Landscape-level variation in stable isotopes of carbon and nitrogen across aquatic ecosystems in New Zealand

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Abstract

The stable isotope ratios of carbon (δ^{13}C) and nitrogen (δ^{15}N) are frequently used in food web studies as natural biological indicators for tracking ecological processes, interactions, and energy flows from the basal plant and detrital energy sources to the primary and secondary consumers. The spatial heterogeneity in the ecological, biogeochemical, hydrological, and geomorphological processes of aquatic ecosystems can influence the δ^{13}C and δ^{15}N isotopic baseline of food webs, which directly affect the isotopic composition of primary and secondary consumers. A better understanding of the significant drivers of δ^{13}C and δ^{15}N of aquatic organisms at the landscape scale would facilitate and improve the accuracy of the use of these isotopic ratios as natural tracers.

The main objective of this study was to identify the leading mechanisms influencing the spatial variability of δ^{13}C and δ^{15}N across New Zealand aquatic ecosystems at the landscape scale. The aims of this study were; (1) to review and collate the findings from published stable isotopic studies that investigated the effect of environmental factors on δ^{13}C and δ^{15}N of aquatic organisms, (2) to identify the sources and scales of δ^{13}C and δ^{15}N variability for top-predatory fish across New Zealand lake environments and, (3) to identify the sources and scales of δ^{15}N variability for top-predatory fish and invertebrates (predatory and non-predatory) across New Zealand stream and river environments. Lake and stream δ^{13}C and δ^{15}N data of aquatic organisms were collated from a range of published and unpublished scientific literature across New Zealand. Data from a total of 88 lakes sourced from 11 publications and 475 stream sites from 15 publications were assessed to investigate the direct and indirect effects of environmental factors on the spatial variability of stable isotopic ratios across lakes and streams. To do so, a combination of correlation analyses, principal component analysis (PCA), and piecewise structural equation modelling (pSEM) were carried out.

The variability of top-predatory fish δ^{13}C values across New Zealand lakes was best explained by the morphology of the lake. Fish sampled from lakes with large surface areas and volumes had higher δ^{13}C signatures from lakes of smaller morphological size. I hypothesised that larger lakes allow for more influence of δ^{13}C-enriched atmospheric CO_2 due to greater equilibration through gas exchange processes and turbulence regimes, contributing to the observed increase in enrichment of fish δ^{13}C with lake surface area. The top-predatory fish δ^{15}N most strongly reflected the watershed land use and showed no apparent relationship with lake size. The variability of non-predatory and predatory invertebrates, and top-predatory fish δ^{15}N across New Zealand streams was best explained by the local land use in the upstream catchment and the nutrient concentrations, i.e., the total nitrogen and total phosphorus concentrations.

Our findings suggest that δ^{13}C and δ^{15}N respond to different environmental drivers. Carbon isotopic ratios tend to reflect the natural ecosystem properties, whereas nitrogen isotopic ratios respond more readily to the watershed land use. The findings in this study were consistent with previous stable isotopic research conducted in North American and European aquatic ecosystems, which have examined the effects of environmental factors on C and N isotopic ratios among aquatic organisms. An enhanced understanding of the natural ecosystem processes and anthropogenic influences, contributing to the spatial distribution of δ^{13}C and δ^{15}N of aquatic organisms, can improve the study design and interpretation of stable isotopes as a tracer in food web and ecosystem ecology, by taking into account the natural and human-induced spatial and trophic subsidies.

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Chapter 1

Introduction

1.1 Introduction to stable isotopes

Most elements exist in several different mass forms, known as isotopes, due to differences in the number of neutrons within the nucleus. Isotopes are atoms of the same element thus exhibit the same chemical properties but differ in atomic masses, so display subtle differences in their nuclear properties, principally in their radius of bending in a magnetic field as charged ions. Isotopes can be either stable or unstable depending on their nuclear properties. For example, both carbon-12 (^{12}C), and carbon-13 (^{13}C), are stable isotopes of carbon. However, when there are too many or too few neutrons within the nucleus, such as in the case of carbon-14 (^{14}C), the isotope becomes unstable, i.e., radioactive, and will spontaneously undergo beta decay (β-decay), in which a beta particle (a fast energetic electron) is emitted from the atomic nucleus to form a stable isotope of a different element, ^{14}N (Rasgon 2008).

1.1.1 Terminology and measurement of C and N isotopes

Both lighter stable C and N isotopic forms have significantly higher average terrestrial abundances compared to the heavier stable isotopes. The natural abundance of the stable isotopes of carbon,^{12}C and ^{13}C, are 98.9% and 1.1% respectively, while the natural abundance of the stable isotopes of nitrogen, ^{14}N and ^{15}N, are 99.6% and 0.4% respectively (Ehleringer and Rundel 1989) (Table 1.1).

Table 1.1. Natural abundances of carbon and nitrogen stable isotopes (adapted from Ehleringer and Rundel 1989).

Element	Isotope	Natural abundance (atom %)
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37

As the absolute composition differences between two samples are normally small, on the order of one or two percent (Ehleringer and Rundel 1989), isotopic measurements are instead expressed in the differential (‘delta’) notation (δ), in parts per thousand (‰):

$$\delta^{13}\text{C or } \delta^{15}\text{N (‰)} = \left[\frac{R_{sample}-R_{standard}}{R_{standard}} \right] \text{ x } 1000$$

where R is equal to the ratio of the heavy to light isotope (either ^{13}C / ^{12}C or ^{15}N / ^{14}N). The δ value signifies the relative deviation in isotope composition of a sample from a defined standard. Peedee belemnite (PDB) and atmospheric nitrogen are recognised as the reference standards for carbon and nitrogen respectively (Ehleringer and Rundel 1989). The R_{standard} for PDB is 1.1237 atom % ^{13}C, and the R_{standard} for N_2 in air is 0.3663 atom % ^{15}N (Mariotti 1983). Samples that are enriched in the heavier isotopes of ^{13}C and ^{15}N relative to the standard will have higher (more positive) δ^{13}C and δ^{15}N values respectively, whereas samples enriched in the lighter isotopes of ^{12}C and ^{14}N will have lower (more negative) δ^{13}C and δ^{15}N values (Peterson and Fry 1987) (Fig. 1.1).

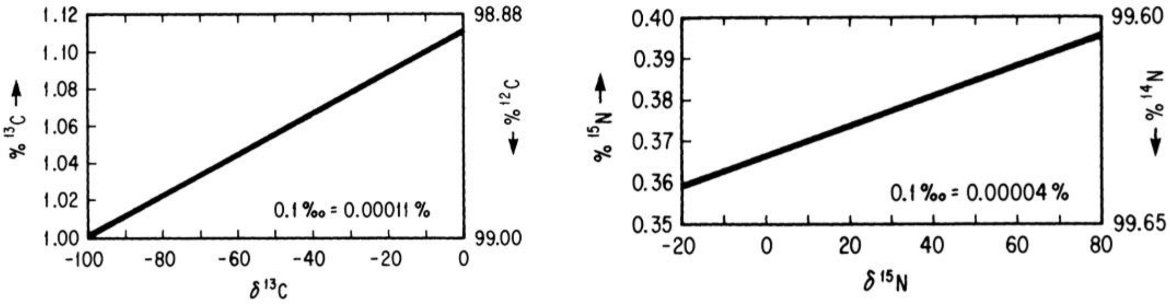


Figure 1.1. Positive linear relationship between ^{13}C content (%) and δ^{13}C (left), and ^{15}N content (%) and δ^{15}N (right) (Source: Peterson and Fry 1987).

1.1.2 Effects of dietary isotopic fractionation

Differences in the chemical and physical properties of an element arising from the interaction between the atomic mass of an isotope and reaction kinetics are called the isotope effect (or fractionation factors) (Griffiths 1991). As the relative mass difference decreases as atomic number increases, only lighter elements such as H, C, N, O and S display distinctive isotope effects (Fitzgerald 1996). Ratios of these stable isotopes can change between diet and consumer due to differential digestion (or fractionation) during assimilation and metabolic processes.

Isotopic fractionation processes can be separated into two main categories, i.e., isotope exchange equilibrium reactions and kinetic processes. The equilibrium fractionation processes ensure that the forward and reverse reaction rates during isotopic exchange remain balanced, such in the maintenance of a constant carbon isotope ratio of atmospheric CO_2 across the globe (Fitzgerald 1996; Wada 2009).

$$^{13}CO_{2(air)} + H^{12}CO_3{}^{-}{}_{(sea)} \rightleftharpoons {}^{12}CO_{2(air)} + H^{13}CO_3{}^{-}{}_{(sea)}$$

During kinetic fractionation processes, the lighter isotopes (^{12}C and ^{14}N) react or diffuse more rapidly and form weaker bonds compared to the heavier isotopes (^{13}C and ^{14}N), and therefore numerous chemical and physical processes, such as diffusion and enzymatic incorporation, will discriminate against the heavier isotopes (Griffiths 1991).

Stomach content analyses are commonly undertaken as the standard practice to explore food web structures, trophic interactions, and community dynamics in aquatic ecosystems, by quantifying the relative diet composition, prey selectivity and food consumption rate (Amundsen and Sánchez-Hernández 2019). However, multiple limitations regarding the application of this method have provided numerous challenges for accurate interpretation, including differential digestion rates, daily and seasonal variability of diets, and the difficulty associated in the identification of smaller organisms (Hyslop 1980). Stomach content analyses also can show a bias towards recent dietary items and can underestimate the quantity of soft-bodied prey to an individual's diet (Polito et al. 2011). Due to this high temporal variability, the analysis of gut contents has been described as providing only a 'snap-shot' of an individual's recent diet (between 8-16 hours) and the food web across a short time frame, often overlooking key energetic dynamics and trophic relationships (Polito et al. 2011; McBride 2005).

Stable isotope analysis (SIA) has evolved to become a popular and powerful tool for ecosystem analysis, allowing for trophic relationships to be explored and understood at the molecular level. SIA provides a time-integrated method which combines the benefits of both trophic-level and food web paradigms (Post 2002). SIA studies are especially useful for exploring relationships and energy flow in freshwater food webs, due to the complex mixture of energy sources and considerable spatial and temporal variation of aquatic ecosystems (Finlay and Kendall 2007; Allan and Castillo 2021). As opposed to stomach content analyses, stable isotopes integrate data across spatial and temporal scales, and have therefore become a common tool for evaluating food web dynamics in aquatic systems, such as for modelling trophic structure (Vander Zanden and Rasmussen 1999; Hicks 1997), niche overlap (Collier et al. 2018), and habitat use (Fry 2002). The stable isotope ratios of carbon, $\delta^{13}C$, (^{13}C / ^{12}C) and nitrogen, $\delta^{15}N$, (^{15}N / ^{14}N) in particular, are frequently utilised in food web studies as natural biological indicators for tracing ecological processes, interactions, and energy flows from the basal plant and detrital sources to the primary and secondary consumers.

The preferential respiration and excretion of the lighter isotopes causes predictable changes to the isotopic ratio of the consumer relative to the diet at each trophic level. The $\delta^{15}N$ of a consumer is typically enriched by excretion by 3 – 4‰ relative to the source material (Peterson and Fry 1987; DeNiro and Epstein 1981) and is therefore useful for predicting trophic positions within aquatic food webs. In contrast, respiration causes carbon isotopic signatures to change relatively little from diet to consumer, due to the lower isotopic fractionation across trophic levels (with an average enrichment of +1‰ per trophic level) (DeNiro and Epstein 1978; Fry 1991) and therefore $\delta^{13}C$ instead becomes useful for determining the primary carbon sources and their relative importance to any given consumer, rather than indicating trophic level (Fig. 1.2). As there is little isotopic shift between trophic levels for carbon isotopes, but a relatively large trophic shift for nitrogen isotopes, analysing $\delta^{13}C$ and $\delta^{15}N$ values together can explore both organic matter transfer and trophic structure across an ecosystem (Hershey et al. 2007). A dual isotope approach can therefore assist in the identification of potential food sources when isotopic ratios overlap to food web analyses (France 1997; Finlay 2001).

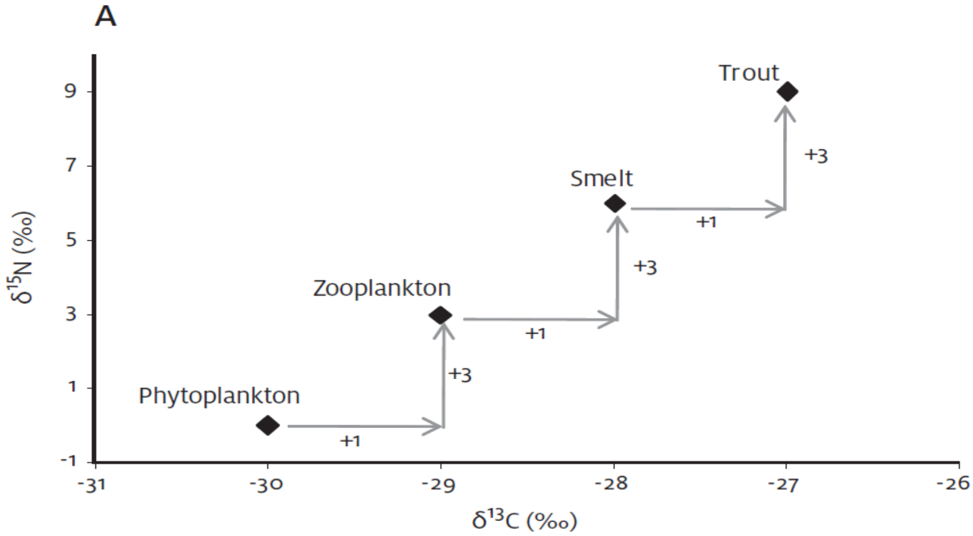


Figure 1.2. Hypothetical aquatic food webs with four trophic levels illustrating the predictable 1‰ increase in δ^{13}C and 3‰ increase in δ^{15}N between diet and consumer (Source: Hicks 2010).

1.2 Natural abundance of C and N isotopic compositions

1.2.1 δ^{13}C distribution in ecosystems

The carbon cycle involves active exchanges of CO_2 between the atmosphere, terrestrial ecosystems, and surface ocean (Fig. 1.3). Atmospheric CO_2 had a δ^{13}C value of approximately −7‰ in 1987, which in response to the input of ^{13}C depleted-CO_2 from fossil fuels plus biomass burning and decomposition, has been decreasing by almost 1‰ over the last 100 years (Peterson and Fry 1987) and is now closer to −8‰ because of CO_2 emissions (NOAA, https://gml.noaa.gov/ccgg/isotopes/c13tellsus.html). One of the most important reactions governing the characteristic isotopic distribution across ecosystems is photosynthesis (Fry 2006 pg. 44). Terrestrial plants with different photosynthetic pathways can be differentiated using δ^{13}C, as the carbon isotopic signatures of plant material vary depending on the characteristic fractionation and subsequent discrimination against ^{13}C (Ehleringer and Rundel 1989). The majority of plants follow the C_3 pathway, where a three-carbon molecule (hence the C_3 name) is produced during the first step of the Calvin cycle, in which carbon dioxide is fixed by the enzyme, rubisco (ribulose-1,5-bisphosphate carboxylase-oxygenase). The rubisco catalyst involved in the C_3 pathway strongly discriminates against $^{13}CO_2$ (net fractionation of approximately 21‰; Peterson and Fry 1987), thus resulting in low δ^{13}C values for C_3 plants, typically ranging between −23 to −34‰ (Fitzgerald 1996). However, plants that follow the C_4 photosynthetic pathway use PEP carboxylase (phosphoenolpyruvate carboxylase) to initially produce a four-carbon molecule (hence the C_4 name), with less isotopic discrimination than the C_3

pathway, resulting in ^{13}C discrimination and carbon fractionation of approximately −6‰ (Peterson and Fry 1987) and subsequently higher δ^{13}C values for C_4 plants, typically ranging between −7 and −23‰ (Fig. 1.3; Hecky and Hesslein 1995; Fitzgerald 1996).

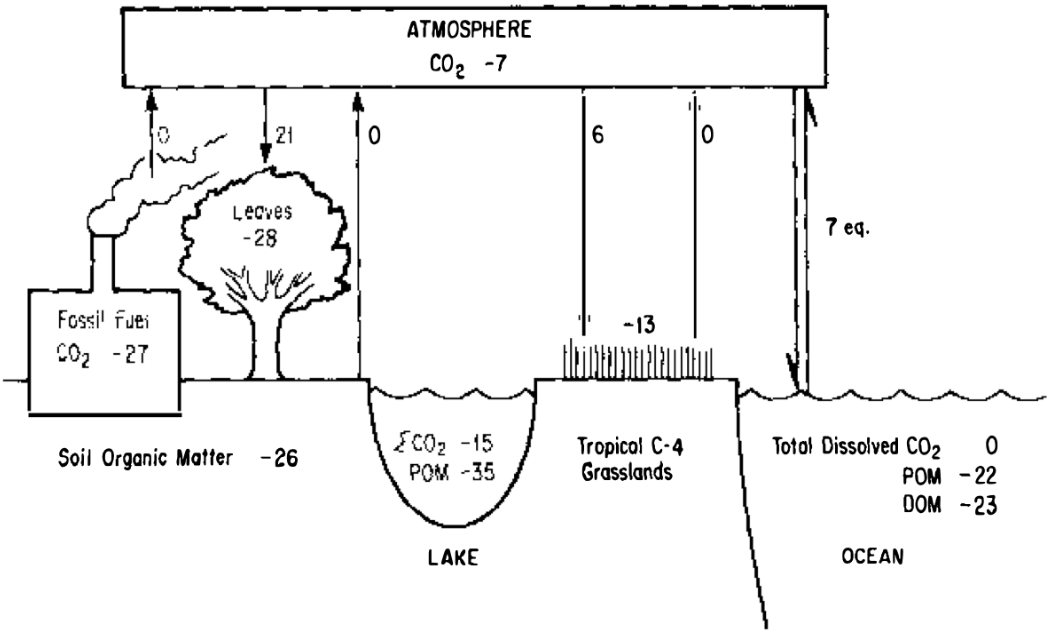


Figure 1.3. The distribution of δ^{13}C in ecosystems (Source: Peterson and Fry 1987).

The δ^{13}C of components of the carbon cycle in freshwater ecosystems vary depending on the sources of dissolved CO_2 in the water and the processes which control the exchange of CO_2 gas with the atmosphere. The major sources of dissolved CO_2 in streams and rivers are carbonate rock weathering, mineral springs, atmospheric CO_2, and organic matter respiration (Fry 2006 pg. 46). The equilibrium fractionation factor between dissolved inorganic carbon (DIC) and gaseous CO_2 is dependent on the relative proportions of the carbonate species ($CO_{2(aq)}$, HCO_3^-, CO_3^{2-}) within the dissolved inorganic pool (Zhang et al. 1995). During CO_2 transfer between water and the atmosphere, dissolved $CO_{2(aq)}$ reacts with H_2O to form the weak inorganic acid, carbonic acid (H_2CO_3), which further dissociates to form bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}) (Allan and Castillo 2021).

$$CO_2 + H_2O = H_2CO_3 = H^+ + HCO_3^-$$

When the pH value of the water is very low (i.e., below 4.5), only CO_2 and H_2CO_3 will be present, with almost no HCO_3^- or CO_3^{2-} detected (Allan and Castillo 2021). Conversely, at higher pH values, H_2CO_3 begins to dissociate, thus causing the bicarbonate and carbonate ions to dominate, with very little CO_2 and H_2CO_3 present (Allan and Castillo 2021). When the pH value is intermediate, HCO_3^- dominates (Fig. 1.4).

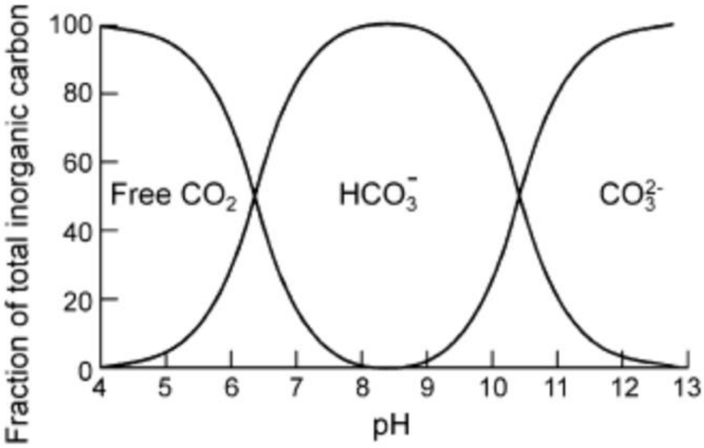


Figure 1.4. Relationship between the pH and the relative proportions of inorganic carbon species in solution (Source: Wetzel 2001).

Bicarbonate and carbonate ions have enriched (more positive) δ^{13}C values by up to 10‰ compared to the atmosphere (Oczkowski et al. 2018), and therefore the use of HCO_3^- and CO_3^{2-} as a carbon substrate will result in higher δ^{13}C values. As the pH of the stream increases (i.e., becomes more basic) and HCO_3^- becomes the dominant carbon species, the δ^{13}C values of DIC will become more positive (Oczkowski et al. 2014). The process of carbonate weathering can also act to increase the δ^{13}C of DIC, as a result of the formation of enriched ^{13}C carbonates, whose δ^{13}C signatures reflect those of carbonate rocks (Fitzgerald 1996). The influx of groundwater also can be an influential source of δ^{13}C variation for river and stream environments. Groundwaters are typically supersaturated with $CO_{2(aq)}$, as most of the CO_2 will be derived from plant root respiration, and to a lesser extent, the oxidation of organic matter (Fitzgerald 1996; Jones and Mulholland 1998; Finlay 2003).

The rate of isotopic exchange with the atmosphere largely is dictated by the contact surface area and residence time. Large water bodies characterised with long residence times tend to be in equilibrium with atmospheric CO_2 (Hendy 1971; Fitzgerald 1996). For example, the δ^{13}C of DIC in Lake Taupo, the largest lake in New Zealand (area of 613 km^2) with a long estimated residence time of 13 years, was close to equilibrium with atmospheric CO_2 (mean δ^{13}C-DIC −0.25‰) (Fitzgerald 1996). Conversely, due to the faster flowing waters and subsequent shorter residence times, stream and river environments tend not to be in equilibrium with atmospheric CO_2.

1.2.2 δ^{15}N distribution in ecosystems

The δ^{15}N value of atmospheric N_2 is considered to be relatively constant and is the defined zero point (0‰) (Mariotti 1983). The δ^{15}N value of most nitrogenous materials in the biosphere typically falls within the range of −10 to +10‰, as the uptake of N for plant growth and bacterial mineralisation is limited by the rate of nitrogen supply, as opposed to enzymatic uptake (Peterson and Fry 1987) (Fig. 1.5). The δ^{15}N values of terrestrial autotrophs may fall within the range of −9 to 8‰ (mode of −1‰), whereas freshwater autotrophs normally have δ^{15}N values between −3 to 10‰ (mode of 3‰) (France 1995b), allowing for N isotopes to be utilised as source markers for tracking autochthonous or allochthonous N origin.

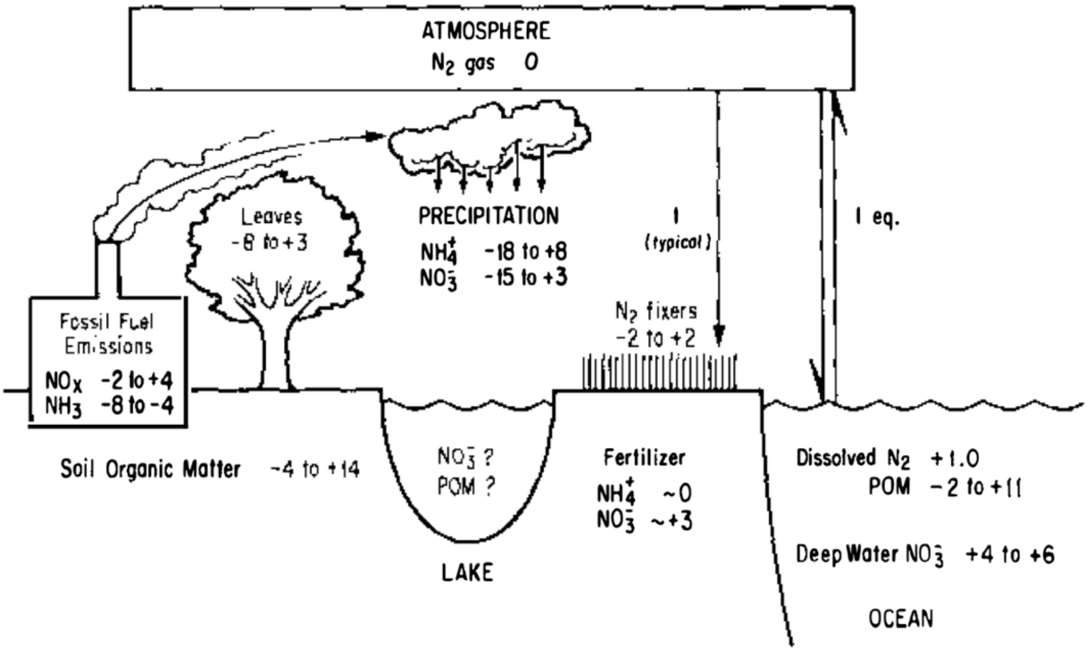


Figure 1.5. The distribution of δ^{15}N in ecosystems (Source: Peterson and Fry 1987).

1.3 Research on δ^{13}C and δ^{15}N variability in aquatic ecosystems

1.3.1 Drivers of δ^{13}C and δ^{15}N variability in streams and rivers

The ecological, biogeochemical, hydrological, and geomorphological processes of stream and river ecosystems experience significant variability across multiple spatial scales from the local habitat to watershed (Allan and Castillo 2021). This spatial heterogeneity can influence the δ^{13}C and δ^{15}N isotopic baseline of food sources, which directly can have an effect on the isotopic composition of primary and secondary consumers. Ratios of stable carbon and nitrogen isotopes for basal resources, primary consumers and top predators, in streams and rivers therefore have been reported to exhibit notable spatial and temporal variability. Natural variations in the isotopic compositions are largely determined by the isotope ratio of the inorganic source elements and their subsequent fractionation during assimilation (Finlay and Kendall 2007). The major environmental drivers of δ^{13}C and δ^{15}N variability in stream and river environments that have been identified in previous research include the extent of canopy cover (e.g., Ehleringer et al. 1986; MacLeod and Barton 1998; Hill et al. 2008), land use (e.g., Hicks 1997; Hicks and Quinn n.d; Espinoza-Toledo et al. 2021), water velocity (e.g., Keeley and Sandquist 1992; Hicks 1997; Finlay et al. 1999; France and Cattaneo 1998; Trudeau and Rasmussen 2003), watershed area (e.g., Finlay et al. 1999; Finlay 2001), and marine-derived nutrient inputs (e.g., Bilby et al. 1998; Helfield and Naiman 2001; Chaloner et al. 2002; Hicks et al. 2005). Seasonal variations in stream isotopic signatures have also been identified (Walcroft et al. 1997). The effect of each of these environmental factors on the carbon and nitrogen isotopic ratios of organisms in streams will be further investigated and discussed in Chapter Two.

1.3.2 Drivers of δ^{13}C and δ^{15}N variability in lakes

For lake environments, the major environmental drivers that have been identified in previous research to contribute to δ^{13}C and δ^{15}N variation include the lake surface area (e.g., McCabe 1985; Post 2002; Bade et al. 2004; Perga and Gerdeaux 2004; McBride 2005), habitat type i.e., littoral vs pelagic (e.g., France 1995a; Vander Zanden and Rasmussen 1999; Post 2002; Vander Zanden and Vadeboncoeur 2002), water depth and stratification (e.g., Fritz and Poplawski 1974; Fitzgerald 1996; McCabe 1985); and nutrient status (e.g., McCabe 1985; Perga and Gerdeaux 2003; Perga and Gerdeaux 2004; McBride 2005). Like with streams, seasonal variations in lake isotopic signatures have also been identified (e.g., Lehmann et al. 2004; Gu et al. 2006; Zeng et al. 2008). The effect of these potential drivers on the δ^{13}C and δ^{15}N values of organisms in lakes will also be further investigated and discussed in Chapter Two.

1.4 Research on δ^{13}C and δ^{15}N variability in New Zealand aquatic ecosystems

Several studies have explored the variability of δ^{13}C and δ^{15}N values across New Zealand aquatic ecosystems. Hicks (1997) reported that for Waikato streams, land use was a significant controlling variable, with pasture and mixed land use sites having higher δ^{15}N values (mean of 6.2‰ to 6.7‰) for basal resources and consumers compared to forested sites (mean of 3.2‰). McBride (2005) identified the nutrient status as an important predictor of intra-spatial isotopic variability among the Rotorua lakes, whereby a strong, positive relationship was reported between the total phosphorus concentration of the lake and the δ^{13}C of primary producers and δ^{15}N of most taxa. McCabe (1985) also found that lake productivity was a powerful predictor of δ^{13}C of primary producers across several lakes in the Waikato.

1.4.1 Meta-analysis of drivers of freshwater δ^{13}C and δ^{15}N variability

Several overseas studies have conducted meta-analysis on existing literature data to examine the dominant controlling factors on δ^{13}C and δ^{15}N variability across different taxonomic levels in various lakes and rivers to elucidate food web dynamics. Ishikawa et al. (2012) studied various regional (biome, season, and watershed area) and local controls (canopy cover, water current velocity, chlorophyll *a* density, and dominant taxa) on the lotic periphyton δ^{13}C, and found strong longitudinal increases in δ^{13}C. Furthermore, the periphyton δ^{13}C was positively correlated to chlorophyll *a* density and negatively correlated to both canopy cover and water current velocity (Ishikawa et al. 2012). Peipoch et al. (2012) explored the main factors contributing to variations in δ^{15}N signatures of basal resources and dissolved inorganic nitrogen (DIN) in streams and rivers. They identified anthropogenic land use in the catchment as the most significant driver of δ^{15}N variability for both the basal resources and DIN. Streams and rivers draining urban (mean δ^{15}N-basal 6.9‰, mean δ^{15}N-DIN 9.94‰) and agricultural catchments (mean δ^{15}N-basal 6.5‰, mean δ^{15}N-DIN 7.6‰) have higher isotopic signatures than those draining forested catchments (mean δ^{15}N-basal 2.4‰, mean δ^{15}N-DIN 2.2‰) (Peipoch et al. 2012). Chappuis et al. (2017) explored the influence of extrinsic (environmental) factors on the variability of δ^{13}C and δ^{15}N for aquatic plants among lakes. The DIC concentration and pH of the water column were the two most significant extrinsic factors driving aquatic plant δ^{13}C variability as they both determined the total amount of inorganic carbon and relative proportions of the carbonate species ($CO_{2(aq)}$, HCO_3^-, CO_3^{2-}) within the dissolved inorganic pool (Chappuis et al. 2017). For the δ^{15}N variability of aquatic plants, the geomorphological type of lake, as well as the basin land use, were reported as the two most significant driving variables (Chappuis et al. 2017). There is, however, a lack of research exploring δ^{13}C and δ^{15}N patterns and their dominant driving forces across New Zealand's freshwater ecosystems.

1.5 Aims, objectives and hypotheses

1.5.1 Aims and objectives

The main objective of this study is to identify the most important mechanisms that influence the spatial differences of stable carbon and nitrogen isotope ratios across New Zealand aquatic ecosystems at the landscape scale. An enhanced understanding of how natural ecosystem processes and anthropogenic influences contribute to the spatial distribution of δ^{13}C and δ^{15}N of both primary producers (invertebrates) and top-predatory fish can improve the use and interpretation of stable isotopes as a tracers in food web and ecosystem ecology.

The main aims of this thesis are as follows:

- To review and collate the findings from published stable isotopic studies which investigated the effect of environmental factors on δ^{13}C and δ^{15}N of aquatic organisms;
- To identify the sources and scales of δ^{13}C and δ^{15}N variability for fish (top predators) and primary consumers across New Zealand stream and river environments;
- To identify the sources and scales of δ^{13}C and δ^{15}N variability for fish (top predators) in New Zealand lake environments.

1.5.2 Hypotheses

We hypothesise that for lakes, variables related to the lake system size, including the volume, catchment area, and surface area will be the most significant predictors of δ^{13}C variability among lakes in New Zealand. Also, the land use of the lake catchment is likely to be the most significant predictor of δ^{15}N variability among lakes in New Zealand. For streams and rivers, we hypothesise that the canopy cover, water temperature, and the land use will be the most significant predictors of δ^{13}C and δ^{15}N variability, contributing to a gradual enrichment of both δ^{13}C and δ^{15}N from upstream to downstream. The stream δ^{15}N values, in particular, will be enriched closer to the sea due to the influence of marine-derived inputs from the diadromous fish.

1.6 Outline of thesis

This thesis comprises the five following chapters: Chapter One (Introduction) provides the general background information on carbon and nitrogen stable isotopes, the use of stable isotopes for ecosystem analysis, as well as the key objectives, aims and hypotheses, and the outline of the thesis. Chapter Two (Sources of variation) is a review of literature which summarises the influence of environmental factors on the δ^{13}C and δ^{15}N values of basal resources and organisms, in both lake and stream environments. Chapter Three (Methods) describes the general study area, summarises the environmental variables utilised in the analysis, and provides the research process and methods. Chapter Four (Results) is separated into three major sections: (4.1) top-predatory fish carbon and nitrogen isotopic ratios in New Zealand lakes, (4.2) top-predatory fish nitrogen isotopic ratios in New Zealand streams, and (4.3) invertebrate nitrogen isotopic ratios in New Zealand streams. Each section provides the key statistical results. Chapter Five (Discussion and conclusion) reviews and summarises the key discussion findings from Chapter Four, evaluates the hypotheses, and presents the final concluding remarks. Several limitations and recommendations for future research are also provided.

Chapter 2

Sources of δ^{13}C and δ^{15}N variation

2.1 δ^{13}C and δ^{15}N variability in lakes

2.1.1 Lake surface area

The lake surface area has been identified as one of the most influential drivers of δ^{13}C spatial variation among different lakes. As the size of the lake increases, the δ^{13}C values also tend to increase and become closer to isotopic equilibrium with the atmosphere (McCabe 1985; Post 2002; Bade et al. 2004; Perga and Gerdeaux 2004; McBride 2005). This positive relationship between the lake surface area and the δ^{13}C have been attributed to changes to the δ^{13}C signature of the dissolved inorganic carbon (DIC) pool, as opposed to alterations to isotopic fractionation (Post 2002; Perga and Gerdeaux 2004). DIC in a lake can originate from three main sources: the dissolution from atmospheric CO_2, the weathering of carbonates within the watershed, and the re-mineralisation (respiration) of organic carbon, each which have isotopically distinct δ^{13}C values (Post 2002; Perga and Gerdeaux 2004). The DIC sourced from the mineralisation process tends to be the lightest isotopically of the three, with low δ^{13}C values ranging between −20‰ to −35‰, reflecting the signature of the parent mineralised organic matter (Post 2002; Perga and Gerdeaux 2004). Alternatively, both DIC originating from atmospheric CO_2 and carbonate weathering processes have higher δ^{13}C signatures of approximately −7‰ and 0‰, respectively (Post 2002). The relative contribution of atmospheric to endogenous carbon in the DIC pool is therefore an important factor that influences the δ^{13}C of lakes, whereby increasing atmospheric carbon increases the overall δ^{13}C of the DIC.

