva, S., Kraus, T., Peek, S., and Guerin, M. 2015. Tracing nutrient and organic matter sources and biogeochemical processes in the Sacramento River and Northern Delta: Proof of concept using stable isotope data. US Geological Survey, Data Release **110**.

Kendel, M., Wielgosz-Collin, G., Bertrand, S., Roussakis, C., Bourgougnon, N., and Bedoux, G. 2015. Lipid composition, fatty acids and sterols in the seaweeds *Ulva armoricana*, and *Solieria chordalis* from Brittany (France): An analysis from nutritional, chemotaxonomic, and antiproliferative activity perspectives. Marine Drugs **13**(9): 5606-5628.

Kennish, M.J. 1996. Practical handbook of estuarine and marine pollution. CRC press.

Kennish, M.J. 2002. Environmental threats and environmental future of estuaries. Environmental Conservation **29**(01): 78-107.

Kennish, M.J. 2016. Anthropogenic Impacts. Encyclopedia of Estuaries: 29-35.

Kennish, M.J., and Paerl, H.W. 2010. Coastal lagoons: critical habitats of environmental change. CRC Press.

Kirchman, D.L. 2018. Processes in microbial ecology. Oxford University Press.

Kline, J., Thomas C. 1999. Temporal and spatial variability of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in pelagic biota of Prince William Sound, Alaska. Canadian Journal of Fisheries and Aquatic Sciences **56**(S1): 94-117.

Kluytmans, J. 1985. Fatty acid synthesis in relation to gametogenesis in the mussel *Mytilus edulis* L. Comp. Biochem. Physiol. **81**: 959-963.

Konlechner, T. 2013. An Evaluation of the Viability of Re-establishing Tidal Exchange in Hoopers Inlet by Mechanical Excavation. Report prepared for the Otago Regional Council.

Kopp, J., Cornette, F., and Simonne, C. 2005. A comparison of growth and biochemical composition of *Mytilus galloprovincialis* (Lmk.) and *Mytilus edulis* (L.) on the West coast of Cotentin, Normandy, France 1999–2000. Aquaculture International **13**(4): 327-340.

Kostka, J.E., Gribsholt, B., Petrie, E., Dalton, D., Skelton, H., and Kristensen, E. 2002. The rates and pathways of carbon oxidation in bioturbated saltmarsh sediments. Limnology and Oceanography **47**(1): 230-240.

Krieger, R.I., Gee, S.J., and Lim, L.O. 1981. Marine bivalves, particularly mussels, *Mytilus sp*., for assessment of environmental quality. Ecotoxicology and Environmental Safety **5**(1): 72-86.

Kumari, P., Reddy, C., and Jha, B. 2011. Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. Analytical Biochemistry **415**(2): 134-144.

Kumari, P., Kumar, M., Gupta, V., Reddy, C., and Jha, B. 2010. Tropical marine macroalgae as potential sources of nutritionally important PUFAs. Food Chemistry **120**(3): 749-757.

Kumari, P., Bijo, A., Mantri, V.A., Reddy, C., and Jha, B. 2013. Fatty acid profiling of tropical marine macroalgae: An analysis from chemotaxonomic and nutritional perspectives. Phytochemistry **86**: 44-56.

Lambert, T., Bouillon, S., Darchambeau, F., Morana, C., Roland, F.A., Descy, J.-P., and Borges, A.V. 2017. Effects of human land use on the terrestrial and aquatic sources of fluvial organic matter in a temperate river basin (The Meuse River, Belgium). Biogeochemistry **136**(2): 191-211.

Lane, E.S., Semeniuk, D.M., Strzepek, R.F., Cullen, J.T., and Maldonado, M.T. 2009. Effects of iron limitation on intracellular cadmium of cultured phytoplankton: Implications for surface dissolved cadmium to phosphate ratios. Marine Chemistry **115**(3-4): 155-162.

Lang, I., Hodac, L., Friedl, T., and Feussner, I. 2011. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology **11**(1): 124.

Lapointe, T., Marcus, N., McGlathery, K., Sharpley, A., and Walker, D. 2000. Nutrient pollution of coastal rivers, bays, and seas. Issues in Ecology **7**: 114.

Larrose, A., Coynel, A., Schäfer, J., Blanc, G., Massé, L., and Maneux, E. 2010. Assessing the current state of the Gironde Estuary by mapping priority contaminant distribution and risk potential in surface sediment. Appl. Geochem. **25**(12): 1912-1923.

La Roche, J. (1983) Ammonium regeneration: its contribution to phytoplankton nitrogen requirements in a eutrophic environment. Marine Biology. 75, 231-240.

Laughlin, R.B., French, W., and Guard, H. 1986. Accumulation of bis (tributyltin) oxide by the marine mussel *Mytilus edulis*. Environmental Science & Technology **20**(9): 884-890.

Lavergne, F.D., Broeckling, C.D., Cockrell, D.M., Haley, S.D., Peairs, F.B., Jahn, C.E., and Heuberger, A.L. 2018. GC-MS Metabolomics to Evaluate the Composition of Plant Cuticular Waxes for Four Triticum aestivum Cultivars. International journal of Molecular Sciences **19**(2): 249.

Lawal-Are, O.A., and Babaranti, O. 2014. Heavy metal concentrations in *Pseudotolithus typus* and *Portunus validus*, water and sediment from Tarkwa Bay, Nigeria. Nigerian Journal of Fisheries **11**(1): 733-744.

Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., and Yeager, L.A. 2012. Applying stable isotopes to examine food-web structure: An overview of analytical tools. Biological Reviews **87**(3): 545-562.

Le, X.C., Lu, X., and Li, X.-F. 2004. Peer reviewed: arsenic speciation. ACS Publications.

Leblond, J.D., Dahmen, J.L., Seipelt, R.L., Elrod-Erickson, M.J., Kincaid, R., Howard, J.C., Evens, T.J., and Chapman, P.J. 2005. Lipid composition of chlorarachniophytes (chlorarachniophyceae) from the genera *Bigelowiella, Gymnochlora, And Lotharella* 1. Journal of Phycology **41**(2): 311-321.

Leeming, R., Ball, A., Ashbolt, N., and Nichols, P. 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water research **30**(12): 2893-2900.

Lehninger, A. 1950. Role of metal ions in enzyme systems. Physiological Reviews **30**(3): 393-429.

Lester, S.E., McLeod, K.L., Tallis, H., Ruckelshaus, M., Halpern, B.S., Levin, P.S., Chavez, F.P., Pomeroy, C., McCay, B.J., and Costello, C. 2010. Science in support of ecosystem-based management for the US West Coast and beyond. Biological Conservation **143**(3): 576-587.

Levin, L.A., Boesch, D.F., Covich, A., Dahm, C., Erséus, C., Ewel, K.C., Kneib, R.T., Moldenke, A., Palmer, M.A., and Snelgrove, P. 2001. The function of marine critical transition zones and the importance of sediment biodiversity. Ecosystems **4**(5): 430-451.

Lewis, G.D., Loutit, M.W., and Austin, F.J. 2010. Human enteroviruses in marine sediments near a sewage outfall on the Otago Coast. New Zealand Journal of Marine and Freshwater Research **19**(2): 187-192.

Li, L.H., Wine, R.N., and Chapin, R.E. 1996. 2-Methoxyacetic acid (MAA)-induced spermatocyte apoptosis in human and rat testes: An in vitro comparison. Journal of Andrology **17**(5): 538-549.

Li, Y.-L., Peacock, A.D., White, D.C., Geyer, R., and Zhang, C.L. 2007. Spatial patterns of bacterial signature biomarkers in marine sediments of the Gulf of Mexico. Chemical Geology **238**(3-4): 168-179.

Litchman, E., Pinto, P.D., Edwards, K.F., Klausmeier, C.A., Kremer, C.T., and Thomas, M.K. 2015. Global biogeochemical impacts of phytoplankton: A trait-based perspective. Journal of Ecology **103**(6): 1384-1396.

Liu, T., Xia, X., Liu, S., Mou, X., and Qiu, Y. 2013. Acceleration of Denitrification in Turbid Rivers Due to Denitrification Occurring on Suspended Sediment in Oxic Waters. Environmental Science & Technology **47**(9): 4053-4061.

Logan, J., Haas, H., Deegan, L., and Gaines, E. 2006. Turnover rates of nitrogen stable isotopes in the salt marsh mummichog, *Fundulus heteroclitus*, following a laboratory diet switch. Oecologia **147**(3): 391-395.

Lomstein, B. and Blackburn, T.H. (1992) Sediment nitrogen cycling in Aarhus Bay, Denmark. Miljoministeriet, Copenhagen.

Longhurst, A., Sathyendranath, S., Platt, T., and Caverhill, C. 1995. An estimate of global primary production in the ocean from satellite radiometer data. Journal of Plankton Research **17**(6): 1245-1271.

Lopes, G., Sousa, C., Bernardo, J., Andrade, P.B., Valentão, P., Ferreres, F., and Mouga, T. 2011. Sterol profiles in 18 macroalgae of the Portuguese Coast 1. Journal of Phycology **47**(5): 1210-1218.

Lorrain, A., Paulet, Y.-M., Chauvaud, L., Savoye, N., Donval, A., and Saout, C. 2002. Differential δ 13 C and δ 15 N signatures among scallop tissues: Implications for ecology and physiology. Journal of Experimental Marine Biology and Ecology **275**(1): 47-61.

Loutit, M. The fate of certain bacteria and heavy metals in sewage discharged through an ocean outfall. *In* 1985 Australasian Conference on Coastal and Ocean Engineering. 1985. Institution of Engineers, Australia. p. 204.

Machás, R., and Santos, R. 1999. Sources of organic matter in Ria Formosa revealed by stable isotope analysis. Acta Oecologica **20**(4): 463-469.

MacKenzie, A.L. 2014. The risk to New Zealand shellfish aquaculture from paralytic shellfish poisoning (PSP) toxins. New Zealand Journal of Marine and Freshwater Research **48**(3): 430-465.

Magni, P., Montani, S., and Tada, K. 2002. Semidiurnal dynamics of salinity, nutrients and suspended particulate matter in an estuary in the Seto Inland Sea, Japan, during a spring tide cycle. Journal of Oceanography **58**(2): 389-402.

Mannino, A., and Harvey, H.R. 2000. Terrigenous dissolved organic matter along an estuarine gradient and its flux to the coastal ocean. Organic Geochemistry **31**(12): 1611-1625.

Maranon, E., Cermeno, P., Huete-Ortega, M., Lopez-Sandoval, D.C., Mourino-Carballido, B., and Rodriguez-Ramos, T. 2014. Resource supply overrides temperature as a controlling factor of marine phytoplankton growth. Plos One **9**(6).

Martínez B, Pato LS, Rico JM. Nutrient uptake and growth responses of three intertidal macroalgae with perennial, opportunistic and summer-annual strategies. Aquatic Botany **96**(1):14-22.

Martins, C.C., Venkatesan, M.I., and Montone, R.C. 2002. Sterols and linear alkylbenzenes in marine sediments from Admiralty Bay, King George Island, South Shetland Islands. Antarctic Science **14**(3): 244-252.

McClelland, J.W., and Valiela, I. 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnology and Oceanography **43**(4): 577-585.

McCorquodale, D.S., Burney, C.M., and Row, M. 1996. Indicators for determining the sources and extent of fecal contamination in coastal waters: An annotated bibliography. Department of Natural Resource Protection.

McCutchan, J.H., Lewis, W.M., Kendall, C., and McGrath, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**(2): 378-390.

Mcdonald, P.S., Essington, T.E., Davis, J.P., Galloway, A.W., Stevick, B.C., Jensen, G.C., Vanblaricom, G.R., and Armstrong, D.A. 2015. Distribution, abundance, and habitat associations of a large bivalve (Panopea generosa) in a eutrophic fjord estuary. Journal of Shellfish Research **34**(1): 137-145.

McLusky, D.S., and Elliott, M. 2004. The estuarine ecosystem: ecology, threats and management. Oxford University Press on Demand.

Metzger, P., and Largeau, C. 2005. *Botryococcus braunii*: A rich source for hydrocarbons and related ether lipids. Applied Microbiology and Biotechnology **66**(5): 486-496.

Michelou, V.K., Caporaso, J.G., Knight, R., and Palumbi, S.R. 2013. The ecology of microbial communities associated with *Macrocystis pyrifera*. PLoS One **8**(6): e67480.

Michener, R.H., and Kaufman, L. 2008. Stable isotope ratios as tracers in marine food webs: An update. Stable Isotopes in Ecology and Environmental Science, Second Edition: 238-282.

Middag, R., de Baar, H.J.W., Klunder, M.B., and Laan, P. 2013. Fluxes of dissolved aluminum and manganese to the Weddell Sea and indications for manganese co-limitation. Limnology and Oceanography **58**(1): 287-300.

Miller, M.R., Pearce, L., and Bettjeman, B.I. 2014. Detailed distribution of lipids in Greenshell™ Mussel (Perna canaliculus). Nutrients **6**(4): 1454-1474.

Miller, R., Carreon, R., Young, J., and McKenna, M. 1982. Toxicity of methoxyacetic acid in rats. Toxicological Sciences **2**(4): 158-160.

Miller, T.W., Brodeur, R.D., and Rau, G.H. 2008. Carbon stable isotopes reveal relative contribution of shelf-slope production to the Northern California Current pelagic community. Limnology and Oceanography **53**(4): 1493-1503.

Mitchell, S. 1989. Primary production in a shallow eutrophic lake dominated alternately by phytoplankton and by submerged macrophytes. Aquatic Botany **33**(1-2): 101-110.

Monismith, S.G., Burau, J.R., and Stacey, M. 1996. Stratification dynamics and gravitational circulation in northern San Francisco Bay. San Francisco Bay: The Ecosystem: 123-153.

Montagna, P., Palmer, T.A., and Pollack, J.B. 2012. Hydrological changes and estuarine dynamics. Springer Science & Business Media.