The size of the lake is an important determinant for the origin of DIC. In small lakes, the production is largely dependent on the recycled carbon sourced from respiration and mineralisation processes, whereas in larger lakes, endogenous-sourced DIC is no longer able to meet the photosynthetic demands of the autotrophs, so production is largely derived from atmospheric CO_2 and carbonate sources (Post 2002). As the size of the lake increases, more production originates from atmospheric CO_2 due to longer retention times and longer periods for gas exchange processes and turbulence regimes (Wanninkhof 1992). The relative inputs of terrestrial allochthonous carbon also varies between different lake sizes, with lakes with small lake area to watershed area typically receiving higher inputs of terrestrial carbon compared to lakes with larger lake area to watershed area (Post 2002; Bade et al. 2004). As a result, as the surface area of the lake increases, the more positive the δ^{13}C values (Fig. 2.1).

There is, however, limited understanding on the effects of lake surface area on δ^{15}N values. For example, through investigations of the physiological controls on the variability of δ^{15}N signatures across numerous Rotorua lakes, no strong correlations were identified between the lake surface area and δ^{15}N (McBride 2005). Alternatively, a literature review of the variability of δ^{15}N of particulate organic matter (POM) across 36 lakes identified that large lakes (>1 km^2) were significantly more enriched in $\delta^{15}N_{POM}$ compared to small lakes, which was attributed to a reduction of nutrient loading (Gu 2009). Subsequently, as the effect of surface area on the δ^{15}N values is not consistent, lake surface area is unlikely to be a dominant control on the δ^{15}N signatures (Fig. 2.1), and thus alternative factors are likely to drive δ^{15}N variability across different lakes.

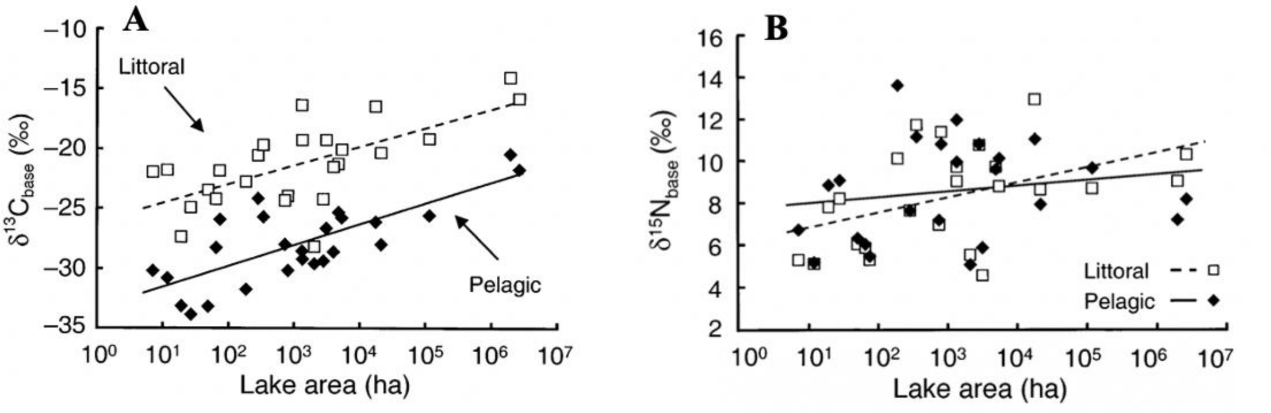


Figure 2.1. Relationship between (A) lake area and $\delta^{13}C_{base}$ and (B) lake area and $\delta^{15}N_{base}$ of littoral and pelagic food webs of eastern North America lakes (Source: Post 2002).

The surface area of the lake is likely to be one of the most significant controlling variables driving the δ^{13}C variability among New Zealand lakes in this study. The larger the lake surface area, the more positive the δ^{13}C values are likely to be. However, as very few studies have discovered consistent patterns between δ^{15}N and lake surface area, we would expect alternative drivers would be influencing any δ^{15}N variability observed among lakes.

2.1.2 Littoral versus pelagic

Habitat-specific variations have also been identified to influence the δ^{13}C patterns due to differences at the base of littoral and pelagic food chains, with pelagic consumers commonly having depleted (more negative) δ^{13}C values relative to the littoral consumers (France 1995; Vander Zanden and Rasmussen 1999; Post 2002; Vander Zanden and Vadeboncoeur 2002). δ^{13}C signatures differ between littoral (near shore) production from attached algae and detritus, and pelagic (open water) production from planktonic algae (Post 2002). As the littoral habitat typically experiences less turbulence than the pelagic habitat, the boundary layer at the cell boundary of producers will be thicker, contributing to limited [CO_2], and reduced isotopic discrimination of ^{13}C (France 1995; Post 2002). Consequently, the base of littoral food webs tend to have higher δ^{13}C values compared to the base of pelagic food webs (France 1995). The consumers thus reflect the pelagic-littoral differentiation in carbon flow, as pelagic consumers rely heavily on ^{13}C-depleted particulate organic matter (POM) as their food base, while littoral consumers depend on a mixture of attached algal and terrestrial detrital carbon (France 1995).

Higher δ^{13}C values for littoral consumers compared to pelagic consumers has been reported in numerous studies. Vander Zanden and Rasmussen (1999) reported statistically significant variations in the isotopic carbon compositions of primary consumers across different lake habitats. δ^{13}C significantly varied between the littoral (mean of −23.8‰), pelagic (mean of −28.4‰), and profundal habitats (mean of −30.5‰) (Vander Zanden and Rasmussen 1999). Vander Zanden and Vadeboncoeur (2002) also identified higher δ^{13}C values in littoral habitats as opposed to pelagic habitats for freshwater fish, and through the use of δ^{13}C mixing models, estimated the mean contribution of littoral carbon to range between 43%, if trophic enrichment is 1‰, to 59% with no trophic enrichment (i.e., $\Delta\delta^{13}C = 0‰$).

Vander Zanden and Rasmussen (1999) also discovered statistically significant variations in the δ^{15}N values between the littoral (mean of 1.58‰), pelagic (mean of 3.05‰) and profundal habitats (mean of 5.17‰). However, there is limited understanding on the controls on δ^{15}N variability between littoral and pelagic consumers, with four potential contributing factors suggested: (1) the incorporation of terrestrial material with more negative δ^{15}N than benthic algae in littoral consumers, (2) the infusion of high ^{15}N waters from the hypolimnion during lake mixing is less influential on the benthic algae compared to phytoplankton, (3) different δ^{15}N values between inorganic N substrates, and (4) differing rates of fractionation depending on the N substrate, algal growth rates, species composition and nutrient concentrations (Vander Zanden and Rasmussen 1999).

Overall, as pelagic consumers commonly have more negative δ^{13}C values relative to the littoral consumers, accounting for in-lake differences in carbon flow is important. Very few datasets included in this study, however, provided the habitat type for each sample, thus future research could quantify the influence of littoral and pelagic habitats on isotopic variability among lakes.

2.1.3 Water depth, stratification, and mixing

The water depth can also contribute to the δ^{13}C and δ^{15}N variation in deep lakes with poor circulation (Fitzgerald 1996). Fritz and Poplawski (1974) demonstrated that surface waters typically have the highest δ^{13}C values (approximately −5 to −9‰), as opposed to the deeper waters (approximately −10 to −20‰), where aerobic decomposition and biological activities dominate. However, this pattern is not observed across all lake systems, for example, the δ^{13}C signatures of surface and deeper waters of a Waikato lake, Lake Arapuni, found no significant difference between water depths (Fitzgerald 1996).

The effect of thermal stratification and mixing regimes in deep lakes have also been identified as a potential source of variation for δ^{13}C values. During a thermal stratification period, in which the hypolimnion experiences a build-up of biogenic (respired) carbon, the surface waters become enriched (more positive) in δ^{13}C and close to an isotopic equilibrium with atmospheric CO_2 (McCabe 1985). As the thermal stratification is broken down in the lake, the inorganic carbon, which is depleted in ^{13}C, will then be mixed throughout the lake (McCabe 1985). For shallow lakes, including Lake Ngaroto, which do not experience prolonged thermal stratification, the δ^{13}C of the surface and deeper waters is similar and remains more or less constant throughout the year (McCabe 1985). As a result, accounting for the depth of lakes is also another important consideration when examining δ^{13}C variability among lakes.

2.1.4 Lake nutrient status

Stable carbon isotopes in lakes are influenced by the lake's nutrient status and rates of primary production; the higher the total phosphorus content (P_{total}), the more positive the

δ^{13}C values tend to be. As the rate of primary production increases, the dissolved inorganic carbon (DIC) concentrations within the epilimnion significantly decrease (McCabe 1985; Perga and Gerdeaux 2004). The declining DIC content in turn reduces the fractionation between ^{12}C and ^{13}C during photosynthesis, thereby contributing to more positive δ^{13}C values (Perga and Gerdeaux 2003; Perga and Gerdeaux 2004). Additionally, higher δ^{13}C signatures are also a consequence of the greater contribution of atmospheric-fixed carbon through photosynthesis (McCabe 1985; Perga and Gerdeaux 2003; Perga and Gerdeaux 2004; McBride 2005). δ^{13}C values for dissolved inorganic carbon (DIC) become depleted when the respired carbon contribution is high, while DIC δ^{13}C becomes more positive when atmospheric CO_2 invasion increases (Perga and Gerdeaux 2003). Low dissolved CO_2 concentrations can increase the importance of ^{13}C-enriched atmospheric CO_2 (McCabe 2005). Eutrophication in large, highly productive lakes therefore leads to greater contribution of atmospheric carbon compared to endogenous carbon, through the increased DIC demand of pelagic primary producers, resulting in more positive phytoplankton δ^{13}C (McCabe 1986; Perga and Gerdeaux 2004).

In a study comprising of over 20 lakes, the variability in δ^{13}C values of the zooplanktivorous whitefish (*Coregonus lavaretus*) was strongly positively correlated with the total phosphorus concentrations and lake productivity ($R^2 = 0.71$) (Perga and Gerdeaux 2004). Additionally, during the recovery period of Lake Geneva, significantly strong correlations ($R^2 = 0.71$) were also identified between the total phosphorus load and isotopic signatures, where P_{Total} decreased from 82 to 34 µg L^{-1}, while δ^{13}C values of the scales of whitefish also declined from −20 to −23‰ (Perga and Gerdeaux 2003). The effect of nutrient status on the δ^{13}C variability in lakes has also been studied in New Zealand, in which a strong, positive relationship between the total phosphorus of a lake and the δ^{13}C of primary producers and δ^{15}N of most taxa was identified in the Rotorua lakes (McBride 2005). Interestingly, macrophyte δ^{13}C displayed strong negative relationships with the P_{Total} content, to the differences attributed to varying photosynthetic fractionation magnitudes and ^{13}C discrimination.

Lake productivity has also been recognised as a significant controller of δ^{15}N variability in lakes due to the decrease in isotopic fractionation during nutrient assimilation. A literature analysis on the variability of δ^{15}N of particulate organic matter (POM) across 36 lakes identified higher mean $\delta^{15}N_{POM}$ values for productive lakes compared to unproductive lakes (Gu 2009). In general, as the lake trophic state increased, the δ^{15}N of

POM also increased, with the exception of lakes dominated by N_2-fixing cyanobacteria (δ^{15}N values close to 0‰) (Gu 2009).

The productivity and nutrient status of lakes is likely to be one of the most significant controlling variables influencing the δ^{13}C variability among lakes in New Zealand, in which the higher the total phosphorus content (P_{Total}), the more positive the δ^{13}C values tend to be.

2.1.5 Gas exchange

Another influential source of δ^{13}C variation in lakes are the sources of dissolved CO_2 in waters and the processes which control the exchange of CO_2 gas with the atmosphere (Fry 2006). Similar to streams, the bicarbonate buffer system plays a significant role in regulating the concentrations of $CO_{2(aq)}$, HCO_3^-, and CO_3^{2-} in the lake, all of which vary in δ^{13}C values, as the relative amounts of carbon substrates is largely dependent on the pH (Hecky and Hesslein 1995). A switch in the carbon balance in a lake from CO_2 to HCO_3^- dominance occurs following a high rate of CO_2 uptake (Gu et al. 2006). Bicarbonate (δ^{13}C of +1‰) and carbonate ions (δ^{13}C of −1‰) have enriched (more positive) δ^{13}C values by up to 6 to 8‰ compared to the atmosphere (δ^{13}C of −8‰) (Hecky and Hesslein 1995), and therefore the use of HCO_3^- and CO_3^{2-} as a carbon substrate will result in higher δ^{13}C values (Vander Zanden and Rasmussen 1999).

In lakes, the rates of photosynthesis and respiration can also influence the δ^{13}C values. During photosynthesis, the rate of CO_2 uptake can exceed the rate of atmospheric invasion, thus depleting the dissolved CO_2 supply, contributing to a reduction in the discrimination against the heavier ^{13}C isotope, and thus more positive δ^{13}C signatures (Hecky and Hesslein 1995). Alternatively, respiration of previously fixed carbon and microbial decomposition of organic matter can contribute to excess $CO_{2(aq)}$, and therefore contributing to an increase in the discrimination against the heavier ^{13}C isotope, and thus more negative δ^{13}C signatures (Hecky and Hesslein 1995).

The δ^{13}C values are therefore likely to vary depending on the origin of dissolved CO_2 in streams and the relative proportions of the carbonate species ($CO_{2(aq)}$, HCO_3^-, CO_3^{2-}). Lakes dominated by CO_3^{2-} and HCO_3^- substrates, both of which are ^{13}C-enriched, would be expected to have more positive δ^{13}C values.

2.1.6 Seasonality

In lakes, stable carbon isotopic signatures have also been reported to vary seasonally, with δ^{13}C values often enriched during summer and depleted during the winter months, due to changes in the water temperature, productivity, and dissolved inorganic carbon (DIC) pool (Lehmann et al. 2004; Gu et al. 2006; Zeng et al. 2008). In general, high water temperatures, high phytoplankton production, high pH, and low DIC concentrations experienced during summer, contribute to more positive δ^{13}C values (Gu et al. 2006). As the surface water temperatures increase during early summer, the rate of phytoplankton photosynthesis is enhanced, in turn promoting low dissolved nutrients and DIC concentration (Zeng et al. 2008). Phytoplankton isotopic fractionation subsequently decreases during these periods of high growth (Gu et al. 2006), thereby increasing the δ^{13}C values.

The build-up and breakdown of thermal stratification and mixing regimes in a lake can result in seasonal δ^{13}C cycles across different regions of the lake (McCabe 1985). During a thermal stratification period, in which the hypolimnion experiences a build-up of biogenic (respired) carbon, the surface waters have more positive δ^{13}C, that are close to an isotopic equilibrium with atmospheric CO_2 (McCabe 1985). As thermal stratification breaks, the hypolimnetic inorganic carbon, which is depleted in ^{13}C, will then be mixed throughout the lake (McCabe 1985). McCabe (1985) found that for the deeper lakes included in the study (i.e., lakes Rotomanuka, Rotoroa, Maratoto and 'D'), the δ^{13}C values for DIC significantly varied throughout the year, becoming depleted in the hypolimnion, and enriched in the surface waters, during thermal stratification in the summer months. McBride (2005) also identified significant seasonal variations in the δ^{13}C signatures of phytoplankton in Lakes Okareka, Rotoehu and Rotoma, with lower δ^{13}C values during spring and winter. For the eutrophic Lake Rotoehu, the δ^{15}N signatures were also strongly correlated to the seasons, with more negative δ^{15}N values during spring (McBride 2005).

The amplitude of seasonal variation has been shown to be significantly dependent on the isotopic turnover rate for different tissues as the δ^{13}C and δ^{15}N reflects the isotopic signature of the consumed food during tissue growth periods. Through analysing the isotopic signatures of δ^{13}C and δ^{15}N for whitefish muscle, Perga and Gerdeaux (2005) discovered that the isotopic values only reflected the prey consumed during the spring and summer growth periods. Isotopic composition of the liver tissue, however, responded faster to isotopic changes of the food sources throughout the year than muscle tissue (Fig. 2.2) (Perga and Gerdeaux 2005).

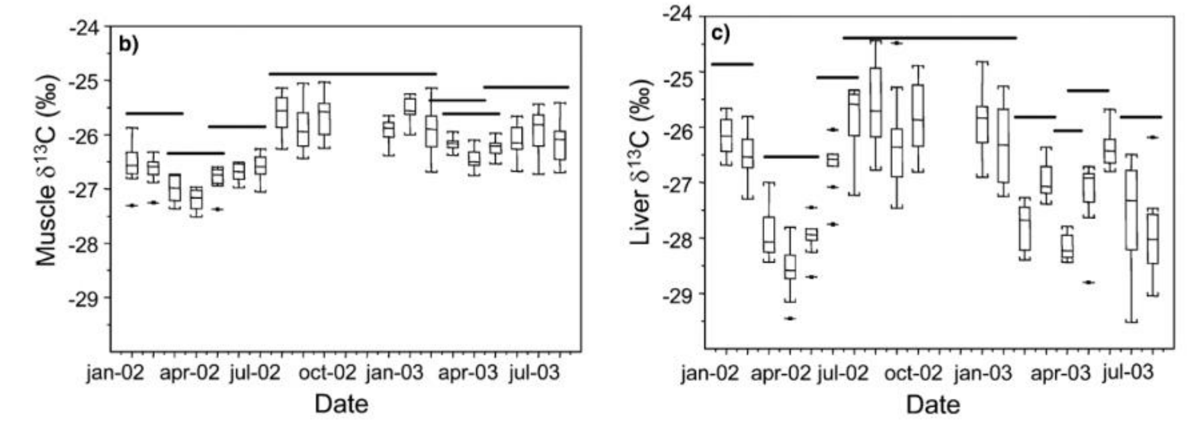
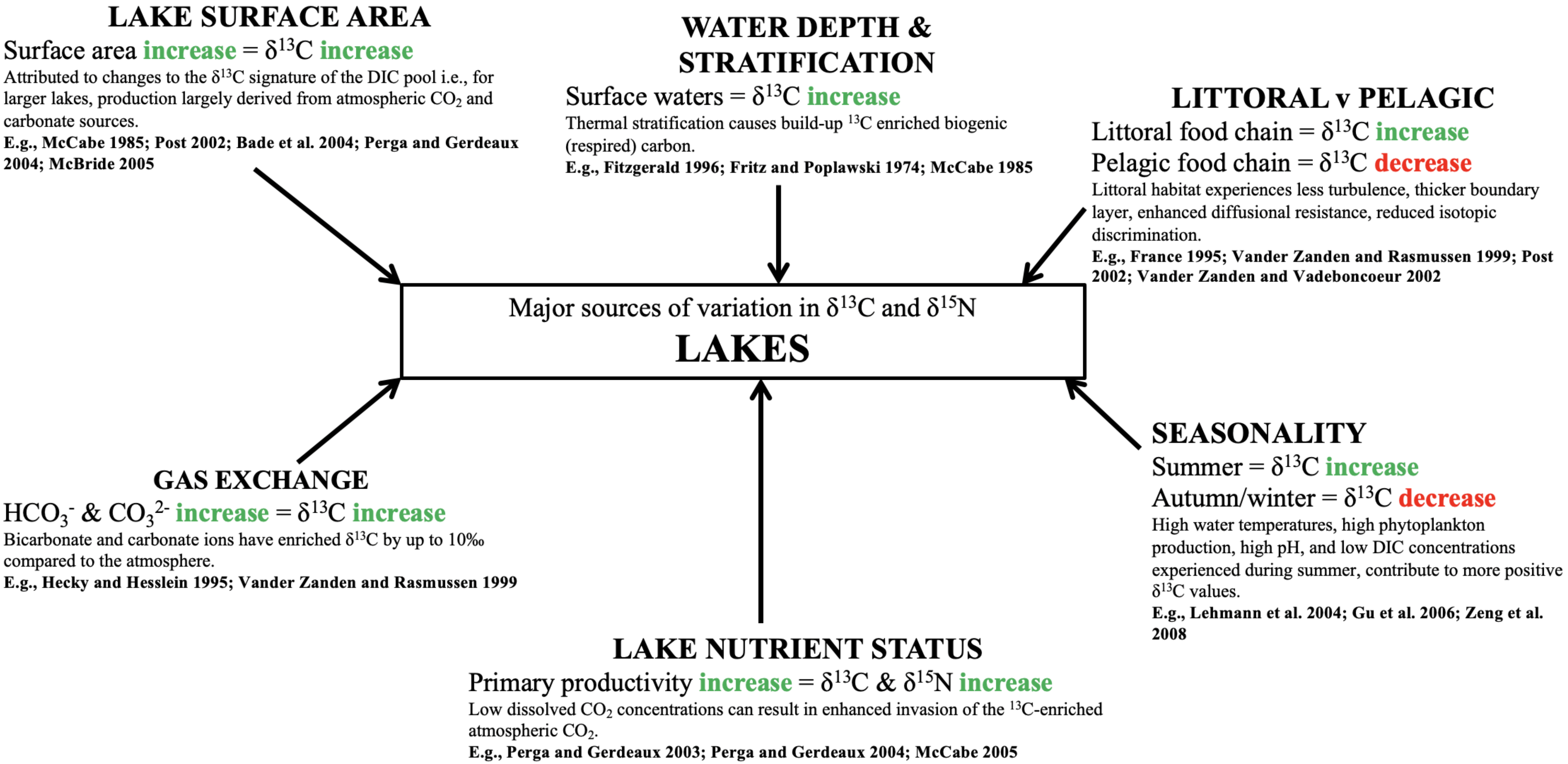


Figure 2.2. Monthly variations of $\delta^{13}C$ of whitefish muscle (left) and whitefish liver (right) (Source: Perga and Gerdeaux 2005).

In small, flood-plain lakes (billabongs) in south-eastern Australia, seasonal variations were also observed, with most sources and consumers being less ^{13}C-depleted and ^{15}N-enriched in later summer compared to spring (Bunn and Boon 1993). Seasonal $\delta^{13}C$ variations also tend to be greatest in smaller-sized lakes, and are most marked when climatic conditions differ significantly between seasons (Fitzgerald 1996).

2.1.7 Summary of environmental controls on $\delta^{13}C$ and $\delta^{15}N$ in lakes

Numerous environmental and climatic factors have been documented to influence the $\delta^{13}C$ and $\delta^{15}N$ values of basal resources and organisms among different lakes (Fig. 2.3). Overall, we hypothesise that in New Zealand, larger lakes with high primary productivity rates and nutrient concentrations, will have the highest $\delta^{13}C$ and $\delta^{15}N$ signatures compared to the smaller, pristine lakes.



LAKE SURFACE AREA

Surface area increase = $\delta^{13}C$ increase

Attributed to changes to the $\delta^{13}C$ signature of the DIC pool i.e., for larger lakes, production largely derived from atmospheric CO_2 and carbonate sources.

E.g., McCabe 1985; Post 2002; Bade et al. 2004; Perga and Gerdeaux 2004; McBride 2005

WATER DEPTH & STRATIFICATION

Surface waters = $\delta^{13}C$ increase

Thermal stratification causes build-up ^{13}C enriched biogenic (respired) carbon.

E.g., Fitzgerald 1996; Fritz and Poplawski 1974; McCabe 1985

LITTORAL v PELAGIC

Littoral food chain = $\delta^{13}C$ increase

Pelagic food chain = $\delta^{13}C$ decrease

Littoral habitat experiences less turbulence, thicker boundary layer, enhanced diffusional resistance, reduced isotopic discrimination.

E.g., France 1995; Vander Zanden and Rasmussen 1999; Post 2002; Vander Zanden and Vadeboncoeur 2002

Major sources of variation in $\delta^{13}C$ and $\delta^{15}N$

LAKES

GAS EXCHANGE

HCO_3^- & CO_3^{2-} increase = $\delta^{13}C$ increase

Bicarbonate and carbonate ions have enriched $\delta^{13}C$ by up to 10‰ compared to the atmosphere.

E.g., Hecky and Hesslein 1995; Vander Zanden and Rasmussen 1999

SEASONALITY

Summer = $\delta^{13}C$ increase

Autumn/winter = $\delta^{13}C$ decrease

High water temperatures, high phytoplankton production, high pH, and low DIC concentrations experienced during summer, contribute to more positive $\delta^{13}C$ values.

E.g., Lehmann et al. 2004; Gu et al. 2006; Zeng et al. 2008

LAKE NUTRIENT STATUS

Primary productivity increase = $\delta^{13}C$ & $\delta^{15}N$ increase

Low dissolved CO_2 concentrations can result in enhanced invasion of the ^{13}C-enriched atmospheric CO_2.

E.g., Perga and Gerdeaux 2003; Perga and Gerdeaux 2004; McCabe 2005

Figure 2.3. Summary of the predicted major sources of variation for $\delta^{13}C$ and $\delta^{15}N$ in New Zealand lake environments.

2.2 δ^{13}C and δ^{15}N variability in streams and rivers

2.2.1 Extent of tree canopy cover

The extent of stream canopy cover, and its direct effect on the degree of light intensity and water temperature, have all been identified to strongly influence the isotopic variability of carbon in streams and rivers. As canopy cover decreases, the δ^{13}C values of primary sources, including periphyton and aquatic plants, can increase (Ehleringer et al. 1986; MacLeod and Barton 1998; Hill et al. 2008). The intercellular CO_2 concentrations, which influence carbon isotopic values, are strongly dependent on both the extent of overhead canopy development and the position of the leaf within the canopy (Ehleringer et al. 1986). With reduced canopy cover contributing to increased light penetration and warmer water temperatures, the photosynthetic rate of primary producers (i.e., the gross primary production) subsequently increases (Doi et al. 2007) (Fig. 2.4). Increased photosynthetic activity causes a decline in the levels of internal CO_2 (i.e., carbon limitation), which in turn reduces the enzymatic discrimination against ^{13}C by Rubisco, as a larger proportion of the available CO_2 is assimilated (MacLeod and Barton 1998; Finlay 2001).

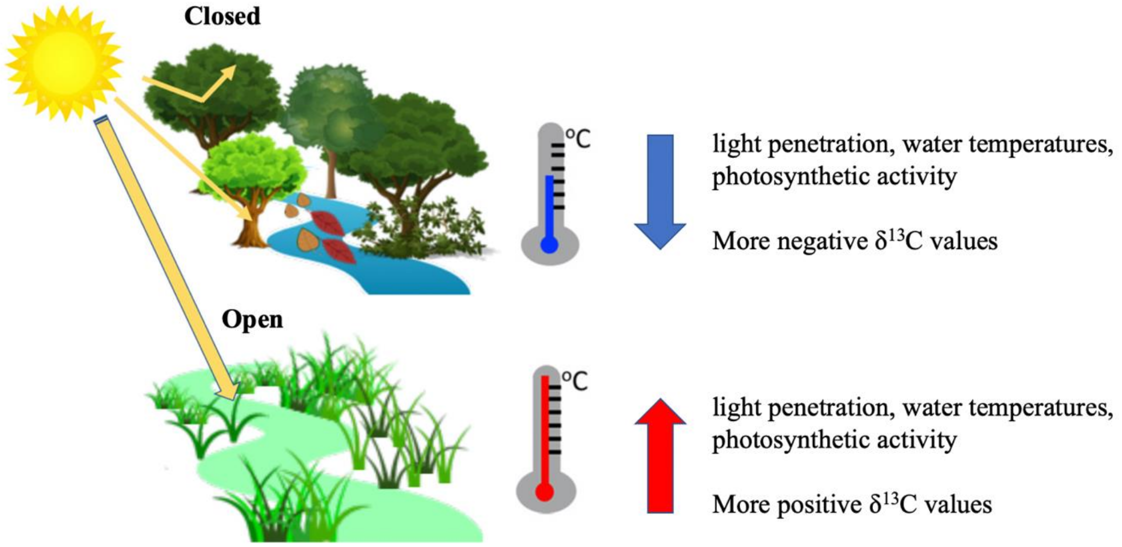


Figure 2.4. Effect of canopy cover on stream primary producer δ^{13}C values (adapted from Espinoza-Toledo et al. 2021).

Warmer water temperatures can also reduce the equilibrium fractionation between HCO_3^- and $CO_{2(aq)}$ by 1-3‰, therefore increasing the δ^{13}C $CO_{2(aq)}$ by up to 3‰ (Mook et al. 1974; Finlay 2001; Hill and Middleton 2006). Aquatic plant and periphyton δ^{13}C signatures are therefore negatively related to canopy cover, resulting in more positive δ^{13}C values for the open stream reaches. The δ^{13}C of terrestrial leaves also can decrease with canopy depth, with leaves in the upper canopy, which receive CO_2 (δ^{13}C of approximately −8‰) directly from the atmosphere, having higher δ^{13}C values compared to leaves in the lower canopy (Ehleringer et al. 1986). While less studied, it is thought that nitrogen reacts in a similar manner (MacLeod and Barton 1998), with more positive aquatic plant and periphyton δ^{15}N values at the open stream reaches which are less constrained by the influences of canopy cover.

A global meta-analysis of the factors controlling C stable isotope ratios of lotic periphyton identified a negative correlation between canopy cover and δ^{13}C values (Fig. 2.5), with periphyton captured in open habitats having significantly higher δ^{13}C signatures compared to periphyton captured in shaded habitats (Ishikawa et al. 2012). The effect of light intensity was also identified as a major driver of periphyton δ^{13}C and δ^{15}N variability in a small headwater stream in Ontario, Canada (MacLeod and Barton 1998). During the summer months under high-light conditions, increased metabolic activity and lower stream NH_4^+ concentrations, both ^{13}C and ^{15}N were enriched in the periphyton, resulting in higher δ^{13}C and δ^{15}N signatures compared to the autumn months under low-light conditions (MacLeod and Barton, 1998).

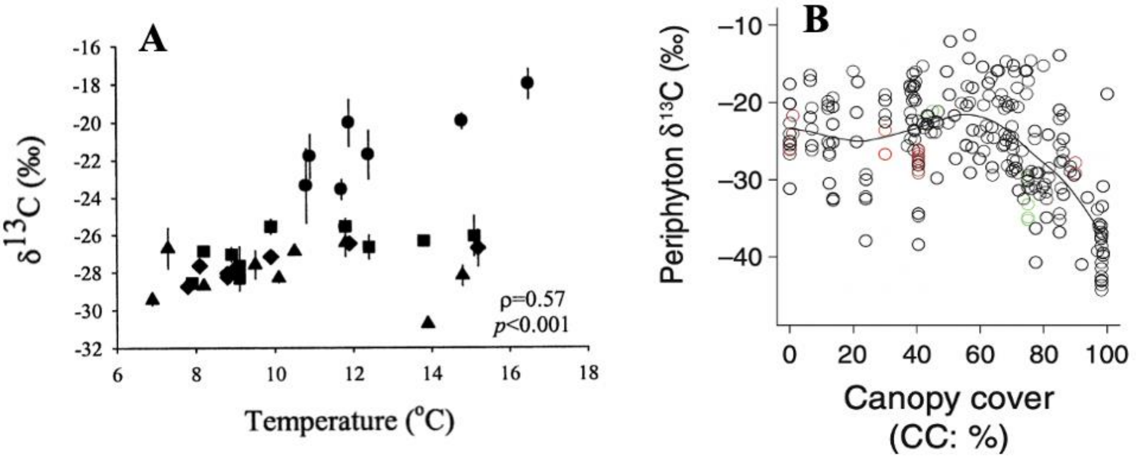


Figure 2.5. Relationship between (A) periphyton δ^{13}C and temperature (retrieved from Hill and Middleton 2006) and (B) periphyton δ^{13}C and canopy cover (Source: Ishikawa et al. 2012).

The extent of stream canopy cover is therefore likely to be one of the most significant controlling variables influencing δ^{13}C variability in streams and rivers across New Zealand. It would be expected that open streams with reduced canopy cover, enhanced light penetration, warmer water temperatures, and increased photosynthetic activity, contribute to carbon depletion and decreased fractionation rates. These open reaches are therefore likely to have more positive δ^{13}C and δ^{15}N values for primary producers, compared to the shaded stream reaches, characterised with enhanced canopy cover, reduced light penetration, cooler water temperatures, and lower rates of photosynthetic activity. While previous studies have largely focussed on the effect of canopy cover on δ^{13}C values of primary sources, we could expect to observe similar enhancement across the higher trophic levels such as fish.

2.2.2 Land use

Land use has also been identified as one of the most influential drivers of δ^{13}C and δ^{15}N variability across stream and river environments, with pasture-influenced streams typically having higher δ^{13}C and δ^{15}N values compared to forested streams (Hicks 1997; Hicks and Quinn n.d; Espinoza-Toledo et al. 2021; Machado-Silva et al. 2022). Land use changes, such as in the form of deforestation or urbanisation, can impose alterations to the natural functioning of stream and river ecosystems, including the fluvial geomorphology, water cycling, river flow regime, and water quality (Wang et al. 2021; Allan et al. 2021). Different land uses can also modify the C and N cycles, thereby changing the δ^{13}C and δ^{15}N signatures of aquatic ecosystem components (Allan 2004; Machado-Silva et al. 2022).

A number of habitat and water quality attributes have been identified that differ between streams draining in forested areas compared to streams draining into pasture in New Zealand. Pasture-influenced streams are much less shaded, with 30% of incident light reaching the stream compared to only 1 - 3% reaching native forest and pine streams, contributing to higher daily maximum (+6 to +7°C) and mean water temperatures (+2.2°C) (Quinn et al. 1997) greater than forest streams. Pasture streams also have 50% higher mean water velocities, 40% narrower reaches, 5-fold higher nitrate concentrations, 30-fold higher algal biomass, and 11-fold higher gross photosynthesis compared to native forest reaches (Quinn et al. 1997).

In New Zealand, the intensification of land-use management regimes has been shown to contribute to increased soil δ^{15}N, with managed cropped soils having the highest mean δ^{15}N value of 6.2‰, followed by pasture under dairy (5.4‰), pasture under drystock (sheep or beef cattle, 3.8‰), plantation forestry (2.8‰), and indigenous forestry (2.1‰)

(Fig. 2.6) (Stevenson et al. 2010). The application of superphosphate fertiliser, which is commonly applied in New Zealand to increase pasture production, indirectly enhances the soil N content by promoting clover growth, which in turn increases the rate of N_2 fixation (Mudge et al. 2013). Positive correlations have been identified between the soil δ^{15}N and the rate of fertiliser application and irrigation frequency, with land receiving higher rates of fertiliser or more frequent irrigation having increasing δ^{15}N values. The pasture age (i.e., the length of time following the conversion of forestry to pasture) has also been found to contribute to soil δ^{15}N enrichment in New Zealand, with soil δ^{15}N highest in the long-term, older pastures compared to recently converted pasture and forested sites (Mudge et al. 2014), with average increases from 0.015 to 0.034‰ yr^{-1} following intensification, due to the increased pasture production, grazing intensity, and N inputs and losses (Mudge et al. 2013). As a result, soils under highly intensive management regimes, which are characterised with high N input through frequent irrigation and fertiliser application, high N cycling and high N loss, are often more enriched with the ^{15}N isotope and therefore have higher δ^{15}N signatures, compared to soils under less intensive management regimes (Mudge et al. 2013).