Moore, C.M., Mills, M.M., Arrigo, K.R., Berman-Frank, I., Bopp, L., Boyd, P.W., Galbraith, E.D., Geider, R.J., Guieu, C., Jaccard, S.L., Jickells, T.D., La Roche, J., Lenton, T.M., Mahowald, N.M., Maranon, E., Marinov, I., Moore, J.K., Nakatsuka, T., Oschlies, A., Saito, M.A., Thingstad, T.F., Tsuda, A., and Ulloa, O. 2013. Processes and patterns of oceanic nutrient limitation. Nature Geoscience **6**(9): 701-710.

Moore, M. 2015. Coastal environment of Otago natural character and outstanding natural features and landscapes assessment. Clutha District Section Report 15 May 2015: 1-89.

Mora, P., Michel, X., and Narbonne, J.-F. 1999. Cholinesterase activity as potential biomarker in two bivalves. Environmental Toxicology and Pharmacology **7**(4): 253-260.

Morand, P., and Merceron, M. 2005. Macroalgal population and sustainability. Journal of Coastal Research: 1009-1020.

Morel, F.M.M. 2013. The oceanic cadmium cycle: Biological mistake or utilization? Proceedings of the National Academy of Sciences of the United States of America **110**(21): E1877-E1877.

Morel, F.M.M., Reinfelder, J.R., Roberts, S.B., Chamberlain, C.P., Lee, J.G., and Lee, D. 1994. Zinc and carbon co-limitation of marine phytoplankton. Nature **369**: 740-742.

Morillo, J., Usero, J., and Gracia, I. 2004. Heavy metal distribution in marine sediments from the southwest coast of Spain. Chemosphere **55**(3): 431-442.

MPI. 2016. New Zealand Total Diet Study. Ministry for Primary Industries, Food Auditing Report, Bureau Veritas–Food **1**(6): 1-106.

Mulholland, P.J., Hall Jr, R.O., Sobota, D.J., Dodds, W.K., Findlay, S.E., Grimm, N.B., Hamilton, S.K., McDowell, W.H., O'Brien, J.M., and Tank, J.L. 2009. Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: Denitrification. Limnology and Oceanography **54**(3): 666-680.

Murphy, K.J., Mooney, B.D., Mann, N.J., Nichols, P.D., and Sinclair, A.J. 2002. Lipid, FA, and sterol composition of New Zealand green lipped mussel (*Perna canaliculus*) and Tasmanian blue mussel (*Mytilus edulis*). Lipids **37**(6): 587-595.

Nasir, M., Saeidnia, S., Mashinchian-Moradi, A., and Gohari, A.R. 2011. Sterols from the red algae, *Gracilaria salicornia* and *Hypnea flagelliformis*, from Persian Gulf. Pharmacognosy Magazine **7**(26): 97.

Nelson, T. A., Nelson, A. V., & Tjoelker, M. (2003). Seasonal and spatial patterns of "green tides" (ulvoid algal blooms) and related water quality parameters in the coastal waters of Washington state, USA. Botonica Marina, **46**(3): 263–275.

Newsome, S.D., Martinez del Rio, C., Bearhop, S., and Phillips, D.L. 2007. A niche for isotopic ecology. Frontiers in Ecology and the Environment **5**(8): 429-436.

Ngugi, C.C., Oyoo-Okoth, E., Gichuki, J., Gatune, C., and Mwangi-Kinyanjui, J. 2017. Fingerprints of upstream catchment land use in suspended particulate organic matter (SPOM) at the river discharge sites in Lake Victoria (Kenya): Insights from element, stable isotope and lipid biomarker analysis. Aquatic sciences **79**(1): 73-87.

Nicholson, C.M., Lewis, G.D., and Loutit, M.W. 1989. Survey of human pathogenic bacteria and viruses in cockle beds at Otakou, Otago Harbour, New Zealand. New Zealand Journal of Marine and Freshwater Research **23**(4): 529-532.

Nielsen, O.I., Kristensen, E., and Holmer, M. 2003. Impact of *Arenicola marina* (Polychaeta) on sediment sulfur dynamics. Aquatic microbial ecology **33**(1): 95-105.

Nielsen, S.A. 1975. Cadmium in New Zealand dredge oysters: Geographic distribution. International Journal of Environmental Analytical Chemistry **4**(1): 1-7.

Nimz, E.L., and Morgan, S.L. 1993. On-line derivatization for complex fatty acid mixtures by capillary gas chromatography/mass spectrometry. Journal of Chromatographic Science **31**(4): 145-149.

Nixon, S., Oviatt, C., Frithsen, J., and Sullivan, B. 1986. Nutrients and the productivity of estuarine and coastal marine ecosystems. Journal of the Limnological Society of Southern Africa **12**(1-2): 43-71.

Nordsieck, R. 2006. The living world of molluscs. On-line). The Common Mussel (*Mytilus edulis*). Accessed December **12**: 2006.

North, J.C., Frew, R.D., and Hale, R.V. 2006. Can stable isotopes be used to monitor landfill leachate impact on surface waters? Journal of Geochemical Exploration **88**(1–3): 49-53.

New Zealand Water Environment Research Foundation (NZ WERF) (2002) New Zealand Municipal Wastewater Monitoring Guidelines. In: Ray D (ed.) Published by the New Zealand Water Environment Foundation, Wellington. ISBN 1-877134-40-6

Oceans, U. 2008. United Nations Atlas of the Oceans (read August 24, 2008).

Odum, E. 2002. Tidal marshes as outwelling/pulsing systems. Concepts and controversies in tidal marsh ecology: 3-7.

Odum, E.P. 1980. The status of three ecosystem-level hypotheses regarding salt marsh estuaries: tidal subsidy, outwelling, and detritus-based food chains. Estuarine Perspectives: 485-495.

Officer, C.B., and Kester, D.R. 1991. On estimating the non-advective tidal exchanges and advective gravitational circulation exchanges in an estuary. Estuarine, Coastal and Shelf Science **32**(1): 99-103.

Ogilvie, D. (1998). National study of the composition of sewage sludge. Drainage Managers Group, a subgroup of the New Zealand Water and Wastes Association.

Ouédraogo, O., Chételat, J., and Amyot, M. 2015. Bioaccumulation and trophic transfer of mercury and selenium in African sub-tropical fluvial reservoirs food webs (Burkina Faso). PloS one **10**(4): e0123048.

Page, H., and Lastra, M. 2003. Diet of intertidal bivalves in the Ria de Arosa (NW Spain): evidence from stable C and N isotope analysis. Marine Biology **143**(3): 519-532.

Pan, J.-F., and Wen-Xiong, W. 2004. Differential uptake of dissolved and particulate organic carbon by the marine mussel *Perna viridis*. Limnology and Oceanography **49**(6): 1980-1991.

Papenbrock, J. 2012. Highlights in seagrasses' phylogeny, physiology, and metabolism: What makes them special? ISRN Botany **2012**.

Park, H., Song, B., and Morel, F.M.M. 2007. Diversity of the cadmium-containing carbonic anhydrase in marine diatoms and natural waters. Environmental Microbiology **9**(2): 403-413.

Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J., and Inger, R. 2013. Bayesian stable isotope mixing models. Environmetrics **24**(6): 387-399.

Patil, V., Källqvist, T., Olsen, E., Vogt, G., and Gislerød, H.R. 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquaculture International **15**(1): 1-9.

Pautler, B.G., Austin, J., Otto, A., Stewart, K., Lamoureux, S.F., and Simpson, M.J. 2010. Biomarker assessment of organic matter sources and degradation in Canadian High Arctic littoral sediments. Biogeochemistry **100**(1-3): 75-87.

Pedersen, M.F., Borum, J., and Fotel, F.L. 2010. Phosphorus dynamics and limitation of fast- and slow-growing temperate seaweeds in Oslofjord, Norway. Marine Ecology Progress Series **399**: 103-115.

Pelch, K.E., Beeman, J.M., Niebruegge, B.A., Winkeler, S.R., and Nagel, S.C. 2011. Endocrine-disrupting chemicals (EDCs) in mammals. *In* Hormones and Reproduction of Vertebrates: Mammals. Elsevier. pp. 329-371.

Peng, X., Zhang, G., Mai, B., Min, Y., and Wang, Z. 2002. Spatial and temporal trend of sewage pollution indicated by coprostanol in Macao Estuary, southern China. Marine Pollution Bulletin **45**(1-12): 295-299.

Peralta-Videa, J.R., Lopez, M.L., Narayan, M., Saupe, G., and Gardea-Torresdey, J. 2009. The biochemistry of environmental heavy metal uptake by plants: implications for the food chain. The International Journal of Biochemistry & Cell Biology **41**(8-9): 1665-1677.

Perez, T., Longet, D., Schembri, T., Rebouillon, P., & Vacelet, J. (2005). Effects of 12 years' operation of a sewage treatment plant on trace metal occurrence within a Mediterranean commercial sponge (*Spongia officinalis*, Demospongiae). Marine Pollution Bulletin, **50**(3), 301-309.

Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. Acta Oecologica **20**(4): 479-487.

Peterson, M.S. 2003. A conceptual view of environment-habitat-production linkages in tidal river estuaries. Reviews in Fisheries Science **11**(4): 291-313.

Phillips, D.H. 1999. Polycyclic aromatic hydrocarbons in the diet. Mutation Research/Genetic Toxicology and Environmental Mutagenesis **443**(1): 139-147.

Phillips, D.L., and Gregg, J.W. 2003. Source partitioning using stable isotopes: Coping with too many sources. Oecologia **136**(2): 261-269.

Pilskaln, C.H., Churchill, J.H., and Mayer, L.M. 1998. Resuspension of sediment by bottom trawling in the Gulf of Maine and potential geochemical consequences. Conservation Biology **12**(6): 1223-1229.

Pinnegar, J., and Polunin, N. 1999. Differential fractionation of δ^{13}C and δ^{15}N among fish tissues: Implications for the study of trophic interactions. Functional Ecology **13**(2): 225-231.

Polis, G.A., Anderson, W.B., and Holt, R.D. 1997. Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**(1): 289-316.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology **83**(3): 703-718.

Post, D.M., Pace, M.L., and Hairston, N.G. 2000. Ecosystem size determines food-chain length in lakes. Nature **405**(6790): 1047-1049.

Povinec, P., De Oliveira, J., Braga, E., Comanducci, J.-F., Gastaud, J., Groening, M., Levy-Palomo, I., Morgenstern, U., and Top, Z. 2008a. Isotopic, trace element and nutrient characterization of coastal waters from Ubatuba inner shelf area, south-eastern Brazil. Estuarine, Coastal and Shelf Science **76**(3): 522-542.

Povinec, P.P., Bokuniewicz, H., Burnett, W.C., Cable, J., Charette, M., Comanducci, J.-F., Kontar, E., Moore, W.S., Oberdorfer, J., and De Oliveira, J. 2008b. Isotope tracing of submarine groundwater discharge offshore Ubatuba, Brazil: Results of the IAEA–UNESCO SGD project. Journal of Environmental Radioactivity **99**(10): 1596-1610.

Prasad, M. 2003. Phytoremediation of metal-polluted ecosystems: Hype for commercialization. Russian Journal of Plant Physiology **50**(5): 686-701.

Preacher, K.J., and Coffman, D.L. 2006. Computing power and minimum sample size for RMSEA. Computer software]. Available from http://quantpsy. org.

Perez, T., Longet, D., Schembri, T., Rebouillon, P., & Vacelet, J. (2005). Effects of 12 years' operation of a sewage treatment plant on trace metal occurrence within a Mediterranean commercial sponge (*Spongia officinalis*, Demospongiae). Marine Pollution Bulletin **50**(3), 301-309.

Priyandoko, D., Ishii, T., Kaul, S.C., and Wadhwa, R. 2011. Ashwagandha leaf derived withanone protects normal human cells against the toxicity of methoxyacetic acid, a major industrial metabolite. Plos One **6**(5): e19552.

Pruell, R.J., Taplin, B.K., Lake, J.L., and Jayaraman, S. 2006. Nitrogen isotope ratios in estuarine biota collected along a nutrient gradient in Narragansett Bay, Rhode Island, USA. Marine Pollution Bulletin **52**(6): 612-620.

Purdom, C., Hardiman, P., Bye, V., Eno, N., Tyler, C., and Sumpter, J. 1994. Estrogenic effects of effluents from sewage treatment works. Chemistry and Ecology **8**(4): 275-285.

Rabalais, N.N., Turner, R.E., Díaz, R.J., and Justić, D. 2009. Global change and eutrophication of coastal waters. ICES Journal of Marine Science: Journal du Conseil **66**(7): 1528-1537.

Rabalais, N.N., Turner, R., Gupta, B.S., Boesch, D., Chapman, P., and Murrell, M. 2007. Hypoxia in the northern Gulf of Mexico: Does the science support the plan to reduce, mitigate, and control hypoxia? Estuaries and Coasts **30**(5): 753-772.

Rainbow, P.S. 1985. The biology of heavy metals in the sea. International Journal of Environmental Studies **25**(3): 195-211.

Rainbow, P.S. 1993. The significance of trace metal concentrations in marine invertebrates. Ecotoxicology of Metals in Invertebrates: 3-23.

Ralston, D.K., and Stacey, M.T. 2005. Stratification and turbulence in subtidal channels through intertidal mudflats. Journal of Geophysical Research: Oceans **110**(C8).

Raven, J.A., and Falkowski, P.G. 1999. Oceanic sinks for atmospheric CO2. Plant Cell and Environment **22**(6): 741-755.

Rayment, W., Dawson, S., and Slooten, E. 2010. Seasonal changes in distribution of Hector's dolphin at Banks Peninsula, New Zealand: implications for protected area design. Aquatic Conservation: Marine and Freshwater Ecosystems **20**(1): 106-116.

RCL. 2000. Otago Regional Council Resource Consent 97530 Condition 8: Rocky Shore Ecological Monitoring-January/February **2000**. Ryder Consulting Ltd. 97530, 195 Rattray St, 9016, Dunedin, New Zealand.