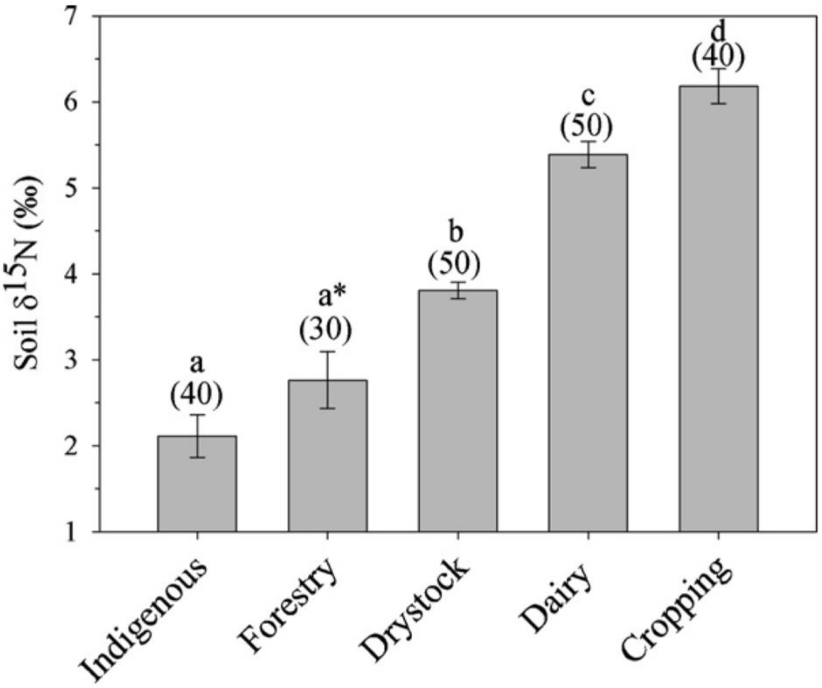


Figure 2.6. Mean soil δ^{15}N values (sampled at depths of 0 – 100mm) for different New Zealand land uses (Source: Stevenson et al. 2010).

The rate of N loss from the soil is a significant contributor to soil δ^{15}N across different land uses. Numerous fractionation processes that contribute to the loss of nitrogen from the soil, including nitrification, ammonia volatilisation, and denitrification, all of which discriminate against the heavier ^{15}N isotope, and therefore contribute to loss of ^{14}N and therefore more positive δ^{15}N signatures (Stevenson et al. 2010; Mudge et al. 2014). Subsequently, as the two major nitrogen inputs to New Zealand soils, N fixation and urea, which both have isotopic signatures of ~0‰, the soil δ^{15}N enrichment with land use intensification is largely a result of the enhanced rate of isotope-fractionating N losses from the soil (Stevenson et al. 2010). With the greater nitrogen inputs, and lower C:N ratios under pasture-dominated land (mean TN of 6.00 mg cm^{-3} and C:N of 11.0) compared to forestry (mean TN of 2.80 mg cm^{-3} and C:N of 18.2), the rate of processes contributing to N losses are higher and thus ^{15}N discrimination becomes enhanced (Stevenson et al. 2010; Mudge et al. 2014). Many studies have therefore identified land use as a significant driver of δ^{15}N variation in streams, with forested streams having more negative δ^{15}N values compared to pasture streams. For example, in Waikato streams, pasture and mixed land use sites had higher δ^{15}N (mean of 6.2‰ to 6.7‰) than in forested sites (mean of 3.2‰) (Hicks 1997; Hicks and Quinn n.d.).

Less, however, is understood regarding the influence of land use on δ^{13}C variability. Like δ^{15}N, the δ^{13}C values in Waikato streams were higher in pasture and mixed land use sites (mean of −25.1‰ to −23.0‰) compared to forested sites (mean of −26.3‰) (Hicks 1997; Hicks and Quinn n.d.). The influence of microbial respiration is thought to contribute to the δ^{13}C variability with land use, by increasing the equilibrium δ^{13}C of the soil reservoir by approximately 4.4.‰ (Amundson et al. 1998; Hicks and Quinn n.d.). Therefore, with increased grazing rates, pasture sites have enhanced soil C stocks, increased soil respiration, and higher soil δ^{13}C values (Hicks and Quinn n.d.). As pasture-influenced streams are also less shaded by the lack of overhead canopy and are more exposed to solar radiation, rates of stream primary productivity are enhanced, thereby reducing ^{13}C discrimination and leading to more positive δ^{13}C values (Machado-Silva et al. 2022; Ishikawa et al. 2012).

The influence of land use is therefore also likely to be one of the most important drivers of δ^{13}C and δ^{15}N variability for ecological components in stream and river ecosystems across New Zealand. As observed across Waikato streams (Hicks 1997; Hicks and Quinn n.d.), it would be expected that pasture-influenced streams have higher δ^{13}C and δ^{15}N values than forested streams.

2.2.3 Water velocity

In stream and river environments, water velocity can also control the carbon isotopic variability of primary producers. In general, as water velocity decreases, the $\delta^{13}C$ values of primary producers tend to increase (Keeley and Sandquist 1992; Hicks 1997; Finlay et al. 1999; France and Cattaneo 1998; Trudeau and Rasmussen 2003). Macrophytes in low energy environments are often more enriched in the heavier ^{13}C isotope, as a consequence of the effects of boundary layer thickness, and therefore have higher $\delta^{13}C$ signatures. As a result of CO_2 uptake by photosynthesis and the slow diffusion of CO_2 in water, (diffusion coefficient of CO_2 is approximately 10,000 times smaller in water than it is in air), the thickness of the boundary layer significantly controls the algae CO_2 supply rate (Keeley and Sandquist 1992; Finlay et al. 1999). The more turbulent the flow, the thinner the boundary layers around the leaves of aquatic plants are, while conversely, the more stagnant the water, the thicker the boundary layers will become (Trudeau and Rasmussen 2003). The enhanced diffusional resistance created by a thicker boundary layer adjacent to the cell membrane in low energy environments, acts to counteract the biochemical discrimination of ^{13}C isotopes by the enzyme, rubsico (Keeley and Sandquist 1992). As a consequence of carbon limitation, the reduced discrimination reduces isotopic fractionation, contributing to more enriched $\delta^{13}C$ signatures in slower flowing rivers (Fig. 2.7) (Finlay et al. 1999).

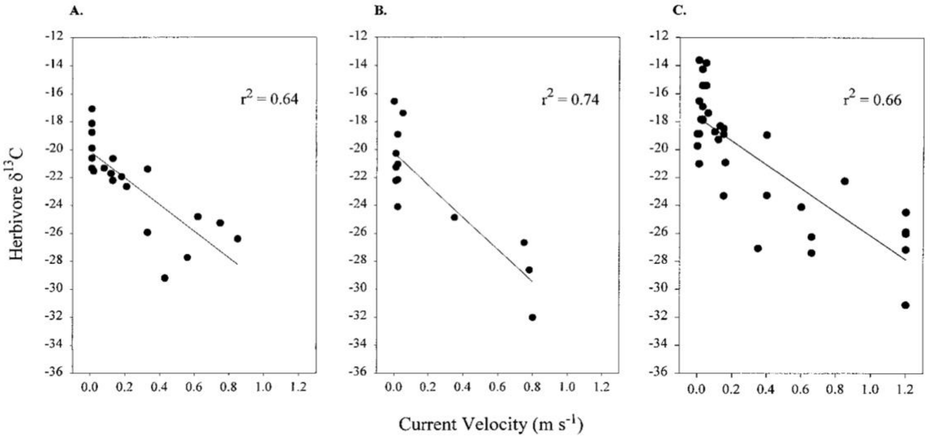


Figure 2.7. Relationship between the current velocity and herbivore $\delta^{13}C$ in three productive rivers: (A) Elder Creek, (B) Ten Mile Creek, and (C) South Fork Eel River, California, USA (Source: Finlay et al. 1999).

The effect of water velocity on the carbon isotopic difference reaches a maximum between stream pool and riffle habitats, with algae and herbivore $\delta^{13}C$ values typically higher by an average of 5 – 8‰ in pools, which generally have laminar flows with velocities < 0.25 m s^{-1}, compared to more turbulent riffles with velocities > 0.3 m s^{-1} (Finlay et al. 1999). For example, in Waikato streams, the $\delta^{13}C$ signatures for filamentous algae were more enriched in pools (mean −23.2‰) than in runs (mean −28.1‰) (Hicks 1997). These effects were most pronounced when photosynthetic rate and primary production rates were high relative to availability of CO_2 (Finlay et al. 1999).

Contrastingly, one field study reported that filamentous benthic algae displayed a positive relationship between the $\delta^{13}C$ values and stream current (France and Cattaneo 1998). With a weak inverse relationship between stream current and water colour (dissolved organic carbon (DOC) concentrations) also identified, it was thought that the photoassimilation of the respired carbon contributed to the lower $\delta^{13}C$ values, and thus overrode the expected influence of water current on the boundary layer thickness (France and Cattaneo 1998). Water velocity also had no detected effect on the isotopic fractionation and variability of periphyton $\delta^{13}C$ in a small headwater stream in Ontario, Canada, but was instead significantly influenced by factors that controlled the rate of metabolic activity, including water temperature and light intensity (MacLeod and Barton 1998).

While less studied, nitrogen isotopes react similarly to water velocity as $\delta^{13}C$, with a progressive enrichment of $\delta^{15}N$ with decreasing water velocity (Trudeau and Rasmussen 2003). However, several studies have identified no significant correlations between the $\delta^{15}N$ variability and water velocity (Hicks 1997; MacLeod and Barton 1998). Subsequently, it can be expected that water velocity may only have a small effect on $\delta^{15}N$ signature variability of aquatic ecosystem components, with other controlling variables of higher importance.

Water velocity has been identified in several studies to influence carbon isotopic signatures of primary producers in streams and rivers, but we predict that its effect on the variability of $\delta^{13}C$ and $\delta^{15}N$ in streams and rivers across New Zealand is not highly significant, and that other controlling variables are likely to be of greater importance.

2.2.4 Watershed area and stream size

The watershed drainage area of the stream has also been identified as a potential controlling variable influencing the variability of $\delta^{13}C$ (Finlay et al. 1999; Finlay 2001). Strong positive correlations have been identified between the watershed area and the $\delta^{13}C$

values for algae, herbivores, and fish, as a consequence of the reduced isotopic discrimination due to the effects of carbon limitation (Finlay 2001). Due to the reduced influence of groundwater inputs, warmer water temperatures and enhanced photosynthetic demand for CO_2, the carbon supply ($CO_{2(aq)}$) within a stream declines with increasing distance downstream (Jones and Mulholland 1998; Finlay 2001). Alternatively, the photosynthetic rates (gross primary production) tend to increase downstream as the stream widens, due to enhanced nutrient loading, alongside reduced canopy cover contributing to increased light penetration and warmer water temperatures (Finlay 2001; Lamberti and Steinman 1997). Therefore, while the $CO_{2(aq)}$ concentration decreases downstream, the demand for $CO_{2(aq)}$ increases, leading to carbon limitation where biochemical discrimination of ^{13}C isotopes by the enzyme, Rubsico is enhanced, contributing to the enrichment of ^{13}C (Finlay et al. 1999). In larger rivers, with deeper waters and self-shading of primary producers, this process may be reversed. Overall, $\delta^{13}C$ values therefore are expected to increase with increasing watershed areas, as small watersheds have a large carbon supply relative to demand, while large watersheds have a small carbon supply relative to demand (Fig. 2.8) (Finlay 2001).

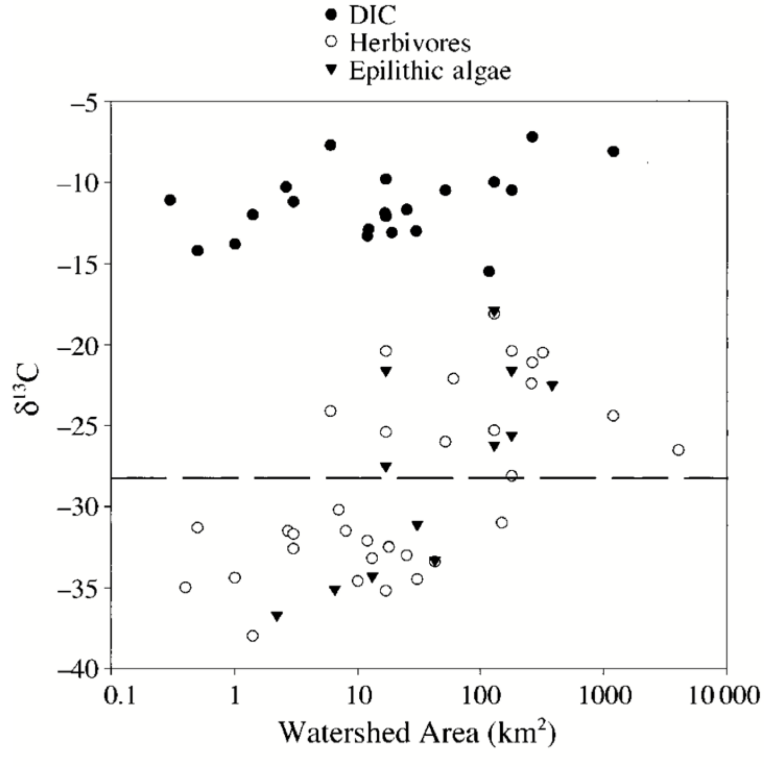


Figure 2.8. Relationship between the watershed area and the $\delta^{13}C$ of DIC, herbivores and epilithic algae (Source: Finlay 2001).

The downstream biogeochemistry changes in inorganic carbon are also thought to potentially contribute to the positive correlations reported between δ^{13}C values and the watershed drainage area (Finlay 2001). If under equilibrium conditions, it is to be expected that the δ^{13}C of $CO_{2(aq)}$ is related to the δ^{13}C of the dominant form of dissolved inorganic carbon (DIC), HCO_3^-. However, the δ^{13}C of $CO_{2(aq)}$ in productive rivers is likely to increase with declining $CO_{2(aq)}$ concentrations downstream, leading to increased contribution of atmospheric CO_2 compared to $CO_{2(aq)}$ (Finlay 2001).

The size of the watershed is likely to be another contributing driver of δ^{13}C variability in streams and rivers across New Zealand. δ^{13}C values across different trophic levels should become more positive with increasing watershed area and increasing distance downstream from the source, as a consequence of the lower carbon supply relative to demand in the larger watersheds.

2.2.5 Marine-derived nutrients

The influence of marine-derived nutrients (MDN) to freshwater habitats is a potential source of δ^{13}C and δ^{15}N variation in streams. The δ^{13}C and δ^{15}N signatures of organic material derived from the marine environment is higher than the organic material from terrestrial and freshwater habitats (Bilby et al. 1998; Helfield and Naiman 2001; Chaloner et al. 2002; Hicks et al. 2005). In general, the tissues of marine animals typically have δ^{15}N values that are 7‰ more positive than those from terrestrial and freshwater habitats (Chisholm et al. 1982; Hicks et al. 2005). Through the deposition of decaying adult salmon carcasses, eggs, and the excretion of waste, all of which are enriched with MDN, anadromous populations can act as an upstream nutrient pump by contributing marine nutrients to freshwater habitats, resulting in an enrichment of isotopic compositions within the freshwater ecosystem (Hicks et al. 2005; Duda et al. 2011).

The effect of marine-originated carbon and nitrogen on the δ^{13}C and δ^{15}N signatures from an anadromous population of coho salmon has been reported in southcentral Alaska (Hicks et al. 2005). As they mature in the ocean, the adult coho salmon incorporate ^{13}C and ^{15}N-enriched MDN that are isotopically more positive than terrestrially derived organic material (Chaloner et al. 2002; Hicks et al. 2005). The adult salmon retain these marine-influenced isotopic compositions as they enter the freshwater-riparian habitat where they spawn and die, thus providing an influx of marine nutrients to the freshwater ecosystems, and enriching the δ^{13}C and δ^{15}N values (Hicks et al. 2005). The presence of dams, however, can directly contribute to the reduction or elimination of MDN through disrupting the migratory pathways for anadromous populations (Duda et al. 2011). The impact of dams on MDN distribution was reported in southwestern Washington, USA, following the removal of the Elwha Dam, whereby within a year, the anadromous Pacific salmon (*Oncorhynchus* spp.) population enriched in MDN returned, contributing to enriched tissue δ^{13}C and δ^{15}N values (Tonra et al. 2015).

The input of marine-derived nutrients is therefore also likely to drive δ^{13}C and δ^{15}N variability in streams across New Zealand. Coastal streams with high marine-derived inputs are expected to have more positive δ^{13}C and δ^{15}N values compared to streams closer to the headwater source. Furthermore, numerous hydroelectric dams constructed in New Zealand waterways, such as the Karapiro Dam, the most downstream dam on the Waikato River, are likely to interrupt the upstream transport of MDN by fish and invertebrates such as shrimp by restricting their natural movement, thereby decreasing the δ^{13}C and δ^{15}N values (Pingram et al. 2012, West et al. 2021).

2.2.6 C_3 and C_4 fractionation

Isotopes in streams and rivers are also influenced by the additions of C from the riparian vegetation. Terrestrial plants with different photosynthetic pathways can be differentiated using δ^{13}C, as the carbon isotopic signatures of plant material vary depending on the characteristic fractionation and subsequent discrimination against ^{13}C (Ehleringer and Rundel 1989). As discussed in Chapter One, C_4 plants have much lower ^{13}C discrimination and carbon fractionation and subsequently higher δ^{13}C values, typically ranging between −7 to −23‰, compared to C_3 plants, which typically range between −23 to −34‰ (Hecky and Hesslein 1995; Fitzgerald 1996). δ^{13}C values have also been found to vary between aquatic (range from −10 to −19‰) and land plants (range from −22 to −32‰), likely due to the lower CO_2 diffusion into the cell in the aquatic medium, compared to in the gaseous phase (Fig. 2.9) (Fitzgerald 1996).

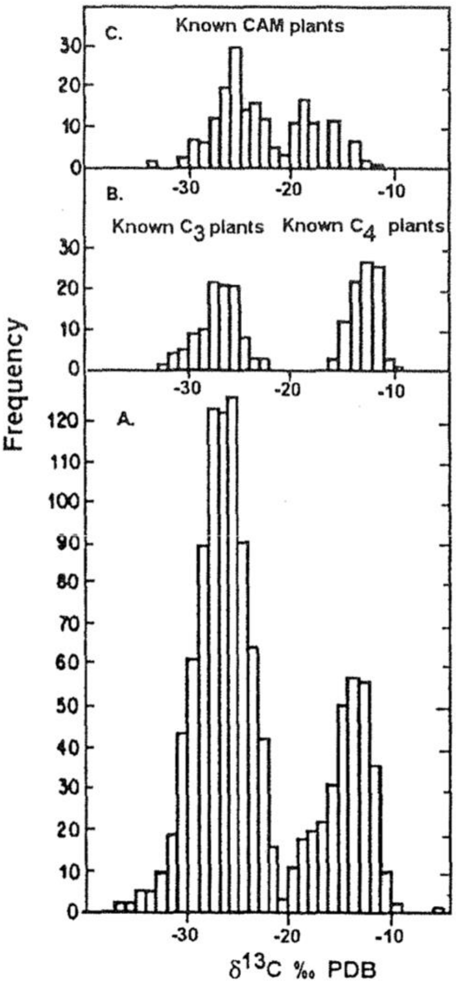


Figure 2.9. Bimodal distribution of plant ^{13}C values (Deines 1981); (A) land plants, (B) known C_3 and C_4 plants, and (C) known CAM plants (Source: Fitzgerald 1996).

However, as C_3 species are the dominant vegetation type across the selected study sites, with C_4 species largely confined to Northland and coastal areas of the northern North Island, the influence of differing photosynthetic pathways on the δ^{13}C and δ^{15}N variation in streams is not expected to be significant.

2.2.7 Gas exchange

Another influential source of δ^{13}C variation for dissolved inorganic carbon (DIC) in stream and river environments are the sources of dissolved CO_2 in waters and the processes which control the exchange of CO_2 gas with the atmosphere. The major sources of dissolved CO_2 in streams are carbonate rock weathering, mineral springs, atmospheric CO_2, and organic matter respiration (Fry 2006). The equilibrium fractionation factor between DIC and gaseous CO_2 is dependent on the relative proportions of the carbonate species ($CO_{2(aq)}$, HCO_3^-, CO_3^{2-}) within the dissolved inorganic pool (Zhang et al. 1995). During CO_2 transfer between water and the atmosphere, dissolved $CO_{2(aq)}$ reacts with H_2O to form the weak inorganic acid, carbonic acid (H_2CO_3), which further dissociates to form bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{2-}) (Allan et al. 2021) according to the reversible dissociation in Eq. 1:

Increasing pH → → → → → → → → → → →

$$CO_2 + H_2O \leftrightharpoons H_2CO_3 \leftrightharpoons H^+ + HCO_3^- \leftrightharpoons 2H^+ + CO_3^{2-} \qquad \text{Eq. 1.}$$

When the pH value of the water is very low (i.e., below 4.5), only CO_2 and H_2CO_3 will be present, with almost no HCO_3^- or CO_3^{2-} (Allan et al. 2021). Conversely, at higher pH values, H_2CO_3 begins to dissociate, thus causing the bicarbonate and carbonate ions to dominate, with very little CO_2 and H_2CO_3 present (Allan et al. 2021). When the pH value is intermediate, HCO_3^- dominates (Fig. 2.10) (Allan et al. 2021). Bicarbonate and carbonate ions have enriched (more positive) δ^{13}C values by up to 10‰ compared to the atmosphere (Oczkowski et al. 2018), and therefore the use of HCO_3^- and CO_3^{2-} as a carbon substrate will result in higher δ^{13}C values. As the pH of the stream increases (i.e., becomes more basic) and HCO_3^- becomes the dominant carbon species, the δ^{13}C values of DIC will become more positive (Oczkowski et al. 2014). The process of carbonate weathering can also act to increase the δ^{13}C of DIC, as a result of the formation of enriched ^{13}C carbonates, whose δ^{13}C signatures reflect those of carbonate rocks (Fitzgerald 1996). The influx of groundwater also can be an influential source of δ^{13}C variation for river and stream environments. Groundwaters are typically supersaturated with $CO_{2(aq)}$, as most of the CO_2 will be derived from plant root respiration, and to a lesser extent, the oxidation of organic matter (Fitzgerald 1996; Jones and Mulholland, 1998; Finlay 2003). The relative groundwater contributions to a stream relative to the stream volume are expected to decline as the stream size increases (Finlay 2003).

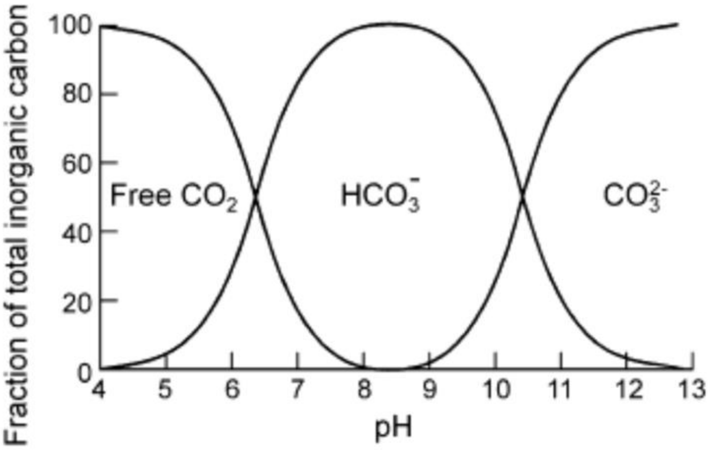


Figure 2.10. Relationship between the pH and the relative proportions of inorganic carbon species in solution (Source: Wetzel 2001).

The δ^{13}C values are therefore likely to vary depending on the origin of dissolved CO_2 in streams and the relative proportions of the carbonate species ($CO_{2(aq)}$, HCO_3^-, CO_3^{2-}). Streams dominated by CO_3^{2-} and HCO_3^- substrates, both of which are ^{13}C-enriched, would be expected to have more positive δ^{13}C values. Thus streams in carbonate-rich rock types, e.g., limestones, should have more ^{13}C-enriched carbon species (Hendy 1971).

2.2.8 Seasonality

In streams and rivers, the primary producer δ^{13}C and δ^{15}N values are also likely to vary seasonally. Seasonal changes by up to 5‰ have been identified in the δ^{13}C values of terrestrial plants, in response to stomatal closure during water stress, due to alterations in the micro-meteorological variables and soil water availability (Walcroft et al. 1997). Changing light levels throughout the year has also been identified to alter the δ^{13}C values of actively growing plants by up to 4.5‰ (McCabe 1985). Seasonal defoliation in deciduous forest rivers during the winter period reduces the extent of canopy cover, thereby increasing light reaching the stream surface, the water temperature, and the rates of gross primary production (Roberts et al. 2007; Ishikawa et al. 2012). Higher waters temperatures contribute to enhanced rates of metabolic and enzymatic activity, and substrate uptake, resulting in a reduction in the discrimination against the heavier isotopes, and therefore higher δ^{13}C signatures. As a result, due to the higher δ^{13}C values of dissolved inorganic carbon (DIC) and reduced ^{13}C discrimination during productive base flow periods, algal δ^{13}C signatures are typically higher during summer and autumn base flow periods, compared to during the winter and spring months (Finlay 2001).

Seasonal patterns in δ^{13}C values can also be attributed to the changes in the atmospheric CO_2 concentrations throughout the year (Fig. 2.11). During the summer growing season, when plant photosynthetic activity is high, the source pool of atmospheric CO_2 becomes depleted. Isotope fractionation during photosynthesis withdraws ^{13}C-depleted carbon from the atmospheric pool, leaving behind the heavier ^{13}C isotope, contributing to more positive δ^{13}C values during the summer months (Fry 2006). Conversely, during the autumn and winter months, the rate of photosynthesis decreases while plant and microbial respiration dominates, allowing for the pool of atmospheric CO_2 to be regenerated with ^{13}C-depleted carbon, contributing to more negative δ^{13}C values during autumn and winter (Fry 2006). Seasonal differences in δ^{13}C are considered to be most significant in smaller streams and rivers (Fitzgerald 1996).

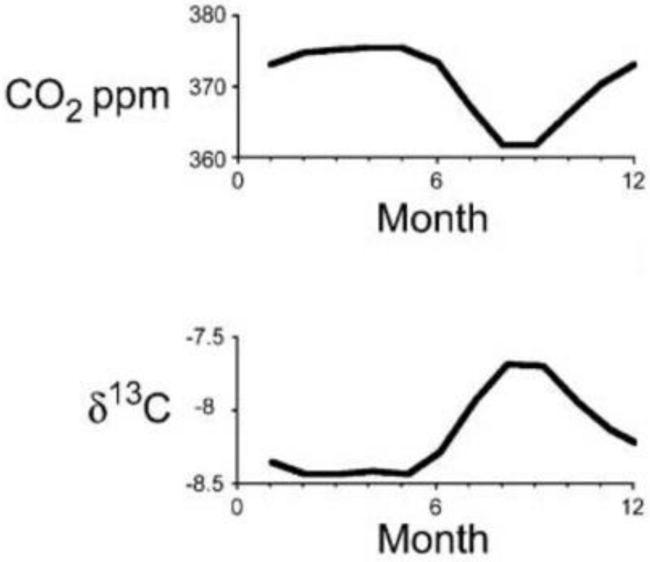


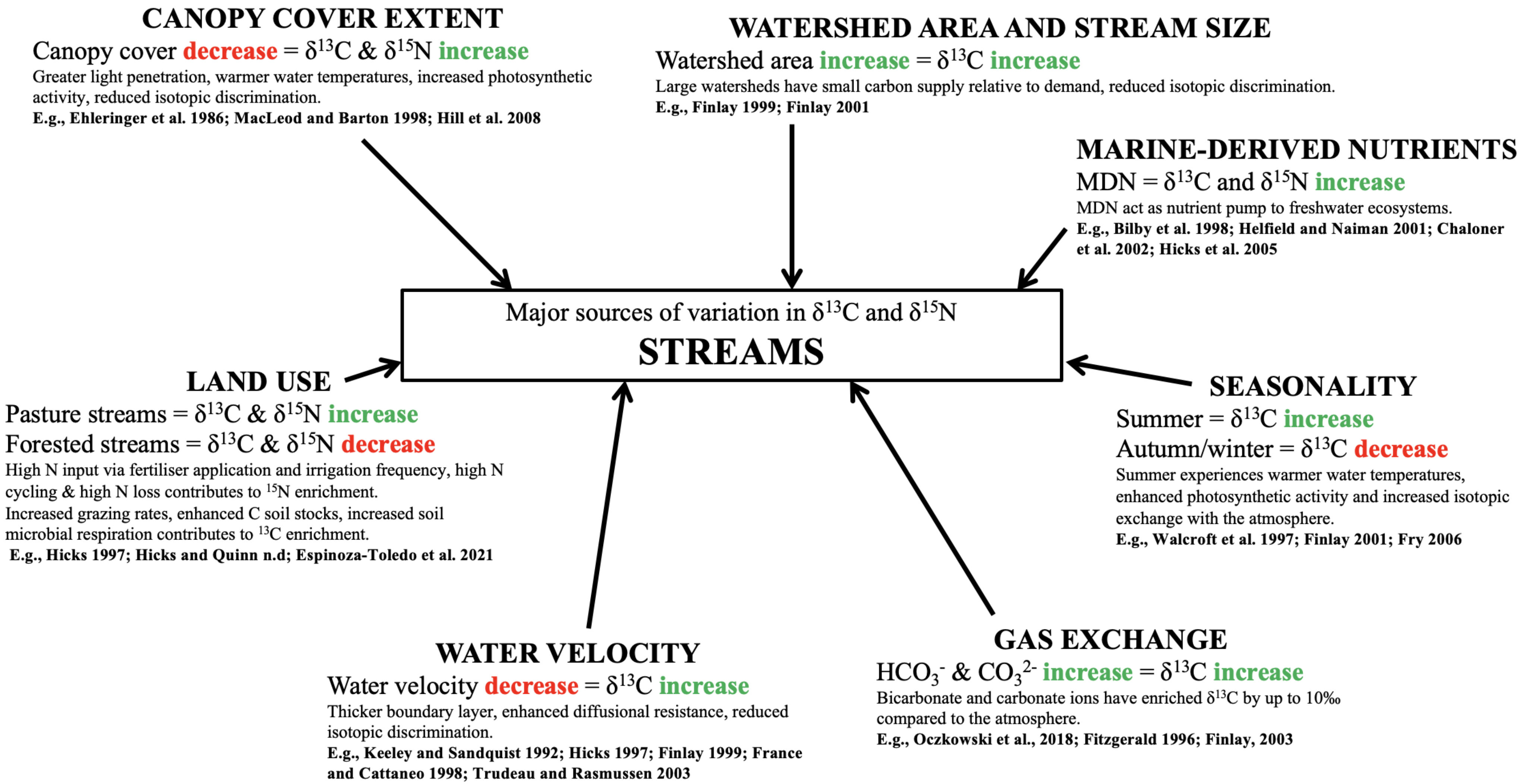
Figure 2.11. Seasonal patterns of atmospheric CO_2 concentration (top) and δ^{13}C values (bottom) during a January (month = 1) to December (month = 12) cycle in the northern hemisphere (Source: Fry 2006).

While the δ^{15}N values are also shown to significantly vary across seasons in streams, the underlying controlling mechanisms are much less understood (Finlay et al. 2002). For example, in the South Fork Eel River, Northern California, USA, the δ^{13}C and δ^{15}N values of juvenile trout (10 – 15 cm standard length) displayed seasonal variability, whereby during early June to late August (the northern hemisphere productive summer period), δ^{13}C values increased by 4 - 6‰, while δ^{15}N decreased by 2 - 3‰ (Finlay et al. 2002). As algal δ^{15}N trends often follow the opposite seasonal pattern to δ^{13}C (higher during winter and spring months compared to summer and autumn base flow periods), changes in the δ^{15}N of dissolved inorganic nitrogen (DIN) or algal discrimination against ^{15}N is thought to contribute to the seasonal algal δ^{15}N patterns (Finlay et al. 2002).

Overall, δ^{13}C values of primary producers are likely to display seasonal patterns, with more positive δ^{13}C during the summer months due to enhanced photosynthetic activity, increased isotopic exchange with the atmosphere, and warmer water temperatures, and less positive δ^{13}C during the autumn and winter months due to regeneration of the atmospheric CO_2 pool through plant and microbial respiration. The differences, however, are unlikely to be significant in the larger rivers, due to the lack of major climatic seasonal differences and the relatively short retention times in the hydro lakes (Fitzgerald 1996). Marked seasonal changes in the isotopic signatures of secondary and tertiary consumers in the Waikato River, however, have been documented (Pingram 2014).

2.2.9 Summary of environmental controls on δ^{13}C and δ^{15}N in streams

Numerous environmental and climatic factors have been documented to influence the δ^{13}C and δ^{15}N values of basal resources and organisms in streams and rivers (Fig. 2.12). Overall, we would hypothesise that for New Zealand streams and rivers, a pronounced upstream-downstream gradient in δ^{13}C and δ^{15}N would exist, in which the headwaters likely would have relatively low isotopic signatures with a gradual enrichment downstream. Open pasture streams, typically located at lower elevations and increased distance from the source, with reduced canopy cover, enhanced light penetration, warmer water temperatures, higher marine-derived inputs, larger watersheds, and increased photosynthetic activity, all can contribute to carbon depletion and decreased fractionation rates. These open reaches are therefore likely to have more positive δ^{13}C and δ^{15}N values for aquatic organisms, compared to the shaded forested stream reaches, typically located at higher elevations, characterised with enhanced canopy cover, reduced light penetration, cooler water temperatures, smaller watersheds, lower rates of photosynthetic activity.



CANOPY COVER EXTENT

Canopy cover **decrease** = $\delta^{13}C$ & $\delta^{15}N$ **increase**

Greater light penetration, warmer water temperatures, increased photosynthetic activity, reduced isotopic discrimination.

E.g., Ehleringer et al. 1986; MacLeod and Barton 1998; Hill et al. 2008

WATERSHED AREA AND STREAM SIZE

Watershed area **increase** = $\delta^{13}C$ **increase**

Large watersheds have small carbon supply relative to demand, reduced isotopic discrimination.

E.g., Finlay 1999; Finlay 2001

MARINE-DERIVED NUTRIENTS

MDN = $\delta^{13}C$ and $\delta^{15}N$ **increase**

MDN act as nutrient pump to freshwater ecosystems.