Rieuwerts, J. 2017. The elements of environmental pollution. Routledge.

Ríos, A.F., Fraga, F., Pérez, F.F., and Figueiras, F.G. 1998. Chemical composition of phytoplancton and Particulate Organic Matter in the Ría de Vigo (NW Spain). Scientia Marina **62**(3): 257-271.

Risk, M.J., Lapointe, B.E., Sherwood, O.A., and Bedford, B.J. 2009. The use of δ^{15}N in assessing sewage stress on coral reefs. Marine Pollution Bulletin **58**(6): 793-802.

Rocha, C., Ibanhez, J., and Leote, C. 2009. Benthic nitrate biogeochemistry affected by tidal modulation of Submarine Groundwater Discharge (SGD) through a sandy beach face, Ria Formosa, Southwestern Iberia. Marine Chemistry **115**(1-2): 43-58.

Rochman, C.M. 2015. The complex mixture, fate and toxicity of chemicals associated with plastic debris in the marine environment. *In* Marine anthropogenic litter. Springer. pp. 117-140.

Rogers, K. 2003. Stable carbon and nitrogen isotope signatures indicate recovery of marine biota from sewage pollution at Moa Point, New Zealand. Marine Pollution Bulletin **46**(7): 821-827.

Rose, S., and Shea, J.A. 2007. Environmental geochemistry of trace metal pollution in urban watersheds. Developments in Environmental Science **5**: 99-131.

Rosner, B. 1975. On the Detection of Many Outliers. Technometrics **17**(2): 221-227.

Rounick, J., and Hicks, B.J. 1985. The stable carbon isotope ratios of fish and their invertebrate prey in four New Zealand rivers. Freshwater Biology **15**(2): 207-214.

Roussiez, V., Ludwig, W., Monaco, A., Probst, J.-L., Bouloubassi, I., Buscail, R., and Saragoni, G. 2006. Sources and sinks of sediment-bound contaminants in the Gulf of Lions (NW Mediterranean Sea): A multi-tracer approach. Continental Shelf Research **26**(16): 1843-1857.

Rüdel, H., Lepper, P., Steinhanses, J., and Schröter-Kermani, C. 2003. Retrospective monitoring of organotin compounds in marine biota from 1985 to 1999: Results from the German Environmental Specimen Bank. Environmental Science & Technology **37**(9): 1731-1738.

Rufino, M.M., Gaspar, M.B., Pereira, A.M., Maynou, F., and Monteiro, C.C. 2010. Ecology of megabenthic bivalve communities from sandy beaches on the south coast of Portugal.

Rullkötter, J. 2006. Organic matter: the driving force for early diagenesis. *In* Marine geochemistry. Springer. pp. 125-168.

Ryder. 2000. Otago Regional Council Resource Consent 97530 Condition 8: Rocky Shore Ecological Monitoring-January/February **2000**. Report 97530 prepared for Dunedin City Council by Ryder Consulting Ltd.

Sakamaki, T., Nishimura, O., and Sudo, R. 2006. Tidal time-scale variation in nutrient flux across the sediment–water interface of an estuarine tidal flat. Estuarine, Coastal and Shelf Science **67**(4): 653-663.

Salas, F., Marcos, C., Neto, J., Patrício, J., Pérez-Ruzafa, A., and Marques, J. 2006. User-friendly guide for using benthic ecological indicators in coastal and marine quality assessment. Ocean & Coastal Management **49**(5): 308-331.

Sánchez-Carrillo, S., and Álvarez-Cobelas, M. 2017. Stable isotopes as tracers in aquatic ecosystems. Environmental Reviews **26**(1): 69-81.

Sánchez-Machado, D., López-Hernández, J., Paseiro-Losada, P., and López-Cervantes, J. 2004. An HPLC method for the quantification of sterols in edible seaweeds. Biomedical Chromatography **18**(3): 183-190.

Santos-Echeandía, J., Vale, C., Caetano, M., Pereira, P., and Prego, R. 2010. Effect of tidal flooding on metal distribution in pore waters of marsh sediments and its transport to water column (Tagus estuary, Portugal). Marine Environmental Research **70**(5): 358-367.

Savage, C. 2005. Tracing the influence of sewage nitrogen in a coastal ecosystem using stable nitrogen isotopes. AMBIO: A Journal of the Human Environment **34**(2): 145-150.

Savage, C., and Elmgren, R. 2004. Macroalgal (*Fucus vesiculosus*) δ^{15}N values trace decrease in sewage influence. Ecological Applications **14**(2): 517-526.

Savage, C., Thrush, S.F., Lohrer, A.M., and Hewitt, J.E. 2012. Ecosystem services transcend boundaries: Estuaries provide resource subsidies and influence functional diversity in coastal benthic communities. PloS One **7**(8): 42708.

Scavia, D., and Bricker, S.B. 2006. Coastal eutrophication assessment in the United States. *In* Nitrogen Cycling in the Americas: Natural and Anthropogenic Influences and Controls. Springer. pp. 187-208.

Scheltinga, D., Counihan, R., Moss, A., Cox, M., and Bennet, J. 2004. User's Guide to Estuarine, Coastal and Marine Indicators for Regional Natural Resource Management. Cooperative Research Centre for Coastal Zone, Estuary and Waterway Management: 4-183.

Schoeller, D.A. 1999. Isotope fractionation: Why aren't we what we eat? Journal of Archaeological Science **26**(6): 667-673.

Schrader, G., Zwijnenburg, A., and Wessling, M. 2005. The effect of colloid stability of wastewater treatment plants effluent on nanofiltration performance. Water Science and Technology **52**(10-11): 345-357.

Schubert, J. 1954. Interactions of metals with small molecules in relation to metal-protein complexes. Chemical Specificity in Biological Interactions, FRN Gurd, Ed., Academic Press, New York.

Schutter, M.E., and Dick, R.P. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Science Society of America Journal **64**(5): 1659-1668.

Schwarzenbach, R.P., Egli, T., Hofstetter, T.B., Von Gunten, U., and Wehrli, B. 2010. Global water pollution and human health. Annual Review of Environment and Resources **35**: 109-136.

Schwarzenbach, R.P., Escher, B.I., Fenner, K., Hofstetter, T.B., Johnson, C.A., Von Gunten, U., and Wehrli, B. 2006. The challenge of micropollutants in aquatic systems. Science **313**(5790): 1072-1077.

Sedell, J.R., Richey, J.E., and Swanson, F.J. The river continuum concept: A basis for the expected ecosystem behavior of very large rivers. *In* Proceedings of the international large river symposium. 1989. Canadian Special Publication of Fisheries and Aquatic Sciences. pp. 49-55.

Seitzinger, S.P., Sanders, R., and Styles, R. 2002. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. Limnology and Oceanography **47**(2): 353-366.

Selvarajah, S. Regulations of Effluent Discharges in the Otago Region. *In* 2008. New Zealand Land Treatment Collective Conference, Queenstown, New Zealand.

Sfriso, A., Pavoni, B., Marcomini, A., and Orio, A. 1992. Macroalgae, nutrient cycles, and pollutants in the Lagoon of Venice. Estuaries and Coasts **15**(4): 517-528.

Shevchenko, N., Burtseva, Y.V., Zvyagintseva, T., Makar, T., Sergeeva, O., Zakharenko, A., Isakov, V., Linh, N.T., Hoa, N.X., and Ly, B.M. 2009. Polysaccharides and sterols from green algae *Caulerpa lentillifera* and *C. sertularioides*. Chemistry of Natural Compounds **45**(1): 1-5.

Shobier, A.H., Ghani, S.A.A., and Barakat, K.M. 2016. GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae. The Egyptian Journal of Aquatic Research **42**(3): 289-299.

Shriver, A.C., Carmichael, R., and Valiela, I. 2002. Growth, condition, reproductive potential, and mortality of bay scallops, Argopecten irradians, in response to eutrophic-driven changes in food resources. Journal of Experimental Marine Biology and Ecology **279**(1-2): 21-40.

Shumway, S.E. 1990. A review of the effects of algal blooms on shellfish and aquaculture. Journal of the World Aquaculture Society **21**(2): 65-104.

Siegenthaler, P.-A., and Trémolières, A. 1998. Role of acyl lipids in the function of photosynthetic membranes in higher plants. *In* Lipids in photosynthesis: Structure, Function and Genetics. Springer. pp. 145-173.

Siegenthaler, P.-A., and Murata, N. 2006. Lipids in photosynthesis: structure, function and genetics. Springer Science & Business Media.

Sigman, D.M., Hain, M.P., and Haug, G.H. 2010. The polar ocean and glacial cycles in atmospheric CO2 concentration. Nature **466**(7302): 47-55.

Simenstad, C.A. 1983. Ecology of estuarine channels of the Pacific Northwest coast: A community profile (No. FWS/OBS-83/05). Washington Univ., Seattle (USA). Fisheries Research Inst.

Simoneit, B.R. 2005. A review of current applications of mass spectrometry for biomarker/molecular tracer elucidations. Mass Spectrometry Reviews **24**(5): 719-765.

Sinsabaugh, R., and Foreman, C. 2003. Integrating dissolved organic matter metabolism and microbial diversity: an overview of conceptual models. *In* Aquatic Ecosystems. Elsevier. pp. 425-454.

SITO. 2006. Seafood Industry Training Organisation- Learning Resource for Unit Standard 16340 v3 "Biology of new zealand green mussels".

Skei, J., Larsson, P., Rosenberg, R., Jonsson, P., Olsson, M., and Broman, D. 2000. Eutrophication and contaminants in aquatic ecosystems. AMBIO: A Journal of the Human Environment **29**(4): 184-194.

Small, C., and Cohen, J. 2004. Continental Physiography, Climate, and the Global Distribution of Human Population 1. Current Anthropology **45**(2): 269-277.

Smith, G., and Parrish, J. 2002. Estuaries as nurseries for the jacks *Caranx ignobilis* and *Caranx melampygus* (Carangidae) in Hawaii. Estuarine, Coastal and Shelf Science **55**(3): 347-359.

Solecki, O., Jeanneau, L., Jardé, E., Gourmelon, M., Marin, C., and Pourcher, A.-M. 2011. Persistence of microbial and chemical pig manure markers as compared to faecal indicator bacteria survival in freshwater and seawater microcosms. Water Research **45**(15): 4623-4633.

Soto-Jiménez, M., Páez-Osuna, F., & Morales-Hernández, F. (2001). Selected trace metals in oysters (Crassostrea iridescens) and sediments from the discharge zone of the submarine sewage outfall in Mazatlán Bay (southeast Gulf of California): chemical fractions and bioaccumulation factors. Environmental Pollution, **114**(3), 357-370.

Sposito, G. 1981. Trace metals in contaminated waters. Environmental Science & Technology **15**(4): 396-403.

Stahl, D.A., Flowers, J.J., Hullar, M., and Davidson, S. 2013. Structure and function of microbial communities. *In* The Prokaryotes. Springer. pp. 3-30.

Standley, L.J., Kaplan, L.A., and Smith, D. 2000. Molecular tracers of organic matter sources to surface water resources. Environmental science & technology **34**(15): 3124-3130.

Stanislav, P., and Cascio, E. 1999. Environmental impact of the offshore oil and gas industry. Offshore-Environment, East Northport, NY.

Staples, C.A., Peterson, D.R., Parkerton, T.F., and Adams, W.J. 1997. The environmental fate of phthalate esters: a literature review. Chemosphere **35**(4): 667-749.

Stedmon, C.A., Thomas, D.N., Granskog, M., Kaartokallio, H., Papadimitriou, S., and Kuosa, H. 2007. Characteristics of dissolved organic matter in Baltic coastal sea ice: allochthonous or autochthonous origins? Environmental Science & Technology **41**(21): 7273-7279.

Steffy, L.Y., and Kilham, S.S. 2004. Elevated δ^{15}N in stream biota in areas with septic tank systems in an urban watershed. Ecological Applications **14**(3): 637-641.

Stołyhwo, A., and Sikorski, Z.E. 2005. Polycyclic aromatic hydrocarbons in smoked fish–A critical review. Food Chemistry **91**(2): 303-311.

Strayer, D. 1983. The effects of surface geology and stream size on freshwater mussel (Bivalvia, Unionidae) distribution in southeastern Michigan, USA. Freshwater Biology **13**(3): 253-264.

Strickland, J., and Parsons, T. 1968. A Practical Handbook of Seawater Analysis. Fisheries Research Board of Canada, Bulletin **167**: 49-56.

Strzepek, R.F., Maldonado, M.T., Hunter, K.A., Frew, R.D., and Boyd, P.W. 2011. Adaptive strategies by Southern Ocean phytoplankton to lessen iron limitation: Uptake of organically complexed iron and reduced cellular iron requirements. Limnology and Oceanography **56**(6): 1983-2002.

Subhashini, P., Dilipan, E., Thangaradjou, T., and Papenbrock, J. 2013. Bioactive natural products from marine angiosperms: abundance and functions. Natural Products and Bioprospecting **3**(4): 129-136.

Sunda, W.G. 2012. Feedback interactions between trace metal nutrients and phytoplankton in the ocean. Frontiers in Microbiology **3**: 204

Sutton, P.J. 2003. The Southland Current: A subantarctic current. New Zealand Journal of Marine and Freshwater Research **37**(3): 645-652.

Syvitski, J.P., Vörösmarty, C.J., Kettner, A.J., and Green, P. 2005. Impact of humans on the flux of terrestrial sediment to the global coastal ocean. Science **308**(5720): 376-380.

Taillefert, M., Neuhuber, S., and Bristow, G. 2007. The effect of tidal forcing on biogeochemical processes in intertidal salt marsh sediments. Geochemical Transactions **8**(1): 6.

Talley, D.M., Huxel, G.R., and Holyoak, M. 2006. Connectivity at the land-water interface. Conservation Biology Series-Cambridge- **14**: 97.