E.g., Bilby et al. 1998; Helfield and Naiman 2001; Chaloner et al. 2002; Hicks et al. 2005

Major sources of variation in $\delta^{13}C$ and $\delta^{15}N$

STREAMS

LAND USE

Pasture streams = $\delta^{13}C$ & $\delta^{15}N$ **increase**

Forested streams = $\delta^{13}C$ & $\delta^{15}N$ **decrease**

High N input via fertiliser application and irrigation frequency, high N cycling & high N loss contributes to ^{15}N enrichment.

Increased grazing rates, enhanced C soil stocks, increased soil microbial respiration contributes to ^{13}C enrichment.

E.g., Hicks 1997; Hicks and Quinn n.d; Espinoza-Toledo et al. 2021

SEASONALITY

Summer = $\delta^{13}C$ **increase**

Autumn/winter = $\delta^{13}C$ **decrease**

Summer experiences warmer water temperatures, enhanced photosynthetic activity and increased isotopic exchange with the atmosphere.

E.g., Walcroft et al. 1997; Finlay 2001; Fry 2006

WATER VELOCITY

Water velocity **decrease** = $\delta^{13}C$ **increase**

Thicker boundary layer, enhanced diffusional resistance, reduced isotopic discrimination.

E.g., Keeley and Sandquist 1992; Hicks 1997; Finlay 1999; France and Cattaneo 1998; Trudeau and Rasmussen 2003

GAS EXCHANGE

HCO_3^- & CO_3^{2-} **increase** = $\delta^{13}C$ **increase**

Bicarbonate and carbonate ions have enriched $\delta^{13}C$ by up to 10‰ compared to the atmosphere.

E.g., Oczkowski et al., 2018; Fitzgerald 1996; Finlay, 2003

Figure 2.12. Summary of the predicted major sources of variation for $\delta^{13}C$ and $\delta^{15}N$ in New Zealand stream environments.

Chapter 3

Methods

3.1 Study area

3.1.1 Isotope data sources

We collated lake and stream δ^{13}C and δ^{15}N data from a range of published and unpublished scientific literature (Table 3.1; Table 3.2) across New Zealand. For each lake and stream site, we recorded the geographic coordinates (in New Zealand Transverse Mercator 2000 (NZTM2000) easting and northing), the date and season of sample collection, the carbon content (% C), nitrogen content (% N), and the C:N ratio where available. As we were largely focussed on analysing the spatial patterns of isotopic values as opposed to the temporal variation, the majority of samples used in the analysis were collected during the New Zealand summer months (December to February) to reduce the potential seasonal variability, particularly for taxa occupying the lower trophic positions (Chapter Two).

A total of 1,757 fish samples from 88 lakes were collated from 11 publications (Table 3.1). The lake fish species were further classified into the four major trophic types; herbivores (n = 40), secondary consumers (n = 713), marine wanderers (n = 117), and top predators (n = 887). A total of 1290 fish samples from 171 stream site locations were collated from 15 publications (Table 3.1). The stream fish species were further classified into three major trophic types; secondary consumers (n = 587), marine wanderers (n = 61), and top predators (n = 629). In combination with the fish data, invertebrate isotopic values were also collated. A total of 2422 invertebrate samples from 426 stream site locations were collated from 25 publications (Table 3.2). As with the fish data, invertebrates were further classified depending on whether they were predominately predatory or non-predatory (i.e., primary versus secondary consumers).

Table 3.1. δ^{13}C and δ^{15}N data sources for New Zealand lakes and the location and food web items provided by each.

Location	Food web items	Citation
Rotorua Lakes District, Bay of Plenty	Invertebrate & fish	McBride CG. 2005. Stable isotopes in the Rotorua lakes: food web structures and relationships to lake morphology and limnology. Unpublished MSc thesis. Hamilton: University of Waikato.
Lake Tarawera, Bay of Plenty	Invertebrate & fish	Pora R. 2021. Seasonal dynamics of food webs in Lake Tarawera using stable isotopes. Unpublished MSc dissertation. Hamilton: University of Waikato.
Canterbury, Otago & Southland	Fish	Drake DC, Kelly D, Schallenberg M. 2011. Shallow coastal lakes in New Zealand: current conditions, catchment-scale human disturbance, and determination of ecological integrity. Hydrobiologia. 658:87-101
Waikato	Fish	Collier KJ, Pingram MA, Francis L, Garrett-Walker J, Melchior M. 2018. Trophic overlap between non-native brown bullhead (*Ameiurus nebulosus*) and native shortfin eel (*Anguilla australis*) in shallow lakes. Ecology of Freshwater Fish. 27: 888-897.
Lake Rotokare, Taranaki	Fish	Smith DR. 2018. The aquatic ecology of Lake Rotokare. MSc thesis. Hamilton: University of Waikato.
Lake Waikare, Waikato	Fish	Hicks BJ, Smith DR, Pingram MA, Kelly DJ, Fraley KM. Conservation of freshwater eels in food-web studies: Non-lethal stable isotope analyses substitute fin for muscle tissue with lipid correction. Ecology of Freshwater Fish 31(3): 515-528.
Waikato (Waikare, Whangape, Waahi)	Invertebrate & fish	Brijs J, Hicks BJ, Powrie W. 2009. Abundance of mysid shrimp (*Tenagomysis chiltoni*) in shallow lakes in the Waikato region and implications for fish diet. CBER Report 107. Prepared for Environment Waikato by Centre for Biodiversity and Ecology Research, University of Waikato.
Turitea Reservoir, Palmerston North	Fish	Hicks BJ, Brijs J, Bell DG, Ling N, Blair JM, Powrie W. 2009. Boat electrofishing survey of the upper Turitea Reservoir, Palmerston North. Prepared for Palmerston North City Council by Centre of Biodiversity and Ecology Research, University of Waikato.

Table 3.1 (continued). δ^{13}C and δ^{15}N data sources for New Zealand lakes and the location and food web items provided by each.

Location	Food web items	Citation
Waikato (Horseshoe lake)	Fish	Hicks BJ. 2005. Boat electrofishing of Waiwhakareke (Horseshoe) Lake in May 2005. Unpublished CBER report.
Canterbury (Lake Coleridge)	Fish	James MR, Hawes I, Weatherhead M, Stanger C, Gibbs M. 2000. Carbon flow in the littoral food web of an oligotrophic lake. Hydrobiologia. 441: 93-106
Waikato (Lake Taupo)	Fish	Stewart SD, Hamilton DP, Baisden WT, Dedual M, Verburg P, Duggan IC, Hicks BJ, Graham BS. 2017. Variable littoral-pelagic coupling as a food-web response to seasonal changes in pelagic primary production. Freshwater Biology. 62(12): 2008-2025

Table 3.2. δ^{13}C and δ^{15}N data sources for New Zealand streams and the location and food web items provided by each.

Location	Food web items	Citation
Waikato	Invertebrate & fish	Hicks BJ. 1997. Food webs in forest and pasture streams in the Waikato region, New Zealand: A study based on analyses of stable isotopes of carbon and nitrogen, and fish gut. New Zealand Journal of Marine and Freshwater Research. 45: 651-664.
Mangaotama Stream, Waikato	Invertebrate & fish	Hicks BJ, Quinn JM. n.d. Stream food webs along a longitudinal gradient: a reconsideration of trophic discrimination factors. New Zealand Journal of Marine and Freshwater Research.
Taranaki	Invertebrate & fish	Witton M. 2022. Fish biomass in Taranaki streams in relation to sources and availability of energy. PhD thesis. Hamilton: University of Waikato.
Canterbury	Fish	Fraley KM, Warburton HJ, Jellyman PG, Kelly D, McIntosh AR. 2018. Responsiveness of fish mass–abundance relationships and trophic metrics to flood disturbance, stream size, land cover and predator taxa presence in headwater streams. Ecology of Freshwater Fish. 27: 999-1014
Waikato	Fish	Brijs J, Hicks BJ, Bell DG. 2008. Electrofishing survey of the fish community in the Whangamarino Wetland. CBER. Contract Report No. 67, Centre for Biodiversity and Ecology Research. Hamilton: University of Waikato.
Waikato River	Invertebrate & fish	Osborne MW. 2006. Ecology of koi carp (*Cyprinus carpio*) in the Waikato River. MSc thesis. Hamilton: University of Waikato.
Waikato River	Fish	DW West. 2007. Responses of freshwater fish to anthropogenic factors in the Waikato River of New Zealand. PhD thesis. Hamilton: University of Waikato.
Waikato	Invertebrate & fish	Watene EM. 1997. The use of stable isotopic analyses to determine the fate of pulp and paper mill effluent in the Waikato River. MSc thesis. Hamilton: University of Waikato.
Waihou and Ohinemuri River	Fish	Hicks BJ. (Unpublished data)
Bay of Plenty, Canterbury, South Otago/Southland	Invertebrate	Clapcott JE, Young RG, Goodwin EO, Leathwick JR. Exploring the response of functional indicators of stream health to land-use gradients. Freshwater Biology. 55: 2181-2199.
Waikato	Invertebrate	Doehring K, Clapcott JE, Young RG. 2019. Assessing the functional response to streamside fencing of pastoral Waikato streams, New Zealand. Water. 11: 1347.

Table 3.2. (continued). δ^{13}C and δ^{15}N data sources for New Zealand streams and the location and food web items provided by each.

Location	Food web items	Citation
Manawatu-Whanganui	Invertebrate	Wagenhoff A, Liess A, Pastor A, Clapcott JE, Goodwin EO, Young RG. 2016. Thresholds in ecosystem structural and functional responses to multiple stressors. Freshwater Science. 36(1): 178-194.
Canterbury	Invertebrate	Greenwood MJ. McIntosh AR, Harding JS. 2010. Disturbance across an ecosystem boundary drives cannibalism propensity in a riparian consumer. Behavioural Ecology. 21(6): 1227-1235.
Canterbury	Invertebrate	Nyström P, McIntosh AR, Winterbourn MJ. 2003. Top-down and bottom-up processes in grassland and forested streams. Community Ecology. 136: 596-608.
Whakakai Stream, Waikato	Invertebrate	Collier KJ, Wright-Stow AE, Smith BJ. 2010. 2004. Trophic basis of production for a mayfly in a North Island, New Zealand, forest stream: Contributions of benthic versus hyporheic habitats and implications for restoration. New Zealand Journal of Marine and Freshwater Research. 38(2): 301-314.
Otago	Invertebrate	Hollows JW, Townsend CR, Collier KJ. 2010. Diet of the crayfish *Paranephrops zealandicus* in bush and pasture streams: insights from stable isotopes and stomach analysis. New Zealand Journal of Marine and Freshwater Research. 36(1): 129-142.
Canterbury	Invertebrate	Greenwood MJ. 2014. More than a barrier: the complex effects of ecotone vegetation type on terrestrial consumer consumption of an aquatic prey resource. Austral Ecology. 39: 941-951.
Canterbury	Invertebrate	Greenwood MJ. (Unpublished data)
Otago	Invertebrate	Lange K, Townsend CR, Matthaei CD. 2019. Inconsistent relationships of primary consumer N stable isotope values to gradients of sheep/beef farming intensity and flow reduction in streams. Water. 11:2239.
Canterbury	Invertebrate & fish	McHugh PA, McIntosh AR, Jellyman PG. 2010. Dual influences of ecosystem size and disturbance on food chain length in streams. Ecology Letters. 13: 881-890.
Canterbury	Invertebrate & fish	McHugh PA, McIntosh AR, Howard S, Budy P. 2012. Niche flexibility and trout–galaxiid co-occurrence in a hydrologically diverse riverine landscape. Biological Invasions. 14: 2393-2406.
Canterbury	Invertebrate & fish	McHugh PA, Thompson RM, Greig HS, Warburton HJ, McIntosh AR. 2015. Habitat size influences food web structure in drying streams. Ecography. 38: 700-712.

Table 3.2 (continued). δ^{13}C and δ^{15}N data sources for New Zealand streams and the location and food web items provided by each.

Location	Food web items	Citation
Waikato	Invertebrate & fish	Parkyn SM, Collier KJ, Hicks BJ. 2001. New Zealand stream crayfish: functional omnivores but trophic predators? Freshwater Biology. 46: 641-652.
Otago	Invertebrate	Simon KS, Niyogi DK, Frew RD, Townsend CR. 2007. Nitrogen dynamics in grassland streams along a gradient of agricultural development. Limnology and Oceanography. 52(3): 1246-1257.
Canterbury	Invertebrate	Stewart S. 2011. Using stable isotopes to trace the sources and fates of nitrate within mixed land-use catchments on the Banks Peninsula, New Zealand. MSc thesis. Christchurch: University of Canterbury.
Canterbury	Invertebrate & fish	Graham BS. (Unpublished data)
Waikato	Invertebrate & fish	Pingram MA. 2014. Food webs in the lower Waikato River and the role of hydrogeomorphic complexity. PhD thesis. Hamilton: University of Waikato.
Canterbury	Invertebrate	Burdon FJ, McIntosh AR, Harding JS. 2019. Mechanisms of trophic niche space compression: evidence from agricultural disturbance. Journal of Animal Ecology. 89(3): 730-744.
Buller-grey / West coast	Invertebrate	Hogsden KL, Harding JS. 2014. Isotopic metrics as a tool for assessing the effects of mine pollution on stream food webs. Ecological Indicators. 36: 339-347.

3.1.2 Study area for lakes

The carbon and nitrogen stable isotopic data used for the analysis was collated from a set of 88 lakes across New Zealand, comprising of 25 lakes in Waikato, 17 in Wellington, 11 in Bay of Plenty, six in Northland, five in Canterbury, five in West coast, three in Hawkes Bay, three in Manawatu-Wanganui, three in Otago, three in Tasman, three in Southland, two in Auckland and two in Taranaki (Figs. 3.1, 3.2, 3.3). The sampled sites also included a range of geomorphic origin lake types (as classified by FENZ (Freshwater Ecosystems of New Zealand)); including aeolian (n = 17), dammed (n = 12), glacial (n = 1), peat (n = 5), riverine (n = 18), shoreline (n = 13), tectonic (n = 2) and volcanic (n = 16) lakes. Many of the lakes were located at low elevations (69.3% at < 100 MASL), were relatively close to the coast (81% were located < 50 km of the coast), and all had a surface area > 1 ha (range of 1.7 ha, Coopers Lagoon, to 61,264.5 ha, Lake Taupo; Table 3.1).

3.1.3 Study area for streams

A total of 475 stream sites were included in the dataset and used for analyses of δ^{13}C and δ^{15}N variability across New Zealand rivers and streams. The stream isotopic samples were collated from a range of stream sites across the country, including in Auckland (n = 2), Waikato (n = 87), Bay of Plenty (n = 15), Taranaki (n = 23), Manawatū-Whanganui (n = 58), West Coast (n = 12), Canterbury (n = 197), Otago (n = 64), and Southland (n = 16) (Fig. 3.4). A large proportion of the available fish isotopic data was collected from studies conducted in low order streams, with 88% of δ^{13}C and δ^{15}N collected from stream orders one, two, three and four. The site locations also varied with land use with just under half (48%) of stream sites situated in catchments with > 50% upstream pasture (Table 3.2).

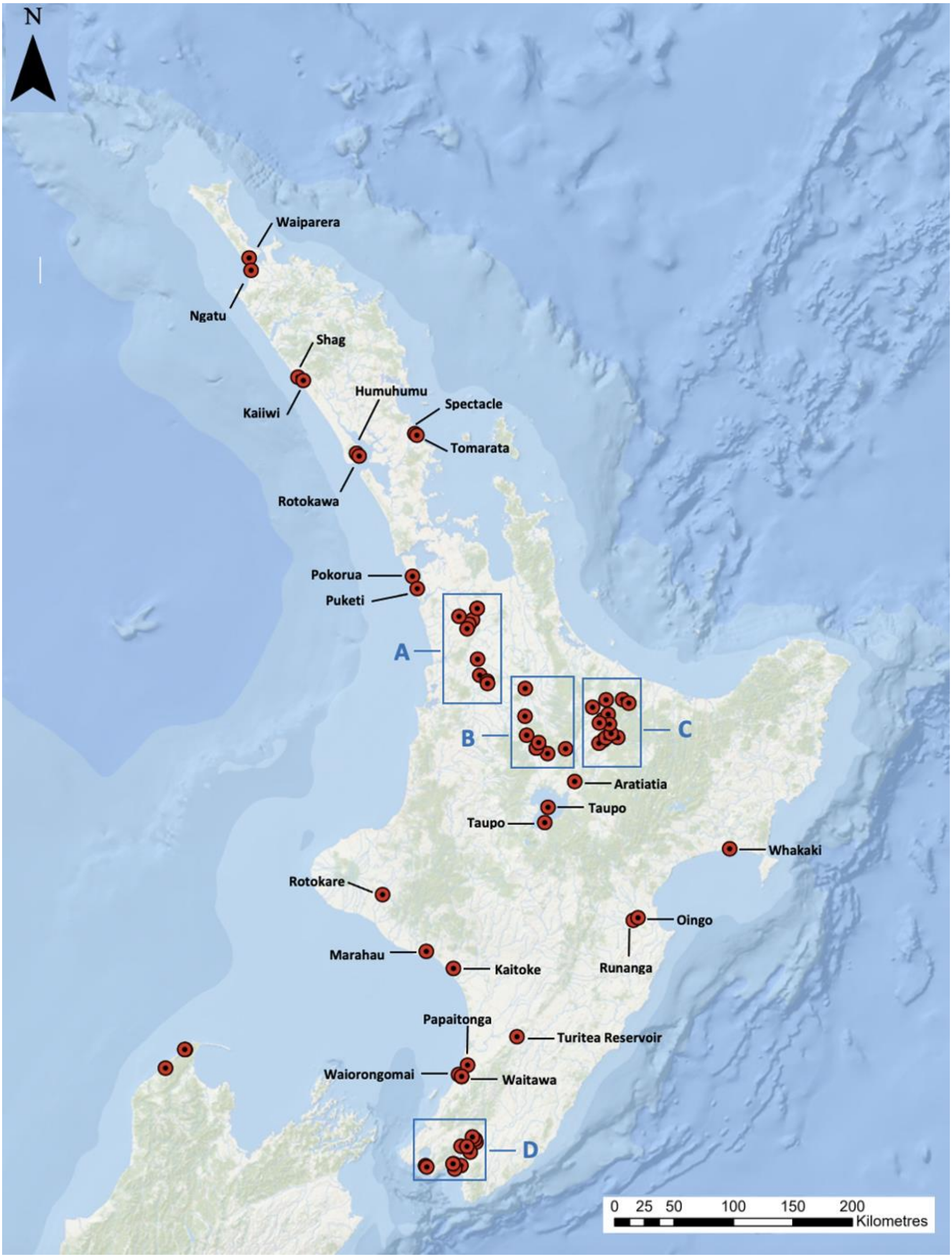


Figure 3.1. Location of lakes (with site names labelled) included in the collated dataset for North Island, New Zealand. Due to clustering of sites, boxes A, B, C and D are provided in Fig. 3.2.

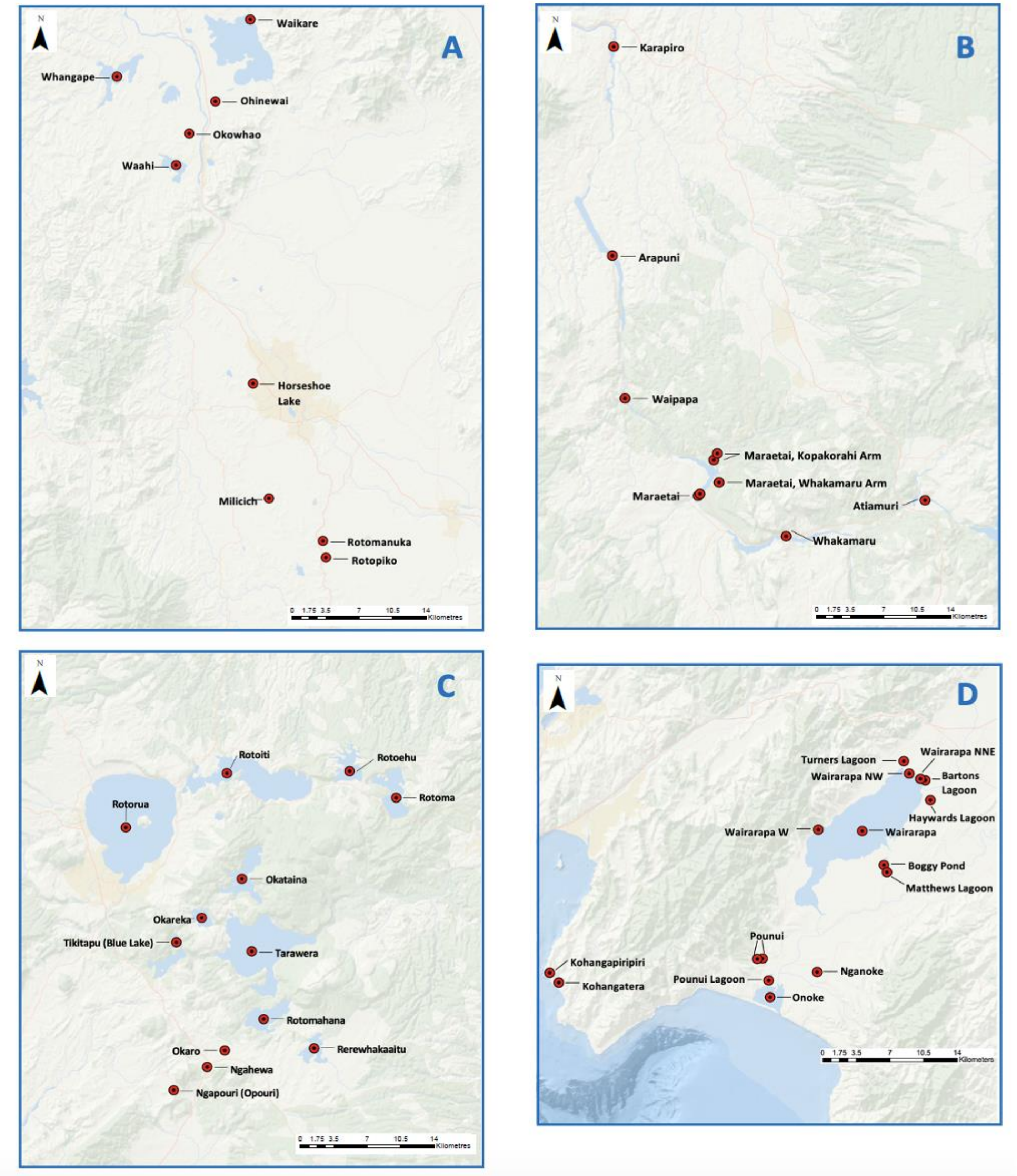


Figure 3.2. Location of lakes (with site names labelled) included in the collated dataset for boxes A (upper Waikato), B (lower Waikato), C (Rotorua) and D (Wellington) from Fig. 3.1.

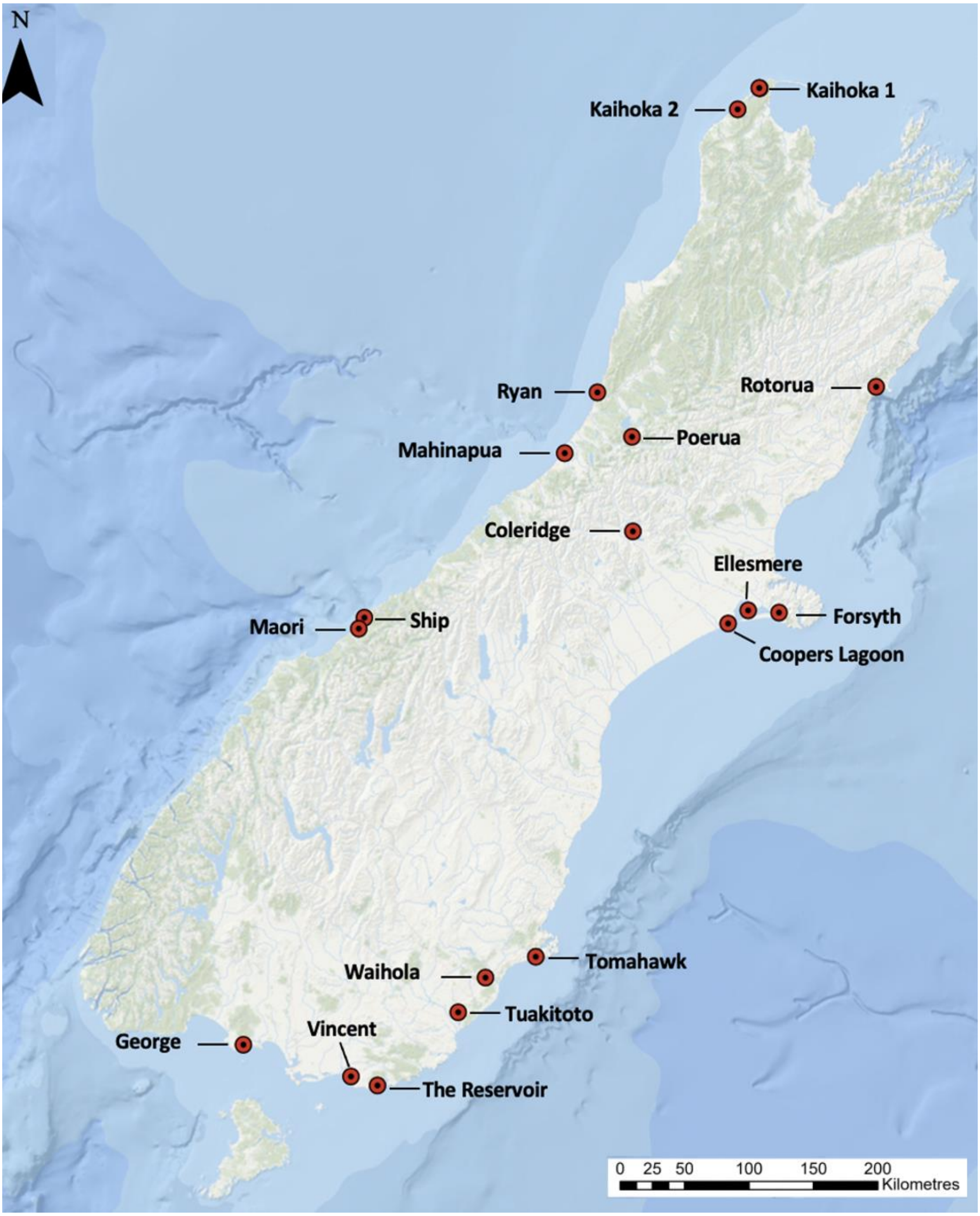


Figure 3.3. Location of lakes (with site names labelled) included in the collated dataset for South Island, New Zealand.

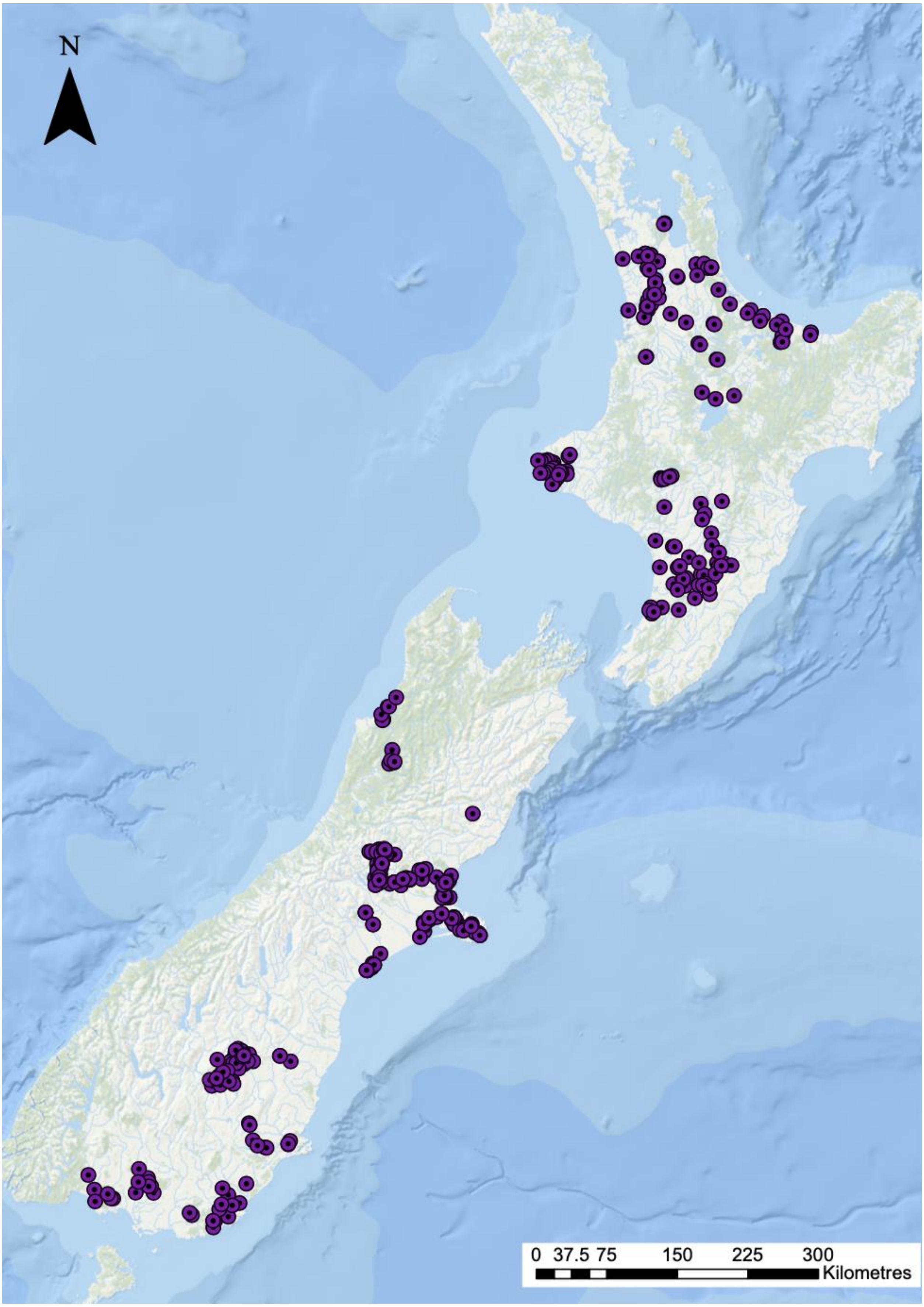


Figure 3.4. Location of streams included in the collated dataset for South Island, New Zealand.

3.2 Environmental variables

To explore the landscape-level patterns in δ^{13}C and δ^{15}N across New Zealand's aquatic ecosystems, 17 environmental variables for lakes and 16 variables for streams, were obtained through remotely sensed and landscape-modelled geospatial data sources.

3.2.1 Environmental variables for lakes

The environmental variables selected to analyse the fish δ^{13}C and δ^{15}N variability in lakes were derived from the Freshwater Ecosystems of New Zealand (FENZ) database (Leathwick et al. 2010) (Table 3.3). The FENZ geodatabase consists of numerous spatial data layers that provide a national representation of the environmental and biological patterns across New Zealand's rivers, lakes, and wetlands (Leathwick et al. 2010). The environmental factors selected for this study included variables associated with the lake system size, such as lake surface area, volume, catchment area, and mean depth, as well as land use influenced impacts, including percent forest and percent pasture in the lake catchment, and nitrogen loading estimates.

3.2.2 Environmental variables for streams

The environmental variables selected to analyse the invertebrate and fish δ^{13}C and δ^{15}N variability in streams were derived from the Freshwater Ecosystems of New Zealand (FENZ) database (Leathwick et al. 2010), the River Environment Classification (REC) database (Snelder and Biggs 2002) and the National Institute of Water and Atmospheric Research (NIWA) New Zealand's river maps (Whitehead and Brooker 2019) (Table 3.4). As mentioned in Chapter Two, the stream water temperature has been observed to contribute to δ^{13}C and δ^{15}N variability across streams (Doi et al. 2007; Espinoza-Toledo et al. 2021). However, very few of the isotope datasets provided recordings of the stream water temperature for each δ^{13}C and δ^{15}N value, plus were difficult to obtain accurate estimations from nationwide geospatial data sources. Subsequently, the mean annual water temperature for each stream study location was predicted using the latitude and altitude of the measurement site following equation 2 from Mosley (1982):

$$T = 95.8 - 46.5 \log(\text{LAT}) - 3.46 \log(\text{MNEL}),$$

where T is the mean temperature, LAT is the latitude in decimal degrees (accounting for 52% of stream water temperature variation), and MNEL is the mean elevation in m of the point, i.e., halfway between the recorder site and the highest point measured along the stream channel (accounting for 21% of variation).

Table 3.3. Summary of variables used for the analysis of aquatic organisms δ^{13}C and δ^{15}N spatial variability in lakes. All variables except for mean lake depth were sourced from the FENZ geodatabase (Leathwick et al. 2010).

Environmental variable	Description	Unit	Range
Elevation	Elevation of lake	m	0.2 – 477.4
Catchment elevation	Mean catchment elevation	m	2.8 – 872.7
Perimeter	Perimeter of lake	m	581.8 – 175,627
Area	Surface area of lake	ha	1.74 – 61,264.5
Volume	Volume of lake	m^3	18,462.6 – 33,246,200,000
Maximum depth	Maximum lake depth	m	0.9 – 200.0
Mean depth	Mean depth of lake (volume / surface area)	m	0.4 – 66.7
Residence time	Estimated residence time (volume / catchment flow)	years	0.81 - 7793.1
Catchment area	Lake catchment area	ha	4.8 – 784,455
Geomorphic origin	Geomorphic formation typology	-	-
Distance to sea	Distance to coast along river channel	km	51.1 – 96,189
Nitrogen load /surface area	Total nitrogen input divided by the lake surface area	kg/year/ha	0.0 – 44.9
Land use pressure	N loading summed for all inflowing tributaries and standardised to water residence time	-	0.0 – 183,261.6
Catchment annual air temperature	Average annual air temperature in the upstream catchment	°C	7.78 – 15.82
Percent forest	Percent forest in lake catchment	%	0.0 – 90.9
Percent grassland	Percent grassland in lake catchment	%	0.0 - 93.2

Table 3.4. Summary of variables used for the analysis of aquatic organisms $\delta^{13}C$ and $\delta^{15}N$ spatial variability in streams.

Environmental variable	Description	Unit	Source	Range
Air temperature (Summer)	January air temperature	°C	FENZ	12.1 – 18.8
Flow	Mean annual flow derived from hydrological models	m^3/s	FENZ	0.009 - 383.7
Slope	Segment slope derived from calculation using length and difference between upstream and downstream elevation	degrees	FENZ	0.0 – 9.35
Riparian shading	Degree of riparian shading derived from satellite imagery and estimates of river size and expected vegetation height	proportion	FENZ	0.0 – 0.8
Native riparian	Native riparian vegetation within 100m buffer of river	%	FENZ	0 – 100
Nitrogen concentration	Nitrogen concentration estimated from CLUES	ppb	FENZ	0.08 – 22.6
US Indigenous forest	Flow-weighted area of indigenous forest in upstream catchment	proportion	FENZ	0 – 1
US Native vegetation	Flow-weighted area of indigenous vegetation in upstream catchment	proportion	FENZ	0 – 1
US Pasture	Flow-weighted area of pasture in upstream catchment	proportion	FENZ	0 – 1
Elevation	Height of reach section in a watershed	m a.s.l.	REC	5.7 – 854.7
Distance to sea	Distance of reach from the coast	km	REC	0.6 – 324.4
Catchment area	Stream catchment area	ha	REC	0.25 – 14,117
Strahler stream order	Numerical position of a tributary or section of river within the entire network	-	REC	-
Total nitrogen	Total nitrogen using unfiltered samples, persulphate digestion to nitrate, nitrate-N by cadmium reduction. Estimate of median of many samples (Whitehead 2019)	mg/m^3	NIWA	49.4 – 5,450
Total phosphorus	Total phosphorus using unfiltered samples, persulphate digestion to orthophosphate, molybdenum blue colorimetry. Estimate of median of many samples (Whitehead 2019)	mg/m^3	NIWA	3.4 – 107.7
Water temperature	Estimated mean annual water temperature	°C	Calculated via eq. 2 in Mosley 1982	9.6 – 20.1

3.2.3 Lipid content correction

The differences in lipid content among various tissues and organisms has the potential to influence the variability of δ^{13}C. Relative to proteins and carbohydrates, lipids are depleted in the heavier ^{13}C isotope, and therefore often have more negative δ^{13}C values (Post et al. 2007). The source of this variation is a result of both the kinetic isotope effects of fractionation during the oxidation of pyruvate to acetyl coenzyme A in lipid synthesis, and the considerable lipid heterogeneity observed among tissues, individuals, and organisms (DeNiro and Epstein 1977; Post et al. 2007; Hicks et al. 2021). This can therefore contribute to false interpretation of aquatic food web studies due to interindividual δ^{13}C variability, imposing a degree of bias into stable isotope analyses (Logan et al. 2008). Subsequently, various lipid correction methods can be applied to reduce the confounding influence of lipids, either by physically removing the lipids or by a form of mathematical normalisation (Post et al. 2007). The requirement to perform lipid correction to the δ^{13}C values is usually estimated based on the carbon-to-nitrogen (C:N) ratios > 3.5.

To reduce the influence of the lipids, and the potential undesirable bias imposed by these ^{13}C-depleted biochemical compounds in this study, δ^{13}C values were lipid-corrected in datasets where either carbon content (% carbon) and nitrogen content (% nitrogen), or C:N ratios were measured and provided. The results from untreated samples with high C:N (> 3.5), were mathematically normalised and adjusted using Equation 3 from Post et al. (2007):

$$\delta^{13}\mathrm{C}_{\text{lipid-corrected tissue}} = (\delta^{13}\mathrm{C}_{\text{untreated tissue}}) - 3.32 + 0.99 \text{ x C:N}$$

3.3 Statistical analyses

3.3.1 Data preparation, transformation, standardisation, and correlations

For the analysis of stream isotopic value variability, the database was homogenised by calculating the means for the invertebrate δ^{15}N values, and the fish δ^{13}C and δ^{15}N values sampled for sites with replicated data from a single sampling data and location. For the lake analysis, the means were calculated for the top predator fish δ^{13}C and δ^{15}N values. Preceding the analysis, where appropriate, the data was transformed using $\log_{10}$-transformations for numerical variables and logit transformations for percentage and proportional variables, in order to meet the assumptions of analyses, reduce skewness and improve heteroscedasticity. When necessary, a constant was added to the δ^{13}C for log-transformations to transform the negative values to positive. Variables were centred (subtracted from sample means) and scaled (divided by the sample standard deviation) for all analyses requiring standardised data (e.g., for Principal Component Analysis and Structural Equation Modelling).

A correlation matrix using Spearman rank correlation was first created to test for the potential correlations between the environmental variables and the stable isotopic ratios, as well as to investigate whether multicollinearity issues existed between the predictor variables. The presence of highly collinear variables can contribute to reduced precision of the estimated regression coefficients, which weakens the statistical power of the model and increases the difficulty for interpreting accurate results. Subsequently variables that were collinear were excluded from further analysis.

3.3.2 Principal component analysis (PCA)

Principal component analysis (PCA) was used to evaluate which environmental predictors best explained the top predator fish δ^{13}C and δ^{15}N variation in lakes, and the top predator fish δ^{13}C and δ^{15}N, and invertebrate δ^{15}N variation in streams. The principal component (PC) axis for the analysis of δ^{13}C and δ^{15}N in lakes consisted of the lake surface area, lake volume, catchment elevation, residence time, and percent grassland in the catchment upstream. The PC axis for streams included the catchment area, stream elevation, proportion of upstream pastoral land, nitrogen load and mean annual water temperature. A two-dimensional PCA biplot and factor loadings were computed and analysed separately, for both lakes and streams. Correlation loadings were considered to be associated with the factor with scores > 0.3. We performed the PCA analysis and created the plots with R version 4.0.4 (2021-02-15) (R Development Core Team 2022), using the ‘stats’ and ‘factoextra’ packages in the R library.

3.3.3 Structural equation modelling (SEM)

Structural equation modelling (SEM) was used to investigate the direct and indirect effects of selected environmental factors on the δ^{13}C and δ^{15}N variability in lakes, and the δ^{15}N in stream food webs. SEM are probabilistic models which combine multivariate relationships (i.e., multiple predictor and response variables) into a single network by constructing a path diagram (Lefcheck 2016). The selection of a parsimonious subset of environmental predictions to retain in the models were identified through forward stepwise selection, using the ‘packfor’ package in R (R Development Core Team 2022). This forward stepwise selection method is based on a permutation procedure using residuals, where the number of permutations was set to 999 (Blanchet et al. 2008).

Two piecewise structural equation models (pSEM) were constructed using the transformed and standardised data. Piecewise structural equation models, as opposed to traditional SEM, prevent model overfitting problems of smaller data sets (Lefcheck 2016). There was insufficient variation when species or sites were imputed as random effects to justify the inclusion of either in a mixed-effects SEM. Subsequently, we fitted a general linear model with only fixed effects to each of the response variables. The collinearity of environmental predictors was investigated by calculating the variance inflation factor (extreme collinearity: VIF > 10), and strongly collinear variables were removed from the SEM analysis when necessary to meet assumptions.

The overall appropriateness of the final pSEM model was assessed using the Fisher’s *C* statistic (where $p > 0.05$ = valid model) (Lefcheck 2016). Fisher’s *C* statistic is used for the test of directed separation to evaluate the hypothesis that specifying missing paths improves the model. If $p > 0.05$ then this hypothesis can be rejected (i.e., fitting the missing relationships does not improve the model fit). To find the most parsimonious model, the goodness of fit of the pSEM model was compared among candidate models using the Akaike information criterion (AIC). The removal of a pathway was accepted if it contributed to a reduction in the AIC value. We selected and presented each pSEM as a path diagram with the lowest AIC value as the best fit model. The standardised model coefficient estimates and *p*-values for the SEM paths were calculated and reported. The significant relationships identified in structural equation modelling of the isotopic ratios were also displayed in the format of a partial residual plot, which corrects for the influence of other predictors. We performed the SEM analysis with R version 4.0.4 (R Development Core Team 2022), using the ‘piecewiseSEM’ package in the R library.

Chapter 4

Stable carbon and nitrogen isotopic ratios in New Zealand

4.1 Fish carbon and nitrogen isotopic ratios in lakes

4.1.1 Variation in δ^{13}C and δ^{15}N values of fish

A total of 1757 samples of fish from 88 lakes across New Zealand were analysed for carbon and nitrogen isotopic ratios. There was a large variation across all fish species and lakes for both the δ^{13}C (−34.49 to −11.46‰; range 23.03‰) (Fig. 4.1A) and δ^{15}N values (0.82 to 18.31‰; range 17.49‰) (Fig. 4.1B). The mean fish δ^{13}C in the whole dataset was −22.72 ± 3.66‰ (mean ± 1 standard deviation, n = 1751), and the mean fish δ^{15}N was 9.85 ± 2.72‰ (mean ± 1 standard deviation, n = 1750) (Table 4.1).

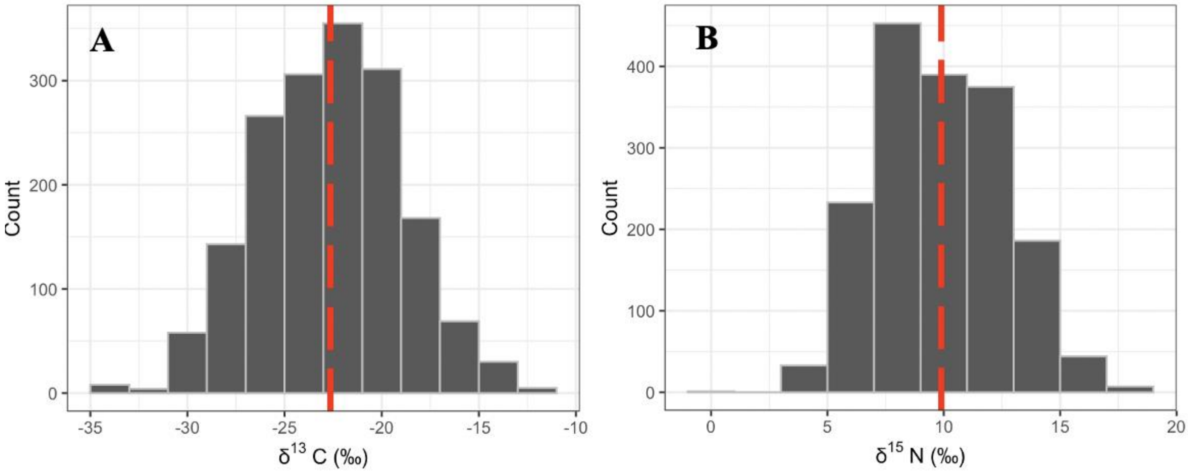


Figure 4.1. Frequency distribution histogram of the (A) mean carbon stable isotope ratios (δ^{13}C) of fish in 88 New Zealand lakes, and (B) mean nitrogen stable isotope ratios (δ^{15}N) of fish in 87 New Zealand lakes. Dotted red line indicates the mean value.

The fish species collated in the dataset were further classified into the four major trophic types; secondary consumers (banded kokopu, bully, catfish, gambusia, goldfish, inanga, koaro, koi carp, and tench), marine wanderers (flounder, smelt, triple fin and grey mullet), herbivores (rudd), and top predators (trout, perch, longfin eel, and shortfin eel) (Table 4.1). Due to the larger number of samples collected for the top predators (i.e., top predators n = 887; secondary consumers n = 713; marine wanderers n = 117; herbivores n = 40), this chapter will largely focus on analysing δ^{13}C and δ^{15}N variability in the organisms occupying the top trophic position of the food web in lakes.

Table 4.1. Mean and standard deviation of stable isotope ratios of C and N for all lake samples. Codes for trophic type: SC (secondary consumer), MW (marine wanderer), HE (herbivore), and TP (top predator).

Species	Trophic type	*n* lakes	δ^{13}C				δ^{15}N			
			n	mean ± SD	min	max	*n*	mean ± SD	min	max
Banded kokopu	SC	1	14	−22.5 ± 0.97	−23.8	−20.9	14	9.84 ± 0.56	8.9	10.7
Bully	SC	58	488	−21.9 ± 3.66	−31.6	−12.4	488	8.66 ± 2.65	0.8	17.0
Catfish	SC	3	10	−29.6 ± 5.10	−34.1	−21.0	10	10.8 ± 1.53	7.3	12.8
Gambusia	SC	6	61	−19.6 ± 2.30	−26.5	−14.7	61	8.22 ± 1.46	4.2	11.5
Goldfish	SC	11	59	−21.2 ± 4.07	−27.9	−13.4	59	7.68 ± 1.80	4.8	12.1
Inanga	SC	12	47	−23.1 ± 3.26	−28.2	−16.6	47	8.21 ± 2.74	4.9	17.3
Koaro	SC	1	1	−22.3	-	-	-	-	-	-
Koi carp	SC	3	23	−20.6 ± 3.53	−27.4	−17.0	23	8.09 ± 1.67	5.8	11.2
Tench	SC	1	7	−23.6 ± 0.47	−24.2	−23.0	7	8.67 ± 1.96	4.2	9.6
Flounder	SC / MW	3	5	−23.0 ± 3.74	−28.0	−19.3	5	11.8 ± 1.73	9.3	13.7
Common smelt	SC / MW	22	91	−22 ± 2.92	−28.2	−14.8	90	9.25 ± 2.85	4.3	15.1
Triplefin	MW	1	8	−22.4 ± 1.23	−24.1	−20.0	8	12 ± 0.25	11.6	12.3
Grey mullet	HE / MW	1	13	−21.4 ± 1.21	−23.7	−19.5	13	12.6 ± 0.40	11.8	13.6
Rudd	HE	7	40	−25.7 ± 3.20	−33.9	−20.2	40	9.34 ± 1.61	5.8	13.4
Brown trout	TP	21	27	−21.3 ± 3.06	−27.4	−15.4	26	10.9 ± 2.30	6.5	13.8
Perch	TP	12	123	−24.6 ± 2.94	−30.5	−18.8	123	10.2 ± 3.18	4.4	17.2
Shortfin eel	TP	49	508	−23.2 ± 3.77	−34.5	−11.5	510	10.8 ± 2.11	6.0	15.8
Longfin eel	TP	38	226	−23.5 ± 2.93	−29.6	−14.6	226	11.7 ± 2.57	7.3	18.3

There was a very small negative correlation (Spearman rank correlation, $r = -0.09$, $p < 0.001$) between the δ^{15}N and δ^{13}C values (Fig. 4.2). A negative δ^{13}C-δ^{15}N relationship was also observed for the periphyton isotopic ratios in the upper Great Lakes (Camilleri and Ozersky 2019), but the statistically significant correlation was attributed to most likely be the consequence of waterbody-specific differences. When all top predator species were pooled however, there was a statistically significant positive correlation (Spearman rank correlation, $r = 0.20$, $p < 0.001$) between the δ^{15}N and δ^{13}C values (Fig. 4.3).

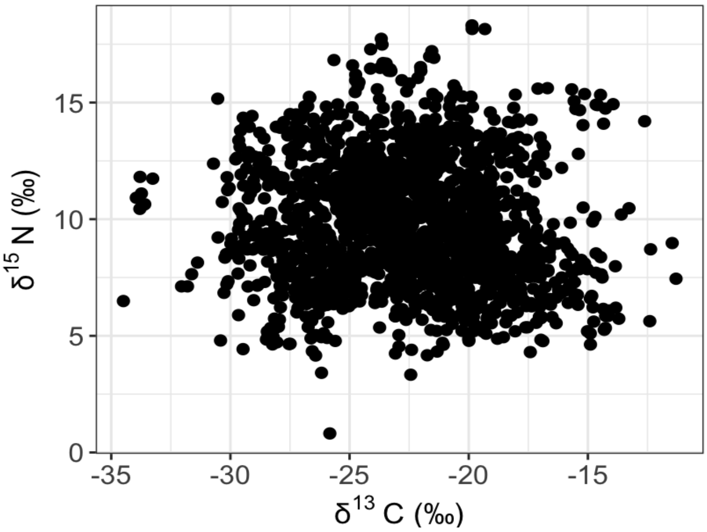


Figure 4.2. Relationship of fish δ^{13}C and δ^{15}N (Spearman rank correlation, $r = -0.09$, $p < 0.001$, $n = 1750$) values from New Zealand lakes. Points are values from the sampled fish species where both δ^{13}C and δ^{15}N were measured.

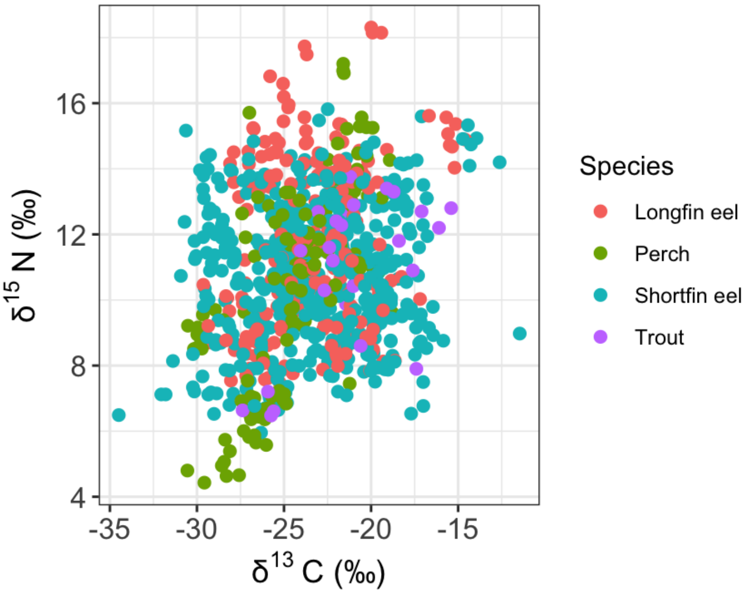


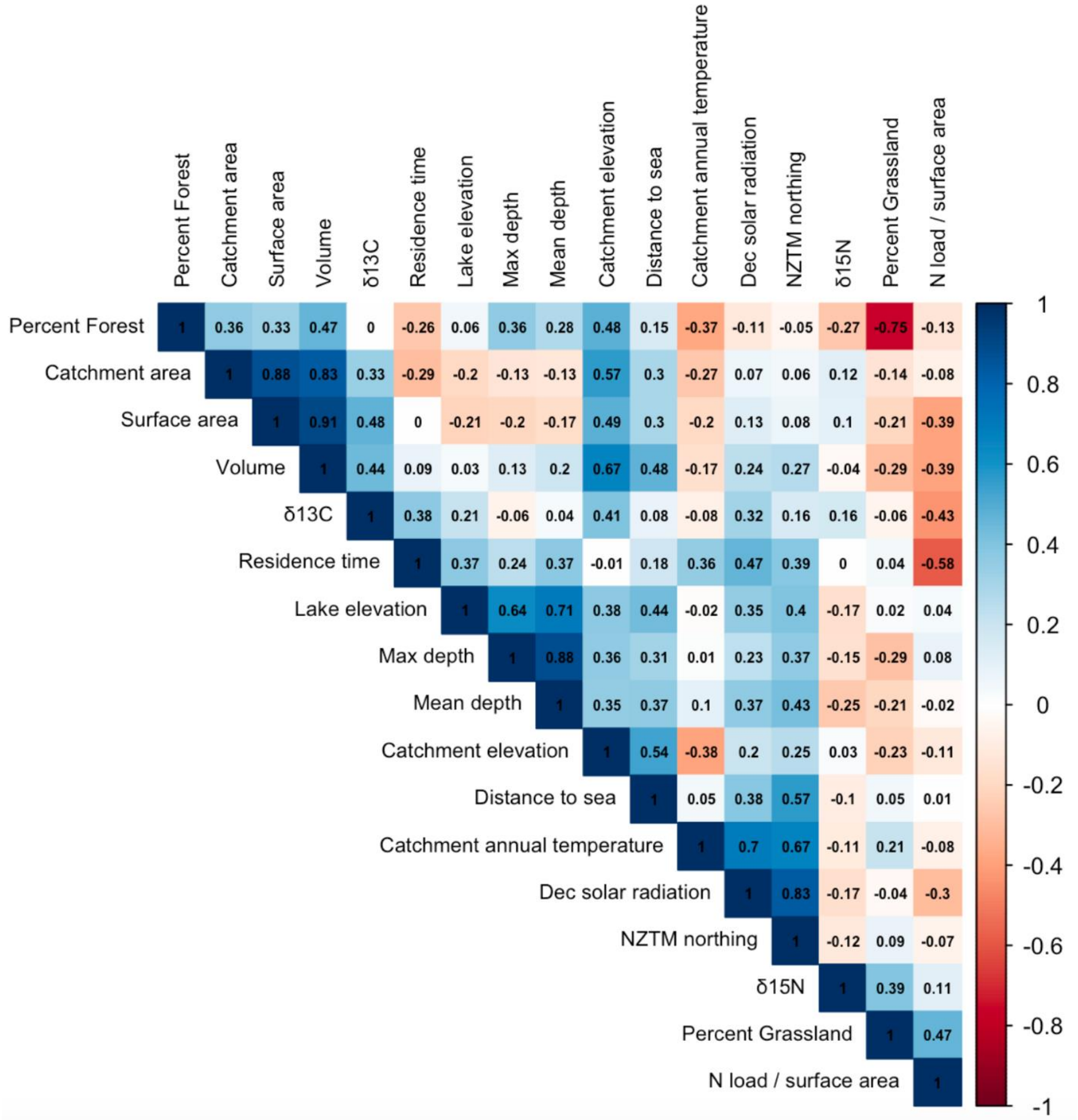
Figure 4.3. Relationship of top predator fish δ^{13}C and δ^{15}N (Spearman rank correlation, $r = 0.20$, $p < 0.001$, $n = 883$) values from New Zealand lakes with colour codes for the fish species, red = longfin eel, green = perch, blue = shortfin eel, and purple = brown trout.

4.1.2 Environmental controls on top predator δ^{13}C and δ^{15}N

The mean δ^{13}C values for top predators were significantly and positively correlated with all the environmental variables related to the system size of the lake, including the lake surface area (Spearman rank correlation, $r = 0.51$, $p < 0.001$), lake volume ($r = 0.48$, $p < 0.001$), catchment elevation ($r = 0.45$, $p < 0.001$), lake perimeter ($r = 0.41$, $p < 0.001$), catchment area ($r = 0.37$, $p < 0.001$), residence time ($r = 0.26$, $p < 0.001$), and the lake elevation ($r = 0.22$, $p < 0.05$) (Table 4.2; Fig. 4.4, Appendix 1). These positive correlations between the lake system size and δ^{13}C values were similar to the findings of Perga and Gerdeaux 2004 and McBride 2005, both of which reported the importance of lake surface area driving δ^{13}C inter-lake variability. There was, however, no relationship between the δ^{13}C and catchment land use variables (i.e., land use pressure, percent forest and percent grassland), with the exception of a weak correlation with the rate of predicted nitrogen loading ($r = 0.18$, $p < 0.05$). Unlike for δ^{13}C, the δ^{15}N values for the lake top predators were not significantly correlated with any of the lake system size variables (all $p > 0.05$). Instead, the δ^{15}N were positively correlated with both the percent grassland in the catchment (Spearman rank correlation, $r = 0.32$, $p < 0.001$), and nitrogen loading ($r = 0.23$, $p < 0.05$), while negatively correlated to the percent forest in the catchment ($r = -0.27$, $p < 0.001$) (Table 4.2; Fig. 4.4; Appendix 1).

Table 4.2. Spearman rank correlation coefficients for δ^{13}C and δ^{15}N of top predators and selected environmental factors from the sampled 88 lakes. * correlation significant at $p \leq 0.05$. ** correlation significant at $p \leq 0.001$. The full spearman correlation matrix for lake top predator isotopic values and selected environmental variables is presented in Appendix 1.

Variable	δ^{13}C	δ^{15}N
Catchment elevation	**0.45****	0.02
Surface area	**0.51****	0.08
Volume	**0.48****	−0.04
Nitrogen load	**0.19***	**0.23***
Percent grassland	−0.06	**0.32****



	Percent Forest	Catchment area	Surface area	Volume	δ13C	Residence time	Lake elevation	Max depth	Mean depth	Catchment elevation	Distance to sea	Catchment annual temperature	Dec solar radiation	NZTM northing	δ15N	Percent Grassland	N load / surface area
Percent Forest	1	0.36	0.33	0.47	0	-0.26	0.06	0.36	0.28	0.48	0.15	-0.37	-0.11	-0.05	-0.27	-0.75	-0.13
Catchment area		1	0.88	0.83	0.33	-0.29	-0.2	-0.13	-0.13	0.57	0.3	-0.27	0.07	0.06	0.12	-0.14	-0.08
Surface area			1	0.91	0.48	0	-0.21	-0.2	-0.17	0.49	0.3	-0.2	0.13	0.08	0.1	-0.21	-0.39
Volume				1	0.44	0.09	0.03	0.13	0.2	0.67	0.48	-0.17	0.24	0.27	-0.04	-0.29	-0.39
δ13C					1	0.38	0.21	-0.06	0.04	0.41	0.08	-0.08	0.32	0.16	0.16	-0.06	-0.43
Residence time						1	0.37	0.24	0.37	-0.01	0.18	0.36	0.47	0.39	0	0.04	-0.58
Lake elevation							1	0.64	0.71	0.38	0.44	-0.02	0.35	0.4	-0.17	0.02	0.04
Max depth								1	0.88	0.36	0.31	0.01	0.23	0.37	-0.15	-0.29	0.08
Mean depth									1	0.35	0.37	0.1	0.37	0.43	-0.25	-0.21	-0.02
Catchment elevation										1	0.54	-0.38	0.2	0.25	0.03	-0.23	-0.11
Distance to sea											1	0.05	0.38	0.57	-0.1	0.05	0.01
Catchment annual temperature												1	0.7	0.67	-0.11	0.21	-0.08
Dec solar radiation													1	0.83	-0.17	-0.04	-0.3
NZTM northing														1	-0.12	0.09	-0.07
δ15N															1	0.39	0.11
Percent Grassland																1	0.47
N load / surface area																	1

Figure 4.4. Spearman correlation matrix for top-predator isotopic values and lake environmental variables.

4.1.3 Principal component analysis (PCA) for top predator δ^{13}C and δ^{15}N in lakes

A principal component analysis (PCA) was carried out to investigate which of the selected environmental predictors (percent grassland, surface area, volume, residence time, catchment elevation) best explained the top predator δ^{13}C and δ^{15}N variation between the sampled New Zealand lakes. Principal component axes one, two and three have eigenvalues greater than 1 (1.67, 1.24, 1.05 respectively), and therefore should be retained according to the Kaiser criterion (Kaiser 1960). The first axis of the PCA accounted for 39.6% of the total variance, second axis accounted for 21.9% of the total variance, while the third axis accounted for 15.7% of the total variance (Table 4.3). As a result, up to 61.5% of the total variance can be explained by the first two principal components, while up to 77.3% can be explained by the first three principal components.

Table 4.3. Eigenvalues and percent variation for first four principal component (PC) axis for lake environmental variables.

PC axis	Eigenvalue	Percent variation	Cumulative percent variation
1	1.67	39.64	39.64
2	1.24	21.89	61.52
3	1.05	15.73	77.26
4	0.755	8.15	85.41

The first PC axis was negatively related to the percent grassland in the lake catchment while positively related to the isotopic signatures of the lake top predators, as well as the variables associated with the physical characteristics of the lake, i.e., the catchment elevation, surface area, lake volume and residence time (Fig. 4.5). PC axis two was weakly negatively related to the catchment elevation, surface area and lake volume, while was positively related to both C and N isotopic signatures of lake top predators, percent grassland and residence time.

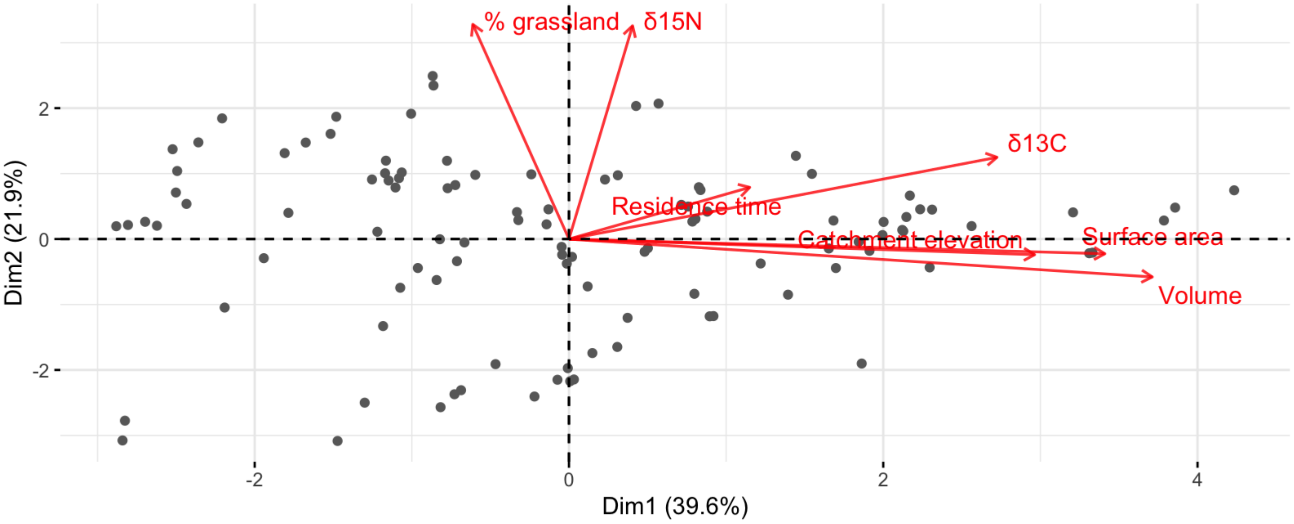


Figure 4.5. PCA biplot of the first two principal components (Dim1 and Dim2) for top predator δ^{13}C and δ^{15}N and the five environmental predictors; surface area, catchment elevation, lake volume, residence time and percentage of grassland in lake catchment.

According to the factor loadings for each principal component axis, PC axis one largely represented the variables associated with the lake physical attributes, including lake volume, surface area and catchment elevation, as well as the δ^{13}C of the lake top predators (Table 4.4; Fig. 4.6). PC axis two strongly represented the δ^{15}N values of the lake top predators and the catchment land use (i.e., the percentage of grassland) (Fig. 4.7). PC axis three largely represented the top predator δ^{13}C values and the estimated lake residence time. The PCA results provide further evidence that the carbon isotopic ratios tended to reflect the natural physical traits of the lake ecosystem, whereas the nitrogen isotopic ratios responded more readily to the watershed land use.

Table 4.4. Factor loadings for the top predator δ^{13}C and δ^{15}N and each of the five environmental predictors. Values > 0.3 and < −0.3 are shown in bold.

Variable	Loadings		
	PC1	PC2	PC3
δ^{13}C	**0.413**	0.255	**−0.320**
δ^{15}N	0.061	**0.665**	0.250
Catchment elevation	**0.449**	−0.049	0.162
Surface area	**0.517**	−0.046	0.286
Volume	**0.563**	−0.118	0.096
Residence time	0.174	0.161	−0.838
Percent grassland	−0.093	**0.670**	0.124

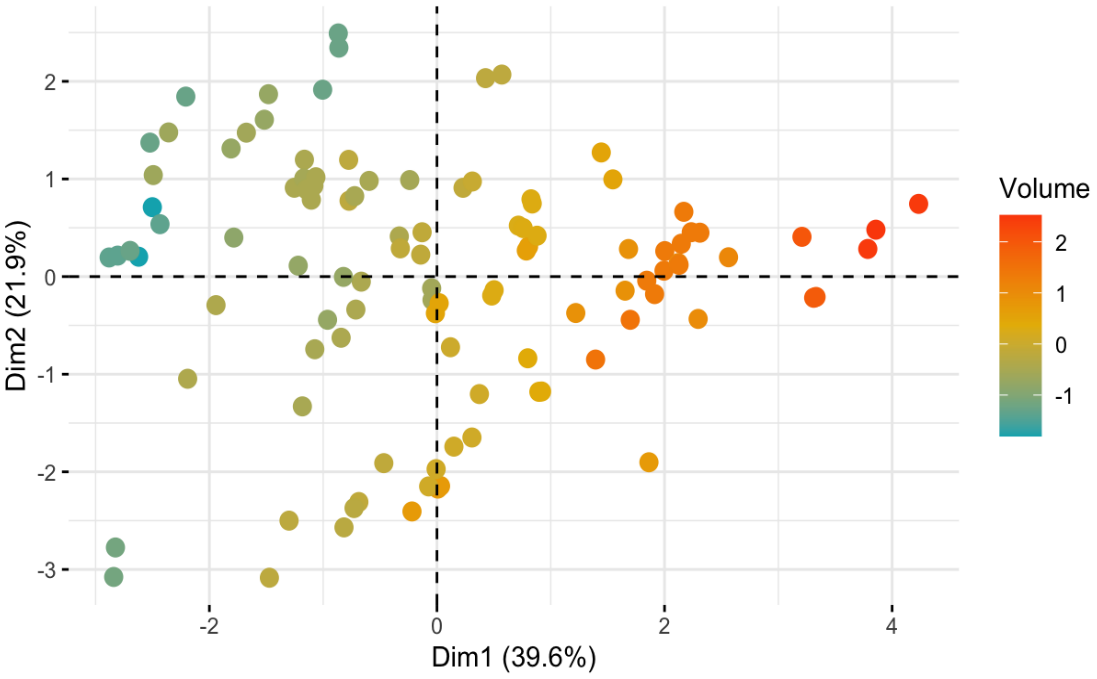


Figure 4.6. PCA plot for the strongest related loading factor, standardised lake volume (loading = 0.563), for PC axis one (Dim1).

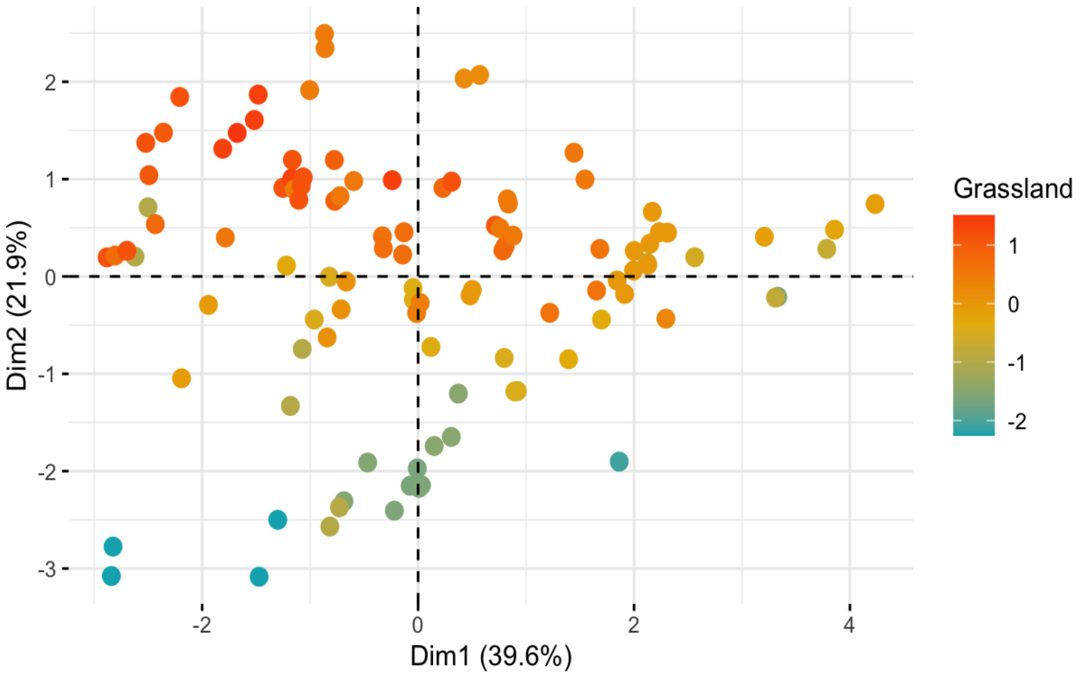


Figure 4.7. PCA plot for the strongest related loading factor, standardised percent grassland (loading = 0.670), for PC axis two (Dim2).