Tallis, H., Levin, P.S., Ruckelshaus, M., Lester, S.E., McLeod, K.L., Fluharty, D.L., and Halpern, B.S. 2010. The many faces of ecosystem-based management: making the process work today in real places. Marine Policy **34**(2): 340-348.

Taniguchi, M., Stieglitz, T., and Ishitobi, T. 2008. Temporal variability of water quality of submarine groundwater discharge in Ubatuba, Brazil. Estuarine, Coastal and Shelf Science **76**(3): 484-492.

Tanna, B., Choudhary, B., and Mishra, A. 2018. Metabolite profiling, antioxidant, scavenging and anti-proliferative activities of selected tropical green seaweeds reveal the nutraceutical potential of Caulerpa spp. Algal Research **36**: 96-105.

Teklehaimanot, G.Z., Coetzee, M.A., and Momba, M.N. 2014. Faecal pollution loads in the wastewater effluents and receiving water bodies: a potential threat to the health of Sedibeng and Soshanguve communities, South Africa. Environmental Science and Pollution Research **21**(16): 9589-9603.

Thompson, D., Phillips, R., Stewart, F., and Waldron, S. 2000. Low δ^{13}C signatures in pelagic seabirds: lipid ingestion as a potential source of ^{13}C-depleted carbon in the Procellariiformes. Marine Ecology Progress Series **208**: 265-271.

Thornton, S., and McManus, J. 1994. Application of organic carbon and nitrogen stable isotope and C/N ratios as source indicators of organic matter provenance in estuarine systems: Evidence from the Tay Estuary, Scotland. Estuarine, Coastal and Shelf Science **38**(3): 219-233.

Thorp, J.H., and Delong, M.D. 1994. The riverine productivity model: An heuristic view of carbon sources and organic processing in large river ecosystems. Oikos: 305-308.

Thrush, S.F., Townsend, M., Hewitt, J.E., Davies, K., Lohrer, A.M., Lundquist, C., and Cartner, K. 2013. The many uses and values of estuarine ecosystems. Ecosystem services in New Zealand–conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand.

Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., and Slade, N.A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet. Oecologia **57**(1-2): 32-37.

Tolosa, I., De Mora, S.J., Fowler, S.W., Villeneuve, J.-P., Bartocci, J., and Cattini, C. 2005. Aliphatic and aromatic hydrocarbons in marine biota and coastal sediments from the Gulf and the Gulf of Oman. Marine Pollution Bulletin **50**(12): 1619-1633.

Toran, L. 1982. Isotopes in ground-water investigations. Groundwater **20**(6): 740-745.

Tran, D., Boudou, A., and Massabuau, J.C. 2002. Relationship between feeding-induced ventilatory activity and bioaccumulation of dissolved and algal-bound cadmium in the Asiatic clam *Corbicula fluminea*. Environmental Toxicology and Chemistry **21**(2): 327-333.

Trusewich, B., Sim, J., Busby, P., and Hughes, C. 1996. Management of marine biotoxins in New Zealand. Harmful and Toxic Algal Blooms.: 27-30.

Tucker, J., Sheats, N., Giblin, A., Hopkinson, C., and Montoya, J. 1999. Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. Marine Environmental Research **48**(4-5): 353-375.

Turner, R.E., Rabalais, N.N., and Justic, D. 2008. Gulf of Mexico hypoxia: Alternate states and a legacy. Environmental Science & Technology **42**(7): 2323-2327.

Tyler, A.C., McGlathery, K.J., and Macko, S.A. 2005. Uptake of urea and amino acids by the macroalgae *Ulva lactuca* (Chlorophyta) and *Gracilaria vermiculophylla* (Rhodophyta). Marine Ecology Progress Series **294**: 161-172.

Uerpmann-Wittzack, R. 2017. European Directorate for the Quality of Medicines and Healthcare (EDQM).

Uncles, R., and Stephens, J. 2000. Observations of currents, salinity, turbidity and intertidal mudflat characteristics and properties in the Tavy Estuary, UK. Continental Shelf Research **20**(12-13): 1531-1549.

USEPA. 2000. Risk-based concentration table. United States Environmental Protection Agency Washington, DC, USA.

USEPA. 2013. Regional screening level (RSL) summary table (TR= 1E− 6, HQ= 1).

USEPA. 2017. Regional Screening Level (RSL) Summary Table (TR= 1E–6, HQ= 1), June 2017. US Environmental Protection Agency Washington, DC.

Van Dover, C.L., Grassle, J., Fry, B., Garritt, R.H., and Starczak, V.R. 1992. Stable isotope evidence for entry of sewage-derived organic material into a deep-sea food web. Nature **360**(6400): 153.

van Leussen, W., Radach, G., van Raaphorst, W., Colijn, F., and Laane, R. 1996. The North-West European Shelf Programme (NOWESP): integrated analysis of shelf processes based on existing data sets and models. ICES Journal of Marine Science: Journal du Conseil **53**(6): 926-932.

Vannote, R.L., Minshall, G.W., Cummins, K.W., Sedell, J.R., and Cushing, C.E. 1980. The river continuum concept. Canadian journal of Fisheries and Aquatic Sciences **37**(1): 130-137.

Vaughn, C.C., and Hakenkamp, C.C. 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biology **46**(11): 1431-1446.

Vázquez-Luis, M., Morató, M., Campillo, J.A., Guitart, C., and Deudero, S. 2016. High metal contents in the fan mussel *Pinna nobilis* in the Balearic Archipelago (western Mediterranean Sea) and a review of concentrations in marine bivalves (Pinnidae). Scientia Marina **80**(1): 111-122.

Velegrakis, A., Gao, S., Lafite, R., Dupont, J., Huault, M., Nash, L., and Collins, M. 1997. Resuspension and advection processes affecting suspended particulate matter concentrations in the central English Channel. Journal of Sea Research **38**(1-2): 17-34.

Venkatesan, M., and Santiago, C. 1989. Sterols in ocean sediments: novel tracers to examine habitats of cetaceans, pinnipeds, penguins and humans. Marine Biology **102**(4): 431-437.

Verney, R., Lafite, R., and Brun-Cottan, J.-C. 2009. Flocculation potential of estuarine particles: The importance of environmental factors and of the spatial and seasonal variability of suspended particulate matter. Estuaries and Coasts **32**(4): 678-693.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., and Tilman, D.G. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecological Applications **7**(3): 737-750.

Voigt, C.C., Matt, F., Michener, R., and Kunz, T.H. 2003. Low turnover rates of carbon isotopes in tissues of two nectar-feeding bat species. Journal of Experimental Biology **206**(8): 1419-1427.

Vongsvivut, J., Heraud, P., Gupta, A., Thyagarajan, T., Puri, M., McNaughton, D., and Barrow, C.J. 2015. Synchrotron-FTIR Microspectroscopy enables the distinction of

lipid accumulation in thraustochytrid strains through analysis of individual live cells. Protist **166**(1): 106-121.