4.1.4 Top predator δ^{13}C and δ^{15}N structural equation modelling

Fisher's *C* statistic confirmed that the piecewise structural equation model constructed for the isotopic ratios and lake environmental predictors (Fig. 4.8) was appropriate for the data ($p = 0.604$; above the 0.05 significance threshold) (Lefcheck 2016). The pSEM results showed that the volume of the lake had the strongest impact on the top predator δ^{13}C values, but, while still statistically significant ($p < 0.05$), had a much weaker impact on the top predator δ^{15}N values (Table 4.5). Both the lake residence time and catchment elevation had significant positive effects on the δ^{13}C values. For the δ^{15}N signatures, the percentage of grassland in the lake catchment was the most significant predictor and had a negative effect on the nitrogen isotopic values. While still significant, the percentage of grassland explained a significantly lower amount of the variation in δ^{13}C. This again suggests that the fish δ^{13}C and δ^{15}N in lakes are responding to different drivers, with the catchment land use being a significant driver of δ^{15}N variability but not for δ^{13}C. Interestingly, the distance to sea had a significant negative effect on both the δ^{13}C and δ^{15}N values ($p = 0.32$). There was no significant pathway between the δ^{13}C and δ^{15}N values of the lake top predator fish. The R^2 for the top predator δ^{13}C (0.46) was slightly higher than that for the top predator δ^{15}N (0.32), therefore indicating that the selected environmental variables explained a greater amount of the total variance for the carbon isotopic ratios compared to nitrogen for the compiled dataset.

Table 4.5. Standardised component model results from the piecewise structural equation model (pSEM) for top predator fish δ^{13}C and δ^{15}N from sampled New Zealand lakes (n = 107). The best-fit model presented had the lowest AIC score (Fisher's C test = 2.729, p = 0. 604 on 4 df). Statistically significant relationships between the response and predictor are shown in bold (i.e., $p < 0.05$).

Response	R^2	Predictor	Standardised parameter estimate	Standard error	p value
Top predator δ^{13}C	0.46	Percent grassland	0.213	0.08	**0.007**
		Catchment elevation	0.304	0.10	**0.003**
		Lake volume	0.432	0.11	**<0.001**
		Residence time	0.369	0.09	**<0.001**
		Distance to sea	−0.425	0.09	**<0.001**
Top predator δ^{15}N	0.32	Percent grassland	0.567	0.09	**<0.001**
		Distance to sea	−0.369	0.10	**<0.001**
		Lake volume	0.290	0.10	**0.004**

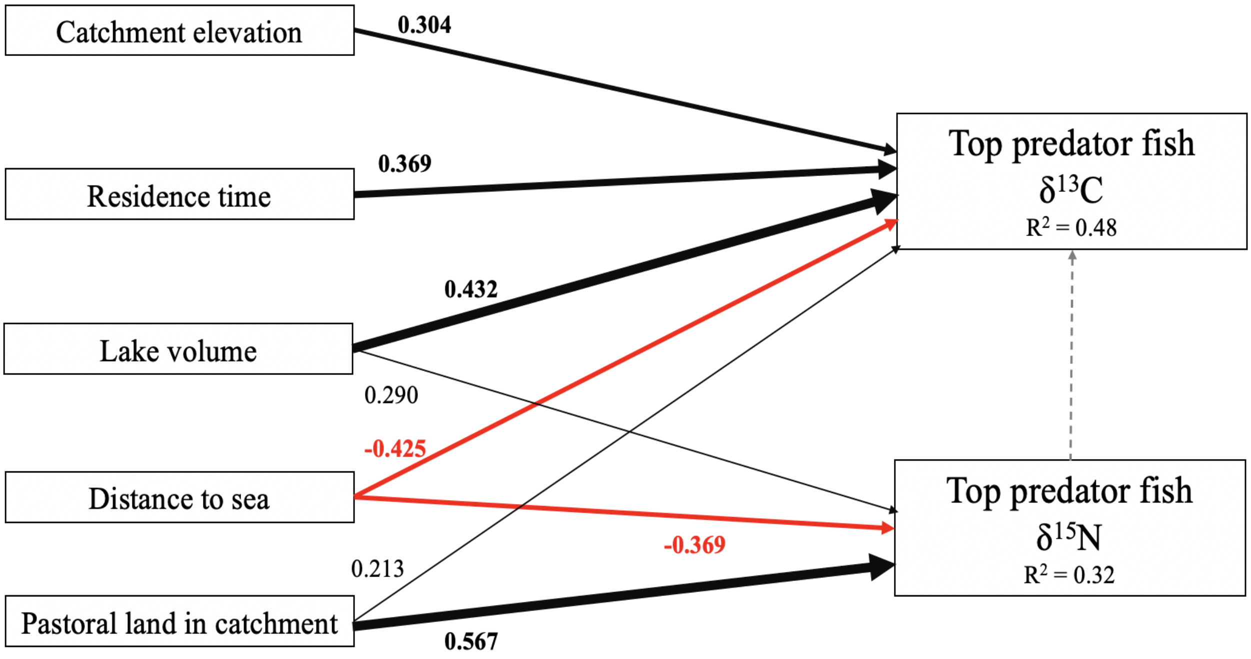


Figure 4.8. Results from piecewise structural equation modelling of top predator fish δ^{13}C and δ^{15}N interrelationships. Solid black arrows indicate significant positive effect, solid red arrows indicate a significant negative effect, and dashed grey arrows denote a non-significant effect ($p \geq 0.05$). The thickness of the significant paths reflects the magnitude of the standardised regression coefficient. R^2 are reported for the endogenous variables (within boxes), and standardised parameter estimates are reported for significant model pathways. Numbers above arrows are correlation coefficients.

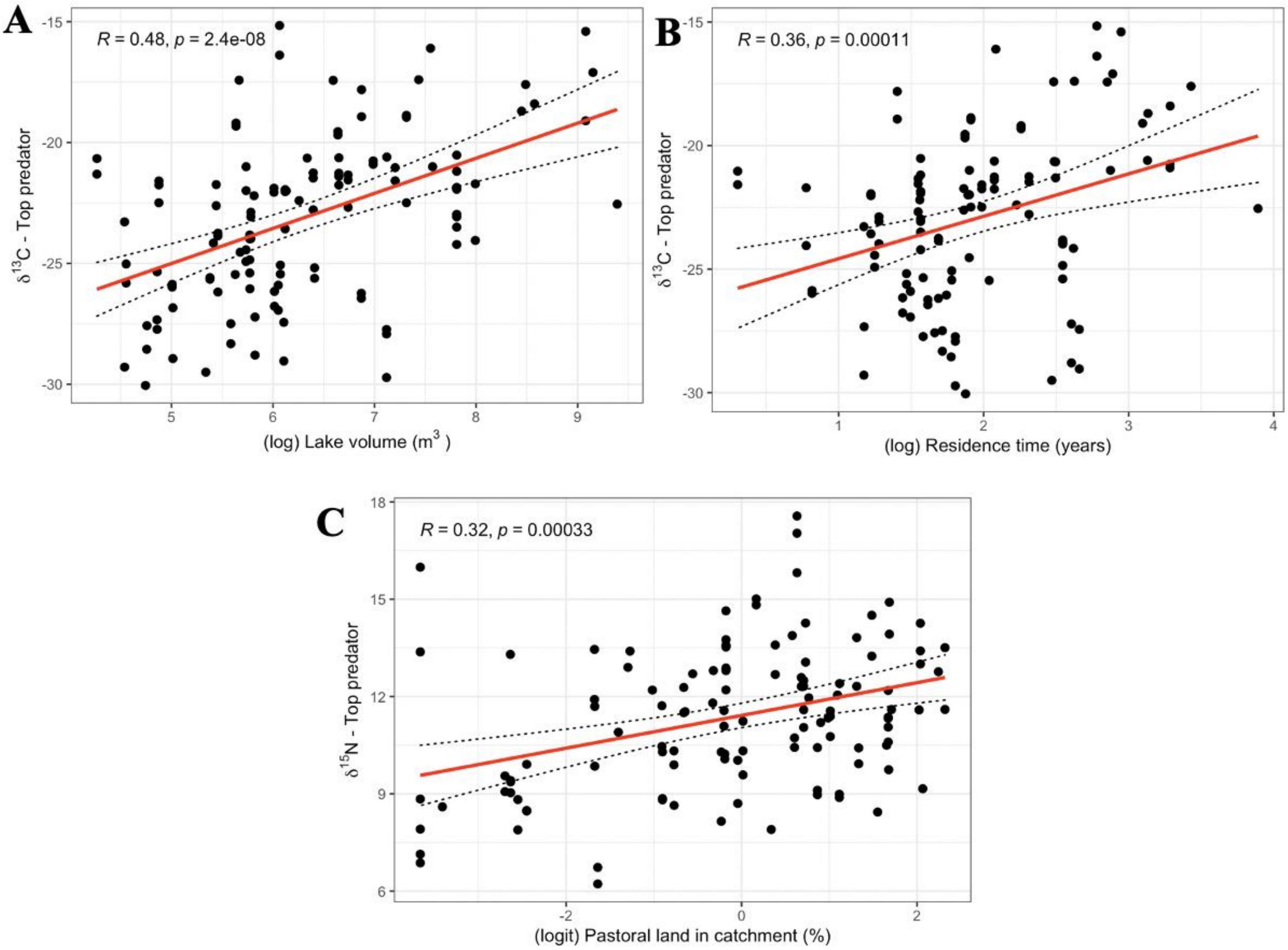


Figure 4.9. Plots of significant relationships ($p < 0.05$) found from the structural equation modelling of top predator δ^{13}C and δ^{15}N, including (A) the effect of lake volume on δ^{13}C, (B) the effect of residence time on δ^{13}C, and (C) the effect of pastoral land in catchment on δ^{15}N. The Spearman rank correlation *r* and *p* values are included in each plot, the red lines are the predicted linear regressions, and dashed black lines indicate 95% confidence intervals for the linear regressions.

4.2 Fish carbon and nitrogen isotopic ratios in streams

4.2.1 Variation in δ^{13}C and δ^{15}N values of fish

A total of 1290 samples of fish from 171 stream site locations were analysed for carbon and/or nitrogen isotopic ratios. There was a large variation across all fish species and streams for both the δ^{13}C (−39.74 to −15.22‰; range 24.52‰) (Fig. 4.10A) and δ^{15}N values (2.17 to 18.05‰; range 15.88‰) (Fig. 4.10B). The mean fish δ^{13}C in the whole dataset was −24.61 ± 3.91‰ (mean ± 1 standard deviation, n = 711), and the mean fish δ^{15}N was 8.61 ± 2.74‰ (mean ± 1 standard deviation, n = 1291) (Table 4.6).

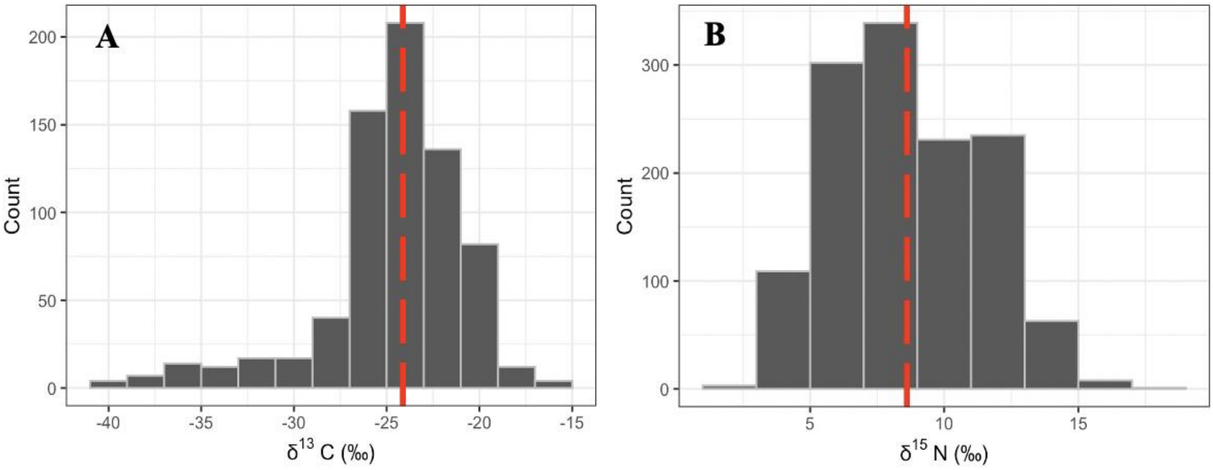


Figure 4.10. Frequency distribution histogram of the (A) mean carbon stable isotope ratios (δ^{13}C) of fish in New Zealand streams (n = 711), and (B) mean nitrogen stable isotope ratios (δ^{15}N) of fish in New Zealand streams (n = 1290). Dotted red line indicates the mean value.

The fish species collated in the dataset were further classified into the three major trophic types; secondary consumers (banded kokopu, bully, galaxias, goldfish, inanga, koaro, koi carp, and torrentfish), marine wanders (grey mullet and smelt), and top predators (longfin eel, shortfin eel, and trout) (Table 4.6). As with lakes, due to the larger number of samples collected for the top predators (i.e., secondary consumer, n = 587; marine wanderer, n = 61; top predators, n = 629), this chapter will largely focus on analysing δ^{13}C and δ^{15}N variability across the organisms occupying the top position of the food web in streams.

Table 4.6. Mean and standard deviation of (non-lipid corrected) stable isotope ratios of C and N for all stream samples. Codes for trophic type: SC (secondary consumer), MW (marine wanderer), and TP (top predator).

Species	Trophic type	*n* stream sites	δ^{13}C				δ^{15}N			
			n	mean ± SD	min	max	*n*	mean ± SD	min	max
Banded kokopu	SC	14	14	−24.2 ± 1.36	−26.1	−20.3	14	5.7 ± 1.27	3.9	8.1
Bully	SC	278	169	−24.4 ± 5.14	−37.2	−15.2	278	8.9 ± 3.06	2.2	16.2
Galaxiids	SC	209	-	-	-	-	209	6.2 ± 1.27	3.1	9.6
Goldfish	SC	59	59	−27.5 ± 3.58	−36.4	−21.8	59	9.9 ± 0.90	8.6	12.5
Inanga	SC	4	1	−21.2	-	-	4	11.0 ± 1.59	8.8	12.5
Koaro	SC	4	4	−27.3 ± 0.30	−27.5	−26.9	4	4.1 ± 0.24	3.8	4.4
Koi carp	SC	5	5	−31.3 ± 5.48	−36.2	−25.3	5	11.3 ± 1.04	9.8	12.2
Longfin eel	TP	291	236	−23.9 ± 2.19	−29.4	−18.7	291	9.1 ± 2.59	3.3	18.1
Grey mullet	MW	46	-	-	-	-	46	11.8 ± 1.21	10.2	14.5
Chinook salmon	MW	15	-	-	-	-	15	7.7 ± 1.92	5.3	11.4
Shortfin eel	TP	178	170	−24.0 ± 2.76	−34.3	−17.3	178	10.0 ± 2.23	3.0	15.6
Common smelt	MW	17	17	−23.2 ± 1.52	−25.2	−19.8	17	10.4 ± 1.86	8.6	16.2
Torrentfish	SC	10	10	−22.6 ± 2.12	−25.9	−19.8	10	9.7 ± 0.95	8.2	11.7
Brown and rainbow trout	TP	160	25	−30.3 ± 7.14	−39.7	−19.5	160	7.4 ± 2.26	3.6	14.6

There was a positive correlation (Spearman rank correlation, $r = 0.29$, $p < 0.001$) between the δ^{15}N and δ^{13}C values (Fig. 4.11). When all top predator species were pooled, again a significant and stronger positive correlation was identified (Spearman rank correlation, $r = 0.45$, $p < 0.001$), in which as the δ^{13}C value of a top predator species increases, the δ^{15}N also tended to increase (Fig. 4.12).

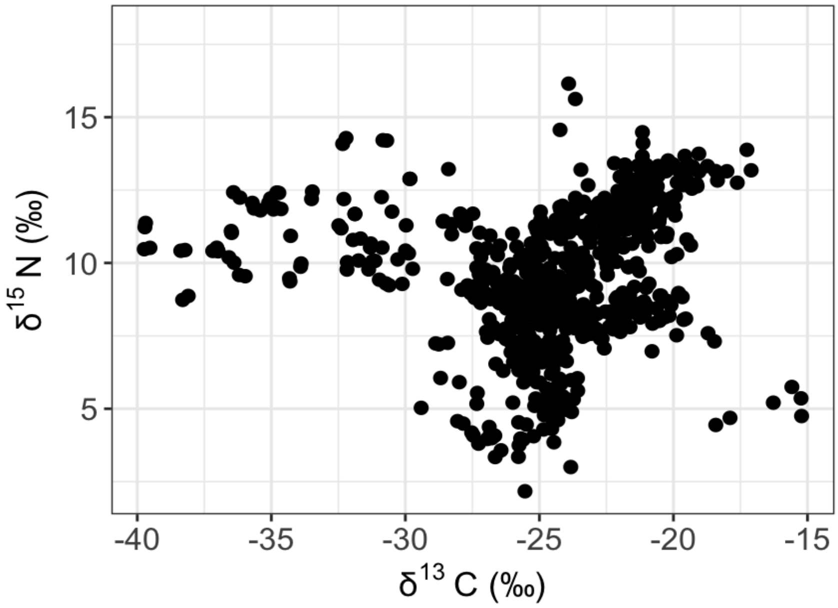


Figure 4.11. Relationship of fish δ^{13}C and δ^{15}N (Spearman rank correlation, $r = 0.29$, $p < 0.001$, $n = 711$) values from New Zealand streams. Points are values from the sampled fish species where both δ^{13}C and δ^{15}N were measured.

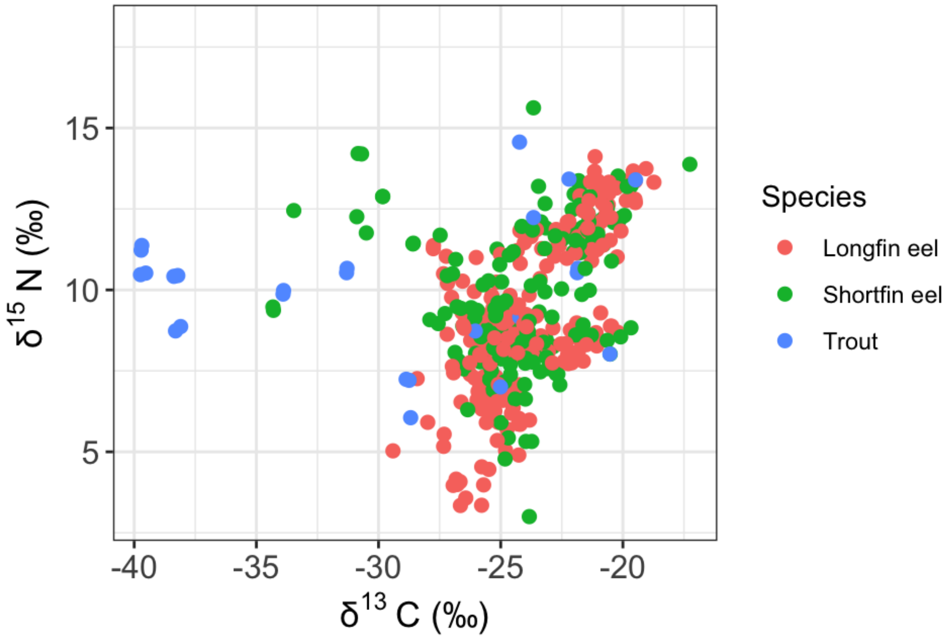


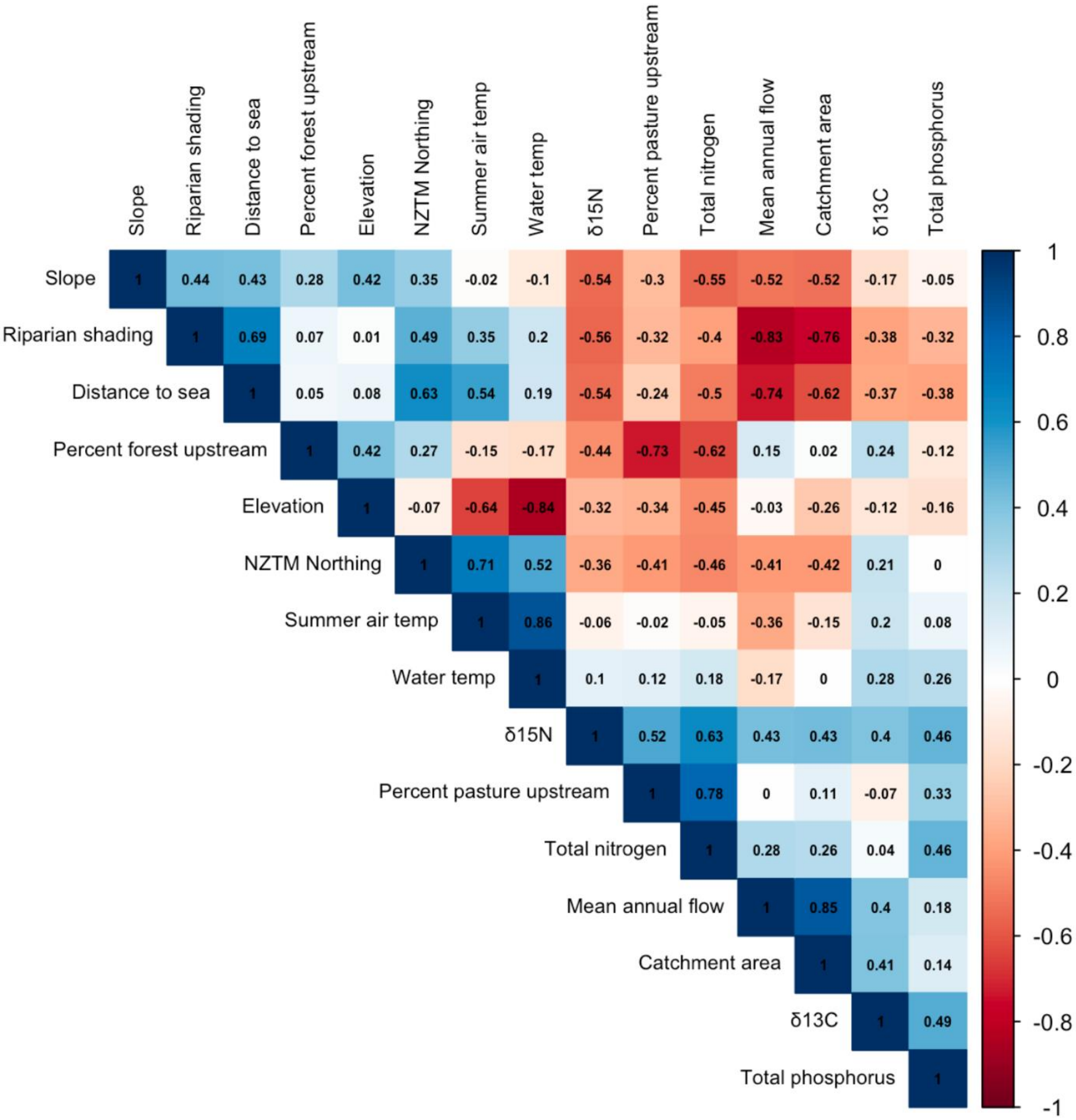
Figure 4.12. Relationship of top predator fish δ^{13}C and δ^{15}N (Spearman rank correlation, $r = 0.45$, $p < 0.001$, $n = 431$) values from New Zealand streams with colour codes for the fish species, red = longfin eel, green = shortfin eel, and blue = rainbow and brown trout.

4.2.2 Environmental controls on top predator δ^{13}C and δ^{15}N

Mean δ^{13}C values for the top predators in streams were significantly and positively correlated with the summer air temperature (Spearman rank correlation, $r = 0.21$, $p < 0.001$), and water temperature ($r = 0.21$, $p < 0.001$), mean annual flow ($r = 0.41$, $p < 0.001$), catchment area ($r = 0.41$, $p < 0.001$), and percent indigenous forest in the upstream catchment ($r = 0.22$, $p < 0.05$) (Table 4.7; Fig. 4.13; Appendix 2). Surprisingly, the top predator species were significantly and positively correlated with the total phosphorus concentrations ($r = 0.47$, $p < 0.001$), but had no significant relationship with the total nitrogen ($r = 0.06$, $p = 0.60$). The percentage of riparian shading and the distance to sea were both statistically and negatively correlated with the δ^{13}C values. The mean top predator δ^{15}N values in streams were significantly correlated with all the selected environmental variables except for the mean annual flow of the studied stream site ($r = 0.14$, $p = 0.08$) (Table 4.7; Fig. 4.13; Appendix 2).

Table 4.7. Spearman rank correlation coefficients for δ^{13}C and δ^{15}N of top predators and selected environmental factors from the sampled stream sites. * correlation statistically significant at $p \leq 0.05$. ** correlation significant at $p \leq 0.001$ level. The full spearman correlation matrix for stream top predator isotopic values and selected environmental variables is presented in Appendix 2.

Variable	δ^{13}C	δ^{15}N
Water temperature	**0.29****	**0.44****
Elevation	−0.15	**−0.61****
Catchment area	**0.41****	**−0.19***
N loading	0.06	**−0.71****
Percent pasture upstream	−0.05	**0.64****



	Slope	Riparian shading	Distance to sea	Percent forest upstream	Elevation	NZTM Northing	Summer air temp	Water temp	δ15N	Percent pasture upstream	Total nitrogen	Mean annual flow	Catchment area	δ13C	Total phosphorus
Slope	1	0.44	0.43	0.28	0.42	0.35	-0.02	-0.1	-0.54	-0.3	-0.55	-0.52	-0.52	-0.17	-0.05
Riparian shading		1	0.69	0.07	0.01	0.49	0.35	0.2	-0.56	-0.32	-0.4	-0.83	-0.76	-0.38	-0.32
Distance to sea			1	0.05	0.08	0.63	0.54	0.19	-0.54	-0.24	-0.5	-0.74	-0.62	-0.37	-0.38
Percent forest upstream				1	0.42	0.27	-0.15	-0.17	-0.44	-0.73	-0.62	0.15	0.02	0.24	-0.12
Elevation					1	-0.07	-0.64	-0.84	-0.32	-0.34	-0.45	-0.03	-0.26	-0.12	-0.16
NZTM Northing						1	0.71	0.52	-0.36	-0.41	-0.46	-0.41	-0.42	0.21	0
Summer air temp							1	0.86	-0.06	-0.02	-0.05	-0.36	-0.15	0.2	0.08
Water temp								1	0.1	0.12	0.18	-0.17	0	0.28	0.26
δ15N									1	0.52	0.63	0.43	0.43	0.4	0.46
Percent pasture upstream										1	0.78	0	0.11	-0.07	0.33
Total nitrogen											1	0.28	0.26	0.04	0.46
Mean annual flow												1	0.85	0.4	0.18
Catchment area													1	0.41	0.14
δ13C														1	0.49
Total phosphorus															1

Figure 4.13. Spearman correlation matrix for top predator isotopic values and stream environmental variables.

4.2.3 Principal component analysis (PCA) for top predator δ^{15}N in streams

A PCA was carried out to investigate which of the selected environmental predictors (nitrogen load, percent pasture upstream, elevation, catchment area, and water temperature) best explained the top predator δ^{15}N variation between the sampled New Zealand streams. Principal component axes one and two have eigenvalues greater than 1 (1.92 and 1.03 respectively), and therefore should be retained according to the Kaiser criterion (Kaiser 1960). The first axis of the PCA accounted for 61.7% of the total variance, while the second axis accounted for 17.5% (Table 4.8). As a result, up to 79.2% of the total variation can be explained by the first two principal components.

Table 4.8. Eigenvalues and percent variation for first three principal component (PC) axes for the selected stream environmental variables.

PC axis	Eigenvalue	Percent variation	Cumulative percent variation
1	1.92	61.65	61.65
2	1.03	17.49	79.15
3	0.80	10.65	89.80

The first PC axis was strongly negatively related to the elevation of the stream site while was positively related to the top predator δ^{15}N values, as well as the percent pasture upstream in the stream catchment, the rate of nitrogen loading, mean annual water temperature and the catchment area (Fig. 4.14). PC axis two was negatively related to the catchment area and δ^{15}N signatures while negatively related to the percent pasture, nitrogen load, elevation and water temperature.

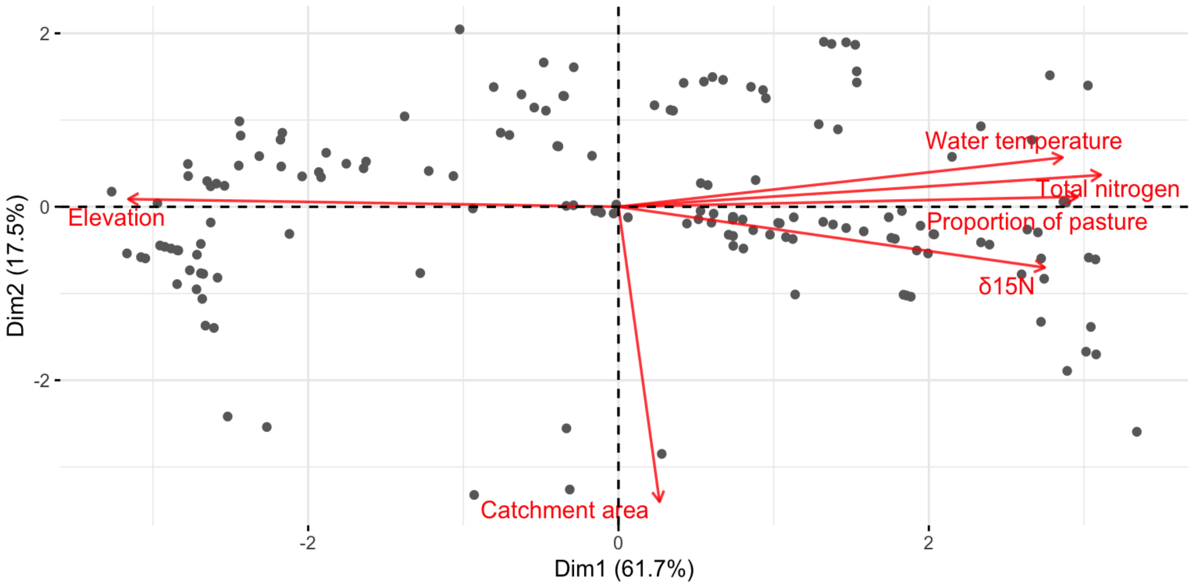


Figure 4.14. PCA biplot of the first two principal components (Dim1 and Dim2) for top predator δ^{15}N and the five environmental predictors; catchment area, water temperature, percent pasture upstream, elevation, and nitrogen loading.

According to the factor loadings for each principal component, PC axis one largely represented the δ^{15}N signatures of the stream top predators, as well as all the environmental predictors (percent pasture upstream, elevation, total nitrogen and water temperature) except for the catchment area (Table 4.9). Contrastingly, PC axis two strongly represented only the catchment area. The PCA results therefore provide evidence that the δ^{15}N values of top predators respond more readily to the watershed land use than alternative sources.

Table 4.9. Factor loadings for the top predator δ^{15}N and each of the five environmental predictors. Values > 0.3 and < −0.3 are shown in bold.

Variable	Loadings	
	PC1	PC2
δ^{15}N	**0.413**	−0.198
Percent pasture upstream	**0.445**	0.033
Elevation	**−0.475**	0.025
Catchment area	0.040	**−0.960**
Total nitrogen	**0.466**	0.104
Water temperature	**0.430**	0.161

4.2.4 Top predator δ^{15}N structural equation modelling

Fisher's *C* statistic confirmed that the piecewise structural equation model constructed for the isotopic ratios and stream environmental predictors (Fig. 4.15) was appropriate for the data ($p = 0.152$, above the 0.05 significance threshold) (Lefcheck 2016). The pSEM results showed that the total phosphorus and proportion of indigenous forest were the two strongest predictors of top predator δ^{15}N values in streams (Table 4.10). The total phosphorus strongly and positively affected the δ^{15}N signatures, while the proportion of indigenous forest strongly negatively impacted δ^{15}N. The slope of the sampled stream reach also negatively affected the top predator δ^{15}N, while the catchment area had a small positive effect on δ^{15}N. Surprisingly, the total nitrogen concentrations did not significantly affect the top predator δ^{15}N from the study dataset. The R^2 for the top predator δ^{13}C (0.62) indicated that the selected environmental variables explained a reasonable amount of the total variance for the nitrogen isotopic ratios.

Table 4.10. Standardised component model results from the piecewise structural equation model (pSEM) for top predator fish δ^{15}N from sampled New Zealand streams ($n = 151$). The best-fit model presented had the lowest AIC score (Fisher's *C* test = 3.766, $p = 0.152$ on 2 df). Statistically significant relationships between the response and predictor are shown in bold (i.e., $p < 0.05$).

Response	R^2	Predictor	Standardised parameter estimate	Standard error	*p* value
Top predator δ^{15}N	0.62	Slope	−0.209	0.059	**0.0004**
		Percent indigenous forest upstream	−0.420	0.052	**<0.0001**
		Catchment area	0.202	0.058	**0.0005**
		Total phosphorus	0.569	0.054	**<0.0001**
Total nitrogen	0.66	Percent indigenous forest upstream	−0138	0.050	**0.0064**
		Elevation	−0.577	0.057	**<0.0001**
		Slope	−0.331	0.060	**<0.0001**
		Catchment area	−0.201	0.053	**0.0002**
Total phosphorus	0.60	Percent indigenous forest upstream	0.193	0.054	**0.0005**
		Catchment area	−0.266	0.053	**<0.0001**
		Elevation	−0.769	0.056	**<0.0001**

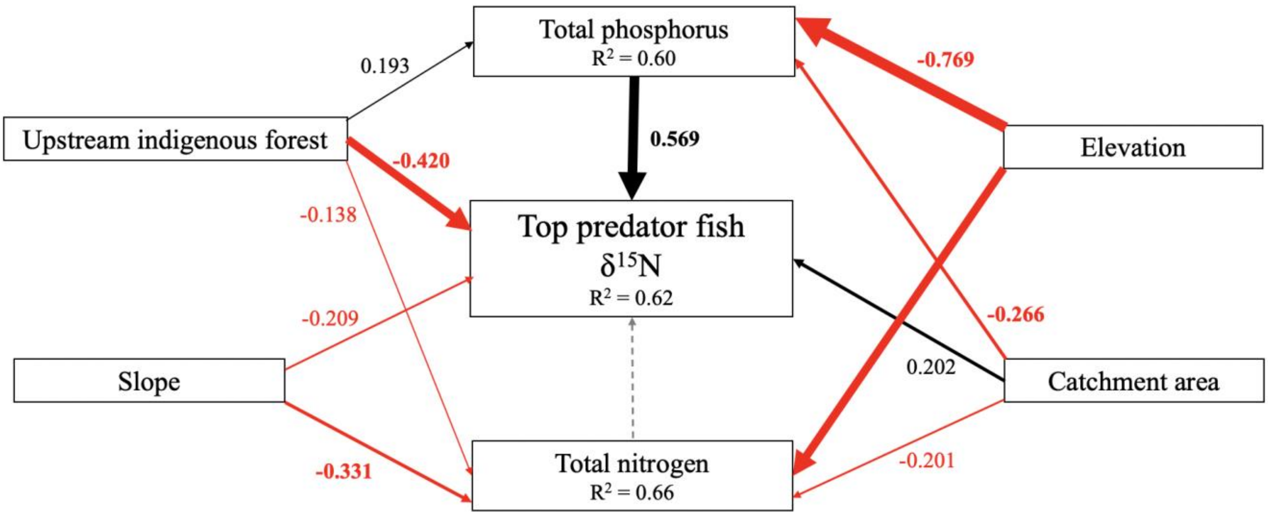


Figure 4.15. Results from piecewise structural equation modelling of top predator fish δ^{15}N interrelationships. Solid black arrows indicate significant positive effect, solid red arrows indicate a significant negative effect, and dashed grey arrows denote a non-significant effect ($p \geq 0.05$). The thickness of the significant paths reflects the magnitude of the standardised regression coefficient. R^2 are reported for the endogenous variables (within boxes), and standardised parameter estimates are reported for significant model pathways. Numbers above arrows are correlation coefficients.

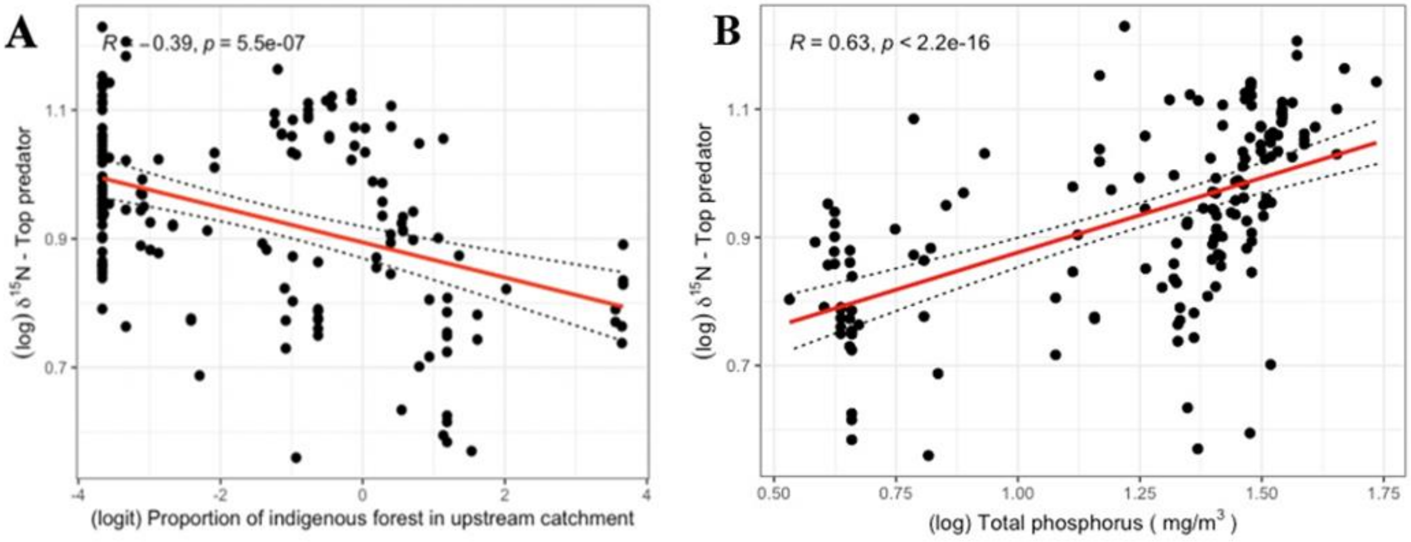


Figure 4.16. Plots of significant relationships ($p < 0.05$) found from the structural equation modelling of top predator δ^{15}N, including (A) the effect of indigenous forest in the upstream catchment on δ^{15}N, and the (B) effect of total phosphorus on δ^{15}N. The Spearman rank correlation r and p values are included in each plot, the red lines are the predicted linear regressions, and dashed black lines indicate 95% confidence intervals for the linear regressions.

4.3 Invertebrate nitrogen isotopic ratios in streams

4.3.1 Variation in δ^{13}C and δ^{15}N values of invertebrates

A total of 2422 samples of predatory and non-predatory invertebrates from 426 stream site locations were analysed for carbon and/or nitrogen isotopic ratios. There was a large variation in invertebrate isotopic values across all species and streams for both the δ^{13}C (−40.43 to −13.09‰; range of 27.34‰) (Fig. 4.17A), and δ^{15}N values (−4.50 to 18.21‰, range of 22.71‰) (Fig. 4.17B). The mean invertebrate δ^{13}C in the whole dataset was −26.30 ± 3.28‰ (mean ± 1 standard deviation, $n = 1045$), and the mean invertebrate δ^{15}N was 5.06 ± 3.71‰ (mean ± 1 standard deviation, $n = 2419$).

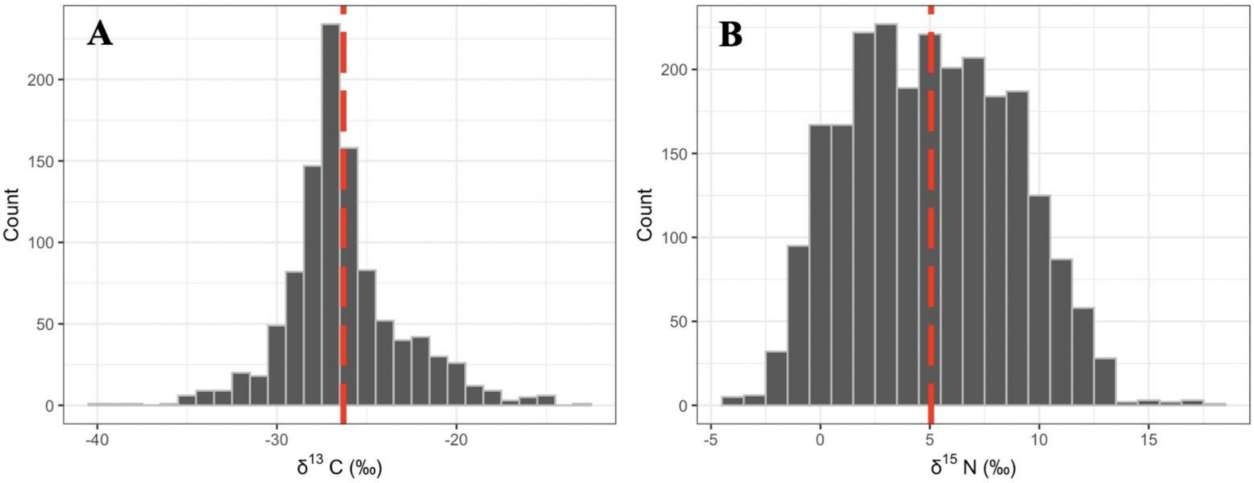


Figure 4.17. Frequency distribution histogram of the (A) mean carbon stable isotope ratios (δ^{13}C) of invertebrates in New Zealand streams ($n = 1045$), and (B) mean nitrogen stable isotope ratios (δ^{15}N) of primary consumers in New Zealand streams ($n = 2419$). Dotted red line indicates the mean value.

As significantly more of the isotopic data collated from stream studies provided only the δ^{15}N values, compared to the δ^{13}C values, more focus will be on the variability of the mean δ^{15}N of invertebrates. There was a very strong positive correlation between the mean δ^{15}N values for the invertebrates and top predators at each stream site (Spearman rank correlation, $r = 0.93$, $p < 0.001$). As was expected, as the δ^{15}N of the invertebrates at a site increase, the δ^{15}N of the top predators also increase (Fig. 4.18).

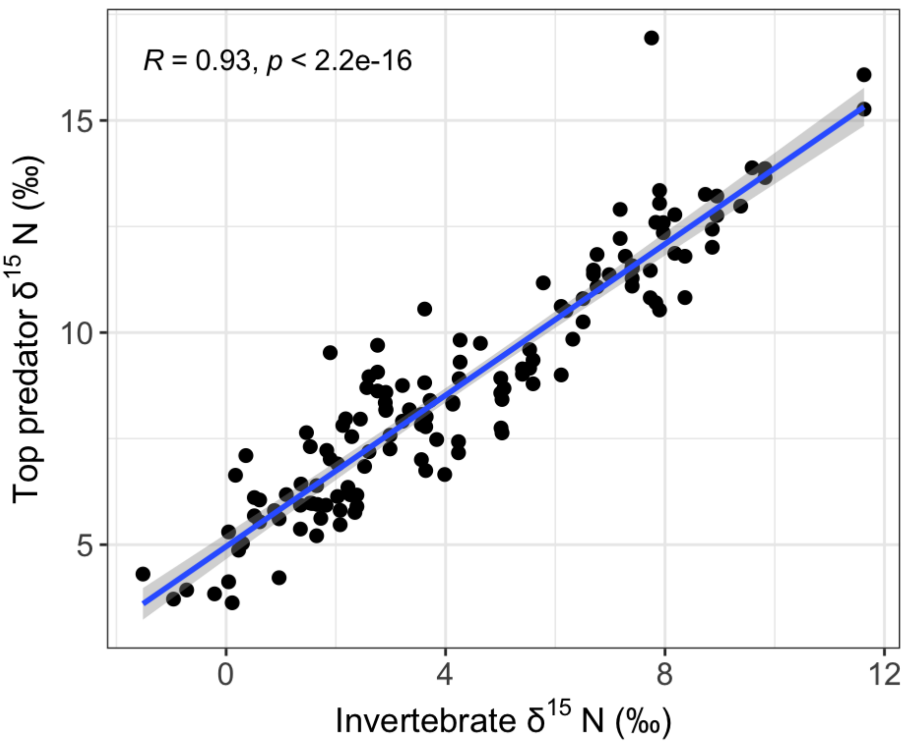


Figure 4.18. Relationship between the mean δ^{15}N values of invertebrates and mean δ^{15}N values of top predators across stream sites ($r = 0.93$, $p < 0.001$, $n = 140$).

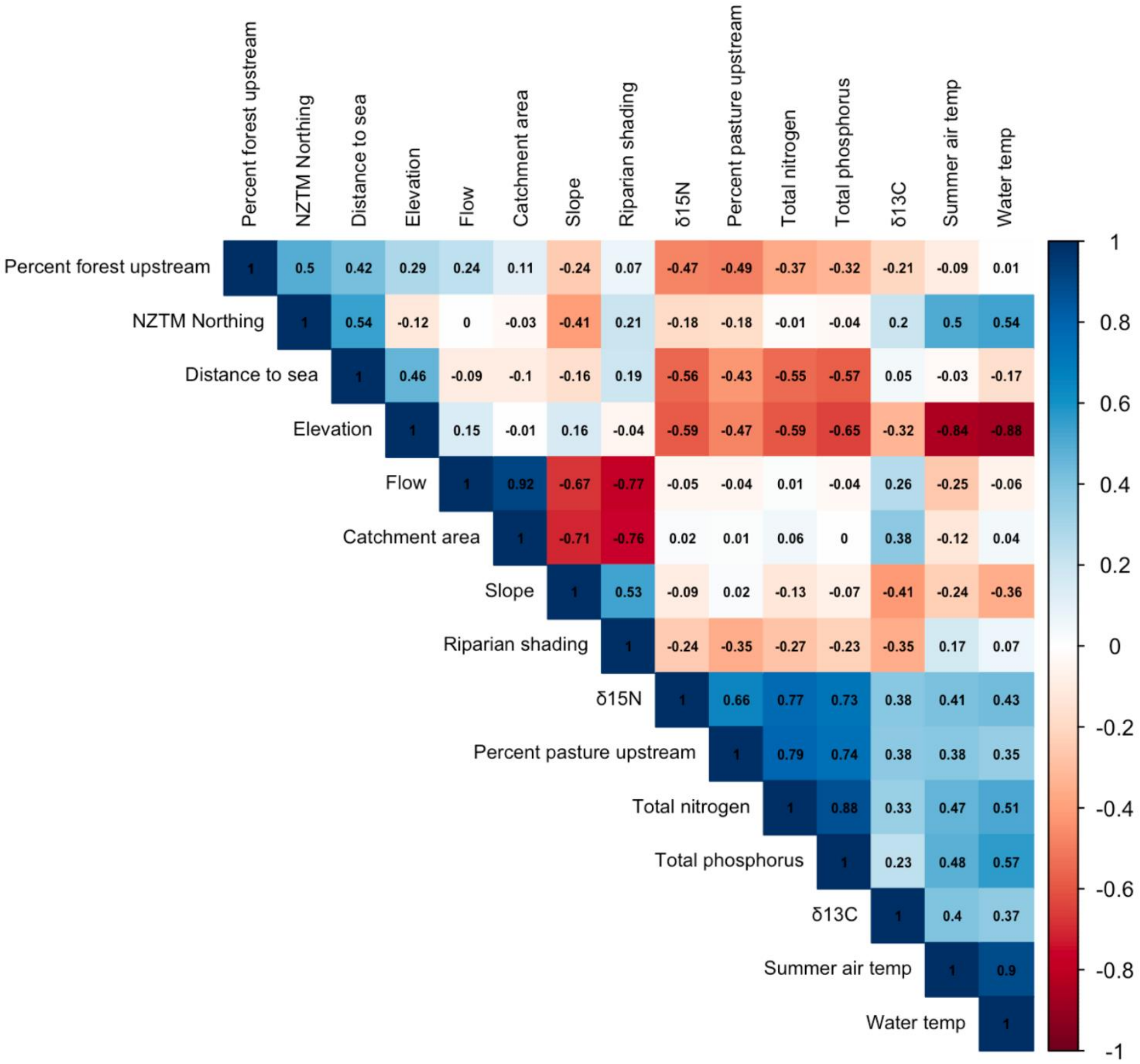
The invertebrate δ^{15}N isotopic values collated from various stream studies included both non-predatory (mean 4.66 ± 3.60‰, $n = 1793$) and predatory (mean 6.19 ± 3.78‰, $n = 627$) invertebrate species. There was a significant difference between the invertebrate δ^{15}N values of predatory and non-predatory species (one-way ANOVA, $F_{1, 2416} = 82$, $p < 0.001$), where as expected, the predatory invertebrates tend to have more positive δ^{15}N in streams compared to the non-predatory invertebrates. As a consequence of this difference, stream invertebrates were pooled into predatory and non-predatory groups, in order to assess the variance accounted for by spatial factors, as opposed to variation among species of invertebrates.

4.3.2 Environmental controls on invertebrate δ^{15}N

Both the non-predatory and predatory invertebrate δ^{15}N values in streams were statistically significantly correlated with a number of environmental predictors. The mean invertebrate δ^{15}N values were significantly and positively correlated with the land-use influenced variables including the total nitrogen concentrations (non-predatory $r = 0.73$, $p < 0.001$; predatory $r = 0.78$, $p < 0.001$), total phosphorus concentrations (non-predatory $r = 0.69$, $p < 0.001$; predatory $r = 0.77$, $p < 0.001$), and percent pasture upstream (non-predatory $r = 0.69$, $p < 0.001$; predatory $r = 0.70$, $p < 0.001$) (Table 4.11; Fig. 4.19; Appendix 3). Stream invertebrate δ^{15}N were also significantly and positively correlated with the mean annual water temperature (non-predatory $r = 0.41$, $p < 0.001$; predatory $r = 0.68$, $p < 0.001$), while were negatively related to the stream reach elevation (non-predatory $r = -0.56$, $p < 0.001$; predatory $r = -0.77$, $p < 0.001$).

Table 4.11. Spearman rank correlation coefficients for δ^{15}N of non-predatory (primary consumer) and predatory (secondary consumer) invertebrates and selected environmental factors from the sampled stream sites. * correlation significant at $p \leq 0.05$ level. ** correlation significant at $p \leq 0.001$. The full spearman correlation matrix for stream invertebrates isotopic values and selected environmental variables is presented in Appendix 3.

Variable	Non-predatory invertebrate δ^{15}N	Predatory invertebrate δ^{15}N
Water temperature	**0.41****	**0.68****
Elevation	**−0.56****	**−0.77****
Catchment area	**0.21****	**0.16****
Total nitrogen	**0.73****	**0.78****
Total phosphorus	**0.69****	**0.77****
Percent pasture upstream	**0.69****	**0.70****



	Percent forest upstream	NZTM Northing	Distance to sea	Elevation	Flow	Catchment area	Slope	Riparian shading	δ15N	Percent pasture upstream	Total nitrogen	Total phosphorus	δ13C	Summer air temp	Water temp
Percent forest upstream	1	0.5	0.42	0.29	0.24	0.11	-0.24	0.07	-0.47	-0.49	-0.37	-0.32	-0.21	-0.09	0.01
NZTM Northing		1	0.54	-0.12	0	-0.03	-0.41	0.21	-0.18	-0.18	-0.01	-0.04	0.2	0.5	0.54
Distance to sea			1	0.46	-0.09	-0.1	-0.16	0.19	-0.56	-0.43	-0.55	-0.57	0.05	-0.03	-0.17
Elevation				1	0.15	-0.01	0.16	-0.04	-0.59	-0.47	-0.59	-0.65	-0.32	-0.84	-0.88
Flow					1	0.92	-0.67	-0.77	-0.05	-0.04	0.01	-0.04	0.26	-0.25	-0.06
Catchment area						1	-0.71	-0.76	0.02	0.01	0.06	0	0.38	-0.12	0.04
Slope							1	0.53	-0.09	0.02	-0.13	-0.07	-0.41	-0.24	-0.36
Riparian shading								1	-0.24	-0.35	-0.27	-0.23	-0.35	0.17	0.07
δ15N									1	0.66	0.77	0.73	0.38	0.41	0.43
Percent pasture upstream										1	0.79	0.74	0.38	0.38	0.35
Total nitrogen											1	0.88	0.33	0.47	0.51
Total phosphorus												1	0.23	0.48	0.57
δ13C													1	0.4	0.37
Summer air temp														1	0.9
Water temp															1

Figure 4.19. Spearman correlation matrix for invertebrate isotopic values and stream environmental variables.

4.3.3 Principal component analysis for invertebrate δ^{15}N in streams

A PCA was carried out to investigate which of the selected environmental predictors (nitrogen load, proportion of upstream pasture, elevation, catchment area, and water temperature) best explained the non-predatory and predatory invertebrate δ^{15}N variation between the sampled New Zealand streams. Principal component axes one and two have eigenvalues greater than 1 (2.1 and 1.0 respectively), and therefore should be retained according to the Kaiser criterion (Kaiser 1960). The first axis of the PCA accounted for 63.7% of the total variance, while the second axis accounted for 14.1% of the total variance (Table 4.12). As a result, up to 77.9% of the total variance can be explained by the first two principal components.

Table 4.12. Eigenvalues and percent variation for first three principal component (PC) axes for stream environmental variables.

PC axis	Eigenvalue	Percent variation	Cumulative percent variation
1	2.11	63.69	63.69
2	1.00	14.16	77.85
3	0.93	8.33	86.17

The first PC axis was strongly negatively related to the elevation of the stream site while was positively related to both the stream non-predatory and predatory invertebrates δ^{15}N values, as well as the percent pasture upstream in the stream catchment, the rate of nitrogen loading, the catchment area and the mean annual water temperature (Fig. 4.20). PC axis two was strongly negatively related to the water temperature and catchment area of the stream site, while positively related to the δ^{15}N signatures, percent pasture upstream, nitrogen load and elevation.

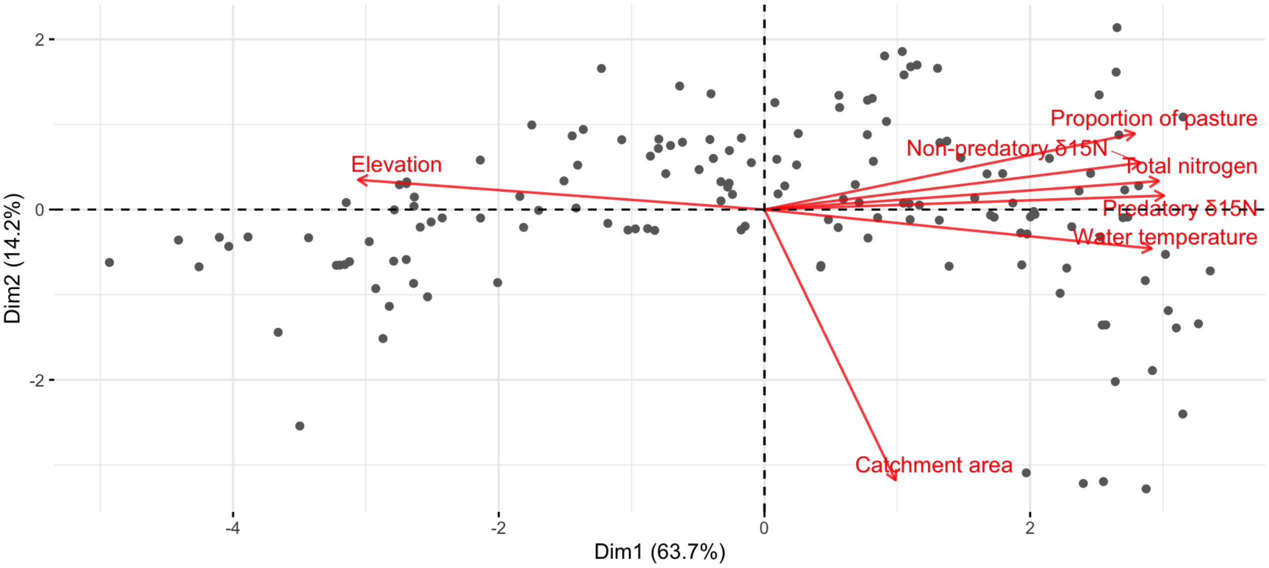


Figure 4.20. PCA biplot of the first two principal components (Dim1 and Dim2) for the non-predatory and predatory invertebrate δ^{15}N and the five environmental predictors; catchment area, water temperature, percent pasture upstream, elevation, and nitrogen loading.

According to the factor loadings for each principal component, PC axis one largely represented the δ^{15}N signatures of the stream non-predatory and predatory invertebrates, as well as all the environmental predictors (proportion of pasture, elevation, total nitrogen and water temperature) except for the catchment area (Table 4.13). Contrastingly, PC axis two only strongly represented the catchment area. Similar to the top predator δ^{15}N findings, the PCA results provide evidence that the δ^{15}N values of both non-predatory and predatory invertebrates also respond more readily to the watershed land use than alternative sources of variation.

Table 4.13. Factor loadings for the non-predatory and predatory invertebrate δ^{15}N and each of the five environmental predictors. Values > 0.3 and < −0.3 are shown in bold.

Variable	Loadings	
	PC1	PC2
Non-predatory δ^{15}N	**0.391**	0.161
Predatory δ^{15}N	**0.415**	0.048
Percent pasture upstream	**0.385**	0.262
Elevation	−**0.422**	0.103
Total nitrogen	−**0.410**	0.098
Water temperature	**0.402**	−0.134
Catchment area	0.136	−**0.930**

4.3.4 Invertebrate δ^{15}N structural equation modelling

Fisher's *C* statistic confirmed that the piecewise structural equation model constructed for the isotopic ratios and stream environmental predictors (Fig. 4.21) was appropriate for the data (p = 0.233, above the 0.05 significance threshold) (Lefcheck 2016). The pSEM results showed that the total phosphorus concentration was the strongest predictor of both non-predatory and predatory invertebrate δ^{15}N values in streams (Table 4.14). The proportion of pasture also significantly and positively affected the invertebrate δ^{15}N signatures, while elevation negatively impacted the C and N isotopic ratios. The R^2 for the predatory invertebrate δ^{15}N (0.57) was slightly higher than that for the non-predatory invertebrate δ^{15}N (0.49), therefore indicating that the selected environmental variables explained a greater amount of the total variance for the predatory invertebrates compared to non-predatory invertebrates for the compiled dataset.

Table 4.14. Standardised component model results from the piecewise structural equation model (pSEM) for non-predatory and predatory invertebrates δ^{15}N from sampled New Zealand streams (n = 138). The best-fit model presented had the lowest AICc score (Fisher's *C* test = 10.479, p = 0.233 on 8 df). Statistically significant relationships between the response and predictor are shown in bold (i.e., $p < 0.05$).

Response	R^2	Predictor	Standardised parameter estimate	Standard error	p value
Non-predatory invertebrate δ^{15}N	0.49	Total phosphorus	0.380	0.118	**0.002**
		Percent pasture upstream	0.195	0.101	**0.0246**
		Elevation	−0.210	0.113	**0.0241**
Predatory invertebrate δ^{15}N	0.57	Total phosphorus	0.314	0.082	**0.0007**
		Percent pasture upstream	0.294	0.070	**0.0003**
		Elevation	−0.245	0.078	**0.0046**
Total nitrogen	0.74	Proportion of pasture	0.622	0.052	**<0.0001**
		Elevation	−0.203	0.057	**0.0009**
		Slope	−0.210	0.047	**<0.0001**
Total phosphorus	0.61	Percent pasture upstream	0.364	0.067	**<0.0001**
		Elevation	−0.502	0.069	**<0.0001**

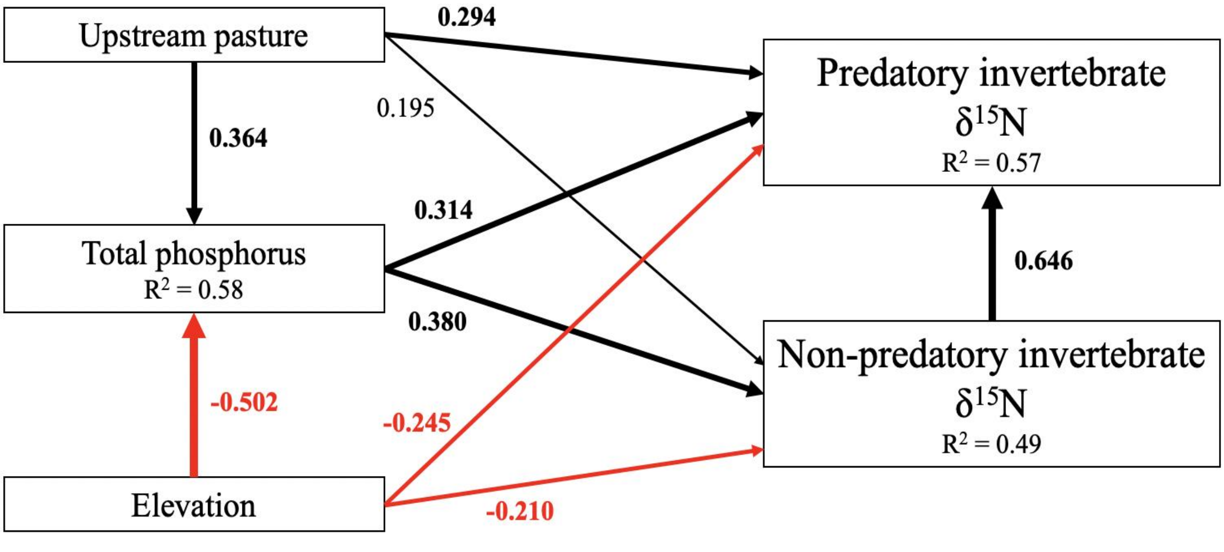


Figure 4.21. Results from piecewise structural equation modelling of invertebrate δ^{15}N interrelationships. Solid black arrows indicate significant positive effect, solid red arrows indicate a significant negative effect, and dashed grey arrows denote a non-significant effect ($p \geq 0.05$). The thickness of the significant paths reflects the magnitude of the standardised regression coefficient. R^2 are reported for the endogenous variables (within boxes), and standardised parameter estimates are reported for significant model pathways. Numbers above arrows are correlation coefficients.

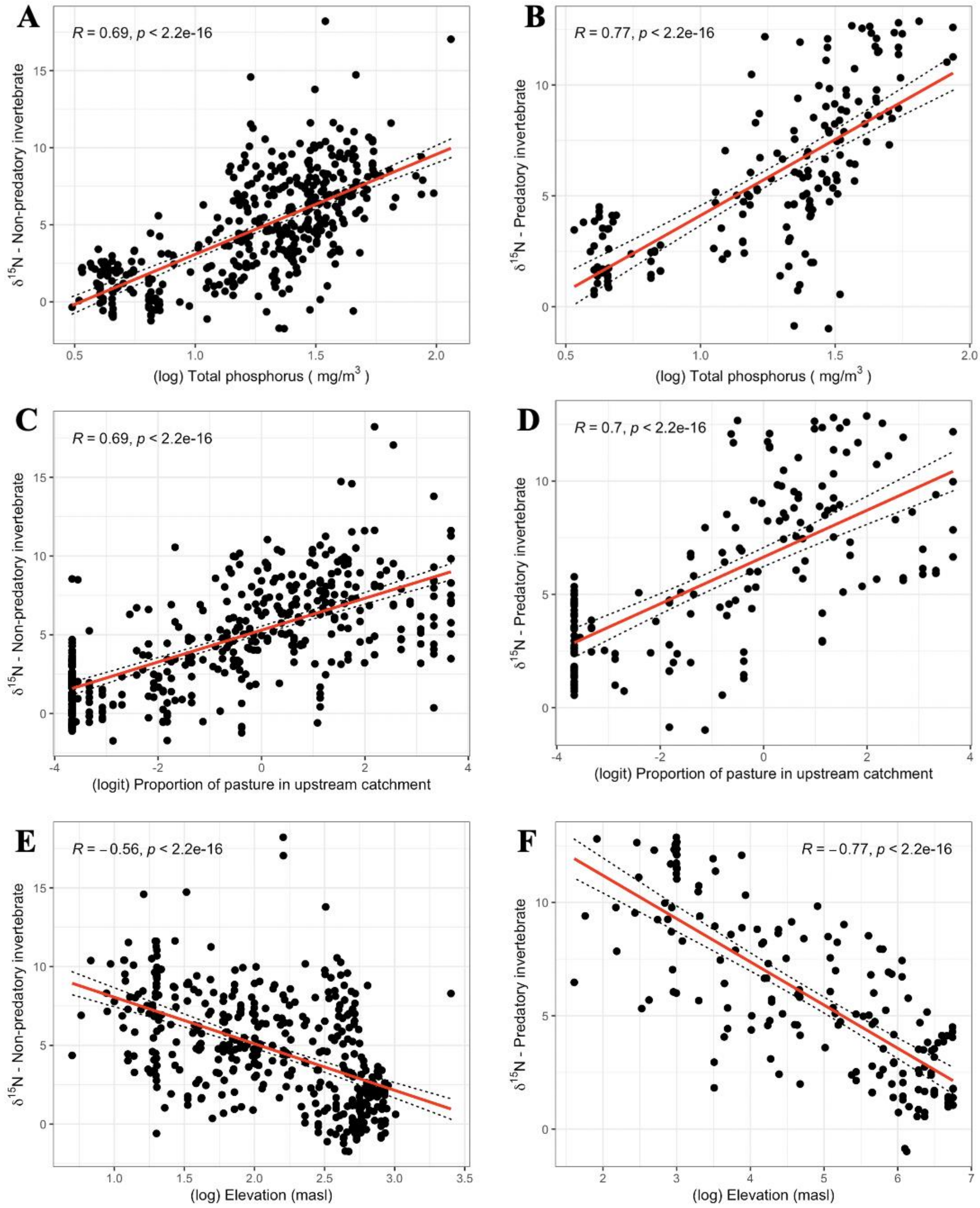


Figure 4.22. Plots of significant relationships ($p < 0.05$) found from the structural equation modelling of invertebrate δ^{15}N, including (A) the effect of total phosphorus on non-predatory invertebrate δ^{15}N, (B) effect of total phosphorus on predatory invertebrate δ^{15}N, (C) effect of pasture in the upstream catchment on non-predatory invertebrate δ^{15}N, (D) effect of pasture in the upstream catchment on predatory invertebrate δ^{15}N, (E) effect of elevation on non-predatory invertebrate δ^{15}N, and (F) effect of elevation on predatory invertebrate δ^{15}N. The Spearman rank correlation *r* and *p* values are included in each plot, the red lines are the predicted linear regressions, and dashed black lines indicate 95% confidence intervals for the linear regressions.

Chapter 5

Discussion and conclusion

5.1 δ^{13}C variability of top predators in lakes

The variability of δ^{13}C values in top-predatory fish in New Zealand lakes was best explained by the morphology of the lake, as shown in the correlations, PCA and pSEM outputs. Fish sampled from a lake with a large surface area and volume had higher δ^{13}C values than individuals from lakes of smaller size, thus meeting our hypothesis that lake size was the most significant predictor of δ^{13}C variability among lakes in New Zealand. In general, as the lake size increased, the δ^{13}C values also tended to become more positive and closer to isotopic equilibrium with the atmosphere because of longer detention times. The observed patterns in top predator δ^{13}C were consistent with other isotopic studies, such as for periphyton (McCabe 1985), seston (Post 2002), dissolved inorganic carbon (DIC) (Bade et al. 2004), and a number of fish species, including whitefish (*Coregonus lavaretus*) (Perga and Gerdeaux 2004), rainbow trout (*Oncorhynchus mykiss*), common smelt (*Retropinna retropinna*), common bully (*Gobiomorphus cotidianus*) (McBride 2005), black bullhead (*Ameiurus melas*), fathead minnow (*Pimephales promelas*), and common carp (*Cyprinus carpio*) (Langer et al. 2022).