Vuorio, K., Meili, M., and Sarvala, J. 2006. Taxon-specific variation in the stable isotopic signatures (δ^{13}C and δ^{15}N) of lake phytoplankton. Freshwater Biology **51**(5): 807-822.

Waalkes, M.P. 2000. Cadmium carcinogenesis in review. Journal of Inorganic Biochemistry **79**(1-4): 241-244.

Waldron, S., Tatner, P., Jack, I., and Arnott, C. 2001. The Impact of Sewage Discharge in a Marine Embayment: A Stable Isotope Reconnaissance. Estuarine, Coastal and Shelf Science **52**(1): 111-115.

Walker, R.W., Wun, C.K., Litsky, W., and Dutka, B. 1982. Coprostanol as an indicator of fecal pollution. Critical Reviews in Environmental Science and Technology **12**(2): 91-112.

Wang, W.-X., and Fisher, N.S. 1999. Delineating metal accumulation pathways for marine invertebrates. Science of The Total Environment **237-238**: 459-472.

Wang, W.-X., and Lu, G. 2017. Heavy Metals in Bivalve Mollusks. *In* Chemical Contaminants and Residues in Food (Second Edition). Elsevier. pp. 553-594.

Wang, W.-X., Fisher, N.S., and Luoma, S.N. 1995. Assimilation of trace elements ingested by the mussel Mytilus edulis: effects of algal food abundance. Marine Ecology Progress Series **129**: 165-176.

Warton, D.I., Wright, I.J., Falster, D.S., and Westoby, M. 2006. Bivariate line-fitting methods for allometry. Biological Reviews **81**(2): 259-291.

Wear, S.L., and Thurber, R.V. 2015. Sewage pollution: mitigation is key for coral reef stewardship. Annals of the New York Academy of Sciences **1355**(1): 15-30.

Webb, K.L. 1981. Conceptual models and processes of nutrient cycling in estuaries. *In* Estuaries and nutrients. Springer. pp. 25-46.

Weiss, E.T., Carmichael, R.H., and Valiela, I. 2002. The effect of nitrogen loading on the growth rates of quahogs (*Mercenaria mercenaria*) and soft-shell clams (*Mya arenaria*) through changes in food supply. Aquaculture **211**(1-4): 275-289.

Wells, A., and Young, J. 1992. Long-term variability and predictability of Hudson River physical and chemical characteristics. Estuarine Research in the 1980s: 29-58.

Wepener, V. 2008. Application of active biomonitoring within an integrated water resources management framework in South Africa. South African Journal of Science **104**(9-10): 367-373.

Werner, S. 1981. Aquatic chemistry: An introduction emphasizing chemical equilibria in natural waters.

Wheeler, D., Sparling, G., and Roberts, A. 2004. Trends in some soil test data over a 14-year period in New Zealand. New Zealand Journal of Agricultural Research **47**(2): 155-166.

Williams, P.l.B. 2000. Heterotrophic bacteria and the dynamics of dissolved organic material. Microbial Ecology of the Oceans. Wiley-Liss: 153-200.

Wolanski, E., and Elliott, M. 2015. Estuarine Ecohydrology: An Introduction. Elsevier.

Wold, S., Esbensen, K., and Geladi, P. 1987. Principal component analysis. Chemometrics and Intelligent Laboratory Systems **2**(1-3): 37-52.

Wolf, B.O., and Del Rio, C.M. 2000. Use of saguaro fruit by white-winged doves: isotopic evidence of a tight ecological association. Oecologia **124**(4): 536-543.

Wuana, R.A., and Okieimen, F.E. 2011. Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. Ecology **2011**.

Wunderlin, P., Lehmann, M.F., Siegrist, H., Tuzson, B.l., Joss, A., Emmenegger, L., and Mohn, J. 2013. Isotope signatures of N_2O in a mixed microbial population system: Constraints on N_2O producing pathways in wastewater treatment. Environmental Science & Technology **47**(3): 1339-1348.

Xie, R.C., Galer, S.J.G., Abouchami, W., Rijkenberg, M.J.A., De Jong, J., de Baar, H.J.W., and Andreae, M.O. 2015. The cadmium–phosphate relationship in the western South Atlantic — The importance of mode and intermediate waters on the global systematics. Marine Chemistry **177, Part 1**: 110-123.

Xu, Q., and Yang, H. 2007. Food sources of three bivalves living in two habitats of Jiaozhou Bay (Qingdao, China): indicated by lipid biomarkers and stable isotope analysis. Journal of Shellfish Research **26**(2): 561-567.

Xu, Y., Shi, D.L., Aristilde, L., and Morel, F.M.M. 2012. The effect of pH on the uptake of zinc and cadmium in marine phytoplankton: Possible role of weak complexes. Limnology and Oceanography **57**(1): 293-304.

Yang, X.-e., Wu, X., Hao, H.-l., and He, Z.-l. 2008. Mechanisms and assessment of water eutrophication. Journal of Zhejiang University Science B **9**(3): 197-209.

Yasuda, S. 1978. Steroids in the feces of a marine bivalve. Bulletin of the Japanese Society of Scientific Fisheries **44**(5): 525-528.

Yeats, P., Gagné, F., and Hellou, J. 2008. Body burden of contaminants and biological effects in mussels: An integrated approach. Environment international **34**(2): 254-264.

Yokoyama, H., and Ishihi, Y. 2006. Variation in δ^{13}C and δ^{15}N among different tissues of three estuarine bivalves: Implications for dietary reconstructions. Plankton and Benthos Research **1**(4): 178-182.

Zar, J.H. 2013. Biostatistical analysis: Pearson new international edition. Pearson Higher Ed.

Zarnetske, J.P., Haggerty, R., Wondzell, S.M., and Baker, M.A. 2011. Dynamics of nitrate production and removal as a function of residence time in the hyporheic zone. Journal of Geophysical Research: Biogeosciences **116**(G1).

Zhang, L., Ye, X., Feng, H., Jing, Y., Ouyang, T., Yu, X., ... & Chen, W. (2007). Heavy metal contamination in western Xiamen Bay sediments and its vicinity, China. Marine Pollution Bulletin, **54**(7), 974-982.

Zhang, M., Li, C., Benjamin, M.M., and Chang, Y. 2003. Fouling and natural organic matter removal in adsorbent/membrane systems for drinking water treatment. Environmental Science & Technology **37**(8): 1663-1669.

Zhou, Q., Zhang, J., Fu, J., Shi, J., and Jiang, G. 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Analytica Chimica Acta **606**(2): 135-150.

Zia, H., Harris, N.R., Merrett, G.V., Rivers, M., and Coles, N. 2013. The impact of agricultural activities on water quality: A case for collaborative catchment-scale management using integrated wireless sensor networks. Computers and Electronics in Agriculture **96**: 126-138.

Zonneveld, K.A.F., Versteegh, G.J.M., Kasten, S., Eglinton, T.I., Emeis, K.C., Huguet, C., Koch, B.P., de Lange, G.J., De Leeuw, J.W., and Middelburg, J.J. 2010. Selective preservation of organic matter in marine environments; processes and impact on the sedimentary record. Biogeosciences **7**(2): 483-511.