This positive relationship between the lake size and δ^{13}C values of aquatic organisms suggests differences among lakes of different size classes in their DIC pool (Post 2002). In small lakes, the autochthonous production is largely dependent on the recycled carbon sourced from respiration and mineralisation processes (Perga and Gerdeaux 2004). The DIC from these mineralisation processes tended to be the lightest isotopically, with low δ^{13}C values ranging between −35‰ to −20‰, reflecting the signature of the parent mineralised organic matter (Post 2002; Perga and Gerdeaux 2004). In larger lakes with long retention time, however, the DIC has longer to equilibrate with atmospheric CO_2 so is more positive than in small lakes, and therefore the autochthonous production is largely derived from atmospheric CO_2 and carbonate sources (Post 2002; Langer et al. 2022). DIC originating from atmospheric CO_2 and carbonate weathering processes have higher δ^{13}C signatures of approximately −7‰ and 0‰, respectively (Post 2002). As a result, as a large lake becomes more autotrophic, this allows for more production to originate from atmospheric CO_2, due to the enhanced gas exchange processes and turbulence regimes (Wanninkhof 1992), which could be a contributing factor to the observed enrichment of fish δ^{13}C with lake size. The significant positive correlation between the lake size and δ^{13}C values has also been suggested to be a result of the higher terrigenous organic carbon inputs, with more negative δ^{13}C, into smaller lakes (Post et al. 2002; Bade et al. 2004).

Several studies have identified the nutrient status and rates of primary production to be a significant driver of δ^{13}C in lakes, for example, in a study comprising of over 20 lakes, the variability in δ^{13}C values of the zooplanktivorous whitefish was strongly positively correlated with the total phosphorus concentrations and lake productivity ($R^2 = 0.71$) (Perga and Gerdeaux 2004). This was not the case in New Zealand where neither the watershed land use nor the total nitrogen concentrations had a significant detectable effect on the δ^{13}C values of top predators when evaluated by correlation, PCA, or piecewise SEM. Any potential differences in δ^{13}C attributed to anthropogenically-induced impacts were most likely masked by the much stronger influence of lake size on the C isotopic signatures of aquatic organisms.

5.2 δ^{15}N variability of top predators in lakes

The variability of δ^{15}N values of top-predatory fish in New Zealand lakes was not driven by lake size, as it was for the δ^{13}C ratios, but rather was best explained by the dominant land use within the lake catchment, as shown in the correlations, PCA and pSEM outputs. Top-predatory fish sampled from lakes dominated by pastoral land within the watershed typically recorded higher δ^{15}N signatures compared to lakes with catchments dominated by indigenous forest, thereby meeting our hypothesis that catchment land use was one of the most significant predictors of δ^{15}N variability in streams. These patterns observed in δ^{15}N were again consistent with those in other isotopic studies, including for benthic macroinvertebrates (Vander Zanden et al. 2005), and a number of fish species, such as black bullhead, fathead minnow, and common carp (Langer et al. 2022).

High agricultural activity within a lake catchment is known to be a dominant source of total nitrogen and phosphorus loading into a lake, which was consistent with our findings, in which the percentage of grassland in the lake catchment was significantly correlated with the nitrogen load per surface area (Spearman rank correlation, $r = 0.42$, $p < 0.001$). This confirms that for the New Zealand lakes included in this analysis, the higher the percentage of pastoral land in the catchment, the greater the input of N relative to the lake surface area. Larger nitrogen inputs to the lake in turn result in higher denitrification rates

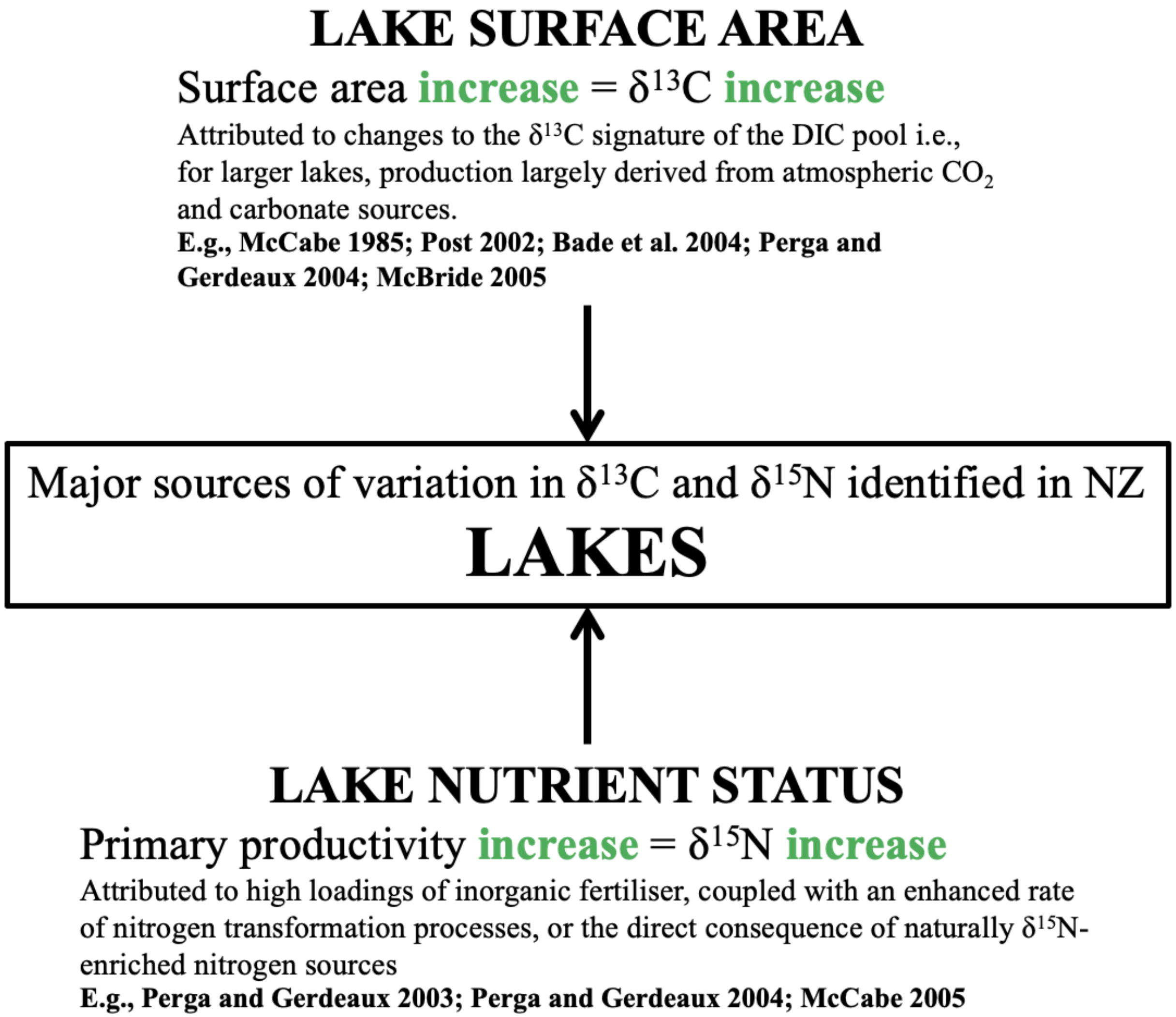
(Vander Zanden et al. 2005), subsequently enriching the isotopic signatures of the dissolved inorganic nitrogen (DIN) pool.

δ^{15}N signatures, however, not only reflect the total amount of nitrogen loading to which the lake receives, but also the source of this nitrogen (Vander Zanden et al. 2005). Nitrogen originating from commercial inorganic fertilisers tend to have relatively low δ^{15}N values (~0‰), whereas animal waste, such as through the application of manure, is known to have enriched δ^{15}N signatures (~+10 – +20‰) (Heaton 1986; Langer et al. 2022). Despite their low δ^{15}N signature, commercial fertilisers can contribute to an enrichment in the δ^{15}N of the aquatic biota, when application and subsequent denitrification rates are high. Positive correlations have previously been identified between the soil δ^{15}N and the rate of fertiliser application and irrigation frequency in New Zealand, with land receiving higher rates of fertiliser or more frequent irrigation having elevated δ^{15}N values (average increase from 0.015 to 0.034‰ yr^{-1}), due to the increased pasture production, grazing intensity, and N inputs and losses (Mudge et al. 2013). In this study, the specific origin of agricultural N inputs was unknown for each lake.

Subsequently, the significant relationship between the percentage of pastoral land in the watershed and the top predator fish δ^{15}N is likely to either be the result of high loadings of inorganic fertiliser, coupled with an enhanced rate of nitrogen transformation processes, or the direct consequence of naturally δ^{15}N-enriched nitrogen sources. Overall, our findings confirm that across multiple lakes in New Zealand, agricultural activity in the catchment is the primary driver of variability in δ^{15}N of top predators, and thus δ^{15}N ratios are likely to strongly reflect the input of anthropogenic nutrients and dominant land use in the watershed.

In our dataset, the relatively low R^2 values for both δ^{13}C and δ^{15}N of top-predatory fish ($R^2 = 0.48$ and 0.32 respectively) of the piecewise structural equation model suggests that a large amount of isotopic variation remains unexplained. Short-term variations in environmental conditions could be important to both δ^{13}C and δ^{15}N values, however, may not be captured in this study, and thus their significance was not evaluated. Finer seasonal resolution would therefore be important to obtain a greater understanding of local controls on the isotopic values.

The fish occupying the top trophic position can be considered as an accurate representation of a lake food web in New Zealand. Macrophytes (primary producers) on the other hand, often have no meaningful contribution to New Zealand lake food webs, therefore having disjointed isotopic signatures and appearing to be disassociated with the other organisms in the food web. For example, in Lake Rotokare, Taranaki, the charophytes had significantly depleted δ^{15}N signatures compared to the rest of the food web (Smith 2018), while in Lake Tikitapu, Rotorua, the macrophytes had significantly enriched δ^{13}C signatures relative to the other organisms (McBride 2005). These results suggest that macrophytes are often not a significant source of energy and are not generally incorporated much into in lake food webs in New Zealand.



LAKE SURFACE AREA

Surface area **increase** = $\delta^{13}C$ **increase**

Attributed to changes to the $\delta^{13}C$ signature of the DIC pool i.e., for larger lakes, production largely derived from atmospheric CO_2 and carbonate sources.

E.g., McCabe 1985; Post 2002; Bade et al. 2004; Perga and Gerdeaux 2004; McBride 2005

Major sources of variation in $\delta^{13}C$ and $\delta^{15}N$ identified in NZ

LAKES

LAKE NUTRIENT STATUS

Primary productivity **increase** = $\delta^{15}N$ **increase**

Attributed to high loadings of inorganic fertiliser, coupled with an enhanced rate of nitrogen transformation processes, or the direct consequence of naturally $\delta^{15}N$-enriched nitrogen sources

E.g., Perga and Gerdeaux 2003; Perga and Gerdeaux 2004; McCabe 2005

Figure 5.1. Summary of the major sources of variation for top-predatory fish $\delta^{13}C$ and $\delta^{15}N$ in New Zealand lake environments.

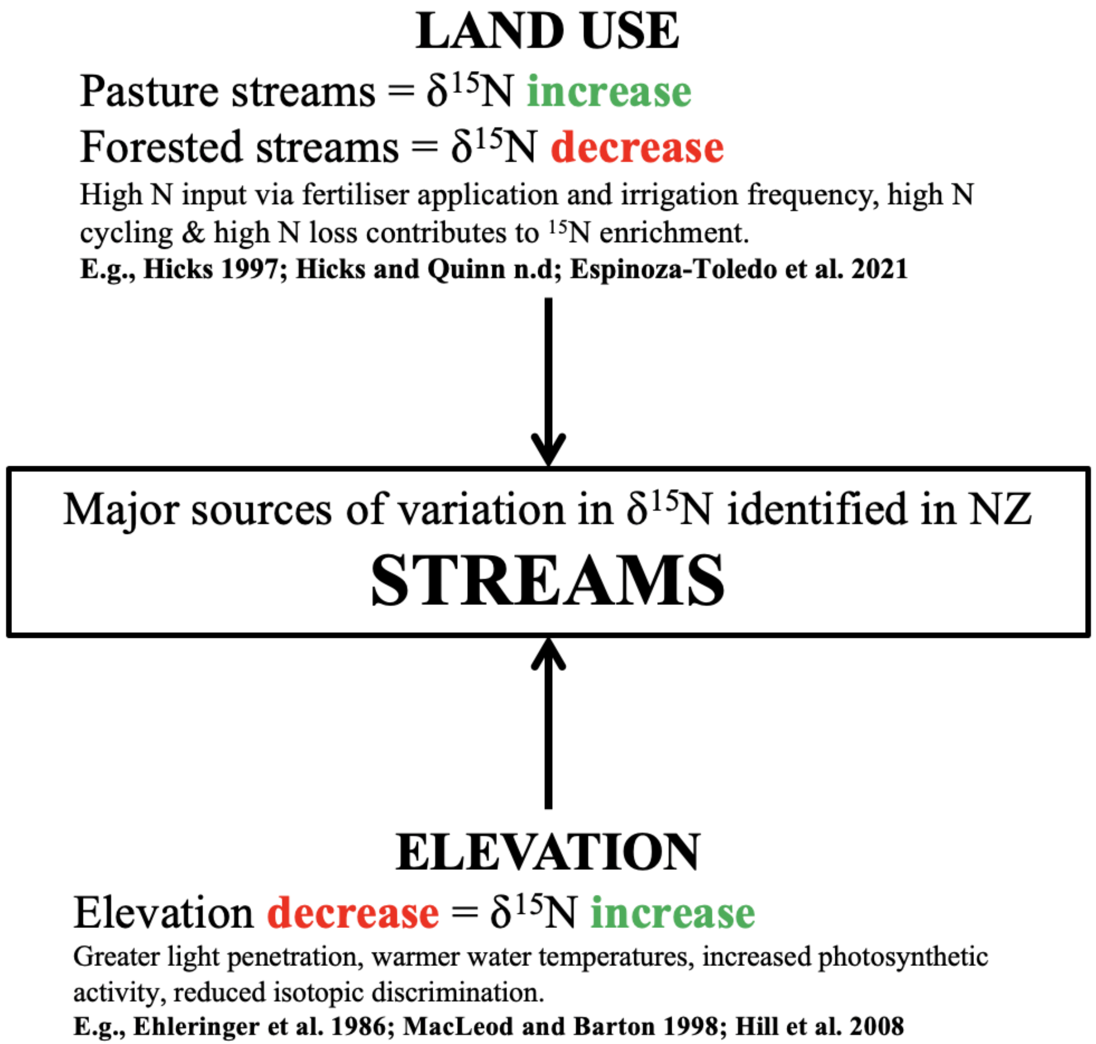
5.3 δ^{15}N variability of invertebrate and top predators in streams

The variability of δ^{15}N in non-predatory and predatory invertebrates, and top-predatory fish in New Zealand streams was best explained by the local land use in the upstream catchment, and the nutrient concentrations, i.e., the total nitrogen and total phosphorus concentrations, as shown in each of the correlation analysis, PCA and pSEM outputs. In general, aquatic organisms sampled from streams draining catchments with high agricultural activity had higher δ^{15}N signatures, compared to individuals sampled from streams draining forested catchments. This strong influence of land use on the δ^{15}N of aquatic freshwater consumers is consistent with previous isotopic studies, whereby pasture-influenced streams have higher δ^{13}C and δ^{15}N values compared to forested streams (Hicks 1997; Hicks unpublished data; Harrington et al. 1998; Clapcott et al. 2010; Espinoza-Toledo et al. 2021; Machado-Silva et al. 2022). These findings support the hypothesis that anthropogenic inputs, such as land-use intensification in pasture-dominated watersheds, drive an enrichment of δ^{15}N signatures of stream aquatic organisms, similar to the variability identified for New Zealand lakes.

The enrichment of δ^{15}N in stream sites with higher proportions of pastoral cover in the watershed is likely to be a consequence of the enhanced nitrogen cycling brought about by the higher rates of primary productivity. Anthropogenic land use, including agricultural practices, promotes denitrification, and in turn, contributes to enrichment of ^{15}N in organisms (Hoffman et al. 2012; Davias et al. 2014). Stevenson et al. (2010) documented how the intensification of land use management regimes in New Zealand can contribute to increased soil δ^{15}N, with indigenous forestry soils having the lowest mean δ^{15}N value of 2.1‰, followed by plantation forestry (2.8‰), pasture under drystock (sheep or beef cattle) (3.8‰), pasture under dairy (5.4‰), and managed cropped soils (6.2‰). Enrichment of δ^{15}N with increasing pastoral land use intensity could be attributed to the numerous fractionation processes that contribute to increased loss of ^{14}N from the soil, including nitrification, ammonia volatilisation, and denitrification following the application of N through fertiliser and animal manure (Anderson and Cabana 2005). These N transformation processes discriminate against the heavier ^{15}N isotope, and therefore N species lost from the soil, e.g., NO_3^-, NH_3, N_2, and N_2O, are depleted in ^{15}N, contributing to more positive δ^{15}N signatures for the remaining N substrates, e.g., NH_4^+ and organic N (Stevenson et al. 2010; Mudge et al. 2014). As the two major nitrogen inputs to New Zealand soils, N fixation and urea, both have isotopic signatures of 0‰, the soil δ^{15}N enrichment with land use intensification is largely a result of the enhanced rate of nitrogen cycling and isotope fractionating N losses from the soil (Stevenson et al. 2010). The introduced agricultural run-off is therefore thought to contribute to the enrichment of N isotopes across the aquatic food web, as seen by the higher δ^{15}N values for invertebrates and fish recorded for New Zealand streams in this study.

Among all the environmental and climatic variables analysed in the piecewise structural equation modelling, however, the estimated concentration of total phosphorus in the stream reach was the most important predictor of δ^{15}N spatial variability for non-predatory invertebrates, predatory invertebrates, and top predator fish species. The observed enrichment of ^{15}N in pastoral-dominated stream reaches would therefore, likely be a result of the enhanced rate of primary production simulated by greater phosphorus availability. Under conditions of high total phosphorus concentrations at the pasture-dominated sites, the demand for N is high, contributing to lower ^{15}N fractionation and thus enriched δ^{15}N signatures of aquatic organisms. Conversely, under conditions of low total phosphorus concentrations and subsequent P stress, greater ^{15}N fractionation by primary producers is likely to occur during the uptake of dissolved inorganic nitrogen (DIN) (Hong and Gu 2020). A recent stable isotope study also identified the total phosphorus concentrations to be the limiting factor driving ecosystem primary productivity and fish δ^{15}N ratios in south Florida wetlands (Hong and Gu 2020). In our study, the relationship between the total phosphorus and stream δ^{15}N signatures was stronger for the top-predatory fish than for invertebrates. This suggests that δ^{15}N in top predators may be a more effective environmental indicator of excessive phosphorus loading and eutrophication compared to the primary and secondary consumer invertebrate species. This is because fish are better known to integrate temporal and spatial variations in N stable isotopic ratios over long term periods, compared to organisms occupying lower trophic positions in the aquatic food web (Cabana and Rasmussen 1996; Vander Zanden et al. 2005; Hong and Gu 2020).

The elevation of the stream site also was significantly related to both the non-predatory and predatory invertebrate δ^{15}N values, such that the δ^{15}N signatures decrease with increasing elevation. This is consistent with the significant correlations identified between pastoral land and δ^{15}N, as lower amounts of pasture typically occur at higher elevations (Spearman rank correlation, $r = 0.60$, $p < 0.001$). The δ^{15}N of top predators, however, did not have as strong a relationship with elevation as the invertebrate δ^{15}N signatures, which may be indicative of the mobile behaviour of fish that have the ability to move between habitats. Streams that drain into pasture-dominated catchments at low elevations are also associated with reduced canopy cover, enhanced light penetration, warmer water temperatures, and increased photosynthetic activity, all of which have been identified to contribute to the enrichment of δ^{15}N in streams (Quinn et al. 1997).



LAND USE

Pasture streams = δ^{15}N **increase**

Forested streams = δ^{15}N **decrease**

High N input via fertiliser application and irrigation frequency, high N cycling & high N loss contributes to ^{15}N enrichment.

E.g., Hicks 1997; Hicks and Quinn n.d; Espinoza-Toledo et al. 2021

Major sources of variation in δ^{15}N identified in NZ

STREAMS

ELEVATION

Elevation **decrease** = δ^{15}N **increase**

Greater light penetration, warmer water temperatures, increased photosynthetic activity, reduced isotopic discrimination.

E.g., Ehleringer et al. 1986; MacLeod and Barton 1998; Hill et al. 2008

Figure 5.2. Summary of the major sources of variation for invertebrates and top-predatory fish δ^{13}C and δ^{15}N in New Zealand stream environments.

5.4 Conclusion

Our study examined the variability of the stable isotopic ratios of C and N across large spatial scales for freshwater aquatic ecosystems in New Zealand and identified whether environmental factors could be driving any potential spatial variation. Our results confirm that for lake environments, as documented by previous stable isotopic studies, δ^{13}C values of top predators largely respond to the morphology of the lake, while δ^{15}N most strongly reflect the watershed land use and showed no apparent relationship with lake size. For stream and river environments, the land use in the upstream catchment and nutrient concentrations were the most significant predictors of the δ^{15}N values for non-predatory invertebrates, predatory invertebrates, and top-predator fish species. Overall, the findings in this study were consistent with previous isotopic research undertaken in North American and European aquatic ecosystems, which have examined the influence of environmental factors on C and N isotopic ratios.

Additional questions regarding the spatial variability of δ^{13}C and δ^{15}N values of organisms in aquatic ecosystems, however, remain. For the top-predatory fish, further variation may be introduced through the biological characteristics of the sampled individual, such as with the age, size, gender, and growth rates, all of which were not considered in this study. Also, as discussed in a previous chapter, stable isotopic signatures have also been documented to vary seasonally in both lakes and streams. As this meta-analysis focussed largely on analysing the spatial patterns of isotopic values, as opposed to the temporal variation, the majority of samples used in the analysis were collected during the New Zealand summer months (December to February), in an attempt to reduce the potential seasonal variability that may exist. However, future research should quantify the degree of seasonal variation for aquatic organism δ^{13}C and δ^{15}N across New Zealand streams and lakes. We also had insufficient evidence to accurately report on the potential effect of marine-derived nutrients on the isotopic signatures of organisms in New Zealand stream food webs, thus future studies could incorporate a larger sample size of coastal streams. Also, further research could go beyond understanding the environmental drivers of variability in stable isotopes, and focus on whether increased production can also lead to longer food chain lengths with more positive δ^{15}N values.

Both the C and N isotopic ratios reflect the necessity to consider the potential differences that may arise at the base of the food web as a consequence of natural ecosystem and watershed characteristics. An enhanced understanding of the natural ecosystem processes and anthropogenic influences, contributing to the spatial distribution of δ^{13}C and δ^{15}N of aquatic organisms, will assist and improve the study design and interpretation of stable isotopes as a tracer in food web and ecosystem ecology, by taking into account the natural and human-induced spatial subsidies. In particular, the δ^{15}N values of non-predatory and predatory invertebrates, as well as top-predatory fish in New Zealand streams should respond most readily to the watershed land use. Our findings provide further evidence that support the importance δ^{15}N signatures in aquatic organisms to monitor improvements in land use management in New Zealand.

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Appendices

Appendix 1. Spearman rank correlations for isotopic values and all selected environmental variables for top predators in lakes. Statistically significant differences ($p < 0.05$) are shown in **red and bold.**

	$\delta^{13}C$	$\delta^{15}N$	Lake elevation	Catchment elevation	Surface area	Volume	Residence time	Catchment area	Distance to sea	Percent forest	Percent grassland	N load / Surface area	Mean depth	Catchment ann temp	NZTM Northing
$\delta^{13}C$	1.00	0.15	**0.21**	**0.45**	**0.51**	**0.48**	**0.36**	**0.37**	0.12	0.06	-0.06	**-0.42**	0.07	-0.12	0.12
$\delta^{15}N$	0.15	1.00	**-0.20**	0.02	0.08	-0.04	0.00	0.12	-0.05	**-0.27**	**0.32**	0.18	**-0.26**	-0.08	-0.12
Lake elevation	**0.21**	**-0.20**	1.00	**0.38**	-0.13	0.09	**0.44**	-0.15	**0.46**	0.07	0.00	-0.09	**0.68**	-0.05	**0.39**
Catchment elevation	**0.45**	0.02	**0.38**	1.00	**0.56**	**0.72**	0.03	**0.62**	**0.56**	**0.48**	**-0.20**	-0.18	**0.39**	**-0.43**	0.18
Surface area	**0.51**	0.08	-0.13	**0.56**	1.00	**0.92**	0.03	**0.88**	**0.36**	**0.37**	-0.17	**-0.41**	-0.07	**-0.25**	0.03
Volume	**0.48**	-0.04	0.09	**0.72**	**0.92**	1.00	0.12	**0.85**	**0.52**	**0.48**	**-0.24**	**-0.40**	**0.26**	**-0.23**	0.19
Residence time	**0.36**	0.00	**0.44**	0.03	0.03	0.12	1.00	**-0.25**	**0.24**	**-0.27**	0.03	**-0.61**	**0.38**	**0.32**	**0.38**
Catchment area	**0.37**	0.12	-0.15	**0.62**	**0.88**	**0.85**	**-0.25**	1.00	**0.36**	**0.38**	-0.08	-0.10	-0.04	**-0.31**	-0.02
Distance to sea	0.12	-0.05	**0.46**	**0.56**	**0.36**	**0.52**	**0.24**	**0.36**	1.00	0.13	0.04	-0.03	**0.38**	0.01	**0.48**
Percent forest	0.06	**-0.27**	0.07	**0.48**	**0.37**	**0.48**	**-0.27**	**0.38**	0.13	1.00	**-0.66**	**-0.19**	**0.27**	**-0.38**	-0.05
Percent grassland	-0.06	**0.32**	0.00	**-0.20**	-0.17	**-0.24**	0.03	-0.08	0.04	**-0.66**	1.00	**0.42**	**-0.20**	**0.20**	0.10
N load / Surface area	**-0.42**	0.18	-0.09	-0.18	**-0.41**	**-0.40**	**-0.61**	-0.10	-0.03	**-0.19**	**0.42**	1.00	-0.07	-0.02	-0.03
Mean depth	0.07	**-0.26**	**0.68**	**0.39**	-0.07	**0.26**	**0.38**	-0.04	**0.38**	**0.27**	**-0.20**	-0.07	1.00	0.03	**0.37**
Catchment ann temp	-0.12	-0.08	-0.05	**-0.43**	**-0.25**	**-0.23**	**0.32**	**-0.31**	0.01	**-0.38**	**0.20**	-0.02	0.03	1.00	**0.66**
NZTM Northing	0.12	-0.12	**0.39**	**0.18**	0.03	**0.19**	**0.38**	-0.02	**0.48**	-0.05	0.10	-0.03	**0.37**	**0.66**	1.00

Appendix 2. Spearman rank correlations for isotopic values and all selected environmental variables for top predators in streams. Statistically significant differences ($p < 0.05$) are shown in **red and bold.**

	δ^{13}C	δ^{15}N	NZTM Northing	Jan air temperature	Flow	Slope	Riparian shade	% Indigenous forest	% Native vegetation	% Pasture	Elevation	Distance to sea	Catchment area	Total nitrogen	Total phosphorus	Water temperature
δ^{13}C	1.00	**0.42**	**0.21**	**0.21**	**0.41**	-0.17	**-0.42**	**0.22**	0.14	-0.05	-0.15	**-0.35**	**0.41**	0.06	**0.47**	**0.29**
δ^{15}N	**0.42**	1.00	-0.14	**0.39**	0.14	**-0.43**	**-0.28**	**-0.39**	**-0.61**	**0.64**	**-0.61**	**-0.61**	**0.19**	**0.71**	**0.63**	**0.44**
NZTM Northing	**0.21**	-0.14	1.00	**0.52**	-0.11	0.14	0.14	**0.58**	0.14	**-0.24**	-0.04	**0.34**	**-0.21**	-0.13	**0.18**	**0.50**
Jan air temperature	**0.21**	**0.39**	**0.52**	1.00	**-0.18**	**-0.24**	**0.16**	0.06	**-0.54**	**0.46**	**-0.80**	-0.10	-0.07	**0.51**	**0.60**	**0.94**
Flow	**0.41**	0.14	-0.11	**-0.18**	1.00	**-0.50**	**-0.84**	**0.27**	0.09	-0.02	0.00	**-0.25**	**0.85**	0.05	-0.04	-0.13
Slope	-0.17	**-0.43**	0.14	**-0.24**	**-0.50**	1.00	**0.39**	0.13	**0.44**	**-0.40**	**0.44**	**0.29**	**-0.42**	**-0.52**	-0.14	**-0.27**
Riparian shade	**-0.42**	**-0.28**	0.14	**0.16**	**-0.84**	**0.39**	1.00	-0.11	-0.02	-0.10	0.01	**0.25**	**-0.74**	-0.11	-0.08	0.12
% Indigenous forest	**0.22**	**-0.39**	**0.58**	0.06	**0.27**	0.13	-0.11	1.00	**0.49**	**-0.43**	**0.17**	**0.19**	0.12	**-0.33**	-0.03	0.08
% Native vegetation	0.14	**-0.61**	0.14	**-0.54**	0.09	**0.44**	-0.02	**0.49**	1.00	**-0.88**	**0.71**	**0.47**	0.01	**-0.84**	**-0.60**	**-0.56**
% Pasture	-0.05	**0.64**	**-0.24**	**0.46**	-0.02	**-0.40**	-0.10	**-0.43**	**-0.88**	1.00	**-0.68**	**-0.51**	0.05	**0.85**	**0.64**	**0.49**
Elevation	-0.15	**-0.61**	-0.04	**-0.80**	0.00	**0.44**	0.01	**0.17**	**0.71**	**-0.68**	1.00	**0.49**	-0.15	**-0.73**	**-0.66**	**-0.87**
Distance to sea	**-0.35**	**-0.61**	**0.34**	-0.10	**-0.25**	**0.29**	**0.25**	**0.19**	**0.47**	**-0.51**	**0.49**	1.00	**-0.18**	**-0.62**	**-0.59**	**-0.23**
Catchment area	**0.41**	**0.19**	**-0.21**	-0.07	**0.85**	**-0.42**	**-0.74**	0.12	0.01	0.05	-0.15	**-0.18**	1.00	0.02	-0.01	-0.04
Total nitrogen	0.06	**0.71**	-0.13	**0.51**	0.05	**-0.52**	-0.11	**-0.33**	**-0.84**	**0.85**	**-0.73**	**-0.62**	0.02	1.00	**0.68**	**0.58**
Total phosphorus	**0.47**	**0.63**	**0.18**	**0.60**	-0.04	-0.14	-0.08	-0.03	**-0.60**	**0.64**	**-0.66**	**-0.59**	-0.01	**0.68**	1.00	**0.65**
Water temperature	**0.29**	**0.44**	**0.50**	**0.94**	-0.13	**-0.27**	0.12	0.08	**-0.56**	**0.49**	**-0.87**	**-0.23**	-0.04	**0.58**	**0.65**	1.00

Appendix 3. Spearman rank correlations for isotopic values and all selected environmental variables for stream invertebrates. Statistically significant differences ($p <$ 0.05) are shown in **red and bold.**

	δ^{13}C	δ^{15}N	NZTM Easting	Jan air temperature	Flow	Slope	Riparian shade	% Indig forest	% Native veg	% Pasture	Elevation	Distance to sea	Catchment area	Total N	Total P	Water temp
δ^{13}C	1.00	**0.35**	**0.29**	**0.45**	**0.30**	**-0.41**	**-0.38**	-0.16	**-0.34**	**0.39**	**-0.36**	0.08	**0.42**	**0.35**	**0.25**	**0.41**
δ^{15}N	**0.35**	1.00	**0.18**	**0.44**	**0.14**	**-0.43**	**-0.20**	**-0.41**	**-0.71**	**0.69**	**-0.57**	**-0.19**	**0.19**	**0.73**	**0.69**	**0.42**
NZTM Easting	**0.29**	**0.18**	1.00	**0.78**	**0.10**	**-0.14**	**-0.17**	**0.18**	**-0.32**	**0.26**	**-0.45**	**-0.21**	**0.12**	**0.27**	**0.42**	**0.73**
Jan air temperature	**0.45**	**0.44**	**0.78**	1.00	**0.13**	**-0.38**	**-0.19**	0.02	**-0.56**	**0.49**	**-0.74**	**-0.24**	**0.16**	**0.51**	**0.59**	**0.88**
Flow	**0.30**	**0.14**	**0.10**	**0.13**	1.00	**-0.60**	**-0.80**	**0.18**	-0.09	**0.11**	**-0.17**	0.07	**0.88**	**0.12**	**0.13**	**0.16**
Slope	**-0.41**	**-0.43**	**-0.14**	**-0.38**	**-0.60**	1.00	**0.52**	**0.12**	**0.49**	**-0.46**	**0.47**	0.05	**-0.57**	**-0.52**	**-0.30**	**-0.38**
Riparian shade	**-0.38**	**-0.20**	**-0.17**	**-0.19**	**-0.80**	**0.52**	1.00	-0.03	**0.20**	**-0.24**	**0.21**	0.01	**-0.73**	**-0.19**	**-0.22**	**-0.21**
% Indig forest	-0.16	**-0.41**	**0.18**	0.02	**0.18**	**0.12**	-0.03	1.00	**0.47**	**-0.45**	0.01	**-0.10**	**0.10**	**-0.35**	**-0.17**	**0.19**
% Native veg	**-0.34**	**-0.71**	**-0.32**	**-0.56**	-0.09	**0.49**	**0.20**	**0.47**	1.00	**-0.92**	**0.63**	**0.21**	**-0.16**	**-0.86**	**-0.74**	**-0.52**
% Pasture	**0.39**	**0.69**	**0.26**	**0.49**	**0.11**	**-0.46**	**-0.24**	**-0.45**	**-0.92**	1.00	**-0.57**	**-0.19**	**0.16**	**0.85**	**0.71**	**0.44**
Elevation	**-0.36**	**-0.57**	**-0.45**	**-0.74**	**-0.17**	**0.47**	**0.21**	0.01	**0.63**	**-0.57**	1.00	**0.61**	**-0.20**	**-0.66**	**-0.66**	**-0.89**
Distance to sea	0.08	**-0.19**	**-0.21**	**-0.24**	0.07	0.05	0.01	**-0.10**	**0.21**	**-0.19**	**0.61**	1.00	**0.11**	**-0.27**	**-0.31**	**-0.51**
Catchment area	**0.42**	**0.19**	**0.12**	**0.16**	**0.88**	**-0.57**	**-0.73**	**0.10**	**-0.16**	**0.16**	**-0.20**	**0.11**	1.00	**0.17**	**0.18**	**0.17**
Total N	**0.35**	**0.73**	**0.27**	**0.51**	**0.12**	**-0.52**	**-0.19**	**-0.35**	**-0.86**	**0.85**	**-0.66**	**-0.27**	**0.17**	1.00	**0.78**	**0.53**
Total P	**0.25**	**0.69**	**0.42**	**0.59**	**0.13**	**-0.30**	**-0.22**	**-0.17**	**-0.74**	**0.71**	**-0.66**	**-0.31**	**0.18**	**0.78**	1.00	**0.61**
Water temp	**0.41**	**0.42**	**0.73**	**0.88**	**0.16**	**-0.38**	**-0.21**	**0.19**	**-0.52**	**0.44**	**-0.89**	**-0.51**	**0.17**	**0.53**	**0.61**	1.00